# Breeding for resistance against angular leaf spot disease of common bean in the Southern Highlands of Tanzania

**by**

**Rose J. Mongi**

**Bsc. Agronomy (University of Missouri – Columbia, USA)**

**MSc. Plant Science (University of Idaho, USA)**

**A thesis submitted in fulfillment of the requirements for the degree of**
**Doctor of Philosophy (PhD) in Plant Breeding**

**School of Agricultural, Earth and Environmental Sciences**
**College of Agriculture, Engineering and Science**
**University of KwaZulu-Natal**
**Pietermaritzburg Campus**
**Republic of South Africa**

January, 2016

# Thesis Abstract

Angular Leaf Spot (ALS) caused by the fungus *Pseudocercospora griseola* is among the major diseases of common bean (*Phaseolus vulgaris* L.) in Tanzania. The fungus is highly variable and has the ability to infect bean varieties of both Andean and Mesoamerican gene pools. Inadequate resistance against the pathogen in the common bean cultivars grown in Tanzania has been associated with yield losses estimated at 80% with a significant negative effect on the livelihoods of the people. Farmers' awareness of the disease and other related socioeconomic aspects have also not been documented in the study area. The objectives of this study were to: 1) assess the common bean farming systems, and farmers' awareness of angular leaf spot disease; 2) examine the economics of yield losses associated with the disease on five selected bean varieties that are commonly grown by farmers; 3) determine the response of common bean landraces widely grown in the Southern Highlands of Tanzania (SHT) against the pathogen and identify promising lines that can be used for resistance breeding; 4) determine genetic diversity and relationships among isolates of *Pseudocercospora griseola,* the fungal pathogen of angular leaf spot; 5) determine combining ability, gene action, and heritability for resistance to angular leaf spot among bean accessions; and 6) determine allelic relationships among the resistance genes present in selected common bean lines widely grown in Tanzania. The results indicated that ALS was widely distributed in the common bean farming systems of the SHT but farmers were unaware of the disease and sources of inocula. Most of the respondents were resource poor and the average bean yield ranged from 200 – 400 kg ha$^{-1}$. Severe yield losses occurred during the heavy rains with yield losses of more than 60%. Favorable environmental conditions coupled with the use of susceptible varieties and lack of knowledge on the disease may be the causes of increased *P. griseola* proliferation. This was supported in part by the twenty races that were characterized through the use of ALS differential cultivars in which race was 63:63 was identified as the most virulent among high numbers of races recorded in Mbeya Stepped Plains, the agro-ecological zone that borders countries of Malawi and Zambia where bean germplasm is exchanged informally between farmers. Three unique bands were found only in specific *P. griseola* isolates that were collected in Mbeya and Nkasi districts . Moderate genetic diversity was observed on the most virulent group of

isolates with a mean of 60.92% polymorphism. Among the one hundred different landraces screened for ALS disease resistance, only three genotypes (SHB002, SHB005 and SHB091) showed resistance to the majority of the races. A half diallel of 8 parents which included resistant and susceptible genotypes while using the most widely distributed and virulent race 63:55   resulted in significant general and specific combining abilities for ALS disease resistance at P≤ 0.05 with additive gene effects preponderant. The predominant additive gene effects for the expression of ALS disease resistance coupled with high heritability (0.97) and high negative heterosis (-29 for better parent) suggested the possibilities of making quick progress to selection within segregating populations in early generations. An allelism study among ALS resistance genes generated 15:1 and 63:1 ratios which suggested the presence of two and three non-allelic genes respectively in the landraces. Based on these results, it can be concluded that smallholder bean farmers lack the knowledge of ALS disease and its causal pathogen. In this regard, it is essential that farmers be trained on disease management strategies in order to reduce yield losses caused by the disease. Since *P. griseola* is highly variable, further studies on the mechanism by which genetic variation arises is necessary to allow prediction of new isolates and type of genes required for deployment in breeding for resistance. Consequently, continuous disease surveys and monitoring of the pathogen are essential. The presence of unique bands revealed during the genetic diversity studies of the pathogen can be used as molecular markers for further studies. In general, there is a potential to breed for ALS disease resistance using common bean landraces that have been kept by farmers for a long time because of the preponderance of additive gene effects. The selected populations from the diallel cross combinations should be advanced to develop pure line cultivars of common bean with ALS resistance and high yields for release in the SHT or similar environments in Tanzania.

# Declaration

I Rose J. Mongi,

1. Declare that the thesis presented herein is my own work and has been generated by me as the result of my original research.
2. Where I have consulted and use published work of others, is clearly referenced.
3. This thesis contain no cut and paste materials from the internet
4. This thesis contains no materials that have been submitted previously in whole or in part for the award of any other academic degree or diploma.

Date: January, 2016

_____

**Rose J. Mongi**

## Supervisors:

_____

**Prof. Pangirayi Tongoona**

_____

**Prof. Hussein Shimelis**

_____

**Dr. Julia Sibiya**

# Acknowledgement

# Dedication

This thesis is dedicated to my family: My husband Paul and dear children Nathan, Shekinah and Shimi. Their encouragement kept me working harder

# Table of Contents

ix

# List of Tables

# List of Figures

# Introduction

Common bean (*Phaseolus vulgaris* L.) is one of the most widely consumed species of the genus *Phaseolus* (Singh, 2001; Freytag and Debouck, 2002). Although beans vary considerably in seed size, shape and color, their nutritional components are remarkably similar (Geil and Anderson, 1994). The edible leaves, pods and seeds are low in fat content but packed with protein, carbohydrates, vitamins and minerals (Lanza et al., 2006). In many parts of the world, beans contribute a substantial portion of the total protein intake for the majority of rural and urban populations. For example, more than 50% of the dietary protein consumed in Sub-Saharan Africa, Latin America and Asia comes from the common bean with per capita consumption of 50 – 60 kg year$^{-1}$ in Eastern Africa and 4 – 17 kg year$^{-1}$ in Latin America (Broughton et al., 2003; Beebe et al., 2013). While the common bean complements cereal diets in terms of protein, carbohydrates (55 – 65% of the dry weight) and minerals in the developing world, beans are consumed at a much lower rate in the developed world despite the crop being highly recommended as a risk reducer to chronic diseases such as cancer, diabetes and cardiovascular diseases (Bobe et al., 2008; Ferlay et al., 2010).

Considering soil health, the common bean has the ability to associate with rhizobia that fix atmospheric nitrogen into the soil making it an essential component of sustainable agriculture. Dry leaves, threshed pods and stalks are also used as animal feed and in some parts of Africa and Asia as a source of fuel for cooking. Additionally, the common bean enhances the welfare of farmers especially when the surplus is sold to generate income that meets household needs. Besides all the benefits the crop can offer, yield per unit area is very low.

Globally, the common bean is cultivated on 28 million ha with an average of 750 kg ha$^{-1}$, while average yields of 500 kg ha$^{-1}$ or lower have been reported in Tanzania and in countries of Uganda, Kenya, Angola, Malawi and Democratic Republic of Congo (Hillocks et al., 2006; Akibode and Meredia, 2011). As a result of these low yields, it is difficult to attain sufficient protein needed for the development of the body, particularly in developing countries where cereal based diets are mainly complimented by proteins from the bean crop. As a result millions of people do not get enough protein and minerals for the normal functioning of the body, causing growth failure, malnutrition and kwashiokor especially to children under five (Lanza et al., 2006).

Although there are several factors that affect yields of the bean crop worldwide, angular leaf spot (ALS) caused by *Pseuodocercospora griseola* is among the major ones that has been reported in more than seventy countries (Guzman et al., 1996). Under favorable conditions (high humidity and optimum temperature), the mode of action of the pathogen can be grouped into five processes: infection, lesion establishment, lesion extension, defoliation of infected leaf and spore dispersal (Allorent and Savary, 2005). The infected host exhibits reduced photosynthetic rate due to abnormalities in form and function of chloroplasts of the diseased tissue followed by decline in photophosphorylation, photochemical reaction and carbon dioxide assimilation reducing physiological performance of the canopy (Daly, 1976; Bergamin Filho et al., 1997; Jesus Junior et al., 2001). The infected plants shows angular shaped lesions on leaves as well as lesions on other aerial parts that include stem, petiole and pods (Cardona-Alvarez and Walker, 1956). Lesion multiplication and extension on the foliage cause premature defoliations that brings the inocula under the crop canopy with a long period of survival. The resultant seeds are shriveled and discolored with the pathogen being embedded within the seed that facilitates its transfer from one place to another and from season to season. In this regard, the primary sources of inocula is the contaminated seed and infected plant debris where the pathogen has been reported to survive up to 19 months in the absence of the living host (Sindhan and Bose, 1979). In Tanzania most bean farmers practice seed recycling sourced from different places, raising questions of whether they are aware of the pathogen itself, the mode of transmission and its adverse effects on the bean crop. Additional question is on the ability of farmers to acquire resources that can be used in the ALS disease management.

Decline in land per household due to increase in population pressure has resulted into continuous and intensive cultivation on the same piece of land that encourages inocula built-ups from defoliated and infected leaves, pods and residuals from previous crops. This makes the cultural practices (crop rotation) that have been highly advocated as the control measure against ALS disease to be not feasible due to limited lands. Since *P. griseola* is a seed borne pathogen that is embedded within the seed, such infections often survives the external fungicide seed treatments that have been recommended, whereas timely spraying requires thorough knowledge of the disease with doubts whether the smallholder farmers who are the majority of the bean producers have that information.

Managing ALS disease through breeding requires diverse sources of resistance genes to counteract the complications associated with genetic variability of the causal pathogen (Alvarez – Ayala and Shwartz, 1979; Srivastava et al., 1995; Pastor – Corrales et al., 1998; Aggarwal et al., 2004). Like any other common bean pathogens, *Pseudocercospora griseola* co-evolved with its hosts and isolates of the fungus have been divided in correspondence with the Mesoamerican and Andean gene pools (Guzman et al., 1995). Those isolates pathogenic on large seeded beans form a group of Andean and those isolates pathogenic on both small and large seeded beans are part of Mesoamerican groups respectively (Mahuku et al., 2002). Since seed varieties from both gene pools are grown in the SHT, and that no precise information is available on the impact of the disease on yields, examining economics associated with yield losses is necessary in recommending proper and feasible control measures against the pathogen.

Much of genetic improvement in common bean has been achieved through conventional breeding techniques. It has been indicated that, resistance to *P. griseola* in common bean is conditioned by major and minor genes, the majority of which are from primary and secondary gene pools. These genes are known to confer high levels of resistance to *P. griseola* compared to the wild relatives (Busogoro et al., 1999 (b); Nietsche et al., 2001). The identified genes have been used extensively in breeding programmes world over including countries of Malawi, Zambia, Mexico and Brazil.

In the Southern Highlands of Tanzania, a bean improvement programme was initiated in 1980 and the major goal was to breed for high yielding and disease resistance. The efforts led to the identification of a few varieties from CIAT germplasm introduction with considerable levels of ALS resistance. Among them were T8 and Kabanima, released in 1980. Since then about five more varieties were released with moderate levels of ALS disease resistance including variety PASI released in 2014 that was developed from crosses made between a local variety and introduced germplasm/line. Although host resistance is the most efficient means of reducing damages caused by *P. griseola*, it is clear that more resistant varieties need to be developed with several genes pyramided in one background as beans in Tanzania are grown in a wide range of agro-ecological zones that may have different races of the pathogen.

Considering the economical importance of angular leaf spot to the bean growers, several well adapted parents containing resistance genes to the diseases must be thought of, to combat a highly variable pathogen like *P. griseola*. Traditional landraces, defined as cultivars that have been grown and maintained by farmers (for various reasons) from generation to generation have been indicated to be potential sources of genes that may contribute valuable resistance for ALS disease (Singh et al., 1991; Geffroy et al., 1999; Melotto et al., 2000). It is estimated that, more than 50% of the cultivated lands in the bean growing agro-ecologies of the zone are sown with seeds obtained from diverse landraces. Therefore, searching for new sources of ALS resistance from the locally adapted germplasm, that are considered to have more desirable genetic background is important in establishing a breeding programme that will generate varieties with acceptable traits. It is therefore expected that the common bean landraces maintained and grown by farmers in the SHT like anywhere else, have resistance genes against *P. griseola* that can be utilized in the breeding program to reduce yields losses caused by the pathogen. However, detailed information on the combining ability and gene action of the parents involved in a cross is required for the identification and selection of superior populations.

Since races of *P. griseola* that exist in a particular area may not be necessarily the same as those found in another area or geographical region (Pastor – Corrales et al., 1998), race identification and studies on virulence patterns is a prerequisite before engaging into any breeding programme against the pathogen. Differential cultivars have been used extensively to discriminate isolates based on phenotypic difference but additional molecular tools are required to better understand the pathogen.  It is with these assumptions that the objectives of this study were

The specific objectives of the study were to:
  i)     assess the common bean farming systems and farmers' awareness of angular leaf spot disease; examine poverty levels within smallholder bean farmers and relate all these to the management of ALS;
  ii)    examine the economics of yield losses associated with the disease on five selected bean varieties that are commonly grown by farmers as an effort towards developing sustainable control measures against *P. griseola;*
  iii)   Determine virulence patterns and pathotype diversity of *Pseudocecospora griseola* of the SHT

iv)      Screening common bean landraces for resistance to angular leaf spot disease

v)      determine genetic diversity and relationships among isolates of *Pseudocercospora griseola,* the fungal pathogen of angular leaf spot of bean from the Southern Highlands of Tanzania; and

vi)      determine combining ability, gene action, heritability, heterosis and allelism of the resistance genes present in selected common bean lines widely grown in Tanzania.

The thesis is organized into eight chapters that are interrelated with a journal paper design. The first chapter presents an overview of common bean as a crop in a form of literature review. An appraisal of common bean farming systems in ALS prone environments is presented in chapter two. Chapter three describes the economic losses associated with ALS disease. Chapter four and five discusses on the virulence trends and genetic diversity among isolates of *P. griseola* collected from the bean growing agro-ecologies of the Southern Highlands which is the major bean production zone of Tanzania. Chapter six presents the response of the common bean landraces to ALS disease infections, while genetic analysis of the resistance present in selected landraces are discussed in chapter seven. The last chapter presents the implications for the research findings in improving the bean crop.

# References

Aggrawal, V.D., M. A. Pastor-Corrales, R. Chirwa and M. Buruchara 2004. Andean beans (*Phaseolus vulgaris* L) with resistance to the angular leaf spot pathogen (*Phaeoisariopsis griseola*) in Southern and Eastern Africa. Euphytica 136: 201-213

Akibode, S. and M. Maredia. 2011. Global and Regional Trends in Production, Trade and Consumption of Food Legume Crops. http://impact.cgiar.org/sites/default/files/images/Legumetrendsv2.pdf Retrieved May, 2013.

Allorent, D and S. Savary. 2005. Epidemiological characteristics of angular leaf spot of bean: a systems analysis. European Journal of Plant Pathology.113:329–341

Alvarez-Ayala, G., and H. F. Schwartz. 1979. Preliminary investigations of pathogenic variability expressed by *Isariopsis griseola*. Annual Report Bean Improvement Cooperative 22:86-88

Beebe, S.E., I. M. Rao, M. W. Blair and J. A. Acosta-Gallegos. 2013. Phenotyping common beans for adaptation to drought. Frontiers in Plant Physiology 4: 1-20

Bergamin Filho, A., S. M. Carneiro, C. V. Godoy, L. Amorim, R. D. Berger and B. Hau, 1997. Angular leaf spot of Phaseolus beans: Relationships between disease, healthy leaf area, and yield. Phytopathology 87:506-515

Bobe, G., K. G. Barrett, R. A. Mentor-Marcel, U. Saffiotti, U, M. R. Young, N. H. Colburn, P. S. Albert, M. R. Bennink and E. Lanza. Dietary cooked navy beans and their fractions attenuate colon carcinogenesis in azoxymethane-induced ob/ob mice. Nutrition and Cancer 2008, 60, 373–381.

Broughton, W.J., G. Hernandez, M. W. Blair, S. E. Beebe, P. Gepts and J. Vanderleyden. 2003. Beans (Phaseolus spp.) – Model Food Legumes. Plant and Soil 252: 55-128.

Busogoro, J. P., M. H. Jijakli and P. Lepoivre. 1996b. Identification of novel sources of resistance to angular leaf spot disease of common bean within the secondary gene pool. Plant Breeding. 118 (5):417 – 427.

Daly, J. M. 1976. The carbon balance of diseased plants: changes in respiration, photosynthesis and translocation. Physiological Plant Pathology 4: 450 – 479.

Ferlay, J., H. R. Sighn, F. Bray, D. Forman, C. Mathers and D. M. Parkin. 2010. Cancer Incidence and Mortality Worldwide. IARC Cancer Base, No. 10; International Agency for Research on Cancer: Lyon, France.

Freytag G. F. and D. G. Debouck. 2002. Taxonomy, distribution and ecology of the genus Phaseolus (Leguminosae – Papilionoideae) in North America, Mexico and Central America. Botanical Research Institute of Texas. Brit Press. Ft. Worth, Texas.

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

Geil, P. G. and J. W.  Anderson. 1994. Nutrition and health benefits of dry beans: A review. Journal of the American College of Nutrition, 13: 549-558.

Guzmán P., R. L. Gilbertson, R. Nodari, W. C. Johnson, S. R. Temple, D. Mandala, A. B. C. Mkandawire, P. Gepts. 1995. Characterization of variability in the fungus *Phaeoisariopsis griseola* suggests co evolution with the common bean (*Phaseolus vulgaris* ). Phytopathology 85: 600–607

Hillocks, R. J., C.S. Madata., R. Chirwa., E. M. Minja and S. Msolla. 2006. Phaseolus bean improvement in Tanzania, 1959 – 2005. DOI: 10.1007/s10681-006-9112-9. Accessed December, 2012

Jesus Junior W.C., F. X. R. Vale, C. A. Martinez, R. R. Coelho, L. C. Costa, B. Hau, L. Zambolim. 2001. Effects of angular leaf spot and rust on leaf gas exchange and yield of common bean (*Phaseolus vulgaris*). Photosynthetica. 39:603–606

Lanza, E., T. J. Hartman, P. S.  Albert, R. Shields, M. Slattery, B.  Caan, E. Paskett, F. Iber, J. W. Kikendall, P. Lance, C. Daston and A. Schatzkin. 2006. High dry bean intake and reduced risk of advanced colorectal adenoma recurrence among participants in the polyp prevention trial.  Journal of Nutrition. 136: 1896-1903.

Mahuku, G.S., C. Jara, C.  Cajiao and S. Beebe. 2003. Sources of resistance to angular leaf spot (*Phaeoisariopsis griseola*) in common bean core collection, wild *Phaseolus vulgaris* and secondary gene pool. Euphytica 130**:**303–313

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

Melotto, M.**,** R. S. Balardin and J. D. Kelly. 2000. Host-pathogen interaction and variability of *Colletotrichum lindemuthianum*. *In*: D. Prusky, S. Freeman, and M.B. Dickman (eds)

*Colletotrichum* host specificity, pathology, and host-pathogen interaction. APS Press, St Paul, MN, USA. pp 346-361

Nietsche, S., A. Borem, G.A. Carvalho, T.J. Paula, C.F. Ferreira, E.G. Barros and M.A Moreira. 2001. Genetic diversity of *Phaeoisariopsis griseola* in the state of Minas Gerais, Brazil. Euphytica 117:77 - 84

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

Sindhan, G.S. and S.K. Bose. 1979. Perpetuation of *Phaeoisariopsis griseola* causing angular leaf spot of French beans. Indian Phytopathology. 32: 252-254.

Singh, S. P., P. Gepts and D. G. Debouck. 1991. Races of common bean (*Phaseolus vulgaris* L., Fabaceae). Economic Botany. 45:379-396

Singh, S. P. 2001. Broadening the genetic base of common bean cultivars: A review. Crop Science. 41: 1659 - 1675

# 1   Literature Review

## 1.1   Introduction

There has been  great concern about the frequent outbreak of angular leaf spot disease of common bean in the Southern Highlands of Tanzania. The question arises on how to better control the disease that devastates the crop with significant yield reduction particularly to smallholder farmers who are the major producers. In order to come up with good and environmentally sound ALS disease control strategies, a thorough understanding of the following is required; importance of common bean in the bean farming community and beyond, the bean farming systems, production constraints associated with the crop, how to recognize and distinguish symptoms of ALS disease on infected bean crop, the pathogen itself and mode of transmission, appropriate methods that can be used to discriminate isolates of the pathogen to detect differences, control measures so far proposed and whether they are applicable in the area of interest, how the pathogen interact with its host, sources of resistance genes and breeding procedures that fits best in deploying resistance genes into the susceptible varieties. This information can be obtained from the literature review on what have been done so far and what are the gaps to be filled during the development of the technology. It is of these reasons this chapter is presented herein.

## 1.2   The common bean and its importance

Common bean (*Phaseolus vulgaris* L.; 2n=2x=22) is one of the food legume crops. It was domesticated some 10,000 years ago in the Andes Mountains of Peru and the Lerma Santiago basin of Mexico (Ladizinsky 1998). It is believed that the common bean began as weeds in fields planted to cassava and sweet potatoes in which selections were done and the best materials domesticated (Toro et al., 1990). Domestication was based on reduced seed dispersal and dormancy, compact growth habit, reduced sensitivity to day length and increase in size of the harvested parts (Papa et al., 2005). During the domestication, morphological, physiological biochemical and genetic changes occurred that resulted into diverse seed color, shapes and sizes (Gepts and Debouk, 1991). Studies conducted on seed protein (phaseolin), DNA variation, nucleotide diversity, geographical distribution of the genes and morphological traits indicated separation of common bean and formation of two gene pools; Andean and Mesoamerican (Singh et al., 1991; Bitocchi et al., 2012; Gepts, 2000). The Mesoamerican gene pool constitutes small

seeded varieties (< 25g/100 seed) domesticated from small seeded wild forms whereas the Andean gene pool has much larger seeded varieties (medium size of 25 – 40 g/100 seed and large seed > 40 g/100 seed) domesticated from large seeded wild forms (Gepts et al., 1986; Smart, 1990).

The genus *Phaseolus* contains five domesticated species in decreasing order of importance, common bean (*Phaseolus vulgaris* L), lima beans (*P. lunatus* L.), runner bean (*P. coccineus* L), tepary bean (*P. acutifolius* A. Grey) and year bean (*P. polyanthus* Greenman), all with distinct adaptation and reproductive systems. *P. vulgaris* belongs to the family Leguminosae, subfamily Papilionoidae, tribe Phaseolinae (Gepts, 2000).

Botanically, the common bean varies in growth habit that ranges from determinate upright bush (Type 1), indeterminate upright bush (Type II), indeterminate prostate none climbing or semi climbing (Type III) and indeterminate strong climbers (Type IV) (Singh, 1982). The leaves are trifoliate, developing from terminal or auxiliary buds but the first two true leaves are unifoliate (Bailey, 1969). Consequently, the plant has fibrous roots with a marked main root and bear nitrogen fixing nodules from a few weeks after emergence through flowering (Miklas and Singh, 2007). Varying root architecture has been observed in certain bean varieties and has been associated with phosphorus use efficiency and drought tolerance (Miller et al., 2003). The flowers of the common bean are about 1 cm long and the structure and shape is of a typical legume flower. The color of the flower varies from pink, purple or white depending on the seed color used as planting material. In each flower, there are five generally fused sepals. The corolla has two petals which are fused forming a prolonged keel, two wing petals and a standard. The keel encloses one vexillary stamen on the upper side and nine stamens united into a long tube around the style. Each stamen has a bilobed anther sac borne on a long filament. The pistil consists of stigma, a style and an ovary containing five to eight or up to ten ovules (Faria et al., 2010). The style is coiled and the stigma is a slightly flattened pad with hairs. The stamen filament follows the stylar coil in such a way that the anther sacs are appressed to the stigma. This type of structure made beans to be considered as cleistogamous and are highly self- pollinated with about 1% out-crossing (Gepts, 2001). Pollination occurs shortly before anthesis resulting in fertilization about eight to nine hours later (Weinsten, 1926). Knuth (1908) stated that, although the anthers surround the style and the pollen is released before the flower opens, the pollen does not get onto the

stigma before tripping occurs. This usually happens when pressure is applied from the tip of the keel by an insect such as bees and large beetles. After pollination, each flower can give rise to one pod which at maturity may be 8 – 20 cm long, containing 4 – 6 beans, with a wide range of shapes and colors (Decoteau, 2000).

Globally the common bean is grown on 28.2 million hectares in 120 countries with a total production of 20.1 million tons while in 2013, 22.8 million metric tons of dry beans were produced (FAOSTAT, 2010; FAOSTAT, 2014). In relation to consumption, common bean is more important than any other legumes to more than 300 million people within eastern and southern Africa, per capital consumption is reported to be as high as 50 kg per person per year reaching 66 kg in the Kisii regions of Kenya (Beebe et al., 2000). Although dietary protein can also take a form of animal products such as eggs, milk and meat, access to meat is limited to a large segment of the population especially in the tropics due to a number of reasons including high prices and scarcity of the products.

In the developed world, bean is gaining importance as it has been associated with the ability to reduce cholesterol and incidences of cancer and diabetes and is considered risk reducer to those chronic diseases (Diaz-Batalla et al., 2006; Thompson et al., 2009). In this regard, beans form a significant part of diets and its protein content play a significant role in human nutrition especially when consumed with carbohydrate staples such as cassava, banana, maize, rice and wheat in a 2:1 ratio (Broughton et al., 2003). Additionally, common bean contains organic and mineral substances (sodium, potassium, calcium, magnesium, iron, boron, copper, zinc); vitamins (A, B, C, E) and fiber (soluble and insoluble) which have important nutritional roles in the body. In the Southern Highlands of Tanzania (SHT), the common bean is the second most important source of vegetable protein and the third most important source of calories, vitamins, zinc and other essential elements. It is one of the best non-meat sources of iron, providing 23 - 25% of daily recommended levels from a single serving when consumed with starchy staples at the recommended ratios (Schwartz et al., 1996).

The roots of the common bean have the ability to fix nitrogen from the atmosphere by developing nodules in symbiosis with compatible rhizobia. In this process of biological nitrogen fixation, the root releases flavonoids and/or isoflavonoids which induce transcription of nodulation genes in rhizobia (Eckardt, 2006). This leads to the formation of lipo-chito-oligosaccharide molecules that

in turn signal the host plant to begin nodule formation (Long, 1996). The center of each mature nodule is packed with billions of di-nitrogen fixing bacteria carrying *nin-fix* genes for nitrogen fixation while the host supplies the energy needed to drive the process (Olivares et al., 2011). All of the nitrogen fixed goes direct into the plants and eventually returns to the soil when the plants die and decompose making it an essential component of sustainable agriculture in rural areas. This is an added advantage to tropical soils which are generally deficient in nitrogen, an element highly needed by plants for increasing yields. Considering the multiple cropping systems traditionally practiced in the Southern Highlands of Tanzania, common bean varieties excluding indeterminate strong climbers, fit well in the cropping system and are highly valued more than any other legume crops as they form a lower canopy structure than maize, banana, cassava and coffee when intercropped, making efficient use of land and other environmental resources such as radiation, water and nutrients (Willey, 1990). Consequently, common bean is highly traded in the regional and international markets increasing the importance of the crop as a source of income to farmers and other stakeholders involved in the bean trading (Broughton et al., 2003).

## 1.3   Faming systems and agroecologies

Farming systems are important components of agriculture. According to FAO, a farming system is defined as a proportion of individual farm systems that have similar resource base enterprise patterns, household livelihoods, and constraints in which similar development strategies and intervention would be appropriate.

Several farming systems have been described in Tanzania but the maize/legume farming systems have been indicated to cover the largest part of the country's agricultural lands (Samki and Harrop, 1984; Ronner and Giller, 2012). The same scenario has been reported in the SHT, the zone which is located between $7^0$ and $11^0$ E and $30^0 - 38^0$ S covering an area of 244,224 km$^2$. The climate in the SHT varies from tropical to temperate with more than 80% of the population engaged in agriculture (Mussei et al., 1997). The annual rainfall ranges from 750 mm to over 2600 mm with temperatures of $4 - 24^0$C in mid altitude and highlands while temperatures of up to $30^0$C have been reported in lowlands. These environments favor the development of various diseases of crops including those caused by bacterial and fungi. Although there are various crops grown in the SHT, common bean occupies the largest area planted to pulses and is mainly grown by smallholder farmers. However, extremly low yields of about 500 kg ha$^{-1}$ have been reported

(Hillocks et al, 2006) suggesting the need of detail studies on the crop and production constraints to improve yields in which majority of the people depend on.

## 1.4   Production constraints to common bean

Common bean is extremely susceptible to both abiotic and biotic stresses and these stresses are more severe under smallholder farming systems where low input production is practiced. The biotic (diseases and pests) and abiotic (drought, heat, nitrogen and phosphorous deficiency; acid soils) stresses are known to lower yields that could otherwise be available to meet the growing demand of many people.  Biotic stresses, in particular diseases caused by bacteria, viruses and fungal are universal constraints to bean production (Wortmann et al., 1998). Although prevalence and importance of each disease varies considerably with season, year and the cultivar grown, fungal diseases are known to be widely distributed and epidemics occur often with yield losses ranging from 80 - 100% (Schwartz and Pastor-Corrales, 1989). Among the top fungal diseases of common bean reported are angular leaf spot, bean anthracnose, bacterial blight and halo blight (Abawi and Pastor – Corrales, 1990).

Angular leaf spot is considered a cosmopolitan disease that has been reported in Africa, Latin America, North America, Asia, Europe and Australia and is ranked as the number one constraint to bean production in many countries. The causal pathogen is favored by a wide range of environmental conditions in the tropic and sub-tropic bean growing agro–ecologies with temperatures that range from $16 – 32^{0}C$ (Verma and Sharma, 1984; Pastor – Corrales et al., 1998; Wortman et al., 1998; Jarvie, 2002; Stenglein et al., 2003). As in any other bean producing countries in the developing world, common bean production in the SHT is to a large extent under small-scale farming systems with diversity of edaphic and climatic conditions that favor angular leaf spot disease. Yield losses due to the disease are expected to increase due to a number of factors including lack of knowledge about the ALS in the bean farming community and climate change particularly the rise in temperature and increase in humidity. Therefore, considerable control measures must be in place to ensure productivity of the crop that contributes significantly to the livelihoods of the people.

## 1.5   Angular leaf spot disease and symptomatology

Angular leaf spot (ALS) is one of many destructive foliar diseases of common bean. The disease was first reported in Italy in 1878 and subsequently from other European countries, Africa, Australia, South America and the Caribbean regions (Sohi and Sharma, 1974). To date, ALS is distributed worldwide in many tropical and sub-tropical bean producing countries where temperature, relative humidity and sunlight are favorable for disease development. Under these environments, abundant inocula is always observed in infected plant debris, volunteer plants, off-season crops and contaminated seed (Mwang'ombe et al., 2007).

The first ALS symptoms on a bean plant appear several days after infection as brown spots with a tan or silvery center that is initially confined to tissues between major veins which give it an angular shape. Lesions then increase in size, coalesce and can cause partial necrosis, thereafter yellowing of the leaves. However, on the primary leaf, lesions are usually rounded, larger than those found on trifoliate leaves and may develop concentric rings within themselves. On pods, lesions appear as brown centers that are sometimes surrounded by darker borders (Correa et al., 1989). Infected pods bear poorly developed or shriveled seeds while in seeds the symptoms can appear as seed discoloration (Barros et al., 1958b). Although all aerial plant parts, including petioles and seed can be infected, lesions on the underside of the leaves are easily distinguishable from other lesions as they are characterized by protruding tiny dark synnemata, which are collections of stocks that produce spores. On the primary leaves, these structures are found on both the upper and lower surfaces. The infected host exhibits a reduced photosynthetic rate due to abnormalities in form and functions of the chloroplasts of the diseased tissue, commonly associated with decline in photosynthetic phosphorylation photochemical reactions and carbon dioxide assimilation (Mathre, 1968; Lopez and Berger, 2001). These changes are frequently associated with reduction in chlorophyll content, decrease in mesophyll conductance, reduced activity of ribulose1, 5 bisphosphatecarboxylase leading to reduction in photosynthesis in plants (Gordon and Duniway, 1982). The results are severe leaf defoliations, the process that transfers sporulation lesions to the bottom of the canopy with strong implications on bean growth and yield.

## 1.6    The angular leaf spot causing pathogen

The ALS causing pathogen in common bean is within the Kingdom fungi; Phylum ascomycota; Order, Moniliales and Family, stilbaceae. The fungus was first discovered by Saccardo, who named it *Isariopsis griseola* (Saccado, 1878). In his research, Ferraris (1909) described four Isariopsis –like species including *I. griseola* Sacc., which were characterized by having synnematous conidiophore fascicles, pigmented conidiophores and conidia. Ferraris (1909) re-named them into the genus *Phaeoisariopsis.* The genus was re-assessed again by Chupp (1954) thereafter by Ellis (1976) based on the structure of geniculate conidiogenous cells. Two genera were created confining species with conspicuously geniculate conidiogenous cells and thickened darkerned scars into *Passalora* Fr and those with inconspicuous conidiogenous cells were re-allocated into *Pseudoscercospora* Speg including *Phaeoisariopsis griseola* (L).  Deighton (1976) reduced *Cercospora solimanii* Speg. to synonymy with Phaeoisariopsis and re-allocated those species with inconspicuous conidial scars originally placed in *Phaeoisariopsis griseola* into *Pseudoscercospora* and later  justified by Braun (2000). Recently Crous et al. (2006) using sequence analysis of the SSU region of nrDNA of the fungus indicated that the genus *Phaeoisariopsis* cannot be distinguished from other hyphomycetes anamorph genera, *Pseudocercospora* and *stigmina.* He suggested the genus to be conserved to *Pseudocercospora* in which the name *Phaeoisariopsis griseola* (Sacc) Ferr was changed to *Pseudocercospora griseola* with two formae speciales namely formae speciales *griseola* and formae speciales *Mesoamericana.*

## 1.7    Genetic variability of *Pseudocercospora griseola*

Knowledge of pathogenic variation among isolates of *P. griseola* is important to any bean breeding programme to guide the deployment of resistance genes to angular leaf spot. In this regard, numerous studies have detailed genetic variability of the pathogen using susceptible and resistant backgrounds using differential cultivars of common bean, isozyme analysis (Correa–Victoria, 1987); random amplified polymorphic DNA (RAPD) markers (Alvarez–Ayala and Schwartz, 1979; Mahuku et al., 2002, Sartorato, 2004;) and inter simple sequence repeat (ISSR) Abadio et al., 2012). The results revealed two major groups of *P. griseola* that are extremely diverse and appear to have co-evolved with the Andean and Mesoamerican gene pools (Buruchara et al., 1988; Mahuku et al., 2002; Stenglein and Balatti, 2006; Abadio et al., 2012). The

*Pseudocercospora griseola* isolates from Andean gene pools are more virulent to Andean common bean whereas the Mesoamerican *P. griseola* isolates have a higher genetic variability and are virulent on common bean from Mesoamerican gene pools with ability to attack Andean beans (Pastor – Coralles and Jara, 1995; Pastor-Corrales et al., 1998; Singh and Schwartz, 2010).

The Andean *P. griseola* isolates have been identified as *P. griseola* fsp. *griseola* and the Mesoamerican *P. griseola* isolates are known as *P. griseola* fsp. *mesoamericana*. The two isolates can co-exist on infected leaves and cannot be differentiated based on symptoms or morphology (Guzman et al., 1999). In this regard, both isolates are expected to be found in the SHT as varieties from both Andean and Mesoamerican gene pools are grown by the farmers and have indicated symptoms of ALS disease.

Although *P. griseola* isolates have a wide range of hosts and can infect *Phaseolus vulgaris*; *P. lunatus*; *P. coccineus*; *P. acutifolius; Vigna mungo; Pisum sativum* and other similar crops, the origin of such wide variability is unclear as no evidence of a sexual life cycle has been detected (Abadio et al., 2012). However, mechanisms such as mutation, parasexual reproduction and migration may have contributed to the great genetic diversity of the pathogen (Mahuku et al., 2002).

### 1.7.1 Biology of *P. griseola* and mode of dispersal

*P. griseola* is found in nature in the form of mycelia or conida on living tissues of the host plant (susceptible on and off-season crop, volunteer plants), undecomposed infected bean residues and infected soils. For the seed, the pathogen is borne internally while external contamination may occur on seed during harvesting and the pathogen has been associated with the hilum area of the seed coat (Correa et al., 1989).

The mode of reproduction in *P. griseola* is mainly asexual and no known sexual cycles have been documented. However, studies conducted on genomics and populations of the kingdom has indicated some form of recombination to all fungal populations and existence of some genes involved in mating, but no inference about the occurrence of recombination in the life of *P. griseola* has been reported (Abadio et al., 2012). As stated earlier, *P griseola* infected bean leaf is characterized by synnemata on the lower surface. The synnemata are composed of loose

conidiophores that grow erect and more or less parallel into sheaf–like structures. They are dark–coloured at the base while gradually becoming lighter towards the tip. The thickness ranges from 20 – 40 μm. Conidia are borne at or near the tip of conidiophores and they are smooth, light gray mostly obclavate, cylindrical, often curved with one or three rarely four septae (Crous et al., 2013). They measure 30 – 70 μm long and 5 – 6 μm thick in the broadest part. Differences in conidial size and amount of septation in *P. griseola* have been related with the variability within the fungus (Buruchara, 1988).

### 1.7.2 Race determination for *P. griseola* using differential cultivars

Physiological races of fungal phytopathogens represent biotypes capable of attacking certain varieties of a susceptible host species as well as affecting the durability of resistance in host plants (Buruchara, 1988). These races are often variable and frequent monitoring is important for a breeding programme aiming at genetic resistance (Leung et al., 1993). To assess the degree of diversity of *P. griseola*, a set of 12 differential cultivars carrying different resistance genes to ALS diseases were proposed in the first ALS workshop held at CIAT in 1993 to standardize the methodology for *P. griseola* pathotype identification. The cultivars and their binary numbers are; Don Timoteo (1), G1179 (2), Bolon Bayo (4), Montcalm (8), Amendoin (16), G5686 (32), PAN 72 (1), G2858 (2) Flor de Mayo (4), Mexico 54 (8), BAT 332 (16) and Cornell 49242 (32). The number or race designation given to an isolate is determined by the cultivars of the differential set that are infected by that isolate. The sum of the numbers assigned to each infected cultivar is the race number separated by a slash or dash to distinguish Andean and Mesoamerican differential cultivars infected (Table 1.1). For example; if an isolate infects Andean cultivar G11796 and Montcalm (binary value, 2 and 8) and the Mesoamerican variety PAN 72 (binary value 1) the race would be designated 10/1. Adopting this system by many researchers working with ALS has revealed high levels of genetic and pathogenic variability in *P. griseola* and in the identification of bean lines carrying genes that could be useful in cultivar development (Mahuku et al., 2002). The use of differential cultivars in *P. griseola* race identification has been reported in Tanzania (Mwalyego, 1987; Ngulu 1999). However for more than a decade there has been no reports in the SHT that shows identification of *P. griseola* race by using differential cultivars or any other method.

**Table 1.1: Characteristics of the common bean differential cultivars for characterizing** *Pseudocercospora griseola* **pathotypes**

| Sr. No. | Name of differential cultivar | Seed size | Gene pool | Race | Binary value |
|---------|-------------------------------|-----------|-----------|------|--------------|
| 1. | Don Timoteo | *G* | Andean | C | 1 |
| 2. | G11796 | G | Andean | P | 2 |
| 3. | Bolon Bayo | G | Andean | P | 4 |
| 4. | Montcalm | G | Andean | NG | 8 |
| 5. | Amendoin | G | Andean | NG | 16 |
| 6. | G5686 | G | Andean | NG | 32 |
| 7. | PAN72 | S | Mesoamerican | M | 1 |
| 8. | G2858 | M | Mesoamerican | D | 2 |
| 9. | Flor de Mayo | S | Mesoamerican | J | 4 |
| 10. | Mexico 54 | M | Mesoamerican | J | 8 |
| 11. | BAT332 | S | Mesoamerican | M | 16 |
| 12. | Cornell 49-242 | S | Mesoamerican | M | 32 |

Seed size: G = large., M= medium., S = Small

Race: C = Chile, P = Peru, NG= Nueva Granada, M = Mesoamerican, D = Durago, J = Jalisco.

## 1.8 Molecular Techniques in *P. griseola* race identification

Although *P. griseola* isolates can be differentiated using a set of differential cultivars, additional use of molecular techniques complements the information obtained on the differential cultivars provide quality data about the variations within pathogen populations that is important in resistance breeding. The advent of molecular techniques has played a significant role in studying and discriminating between and within fungal pathogen populations. The techniques proved successful in identifying differences between micro – organisms including fungal isolates pathogenic to common bean, rice, wheat lentil among others (Marotti et al., 2007; Abadio et al., 2012; Ddamulira et al., 2014; Tsedaley, 2015). Although the degree of aggressiveness of the pathogen cannot be detected using molecular techniques, large number of isolates can be handled in one time reducing labor and the time in eluciding the differences between isolates.

## 1.9    Control strategies of angular leaf spot disease of common bean

Studies on the trends of ALS disease have indicated that the disease used to occur only at the end of the crop cycle with little effect on the yields (Mendonca et al., 2003). However, it was verified later that the pathogen affects beans at any stage of development, causing yield losses estimated at 80%. High levels of inoculum were indicated to be attained either through successive bean crops in the same area or planting infected seed (Nietsche et al., 2001). Considering the importance of the crop in Tanzania and the world as a whole, several control strategies have been recommended that include adoption of appropriate cultural practices for bean production, fungicide application and the use of resistant varieties. However, the growers' acceptance and utilization of some of these strategies are not always possible especially in the case of subsistence farmers. The following sections present strategies used to control angular leaf spot disease of common bean.

### 1.9.1    Cultural control

One of the methods that have been proposed to deal with plant diseases is cultural control that involves planting pathogen free seeds, eradicating infected plants, practicing crop rotation and deep tillage that burry the infected plant debris in the soil. These practices are known to create environmental conditions that are unfavorable to the pathogen, thereby reducing or eliminating the amount of inocula available for the subsequent crop. Cultural disease control methods have been successfully practiced in maize, wheat and potatoes among others. For example; host plant eradications that involve removal of infected individual plants have been practiced in the control of smut diseases of maize where plants with immature galls are removed and incinerated (Froud et al., 2007). On the other hand, crop rotation and deep tillage are currently in use as the control measures against bacterial wilt and patchy stunting of wheat, respectively (Dyk, 2004). However, the use of cultural practices in common bean production particularly under smallholder farming systems is limited due to scarcity of land.  For example, eradication of infected plants is not possible due to the mode of transmission of the pathogen such as wind and splashing water.

Availability of clean seed is difficult as many seed companies hesitate to engage in seed business with self pollinated crops such as beans as the majority of farmers keep recycling seed. Another aspect is the conditions that are required prior to planting clean seed that include the use of fields free from infected plant materials (Liebenberg and Pretorius, 1997). In the SHT, plant residues are

normally left in the field after harvesting and if infected, they will constitute a reservoir for the pathogen. Since severe infections of *P. griseola* on susceptible cultivars is accompanied by premature defoliations of leaves and pods loaded with inocula that, coupled with minimal tillage and planting thrice in the same field due to limited lands, makes it difficult to come –up with fields that are free from infected materials.

## 1.9.2  Chemical control

Angular leaf spot disease can be controlled using fungicides. The common ones are the protectants (Bravo containing chlorothalonil), where the chemical comes in direct contact with the germinating spore or growing mycelium when used as foliar spray during the cropping cycle. Since most of the protectants are not systemic, once an infection has occurred, a lesion may produce spores thus repeated sprays are required during the cropping season. Protectant fungicides have been used to control anthracnose of common bean, whereby all aerial parts of the plants are sprayed with fungicides. The spraying is done 6 – 8 weeks after flowering, mid flowering and end of flowering when pods are at grain filling stage (Sartori and Maringoni, 2008).

Although the majority of smallholder farmers are not aware of the chemical technology in controlling angular leaf spot, a small number of the farmers accept that, fungicides are required to minimize the impact of the disease on the bean crop. However, they lack detailed information on the disease and the technology itself.  Numerous fungicides are effective in controlling angular leaf spot but proper timing of applications requires good disease monitoring and weather forecasting systems (Lindgren et al., 1995). Consequently, effective studies on fungicide rates, efficacy, and impact of the diseases on yield and economics of the fungicides are essential before any recommendations can be given.

## 1.9.3  Deployment of resistant varieties

The use of disease resistant varieties in controlling angular leaf spot is among the most economical and viable methods especially to smallholder bean farmers in reducing bean yield losses. The mechanism of resistance is achieved by regulation of resistance genes that respond to specific signals from the elicitor molecule of the invading pathogen (McDowell and Dangl, 2000).

Cultivation of resistant varieties not only eliminates losses from diseases but also eliminates expenses from sprays and avoids contamination of the environment with toxic chemicals. Because of this, there is a wide adoption and confidence from farmers in using resistant varieties than any other control measures. This has been expressed by farmers in Indonesia and Philippines during the outbreak of rice turgo disease that occurred in 1980s (Leung et al., 2003). In the SHT, a number of released varieties with moderate levels of resistance have been released but does not suffice the demand for resistance varieties due to different variety preferences that exist among stakeholders.

## 1.10 Genetics of host-pathogen interaction

Under natural environments, there are always some varieties or individual plants in a given species with the ability to survive disease epidemics (resistance) while others succumb to infections (susceptible). Disease resistance can be defined as the ability of the host plant to suppress or inhibit the activities of a pathogen that penetrate the host for access of intracellular nutrients (Huckelhoven, 2007). To gain access, the pathogen releases exogenous as well as endogenous elicitors that induce a response by the host (Huckelhoven, 2007). Once the pathogen is recognized, the plant responds by strengthening the cell wall, poisoning the pathogen or inducing a hypersensitive reaction which includes localized cell death that restricts the pathogen movement (He, 1996). Depending on the genetic mechanism involved, the resistant response can either be race-nonspecific or race–specific.

Race-nonspecific or quantitative resistance depends on two or more genes that confer resistance against two or more types of pathotypes or the majority of races of the same pathogen species (Wisser et al., 2005). This type of resistance is also known as horizontal or durable resistance and is characterized by a susceptible infection type in the seedling stage and a slow epidemic development in the adult plant stage (Broers et al., 1997). Unlike the qualitative resistance, the expression of quantitative resistance depends on the action of multiple genes at different loci, each providing a partial increase in resistance (Keller et al., 2000). Quantitative resistance is the most important form of resistance to necrotrophic pathogens including some biotrophic pathogens such as *Xanthomonas oryzae* pv. *oryzicola* that causes rice bacteria streak and *P. griseola* that causes angular leaf spot diseases in common bean (Wisser et al., 2005, Poland et al., 2009). Quantitative inheritance in common bean against ALS has been reported in the common bean

variety CAL143, where several QTLs with variable effects were identified (Oblessuc et al., 2012). Multiple genes or quantitative trait loci (QTLs) that explain >10% of the phenotypic variation are grouped as major QTLs and those that explain <10% of the phenotypic variation are minor QTLs (Krattinger et al., 2009).

The second type of resistance is known as monogenic/qualitative/ vertical resistance and is based on one or two major genes which are simply inherited with a major effect on the phenotype. Monogenic resistance is generally very efficient and in most cases confers complete resistance, but is only active against certain races of the pathogen (Vale et al., 2001). In this regard, the resistance can easily be overcome by either genetic adaptation of the fungus (pathogen), evolution of new virulent races, shifts of virulence of the pathogen and variability of the pathogen population.

Monogenic resistance can be detected at seedling stage, and is known as seedling resistance. Regardless of the stage of development of a plant, resistance may show none to moderate symptoms of infection while susceptible plants show more severe symptoms. The phenotypes of the host plant can be classified into resistant and susceptible classes that are easily distinguishable. Both race specific and race non-specific types of resistance are important in plant breeding as the choice of what types of resistance to be developed depends on the types of pathogen that exists in the area of interest

## 1.11 The gene for gene concept

According to the classical work of Flor (1971), on the genetics of interaction between flax and flax rust, a single dominant resistance gene of a plant specifically recognizes the complementary avirulent genes of the pathogen. In this regard, an avirulent gene in a pathogen encodes a protein product that is recognized by the product of the complimentary resistance gene of the plant (Keller et al., 2000). This recognition triggers physiological defense reaction that inhibits the growth of the pathogen, or accumulation of molecules which are toxic to the pathogen (Lamb, 1994). If a plant does not contain a resistance gene that produces a resistant gene product, or in the absence of the avirulent gene product from the pathogen, there is no recognition of the pathogen by the plant even though it contains an avirulent gene (Thakur et al, 2007). Therefore, resistance and avirulence genes are conditioned by positively acting gene products that interact as

part of a recognition system through the initiation of the transduction pathways that interact with each other to form a complex network leading to defense response (Collinge et al., 1987). In case of mutation either to avirulence or resistance genes, the results will be loss of function as many of the activities are at the level of transcription or translation in the cell (Keller et al., 2000). The gene–for-gene system occurs more clearly and frequently in biotrophic pathosystem such as rust, powdery mildew, anthracnose and angular leaf spot diseases (Flor, 1971). Knowledge of gene for gene is important in developing a breeding program particularly on parental choices in creating resistance against P. *griseola*.

## 1.12 Sources of resistance genes against *Pseudocercospora griseola*

*Pseudocercospora griseola* is among the fungi that presents great genetic variability with several physiological races (Silva et al., 2008). Co-evolution of the pathogen virulence with common bean gene pools has been a major contribution to variability not only to *P. griseola* but also to anthracnose pathogen (*Colletotrichum lindemuthianum* (Sacc. Magnus) Lam. – Scrib., (Balardin and Kelly, 1998), common blight bacteria (*Xanthomonas campestris* pv. *phaseoli* and *X. campestris* pv. *phaseoli* var. *fuscans*) (Mkandawire et al., 2004) and rust pathogen *Uromyces appendiculatus*. Variability of the pathogen virulence within gene pools has an effect on resistance gene deployment strategies and continuous emergence of new races has been linked to frequent breakdown in disease resistance. In this regard, managing the diseases through breeding requires diverse sources of resistance genes. However, incorporation of resistance genes into new varieties or designing deployment strategies using varieties with different resistance genes relies on proper identification of useful genes and a good understanding of host pathogen interaction.

Since breeding for diseases resistance depends on the availability of genetic variability, a number of accessions from primary and secondary sources and wild relatives of *Phaseolus vulgaris* have been evaluated as an effort to search for resistance genes against the pathotypes (Mahuku et al., 2002; Busogoro et al., 1999). Results indicated that primary and secondary (*Phaseolus coccineus* and *Phaseolus polyanthus*) gene pools have high levels of resistance to *P. griseola* compared to the wild relatives and several genes have been identified and described. The first gene was *Phg-1* identified in the variety AND 277, followed by *Phg-2* in the variety Mexico 54 and BATT 332 (Busogoro et al., 1999 and Namayanja et al., 2006). *Phg-3, Phg-4* and *Phg-5* have also been characterized in AND 277, Mexico 54, MAR2 and Cornell 149-242, varieties previously

indicated as containing monogenic resistance. The identified genes have been used extensively in bean breeding programmes the world over including Africa, to develop lines resistant to *P. griseola*.

Although there are good indications that diversity of resistance genes available for use is high, not all of them are effective against all races of *P. griseola* indicating the complexity of the disease. For example: some of the well-known resistance sources that are effective in Mexico may not necessarily be effective in the SHT. CAL 143 is the first CIAT bred Andean cultivar identified with resistance to angular leaf spot diseases in Malawi, South Africa, Tanzania and Zambia, and has eliminated losses caused by the disease where the variety was introduced. However, the same varieties were susceptible in Uganda (Aggrawal et al., 2004) and in high rainfall bean growing agro-ecologies of the Southern Highlands of Tanzania, suggesting presence of different races within and between countries. There is no doubt that many virulence genes in *P. griseola* and resistance genes in *Phaseolus vulgaris* are unknown. Therefore, continual evaluation of germplasm and eventual introgression of diverse genetic resistance is essential.

Successes in developing common bean varieties with improved levels of resistance to *P. griseola* depend mainly on the sources of resistance used in the breeding programme and the types of genes that exist within the genotype. Diverse gene pools from the centers of origin have been used as sources of resistance genes to many crops (Singh et al., 1991; Geffroy et al., 1999). However, limitations of usage have been reported in common bean that include low adaptability and undesirable traits (Beebe et al., 1981; Holbrook et al., 2000). An example is cultivar G11796 which is photoperiod sensitive with difficulties in flowering under eastern African environments, whereas Mexico 54 is known for its climbing growth habits a trait which is not preferred by many common bean farmers (Beebe et al., 1981).

Landrace varieties are defined as a group of crop plants that have been grown and maintained by farmers (for various reasons) from generation to generation and these have great potential in contributing valuable resistance for ALS disease (Singh et al., 1991; Geffroy et al., 1999; Melotto and Kelly, 2000). An example is landrace Oura Negro identified in Brazil as carrying *Phg* genes (Goncalves – Vidigal, 2013) and G5686 that originated from Ecuador, both have been reported to have resistance to *P. griseola* (Mahuku et al., 2009). However, the exploitation of a single

24

genotype in breeding against the pathogen may not be sufficient due to the high degree of genetic variability that exits in *P. griseola*. This is supported by the fact that the number of resistance genes so far reported does not match the number of races that exists in the bean growing environments. In this regard, one must have a good number of parents coupled with a clear understanding of the combining abilities, gene action and heritability before a successful breeding programme against *P. griseola* can be established. Additionally, the type of mating design to be used is important as it determines the genetic parameters that can be estimated, information that is useful for breeding.

## 1.13 The diallel mating design and genetic analysis

Diallel mating designs have been used extensively in developing new varieties of different crops with great successes. The technique is based on the assumption that parents are diploid and homozygous with no reciprocal differences, no epistasis, no multiple allelism and that the genes are independently distributed between the two parents involved in a cross (Jinks and Hyman, 1954). The main advantage of the design is the ability to carry out complex approaches in order to test and analyze parents and progenies involved through general and specific combining abilities and other secondary genetic parameters (Viana et al., 1999). General combining ability is defined as the average performance of a line in a hybrid combination and is associated with additive gene action, whereas specific combining ability indicates the worse or better performance of the combination on the basis of the average performance of the parents involved in a cross and is associated with non-additive gene action.

## 1.13.1 Gene action and heritability

Gene action refers to mode of expression of a gene in a population. Four types of gene action are known and have been used in various breeding program with great successes. These are additive, dominance , overdominance and epistatic. Additive gene action occurs when there are several genes that influence a trait in equal increament while dominance, overdominance and epistatic involve the relationship of allele at the same locus. Heritability on the other hand is the ratio of genetically caused variation within a population, The estimates of heritability associated with the diallel analysis have been indicated to be a useful guide to breeders in estimating the proportion of variation due to genetics and environment. The narrow sense heritability reflects the magnitude

of additive genetic variance of a trait of interest and can form a strong and reliable base on which breeding programme of a self pollinated crop like beans can depend on (Kearsy and Pooni, 1996). Narrow sense heritability values estimate how the character will respond to selection and Griffith used the parameter to indicate additive and dominance interactions (Rainey and Griffiths 2005). On the other hand, broad sense heritability has been used in plant breeding to estimate additive, dominance and epistatic source of genetic variation. Heritability allows comparison of relative importance of the gene and environment to the variation of the trait within and across populations and has been used extensively in various crops to measure the fraction of genetic variations between individuals in a population (Sleper and Poehlman, 2006; Visscher et al., 2008)

## 1.14  Allelism tests in disease resistance breeding

One of the best ways to develop varieties with durable resistance against multiple races of the pathogen is to pyramid the genes in a single variety (Lowe and Dubcovsky, 2011). Allelism studies on resistance genes are known to be important in gene pyramiding especially when dealing with a highly variable pathogen such as *P. griseola.* These types of pathogens are known to have rapid appearance of virulent races requiring multiple resistant genes to counteract the negative effects generated. Allelism offer an opportunity to tests various genes that exists in the parents involved in a cross by examining ratios of resistance to susceptible reactions observed in the $F_2$ segregating populations. Depending on the type of ratio obtained, the trait can be controlled by different allele at the same locus or share a resistance allele from different loci (Dhillon and Dhaliwal, 2011). For example; if a population shows a ratio of 15R:1S the genes involved in a cross has been described to be non allelic (Lowe and Dubcovsky, 2011). Several studies have indicated the importance of allelism in identifying appropriate parents to be involved in disease resistance breeding programme including those done on barley and wheat (Enhdaie and Baker, 1999; Dhillon and Dhaliwal, 2011).

## 1.15  Conclusion

Angular leaf spot disease of common bean causes significant yield losses. The disease is more severe under smallholder bean faming systems where low input agriculture is practiced. Common bean is grown primarily by smallholder farmers, the majority of which are resource poor with

limited knowledge about the adverse effect of angular leaf spot disease on the crop. Cultural and chemical controls methods have been proposed to control ALS disease but may not be appropriate in the SHT due to land scarcity and lack of capital to purchase fungicides. Considering the high variability of *P. griseola* and the ability of the pathogen to attack both Mesoamerican and Andean gene pools, characterizing races that exist in the SHT is important prior to engaging into any breeding efforts. Additionally, it is well known that races that exist in one agro-ecology may not necessarily be the same as those found in another. This makes it difficult to breed against the pathogen without prior assessments of the existing races. There are several *Phg* genes that have been identified as sources of resistance to *P. griseola* from landraces, secondary and tertiary genes pools. However, utilization of these genes in a breeding programme will depend on the mode of inheritance and the background of the cultivar carrying them. Landrace varieties might be excellent sources for resistance breeding against ALS. Landraces are readily available, adapted to the environments and have been kept by farmers because of their desired traits. In this regard, breeding against multi-races of ALS disease is an overriding consideration which requires gene pyramiding that involves several parents. Therefore, appropriate mating design and genetic analysis that will provide information of the best parent in a combination and best selection methods to identifying superior progenies is important. Superior progenies are required for advancement towards developing varieties that are resistant to *P. griseola* and with enhance yields under the smallholder farming systems of the SHT.

**References:**

Abadio, A.K.R., S.S. Lima, M.F. Santana, T.M.F. Salomão, A. Sartorato, E.S.G. Mizubuti, E.F. Araújo and M.V. de Queiroz. 2012. Genetic diversity analysis of isolates of the fungal bean pathogen *Pseudocercospora griseola* from central and southern Brazil. Genetics and Molecular Research 11: 1272 – 1279.

Abawi, G.S. and M. A. Pastor-Corrales.1990. Root rots of beans in Latin America and Africa: diagnosis, research methodologies, and management strategies. Cali Colombia. CIAT.

Aggrawal, V. D., M. A. Pastor – Corrales, R. M. Chirwa and R. A. Buruchara, 2004. Andean beans *(Phaseolus vulgaris* L.) with resistance to the angular leaf spot pathogen *(Phaeoisariopsis griseola*) in southern and eastern Africa. Euphytica 136: 201–210.

Alvarez-Ayala, G and H. F. Schwartz. 1979. Preliminary investigations of pathogenic variability expressed by *Isariopsis griseola.* Annual Report Bean Improvement Cooperative 22:86-88.

Bailey, L.H. 1969. Manual of Cultivated Plants. 11th Edition, Mcmillan Company. New York.

Balardin, R.S and J.D. Kelly**.** 1996. Identification of race 65-epsilon of bean anthracnose *(Colletotrichum lindemuthianum*) in Michigan. Plant Disease. 80:712 - 718.

Barros, O., R. Cardenosa and R. L. Skiles. 1958b. Angular leaf spot of common bean in Columbia. Plant Disease 42:420 -421

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

Beebe, S., A. V. Gonzalez and J. Rengifo. 2000. Research on trace minerals in the common bean. Food Nutrition Bulletin 21:387-91.

Beebe, S.E., F. A. Bliss and H. F Schwartz. 1981. Root rot resistance in common bean germplasm of Latin American origin. Plant Disease. 65: 485-489.

Bitocchi, E., L. Nanni L, E. Bellucci, M. Rossi, A. Giardini, P. Spagnoletti Zeuli, G. J. Logozzo-Stougaard, P. McClean, and G. Attene. 2012. Mesoamerican origin of the common bean *(Phaseolus vulgaris* L.) is revealed by sequence data. Proceedings of the National Academy of Sciences Early Edition.

Braun. U. 2000. Annotated list of *Cercospora* spp. described by C. Spegazzini. Schlechtendalia 5: 57–79.

Broers, L.H.M. 1997. Components of qualitative resistance to yellow rust in ten bread wheat cultivars and their relations with field assessments. Euphytica 96: 215 – 223.

Broughton, W.J,. G. Hernandes, M. Blair, S. Beebe, P. Gepts and J. Vanderleyden. 2003. Beans (*Phaseolus spp.*)- model food legumes. Plant and Soil. 252: 55 – 128

Buruchara, R. A, E. M. Gathuru and D. M. Mukunya. 1988. Disease progress of angular leaf spot caused by *Isariopsis griseola* Sacc. and its implications on resistance of some bean (*Phaseolus vulgaris* L.) cultivars. Acta Horticulturae. 218: 321–328.

Busogoro, J.P., M. H. Jijakli and P. Lepoivr. 1999. Identification of a novel source of resistance to angular leaf spot disease of common bean within the secondary gene pool. Plant Breeding 118: 417–423.

Collinge, D.B., D. E. Milligan, G. Scofield, J. M. Dow and M.J. Daniels. 1987. Gene expression in Brassica compestris showing a hypersensistive response to the incompatible pathogen *Xanthomonas compestris* pv. *Vitians*. Plant Molecular Biology 8:405 – 414.

Correa-Victoria F.J. 1987. Pathogenic variation, production of toxic metabolites, and isoenzyme analysis in *Phaeoisariopsis griseola* (Sacc.) Ferr. Ph.D. thesis, Michigan State University

Correa, V. F., M.A. Pastor- Corrales and A.W. Saettler. 1989. Angular leaf spot. in: H.F.Schwartz, M.A. Pastor Corrales (eds), Bean Production Problems in the Tropics, CIAT, Cali, Colombia, 1989, pp. 59 - 75.

Crous, P.W., M. M. Liebenberg, U. Braun and J. Z. Groenewald. 2006. Re-evaluating the taxonomic status of *Phaeoisariopsis griseola*, the causal agent of angular leaf spot of bean. Studies in Mycology. 55:163-173.

Crous, P.W., U. Braun, G.C. Hunter, M.J. Wingfield, G.J.M. Verkley, H.-D. Shin, C, Nakashima and J. Z. Groenewald. 2013. Phylogenetic lineages in Pseudocercospora. Study Micology 75:37 - 114

Chupp, C. 1954. A monograph of the fungus genus Cercospora. Ithaca, New York.

Deighton, F.C. 1976. Studies on *Cercospora* and allied genera. VI. *Pseudocercospora* Speg., *Pantospora* Cif. and *Cercoseptoria* Petr. Mycological Papers 140: 1–168

Díaz-Batalla, L., J.M. Widholm, G.C. Jr. Fahey, E. Castaño-Tostado and O. Paredes-López. 2006. Chemical components with health implications in wild and cultivated Mexican common bean seeds (*Phaseolus vulgaris* L.). Agriculture and Food Chemistry. 54:2045-2052

Ddamulira, G., C. Mukankusi, M. Ochwo-SSemakula, R. Edema, P. SSeruwagi and P. Gepts.2014. Distribution and variability of *Pseudocercospora griseola* in Uganda. Journal of Agricultural Science 6: 16 – 29.

Dyk van K, 2004. Fungi associated with root and crown rot of wheat and barley in Tanzania. African plant protection 10:118 – 124.

Eckardt, N. A. 2006. The Role of Flavonoids in Root Nodule Development and Auxin Transport in *Medicago truncatula*. Plant cell. 18:1539 - 1540

Ehdaie, B and J. Baker. 1999. Inheritance and allelism for resistance to Russian wheat aphids in Iranian spring wheat. Euphytica. 107:71 - 78

FAOSTAT. 2010. http://faostat.fao.org/site/339/default.aspx, accessed October 23, 2012.

FAOSTAT. 2014. United Nations Food and Agriculture Organization. Dry Bean. Statistical database. http://faostat.fao.org/site/567/default.aspx#ancor. Accessed December 29, 2015.

Faria, J.C., Carneiro, G.E.S and Aragao F. J. L. 2010. Gene flow from transgenic common beans expressing the bar gene. GM crops 1: 2 pp 94-98

Ferraris, T. 1909. Osservazioni micologiche. Su specie del gruppo Hyphales (Hyphomycetae) Annales Mycologici 7: 273–286.

Flor, H.H. 1971. Current status of gene- for gene concept. Annual review. Phytopathology. 9: 275 – 296

Froud, K. J., M. Bullians, M. Braithwaite, M. F. S. Fernando, C. F. Hill and R. Midgley. 2006. New to New Zealand; detection of common smut of corn (Ustilago maydis) from a single cornfield in Gisbone. New Zealand Plant Protection. 59: 373 – 379

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

Gepts P., T. C. Osborn, K. Rashka, F. A. Bliss. 1986. Phaseolin-protein variability in wild forms and landraces of the common bean (*Phaseolus vulgaris*): evidence for multiple centers of domestication. Economic Botany 40: 451-468.

Gepts P and D. Debouk. 1991. Origin, domestication, and evolution of the common bean (*Phaseolus vulgaris* L.) In: Van Schoonhoven A, Voyset O, editors. Common Beans: Research for Crop Improvement. CAB International; Wallingford. pp. 7–53.

Gepts P. 2000. A phylogenetic and genomic analysis of crop germplasm: a necessary condition for its rational conservation and use. In: J. P. Gustafson (ed.), Proceeding Stadler Genetics Symposium, June 8-10, 1998, Columbia, MO. Plenum, pp. 163-181.

Gepts, P. 2001. *Phaseolus vulgaris* (Beans). doi: 10.1006/rwgn.2001.1749. Accessed 26th October 2015

Goncalves – Vidigal, M. C., S. Cruz, G. F. Lacano, P. S. Vidigal, Filho, L. L. Sous, C. M. Pacheco, P. McClean, P. Gepts and M. A. Pastor – Coralles. 2013. Co-segregation analysis and mapping of the anthracnose Co-10 and angular leaf spot Phg-ON disease-resistance genes in the common bean cultivar Ouro Negro. 126: 2245 – 2255

Gonzalez, M., R. Rodríguez, M. E. Zavala, J. L. Jacobo, F. Hernández, J. Acosta, O. Martínez, and J. Simpson. 1998. Characterization of Mexican isolates of *Colletotrichum lindemuthianum* by using differential cultivars and molecular markers. Phytopathology 88:292-299

Gordon, T.R., and J. M. Duniway. 1982. Effects of powdery mildew infection on the efficiency of $CO_2$ fixation and utilization by sugar beet leaves. Plant Physiology 69:139 – 142.

Hayman, B.I., 1954. The theory and analysis of diallel cross-I. Genetics. 32: 789-809

Hillocks, R. J., C.S. Madata., R. Chirwa., E. M. Minja and S. Msolla. 2006. Phaseolus bean improvement in Tanzania, 1959 – 2005. DOI: 10.1007/s10681-006-9112-9. Visited December, 2012

He, S. Y. 1996. Elicitation of Plant Hypersensitive Response by Bacteria. Plant physiology. 112:865 -869

Holbrook, C.C., P. Timper and H. Q. Xue. 2000. Evaluation of the core collection approach for identifying resistance to *Meloidogyne arenaria in* peanut. Crop Science. 40: 1172–1175

Huckelhoven, R. 2007. Cell wall-associated mechanism of disease resistance and susceptibility. Annual Review of Phytopathology. 45: 101 – 127

Jarvie, A. 2002. The status of dry bean diseases as influenced by cultivar changes. http://www.pannar.com/ download/Art1.htm. Accessed on 20th December, 2015

Kearsey, M.J and S. Pooni. 1996. The genetic analysis of quantitative traits. Chapman and Hall, London

Knuth, P. 1908. Handbook of flower pollination. Translation of 1898 Original. Volume 2. Page 703. Clarendon Press, Oxford, UK.

Krattinger. S., E. Lagudah, W. Spielmeyer, R. Singh, J. Huerta-Espino, H. McFadden, E. Bossolini, L. Selter, B. Keller .2009. A putative ABC transporter confers durable resistance to multiple fungal pathogens in wheat. Science. 323:1360–1363

Ladizinsky, G. 1998. Plant Evolution under Domestication. Kluwer Academic Press, Dordrecht. Page 262

Lindgren. D.T., K. M. Escridge, J. R. Steadman and D. M. Schaaf. 1995. A model for dry bean yield loss due to rust. HortTechnology 5: 35–37.

Lamb, C.J. 1994. Plant disease resistance genes in signal perception and transduction. Cell 76:419 – 422.

Leung, H., Y. Zhu., I. J. X. Revilla-Molina, H. C. Fan., I. Pangga, C. Vera Cruz and T. W. Mew.2003. Using genetic diversity to achieve sustainable rice disease management. Plant Disease 87: 1156-1160

Leung, H., R.J. Nelson and J.E Leach. 1993. Population structures of plant pathogenic fungi and bacteria. Advances in Plant pathology. 10:157 – 205

Levine, M.N and E.C. Stakman. 1918. A third biological form of *Puccinia graminis* on wheat. Journal of Agricultural Research 13: 651 – 654

Liebenberg, M.M and Z.A. Pretorius. 1997. A review of Angular leaf spot of common bean (*Phaseolus vulgaris* L.). African Plant Protection. 3: 81 – 106

Long S. R. 1996. Rhizobium symbiosis: nod factors in perspective. Plant Cell 8:1885–1898

Lopes D.B and R. D. Berger. 2001. The effects of rust and anthracnose on the photosynthetic competence of diseased bean leaves. Phytopathology. 91: 212–220

Lowe,I., D. Cantu and J. Dubcovsky 2011. Durable resistance to the wheat rusts: integrating systems biology and traditional phenotype-based research methods to guide the deployment of resistance Genes. Euphytica. 179: 69-79. doi: 10.1007/s10681-010-0311-z

Mahuku, G.S., A. M. Iglesias and C. Jara. 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. G. S., C. Jara, J. B. Cuasquer and G. Castellanos. 2002. Genetic variability within *Phaeoisariopsis griseola* from Central America and its implications for resistance breeding of common bean. Plant Pathology. 51: 594–604

Mahuku G. S., M.A Henriquez., Munoz and R.A. Buruchara. 2002. Molecular markers dispute the existence of the Afro-Andean group of the bean angular leaf spot pathogen, *Phaeoisariopsis griseola*. Phytopathology. 92:580 – 589

Mathre, D. E. 1968. Uptake and binding of oxathiin systemic fungicide by resistance and sensitive fungi. Phtopathology. 58:1464 – 1469

Melotto M., R. S. Balardin and J. D. Kelly. 2000. Host-pathogen interaction and variability of *Colletotrichum lindemuthianum*, in Colletotrichum: Host Specific City, Pathology, and Host-Pathogen Interaction, eds Prusky D., Freeman S., Dickman M. B., editors. (St. Paul, MN: APS Press), 346–361.

Mendonça, H.A., J. B. Santos and M. A. P. Ramalho. 2003. Genetic control of common bean reaction to angular leaf spot. Crop Breeding and Applied Biotechnology 3: 209-226

Miklas, P.N and S. P. Singh. 2007. Common bean. In: Kole C (ed) Genome mapping and molecular breeding in plants. Pulses, Sugar and Tuber Crops, volume 3. Springer, Berlin, page 1–31

Miller, C.R., I. Ochoa, K. L. Nielsen, D. Beck and J. P. Lynch. 2003. Genetic variation for adventitious rooting in response to low phosphorus availability: potential utility for phosphorus acquisition from stratified soil. Functional Plant Biology. 30: 973-985.

Milgroom, M.G and W.E. Fry. 1997. Contributions of population genetics to plant disease epidemiology and management. Advances of Botany Research. 24: 1- 30

Mussei A. N., R. P. Mbwile, J. A. Kamasho, G. J. Ley, R. M. Mghogho and C. M. Mayona. 1997. Agro-ecological zones and farming systems of the Southern Higlands of Tanzania. Ministry of Agriculture and Cooperatives, Southern Highlands Zonal Research Institute.

McCartney, H. A., S. J. Foster, B. A. Fraaije and E. Ward. 2003. Molecular diagnostics for fungal plant pathogens. Pest Management Science. 59:129–142

McDowell, J.M and J. L. Dangle. 2000. Signal transduction in the plant immune response. Trends in Biochemical Sciences 25: 79 – 82

Mkandawire A.B.C., R.B. Mabagala, P. Guzman, P. Gepts and R.L. Gilbertson, 2004. Genetic diversity and pathogenic variation of common blight bacteria (*Xanthomonasaxonopodis* pv. *phaseoli* and *Xanthomonas axonopodis* pv. *phaseoli* var. fuscans) suggest pathogen coevolution with the common bean. Phytopathology 94:593–603.

Mwangombe, A.W., I.N. Wagara, J.W. Kimenju and R.A. Buruchara. 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: 235-241

Namayanja A., R. Buruchara, G. Mahuku, P. Rubaihayo, P. Kimani, S. Mayanja and H. Eyedu. 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

Nietsche, S., A. Borem, G.A. Carvalho, T.J. Paula, C.F. Ferreira, E.G. Barros and M.A Moreira. 2001. Genetic diversity of *Phaeoisariopsis griseola* in the state of Minas Gerais, Brazil. Euphytica 117:77 – 84

Oblessuc, P.R, R M. Baroni, A. A. Franco Garcia, A. F. Chioratto, S. A. Morais Carbonell,L E. Aranha Camargo and L.L Benchimol. 2012. Mapping of angular leaf spot resistance QTL in common bean (*Phaseolus vulgaris* L.) under different environments. BMC Genetics. 13:50 -58

Olivares, J.E., Diaz-Camino, C. Estrada – Navarrete, G.E. Affantranger, X. A. Kessler, M.R. Zamudio, F. Olamendi – Portugal, T. Marquez, Y. Servin, L. E and Sanchez, F. 2011. Nodulin 41, a novel late nodulin of common bean with peptidase activity. BMC Plant Biology 11: 134 - 139

Papa R., L. Nanni. D. Sicard, D. Rau and G. Attene. 2005. The evolution of genetic diversity in phaseolus vulgaris L., In: T.J. Motley, N.Zerega, and H. Cross (eds). Darwins Harvest: New Approaches to the origins. Evolution and conservation of crops. Columbia University Press: USA

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

Poland J.A., P. J. Balint-Kurti, R. J. Wisser, R. C. Pratt and R. J. Nelson .2009. Shades of gray: The world of quantitative disease resistance. Trends in Plant Science 14:21–29

Roelfs, A. P and J.W. Martens. 1988. An International system of nomenclature for *Puccinia graminis f.sp. tritici*. Phytopathology. 78: 526 – 533

Ronner, E. and K. E. Giller. 2012. Background information on agronomy, farming systems and ongoing projects on grain legumes in Tanzania. www.N2Africa.org

Saccardo P.A .1878. Fungi veneti novi vel critici. 8. Michelia 1: 239–275

Samki, J. K. and J. F. Harrop. 1982. Fertilizer recommendations related to ecological zones in Tanzania. Annual progress report. Mlingano agricultural research Institute Annual progress report, Tanga-Tanzania

Sartorato, A. 2004. Pathogenic Variability and Genetic Diversity of *Phaeoisariopsis griseola* Isolates from Two Counties in the State of Goias, Brazil. Phytopathology. 152: 385–390.

Sartori, J. C. and C. A. Maringoni. 2008. Effects of fungicide on colony growth of *Colletotrichum lindemuthianum* (Sacc. & MAGN) Crib. Plant Protection Research. 48:201 - 2012

Silva K.J. D., E. A. Souza, A. Sartorato and C. N. S. Freire. 2008**.** Pathogenic variability of isolates of *Pseudocercospora griseola*, the cause of common bean angular. Phytopathology 156:602-606

Singh S. P. 1982. A key for identification of different growth habits of Phaseolus vulgaris L.Annual Report of Bean Improvement Cooperatives. 25:92–95

Singh S. P., P. Gepts and D. G. Debouck. 1991. Races of common bean (*Phaseolus vulgaris* L., Fabaceae). Economic Botany. 45:379-396.

Singh, S. P and H.F. Schwartz. 2010. Breeding common bean for resistance to diseases: A review. Crop Science 50: 2199 – 2223

Sohi, H.S. and R. D. Sharma. 1974. Mode of survival of *Isariopsis griseola* Sacc., the causal agent of angular leaf spot of beans. Indian journal of Horticulture 31:110-113

Schwartz, H. F and M. A. Pastor Corrales. 1989. Bean production problems in the tropics. 2nd ed. CIAT, Cali, Colombia.

Schwartz, H. F., M. A. Brick, D. S. Nolan and C. D. Franc. 1996. Dry bean production and pest management. Regional Bull. 562A, Colorado State University, University of Nebraska and University of Wyoming

Sleper, D.A and J.M. Poehlman. 2006. Breeding Field Crops. 5th Edition., Wiley-Blackwell Publications. New York, USA. ISBN-13: 9780813824284, Pages. 424- 428.

Stenglein S., L. D. Ploper, O. Vizgarra, P. Balatt. 2003. Angular leaf spot: a disease caused by the fungus *Phaeoisariopsis griseola* (Sacc.) Ferraris on *Phaseolus vulgaris* L. Advances of Applied Microbiology 52: 209–243.

Stenglein S. A and P. A. Balatti. 2006. Genetic diversity of *Phaeoisariopsis griseola* in Argentina as revealed by pathogenic and molecular markers. Physiological and Molecular Plant Pathology 68: 158-167

Sharma, T. R. 2003. Molecular diagnosis and application of DNA markers in the management of fungal and bacterial plant diseases. Indian journal of biotechnology. 2:99-109

Smart, J. 1990. Grain Legumes: Evolution and genetic resources. P.200. Cambridge University Press, Cambridge, UK.

Thakur, R.P. 2007. Host plant resistance to diseases: Potential and limitations. Indian Journal of Plant Protection. 35: 17 -21

Thompson, M., M. A. Brick, J. N. McGinley and H. J. Thompson.2009. Chemical composition and mammary cancer inhibitory activity of dry bean. Crop Science. 49: 179–176.

Toro, O., J. Tohme and I. X. J.  Debouck. 1990. Wild bean *(Phaseolus vulgaris* L.); Description and distribution- International Board for Plant Genetic Resources (IBPGR) and Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia

Tsedaley, B. 2015. A Review on Disease Detection, Pathogen identification and Population Genetics in Fungi. Journal of Biology, Agriculture and Healthcare.5:6 – 20.

Vale, F. X. R and J. E. Parlevliet. 2001. Concepts in plant disease resistance. Fitopatologia Brasileira. 26:577 – 589.

Verma., B.R and S.L. Sharma. 1984. Variability in *Phaeoisariopsis griseola* the causal agent of angular leaf spot of beans. Indian Phytopathology. 37: 580-581.

Viana, J. M. S., C. D. Cruz and A. A. Cardso. 1999. Theory and analysis of partial diallel cross.Genetics and Molecular biology 22: 591 – 599

Visscher, P.M., W.G. Hill and N.R. Wray. 2008. Heritability in the genomics era: Concepts and misconceptions. Nature Reviews Genetics.  9: 255-266.

Weinstein, A. I. 1926. Cytological studies on *Phaseolus vulgaris*. American Journal of Botany.13: 248- 263

Willey, R.W. 1990. Resource use in intercropping systems. Agricultural Water Management, Amsterdam, 17:.215-231

Wilson R.A and N. J. Talbot. 2009. Under pressure: investigating the biology of plant infection by Magnaporthe oryzae. Nature reviews Microbiology. 7: 185–195

Wisser, R.J., Q. Sun, S.H. Hulbert, S. Kresovich and R.J. Nelson. 2005. Identification and characterization of regions of the rice genome associated with broad spectrum, quantitative diseases resistance. Genetics 169:2277 – 2293

Wortmann, C.S., R. A. Kirkby, C. A. Eledu and D. J. Allen. 1998.  Atlas of common bean (*Phaseolus vulgaris* L.)  production in Africa. Cali, Colombia: CIAT

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

# 2   Appraisal of common bean farming systems under angular leaf spot disease prone environments of the Southern Highlands of Tanzania

**Abstract**

Common bean (*Phaseolus vulgaris* L.) is the most important legume crop cultivated by the majority of smallholder farmers in the Southern Highlands of Tanzania (SHT). However, the crop is devastated by angular leaf spot (ALS) disease caused by *Pseudocercospora griseola.* The objective of this study was to assess the common bean farming systems under ALS disease prone environments of the SHT.  A structured questionnaire was designed to collect information from 238 common bean growers sampled from Mbeya, Njombe, Iringa and Rukwa regions. Data collected included population demography, types of bean varieties grown, farmers' awareness of the disease symptoms, yield losses, agronomic practices followed in bean production and satisfaction of basic needs at households as indicators of poverty.  The results showed that ALS disease widely occurred in the common bean farming systems of the SHT and farmers were not aware of the disease and inocula sources. Most of the respondents were poor and the average bean yields ranged from 200 – 400 kg ha$^{-1}$. The use of susceptible bean varieties, the possibilities of introduction of new races from seed sources, lack of knowledge on ALS disease and the presence of favorable environmental conditions increased chances of *P. griseola* proliferation. Fungicides types, rate and application regime practiced by few farmers were not those recommended for controlling ALS. Therefore, creating farmers awareness and practicing integrated ALS disease management is important. Breeding for durable resistance targeting farmers' preferred traits is an overriding consideration towards increasing bean productivity under smallholder farming systems in the SHT.


**Keywords:** Angular leaf spot; common bean; farming systems; smallholder farmers; *Pseudocercospora griseola*

## 2.1  Introduction

Common bean (*Phaseolus vulgaris* L.) is the most important and widely cultivated grain legume globally. It grows in diverse environments of the world, ranging from tropical to temperate regions. In East African countries of Tanzania, Burundi, Kenya, Rwanda and Uganda the crop accounts for about 62% of agricultural production (Broughton et al., 2003). Tanzania is among the top 20 largest producers of common bean in the world and second largest producer in sub Saharan Africa (FAO, 2004). In the Southern Highlands of Tanzania (SHT), the common bean is the most important annual legume crop cultivated by the majority of smallholder farmers who constitute about 85% of the population. The crop occupies the largest area planted to pulses and is highly valued especially by women farmers because of its short growing cycle and the ability to fit in intercropping farming systems. Nutritionally, the common bean is the second most important source of vegetable protein to the Southern Highlanders and the third most important source of calories, vitamins, zinc and other essential elements (Wortmann et al., 1998; Broughton et al., 2003). Additionally, beans enhance the well-being of farmers especially when surpluses are obtained and sold to generate income that can be used to meet other household needs. Despite these benefits, many households do not produce enough beans to feed themselves and the majority live below the poverty line. The average yield of common bean in the SHT is 500 kg ha$^{-1}$, lower than the national average of 741 kg ha$^{-1}$ (FAO, 2008).

Among the major constraints to common bean production is angular leaf spot disease caused by *Pseudocercospora griseola* (Sacc.) Crous & U. Braun. The disease (ALS) is associated with substantial yield losses and reduces seed quality of common bean under the smallholder farming systems across the entire bean growing agro-ecologies of the SHT. Sources of *P. griseola* inocula include infected plant debris where the pathogen has the ability to survive up to twelve months, while on the seed the pathogen can survive for up to 19 months, thus contributing to inoculum build-up and transmission of the pathogen from one location to another (Sindhan and Bose, 1979). Bean production practices that include tillage, sowing, weeding, harvesting and crop residual management may increase the incidences of ALS disease if farmers are not aware of the pathogen, sources of inocula and the response of the host to infection (Liebenberg and Pretorius, 1997; Wagara et al., 2005;). In addition, poverty contributes to vulnerability of smallholder farmers to crop diseases including ALS.

Poverty is characterized by low satisfaction of basic needs that include housing conditions, food, access to health and education services and economic capacity that are associated with the wellbeing of a household/ community. The indicators for basic needs as measures of poverty were agreed upon during the world summit for social development held in Copenhagen in 1995 and have been used in Malaysia, Mexico, Philippines, India, Bhutan, Bolivia, South Africa and Tanzania to examine poverty within different communities including low income households (Alkire and Sarwar, 2009; Mamun and Adaikalam, 2011; World bank, 2012).  It is well known that resource poor farmers generally have limited capacity in managing crop diseases, have poor access to healthy seed and often practice farming with low or no farm inputs. Increased disease incidences as a result of poverty have been reported on angular leaf spot of common bean and fusarium wilt of potatoes (Nyomora, 1990; Hidalgo et al., 2001).

In view of the economic importance of ALS in the SHT, integrated disease management to improve productivity of the crop is necessary. However, such efforts can only be successful if farmers' perceptions, knowledge and their disease management practices are studied either for creating awareness or for improvement.

## 2.2   Objectives

The objective of this study was to evaluate common bean farming systems under angular leaf spot disease prone environments of the SHT through; 1) assessments of farmers' awareness of the disease 2) determine common bean production methods practiced by the smallholder bean farmers and 3) examine poverty levels within smallholder bean farmers and relate all with the occurrence and management of ALS disease.

## 2.3   Materials and methods

### 2.3.1   Study Area

This study was conducted in the Southern Highlands of Tanzania covering a total of seven districts and ten villages that represented common bean growing areas of the zone and Tanzania as a whole. The districts and their respective villages were: Mbeya Rural district − Isangala; Mufindi district − Ihalimba and Vikula; Sumbawanga district − Malonje; Mbozi district − Nambinzo and Mbimba; Wanging'ombe district −Masaulwa; Njombe district − Igosi; Rungwe

district – Kabate and Lukata. Mbeya Rural, Rungwe and Mbozi districts are situated in Mbeya Region, Wanging'ombe and Njombe districts are in Njombe Region, whereas Mufindi, Sumbawanga, Rungwe are in Iringa and Rukwa regions respectively (Fig. 2.1). Generally, more than 30% of the Southern Highlands area is located at an elevation between 1500 to 2500 meters above sea level (Nyomora, 1990). The weather is generally cool with temperatures ranging between a mean maximum of 23°C and mean minimum of 14°C and relative humidity of 60 – 95, conditions that have a direct influence on ALS disease incidences on the bean crop. The SHT is characterized by uni-modal type of rainfall ranging from 600 mm to over 2600 mm annually on the mountains and along Lake Nyasa (David et al., 2001; Hildago et al., 2001; ARI – Uyole., 2012) The rainy season is between the months of November and May, ideal for production of most crops including the common bean.
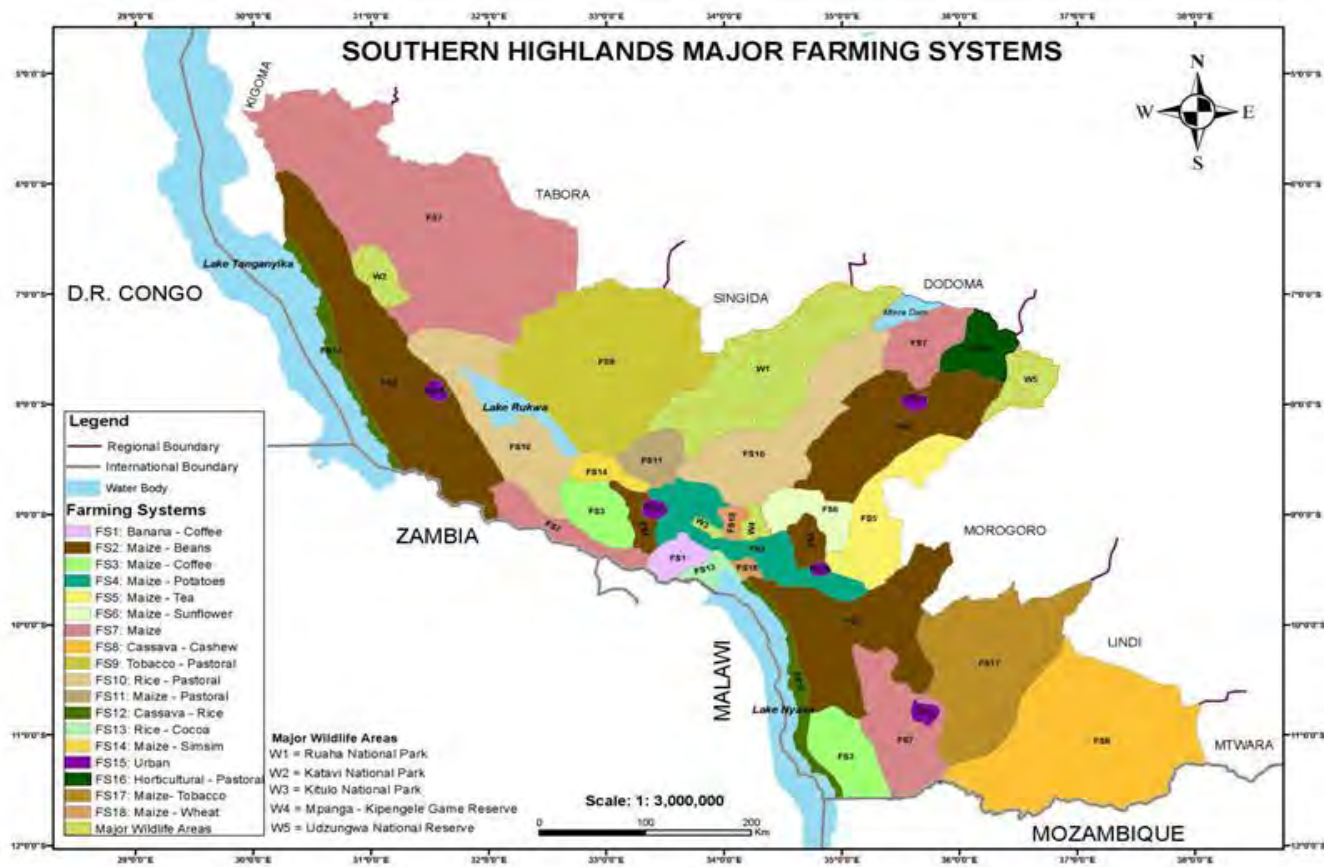


**Figure 2.1: Map of the Southern Highlands of Tanzania showing major farming systems: Source: Department of soil science, The Uyole Agricultural Research Institute, Tanzania**

## 2.3.2 Sampling Technique

Simple random sampling technique was used to draw a sample of 238 respondents who are smallholder bean farmers and stakeholders. Standard questionnaires were prepared, pre-tested and data were collected from respondents across sampled villages.

## 2.3.3 Data Sources

Two types of data were collected; these were primary and secondary data. Secondary data were collected on population demography and types of crops grown from District Agricultural Office reports, discussions with District Agricultural Extension officers, village leaders and the Southern Highland Zone Agricultural Research Institute (ARI, Uyole). A closed ended questionnaire was designed to collect the primary data from the 238 smallholder bean farmers to capture information on gender, awareness of the disease symptoms, sources of information on the disease, agronomic practices that include tillage, field sanitation, bean cropping systems, types of varieties grown, seed sources, weeding, harvesting and seed sorting practices. Each farmer was shown live samples of bean plants with angular leaf spot diseases on leaves, pods, and seed and asked if they had observed the symptoms and what they thought the cause could be (Fig. 2.2). More infected bean samples with other diseases including anthracnose, halo blight, and ascochyta blight were also shown to the farmers to determine whether they normally observed them in their bean fields.

<center>(a)</center>



<center>(b)</center>



<center>(c)</center>



<center>(d)</center>

**Figure 2.2: Angular leaf spot symptom on common bean plants under natural infection at Isangala village, Mbeya district.**
**Note: (a) high ALS disease severity (b) and typical disease symptom (c) Scientist with farmers looking at ALS symptoms in field planted to common bean in Mbozi district (d) Scientist interviewing one of the farmers on bean production constraints in Rungwe district**

To study levels of poverty within the bean farming communities, criteria were established based on low satisfaction of basic needs that included quality of the housing, food, access/satisfaction to education and health-care services (World Bank, 2012).

**2.3.4   Data Analysis**

The Statistical Package for Social Sciences (SPSS, 2012) was used for data analysis. The chi-square statistics with $P \leq 0.05$ were used to determine associations of various data pertinent to

<center>43</center>

awareness of angular leaf spot disease of common bean among districts. Spearman's rank correlation analyses were conducted to determine if there is any association between poverty indicators and the usage of fungicide. Also descriptive statistics with frequency distribution tables, percentages, charts and graphs were used to generate results on farmers 'awareness as well as to rank their preferences on parameters related to angular leaf spot disease such as bean varieties, bean production practices as well as causes of the disease.

## 2.4 Results

### 2.4.1 Demographic characteristics of households

The distribution of respondents on awareness of angular leaf spot (ALS) disease is presented in Fig. 2.3). The largest number of farmers on ALS awareness was recorded at Sumbawanga (24.8%) with the least at Mbeya rural (8.5%) and Njombe districts (8.4%). Of the 238 respondents, 127 (53.4%) were men and 111 (46.4%) women. Farm sizes per household were small ranging from 0.25 – 1.0 ha. According to the respondents more than 50% of the land was allocated to common bean production although the figure varied between households and districts. Most of the bean farmers depended heavily on traditional practices such as hoeing, mixed cropping and seed recycling for crop production with families being the main source of labor while a few had access to hired labor. According to the respondents, bean production in the surveyed villages was critical because it was consumed every day with food staples such as maize, rice, banana, potatoes and cassava.
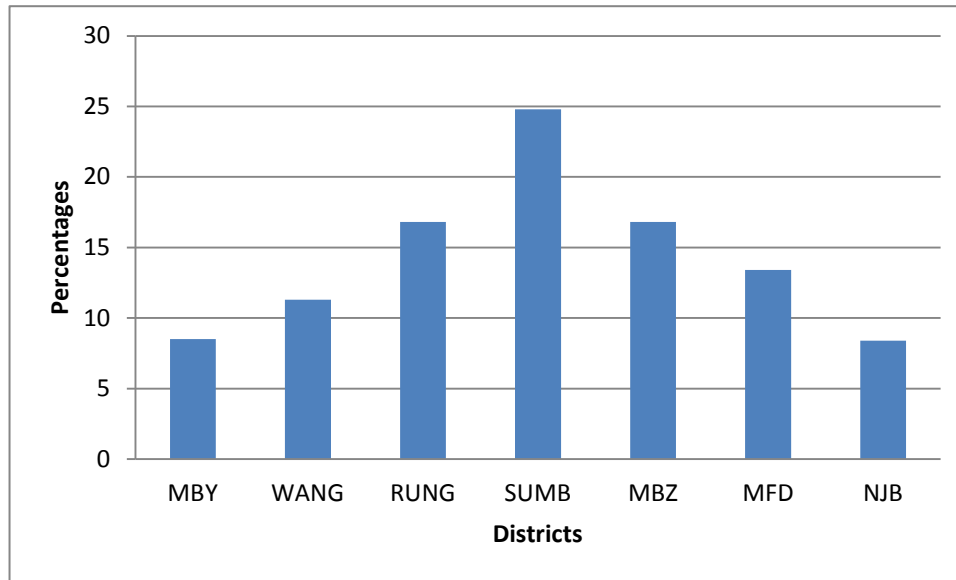
MBY = Mbeya rural, WANG = Wanging'ombe, RUNG = Rungwe, SUMB = Sumbawanga, MBZ = Mbozi, MFD = Mufindi, NJB = Njombe

**Figure 2.3: Percent distribution of respondents on awareness for angular leaf spot disease in the Southern Highlands of Tanzania**

### 2.4.2 Awareness of angular leaf spot and other diseases of common bean

All respondents reported diseases to be among the major constraints to bean production followed by insect pests and low soil fertility (Table 2.1). There was a wide variation in the awareness of bean disease symptoms among districts. About 62.0% of the farmers indicated that they normally observed angular brown spots on bean leaves but had no idea of the causal pathogen while 38.2% indicated that they neither paid attention to the shapes of the spots nor related them to disease infections. The differences between the respondents on awareness of angular leaf spot on leaves were statistically significant as indicated by the chi-square test (Table 2.2).

**Table 2.1: Bean production constraints indicated by respondents in seven districts of the Southern Highlands of Tanzania.**

| Bean production constraint | Percentage[1] |
|---|---|
| Diseases | 100 |
| Insect pest | 56.1 |
| Soil fertility | 49.8 |
| Weather changes | 30.5 |
| Distance to the field | 1.3 |
| Animals (destroying the crop) | 1.0 |

[1]Information obtained in multiple answers

**Table 2.2: Cross tabulation and Chi-square tests of awareness between districts on common bean diseases in the seven districts of the SHT**

| District | Angular brown spots on leaves | | Brown spots on pods | | Seed discoloration | | Halo blight on leaves | | Plant wilting | | Sunken brown spots on leaves | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Yes | No | Yes | No | Yes | No | Yes | No | Yes | No | Yes | No |
| MBY | 16 | 4 | 8 | 12 | 6 | 14 | 0 | 20 | 4 | 16 | 1 | 19 |
| MFD | 17 | 15 | 9 | 23 | 6 | 26 | 8 | 24 | 4 | 28 | 4 | 28 |
| SUMB | 30 | 29 | 19 | 40 | 10 | 49 | 6 | 53 | 10 | 49 | 6 | 53 |
| MBZ | 21 | 19 | 9 | 31 | 0 | 40 | 6 | 34 | 2 | 38 | 7 | 33 |
| NJB | 14 | 6 | 10 | 10 | 7 | 13 | 2 | 18 | 1 | 19 | 2 | 18 |
| RUG | 26 | 14 | 7 | 33 | 0 | 40 | 2 | 38 | 3 | 37 | 2 | 38 |
| WAG | 23 | 4 | 11 | 16 | 7 | 20 | 6 | 21 | 14 | 13 | 6 | 21 |
| Total | 147 | 91 | 73 | 165 | 36 | 202 | 30 | 208 | 38 | 200 | 29 | 209 |
| $X^2$ | 13.17 | | 35.56 | | 115.78 | | 133.12 | | 1.103 | | 139.18 | |
| $p \leq 0.05$ | 0.000 | | 0.000 | | 0.000 | | 0.000 | | 0.000 | | 0.000 | |

MBY = Mbeya; MFD= Mufindi; SUMB = Sumbawanga; MBZ = Mbozi; NJB = Njombe; RUG = Rungwe; WAG = Wanging'ombe

Additionally, 69.3% (165) and 84.8% (202) of farmers in all districts responded 'no' when they were asked to indicate whether the ALS symptoms on pods and seed respectively, shown to them were associated with disease infection. A statistical difference was observed on responses to seed and not on pods. For other bean diseases, the responses were statistically different with the exception of sunken brown spot on leaves (Table 2.2). However all the respondents who answered "no" on the question related to ALS symptoms were categorized into different age groups, as teenage farmers (below 18 years), young farmers (19 – 35 years), middle aged farmers (36 – 50 years), old farmers (51 – 65 years) and elderly farmers with age of over 66 years. Middle aged women farmers (58.1% responses) outnumbered middle aged men (41.8%) responses) on lack of awareness of ALS symptoms on leaves, pods and seed, while women (66.3%) responses in other age categories with the exception of the elderly, outnumbered men (33.6% responses) on lack of awareness of ALS disease on harvested seed. For the young, adult and elderly age categories, women were more aware of ALS symptoms on leaves compared to men. However 91% of the farmers associated the disease with foggy weather conditions which normally occurs during the bean cropping season while 8% related the disease to the effect of frost and very few (1%) to witchcraft. Analysis of other bean diseases particularly halo blight, plant wilting caused by fusarium root rot and sunken brown spot on leaves (anthracnose) indicated the same trend as those observed on ALS disease. The same results were obtained on other angular leaf spot symptoms on pods and harvested seed in which a large proportion of farmers regardless of gender and age indicated lack of awareness. In addition, analysis on other major diseases of common bean; halo blight, plant wilting (fusarium root rot) and sunken brown spots on leaves (anthracnose) indicated the same trend as those observed on angular leaf spot disease.

Considering angular leaf spot, 85% of the respondents did not know the effect of the disease on yield and quality of the harvested bean crop. When the respondents were asked to indicate stages of the bean crop at which ALS symptoms were normally observed, 64% reported the occurrence at three stages of bean development; seedling, vegetative and early podding. However, sources of inocula were not known to the farmers and the majority of them reported that ALS disease was not preventable. In this regard, a large proportion of farmers (80%) were unable to mention any control measures with the exception of 3% who indicated the possibility of using fungicides. Those who mentioned fungicides indicated using Cuprous oxide traded as AGCopp 75 Wpand

this type of fungicide did not match any of those recommended for the control of ALS disease in common bean.

Considering sources of information on bean diseases available in their communities, 90% of respondents were not aware of the sources, while some indicated they acquired information from fellow farmers, agricultural extension staff, relatives, parents, researchers and mass media in particular radio and television broadcasts in descending order (Table 2.3). According to the respondents, the information was very limited with less emphasis on bean disease control measures. In discussing with farmers on the type of new varieties they would prefer, all of them expressed strong preferences for disease resistance including angular leaf spot resistance. Other preferred traits were; earliness, fast cooking and good taste that included tenderness and thick broth. Early maturing varieties were highly preferred as they were the first to be harvested, therefore used to feed the family and meet household cash needs when sold.

**Table 2.3: Possible sources of information for the respondents on angular leaf spot disease to common bean farmers in the Southern Highlands of Tanzania.**

| Source of information | Percentage[1] |
| --- | --- |
| None (respondents could not tell) | 99.5 |
| Fellow farmers | 48.1 |
| Agricultural extension staffs | 21.6 |
| Relatives | 9.6 |
| Parents | 8.0 |
| Researchers | 6.2 |
| Mass media (radio/TV) | 0.6 |

[1]Information obtained in multiple answers

### 2.4.3  Bean production practices under smallholder farming systems

Bean production practices listed by farmers were; tillage, sowing, weeding, crop residual management, crop rotation and harvesting as described below.

### 2.4.3.1 Tillage

Hoeing and the use of ox-drawn ploughs were reported in the surveyed villages as the main methods used to till the land. Hand hoe was mainly used in Rungwe and Mufindi Districts (99%), whereas ox-drawn ploughs were reported in Sumbawanga (72%) and Mbozi (45%) districts. The main source of labor for tilling the land was the family, while hired labor, relatives and self- help groups contributed less labor for the bean production (Table 2.4). A number of farmers (5%) indicated that they practiced zero tillage due to delays caused by various reasons that included selling labor, sickness and attending social events such as weddings and burial ceremonies. In general, 89% of the respondents in this study practiced minimal tillage with huge retention of the previous season's crop residues on the soil surface.

**Table 2.4: Labor sources in common bean production considering tillage practice reported by farmers in bean growing districts of the Southern Highlands of Tanzania.**

| Source of labor | Percentage[1] |
|---|---|
| Family (father, mother and children) | 100.0 |
| Family (father and mother) | 73.4 |
| Male famer | 63.0 |
| Female farmer | 23.1 |
| Hired labor | 7.9 |
| Relatives | 2.3 |
| Self-help groups | 1.4 |

[1]Information obtained in multiple answers

### 2.4.3.2 Sowing

Farmers indicated that they sowed bean seed in a mixed cropping system where the beans were planted together with maize, coffee, banana, cassava and/or cocoyam. Some farmers (15%) planted beans as a sole crop. However, 78% of all farmers had no standard spacing used in planting beans and were not aware of the recommended spacing for the bean crop. Consequently, the amount of seed sown per unit area was very low estimated at 45 kg ha$^{-1}$ under sole crop

compared to recommend seed rates of 90 – 100 kg ha $^{-1}$. About 95% of the women farmers were involved in sowing common bean especially in Rungwe districts where division of labor was evident. In Sumbawanga districts, 58% of the farmers, especially those who owned large fields, broadcasted beans followed by harrowing using oxen.

Most of the farmers regardless of age grew mixtures of local (72.6%) than improved varieties (7.98%). About 19% of the respondents grew both local and improved varieties but in separate fields. Male farmers (97%) grew more of the local varieties than female farmers (79%), while small differences were observed between female (22%) and male (21%) farmers in growing both improved and local varieties. The local varieties grown, constituted a mixture of seeds with different sizes, colors, shapes and growth habits. Elderly farmers maintained and grew diverse varieties not only of beans but also of other crops. The farmers indicated that they chose the varieties based on a number of reasons including taste, color and seed size. Seed coat cleanliness which had an implication of disease free was also indicated as one of the criteria in selecting seed for planting. Multiple needs for consumption were indicated by 82.1% of farmers as one of the reasons farmers maintained a number of bean varieties. For example, some bean varieties were more preferred because of their tasty leaves in making cooked vegetables while others were suitable only for their grains. Furthermore, comparison between age groups indicated that 38.2% of middle aged farmers grew more local varieties compared to the other categories. Only 2.3% and 6.3% of teenage and adult groups, respectively, grew local varieties of beans (Table 2.5).

**Table 2.5: Cross tabulation on the relationship between age and types of bean varieties grown and seed sources in the Southern Highlands of Tanzania.**

| Age (years) | Bean varieties grown | | | Seed sources | | | |
| | Local | Improved | Improved + local | Own saved seed | Friends | Relatives | Local market |
|---|---|---|---|---|---|---|---|
| <18 | 0 | 0 | 0 | 4 | 0 | 0 | 0 |
| 19-35 | 35 | 2 | 9 | 37 | 0 | 0 | 9 |
| 36 - 50 | 91 | 11 | 25 | 96 | 0 | 1 | 23 |
| 51 - 65 | 34 | 5 | 10 | 37 | 7 | 2 | 10 |
| >66 | 11 | 1 | 0 | 10 | 0 | 0 | 2 |
| Total | 173 | 19 | 46 | 184 | 7 | 3 | 44 |

Most of the respondents acquired seed through informal seed systems. Of these, 91.7% used own seed saved from the previous crop, while 25.4% purchased seed from local markets and 3.1% received the seed as gifts, and 3.1% from friends and relatives. The respondents indicated seed recycling to be a continuous process in which farmers planted thrice in a year; twice during the long rains and once during the dry seasons mainly in the valleys and along the banks of the seasonal rivers. Seed purchased from the markets were from different parts of the SHT including neighboring countries such as Malawi and Zambia.

Farmers, who planted their own seeds, were asked whether sorting to remove discolored seed was done before planting. The results indicated that, 85.7% sorted their seed and 14.2% did not practice seed sorting. The middle aged group, regardless of gender outnumbered the teenage group, youth and the elderly groups in sorting seed. The age categories of elderly (above 66 years) and teenage (below 18 years) reported less seed sorting. The amount of seed discarded from the harvested crop in the seed sorting process was determined by the proportion retained for every unit of the harvested seed. This ranged from 5 – 25% during the dry season and about 50 - 90% for the crop planted during the rainy season. Removal of discolored seed was the main criterion indicated by the respondents and the proportion discarded differed between the sexes. Some retention of discolored seed was reported as a result of low yields. However, a higher number of male farmers were discarding seed compared to women and the differences were statistically significant when the Chi-square test was used at $P \leq 0.05$ (Table 2.6).

**Table 2.6: Chi square test between male and female on proportion of seed discarded as a result of sorting in the Southern Highlands of Tanzania.**

| Proportion of respondents who sorted the seed | | | |
|---|---|---|---|
| Variable | Observed | Expected | Chi - square |
| Male | 127 | 119 | 0.063 |
| Female | 111 | 119 | 0.063 |
| $X^2$ | | 0.126  < P 0.05 at 3.84 | |

### 2.4.3.3 Weeding

All the respondents reported hoeing and hand pulling as the only means used to control weeds in bean fields. Eighty six percent weeded their crops once during the season whereas 10% weeded twice and 4% did not weed at all. For those who indicated to weed once, the activity was done just before flowering and the hand hoe was used as the tool in weeding. The main source of labor was the family in which women farmers spent more time in the field compared to men. Weeding in a mixed cropping pattern was done by hand pulling and occasionally by small hand hoes.

When farmers were asked whether crop residue management and crop rotation was practiced for the general control of bean diseases, 89.9% indicated not to practice crop rotation and were not aware of the importance of crop residue management in controlling bean diseases. However, 10.08% of the respondents were aware of the importance of crop rotation but unable to implement the practice. Regarding crop residues, 87.1% of farmers indicated that they left them in fields after harvest and about 12% used the residues as animal feeds. Farmers were subsequently asked whether crop residuals can be burned in the field as a way of controlling pests but all indicated that it is prohibited by Zone by-laws to initiate fire in fields.

## 2.5 Harvesting

The majority of respondents (80%) reported to harvest and thresh the beans in the fields while 20% of the respondents reported that the beans were carried out of the field to the homesteads where threshing was done. The main source of labor for harvesting was the family (82.3%) followed by both family members and casual laborers (7.6 %) and lastly collaboration between family members and self- help groups. When the respondents were requested to estimate yield, 73.6% reported harvesting between 200 – 400 kg ha$^{-1}$ while 26.4% harvested between 500 – 1000 kg ha$^{-1}$.

## 2.6 Poverty and levels of education in relation to awareness of angular leaf spot disease

The surveyed farmers were asked to indicate their main sources of income. Farming activities accounted for 80% while less than 20% derived their income from wage employments, petty

business and livestock keeping. Male farmers (23%) had more access to income generating activities other than crop farming activities compared to their female counterparts (19%).

The levels of poverty within the bean farming communities of the SHT were measured. Significant differences were observed between farmers on satisfaction/ dissatisfaction levels to basic needs such as housing, clothing, access to good health care services and access to education (Table 2.7). Most of the respondents (44.5%) were more satisfied with the availability of food at the household than other traits, and indicated that they had at least two meals a day. Details about the nutritional value of the meals consumed are beyond the scope of this study. On the other hand, 61.3% of the respondents regardless of sex indicated that they were less satisfied by the type and quality of their houses, clothing, and the ability to access health and education services.

**Table 2.7: Comparison between respondents and satisfaction levels to basic needs in the Southern Highlands of Tanzania.**

| | | | Satisfaction levels to basic needs | | |
|---|---|---|---|---|---|
| Response | Housing | Food | Clothing | Healthcare services | Education services |
| Yes | 91 | 114 | 89 | 98 | 146 |
| No | 147 | 124 | 149 | 140 | 92 |
| $X^2$ | 13.17 | 0.42 | 15.12 | 7.41 | 12.25 |
| P< 0.05 | 0.00 | 0.516 | 0.00 | 0.00 | 0.00 |

The levels of education within farming communities have been shown to play a significant role in analyzing farming situations and access to agricultural information, on the potential benefits and drawbacks of farm enterprises. In this study, the majority of sampled farmers indicated to have attained primary and secondary levels of education accounting for 79.4% and 10.9% respectively. The least were farmers with college level of education (0.84%). However, the most educated were male farmers in which 53.97% attained primary education whereas female farmers indicated to have more of the informal knowledge as compared to males (Table 2.8). The high number of respondents that were not satisfied with the basic needs, had low levels of education and could not

differentiate ALS disease symptoms with other abiotic factors such as effect of frost on the bean plants

**Table 2.8: Gender versus levels of education among 238 common bean growing farmers in the Southern Highlands of Tanzania.**

| Gender | Primary school | Secondary school | College education | Informal education |
|---|---|---|---|---|
| Males | 102 | 21 | 2 | 4 |
| Females | 87 | 5 | 0 | 17 |
| Total | 189 | 26 | 2 | 21 |

## 2.7 Discussion

Angular leaf spot was among the major production constraints to common bean in the SHT in this study. The disease was prevalent in all of the seven districts surveyed as indicated by 61.7% of respondents who recognized ALS symptoms on bean leaves in their fields. However, there was a misconception as farmers related the disease symptoms with the effect of frost and foggy weather conditions. The burning effect caused by frost on bean leaves for late planted crops probably explains why farmers failed to associate ALS symptoms with disease, and as such none of them were familiar with the causal agent. It has been reported that, in the presence of a susceptible host, *P. griseola* thrives well at temperatures ranging from $20 - 25^0C$ with adequate moisture characterized by high relative humidity of 95 -100% (Liebenberg and Pretorius, 1997). These conditions are commonly associated with foggy weather of the SHT therefore; fulfilling the critical environmental requirements for successful infection and sporulation of the pathogen. *P. griseola* infects other aerial parts of the bean plant although farmers were unable to relate the symptoms observed on pods and seed with the disease. The majority of the farmers have attained some levels of education regardless of being formal or informal. However, all of them knew little about the sources of inoculum and even less on the role of the bean seed in transmitting the disease. This might be attributed to the lack of information about the disease.

Survival of *P. griseola* on/or within the infected seed is one of the most effective means of transmitting the pathogen. As observed in this study, overdependence on recycled seed from the previous harvest coupled with limited knowledge on ALS disease symptoms created a possibility of accumulating new races of the pathogen. *P. griseola* is highly variable with different pathogenic races that can possibly overcome resistant genes even if they are present in different backgrounds. Since seeds were acquired from various sources including the neighboring countries of Zambia and Malawi, chances of increasing pathogenic races in the zone are high. The seed plays a significant role in increasing the incidence of ALS as seedlings arising from contaminated seed harbor high inoculum levels of the pathogen that can colonize developing leaves and large proportion of seed discolored with a high negative effect on yield and seed quality. Although farmers indicated that they sorted their seed prior to planting, the method had little effect on the pathogen as deep seated infections often go unnoticed. Additionally, lack of enough seed for planting may have forced farmers, particularly females, to retain discolored seeds that were infected. This is supported by the survey data that indicated low yields that ranged from 200 - 500 kg ha$^{-1}$ lower than the national average of 741 kg ha$^{-1}$.

Availability and access to agronomic information related to angular leaf spot is important in reducing the disease incidences in common bean production. In this study, the majority of farmers grew a mixture of local varieties acquired from different local sources and no ALS disease management was implemented. The effectiveness of varietal mixtures in reducing disease epidemics has been indicated as either through eliminating the number of spores landing on the varieties with high levels of resistance in the mixture or by some of the resistant varieties acting as barriers to spore movement due to increased distance between resistance and susceptible plants (Cox et al., 2004). Consequently, the efficiency of the pathogen may be reduced as the inoculum produced in one plant in a mixture may not be virulent on other plants in the population. However, for a mixture of varieties to be effective in controlling disease, cultivars used in a mixture must possess known levels of disease resistance. For the case of bean farmers in the SHT, the varietal mixtures were done according to family preferences and one may mix more than five varieties with no records of resistance or susceptibility to ALS disease. It is evident that there is a need to conduct systematic studies on the levels of susceptibility or resistance within local bean varieties kept by smallholder farmers. Consequently, farmers have their own preferences such as

seed color, growth habit, seed size and taste that must be considered in any efforts of deploying resistance genes in developing new bean varieties.

Bean production methods play a significant role in increasing or decreasing disease incidences in a given locality. In this study farmers indicated that they practiced traditional farming in bean production that included the use of hand hoe in tilling the land. Hoeing and occasionally zero tillage were practiced with huge retention of crop residues on the soil surface. Even though a number of farmers used bean straws as animal feeds, still large quantities of the inoculum may have remained in the fields as ALS infected leaves tend to fall-off prematurely before the crop is harvested while harboring large quantities of inoculum, which can infect the next bean crop. It has been reported that *P. griseola* has the ability to survive for up to 19 months on host plant residues and up to 12 months on infected seed (Mwang'ombe et al., 2007). Considering that beans are planted thrice in a year, high inoculum densities are expected. Survival of the pathogen on crop residues has been reported on other crops including maize. For example, under favorable conditions for disease development, if a farmer leaves as little as 10% crop residues infected with *Cercospora zeae maydis* on the soil surface, high incidences of maize grey leaf spot are expected even for a crop planted in an adjacent field (Nazareno et al., 1993). Available evidence also indicates increase in fusarium head blight caused by *Fusarium graminearum* when sequential wheat to wheat is planted under minimal tillage (Xu and Nicholson, 2009).

Field sanitation includes removal, soil incorporation or burning of residues left in bean fields. These practices have been advocated as some of the cultural control measures in efforts to reduce survival of the crop pathogens including *P. griseola* (Sindhan and Bose, 1979). In this study, the majority of bean farmers were not practicing field sanitation partly due to the lack of awareness and knowledge about the effectiveness of the technology, lack of appropriate tillage implements to plough under the infected crop residues and the local government restrictions on initiating fires in fields. On the other hand, farmers were unable to practice crop rotation which is recommended for the control of ALS as the technology suggests beans to be grown once in three years when the same field is used. As indicated by the respondents, average land holdings whether rented or owned were very small ranging from 0.5 – 1.0 ha, lower than the average landholdings in Africa which is 1.6 ha (African Development Bank, 2011). Such small fields are an indicator of high population pressure that forces farmers to practice intensive cultivation. Since common bean is

the main source of protein that is often consumed with starchy staples of maize and other cereals, practicing crop rotation as it is recommended means lack of protein and relish to the diets of many which will be detrimental to the health of the bean farming communities of the SHT.

As indicated by the respondents, weeding was done once or twice and in some cases no weeding at all. Unweeded fields create microenvironments within the field that improve conditions for disease development as a result of high numbers of plants per unit area that increases dew, humidity and temperature. *P. griseola* depends on free water on the leaf surface of a susceptible host for the spores to germinate, penetrate and establish infection (Allorent and Salvary, 2005). In this regard there was a high possibility of creating ALS diseases infections considering the types of weed management practiced under bean production systems of the SHT.

From the results, the majority of the respondents were poor and not satisfied with the basic necessities that included housing, access to health and education services. Satisfaction of basic needs is often linked with sources of income. The sources, particularly crop farming and occasionally petty businesses, wage employment and livestock keeping were not generating enough income to sustain household needs and farming activities. These results confirm the reports of FAO, 2010 that the numbers of smallholder farmers who can access to additional income apart from farming activities are very low. As a result farmers continued with their traditional methods for bean production with high chances of increasing incidences of angular leaf spot disease. There were a small number of farmers (4%) who associated ALS symptoms with disease infection and they were using fungicides. However, none of them knew about the recommended fungicides for controlling ALS and appropriate time for application. As a result, spraying was done after the crop has been infected. The weak but negative associations between fungicide usage, housing satisfaction and access to education services were good leads that if ALS disease is properly controlled, chances of improving livelihoods through increased bean yields are high.  The information obtained on fungicide usage was important in developing fungicide based management strategies to control ALS as a short term option to bean farmers.

## 2.8 Conclusion

This study established that ALS disease is a problem in the bean farming communities of the Southern Highlands of Tanzania. Majority of farmers were not aware about the disease and its

adverse effect on yield. The traditional bean farming practices could have contributed to the innoculum built ups of the pathogen. It was evident that most farmers were poor as indicated by low satisfaction of basic needs an implication of low purchasing power of farm inputs. Additionally the education levels were low to grab disease control recommendations that are often made in languages other than the local ones. Therefore, breeding disease resistant bean varieties with preferred traits for home consumption and the market will be a long term solution in managing ALS.

# References

African Development Bank. 2011. The Middle of the Pyramid: Dynamics of the Middle Class in Africa. Market Brief. www.afdb.org.

Alkire, I. S and M. B. Sarwar. 2009. Multidimensional Measures of Poverty and well-being Oxford Poverty and Human Development Initiatives. Oxford University. Retrieved from www.ophi.org.uk/wp-content/uploads/OPHI-RP- 6a.pdf.Accessed on 30th May, 2014.

Allorent, D and Savary, S. 2005. Epidemiological characteristics of angular leaf spot of beans. A system analysis. European Journal of Plant Pathology. 113: 324 -341

Broughton, W.J., G. Hernandez, M. Blair, S. Beebe, P. Gepts, J. Vanderleyden. 2003. Beans (*Phaseolus* spp.): model food legumes. Plant Soil 252**:** 55–128.

Cox, C. M., K. A. Garrett, R. L. Bowden, A. K. Fritz, S. P. Dendy and W. F. Heer. 2004. Cultivar mixtures for the simultaneous management of multiple diseases: Tan spot and leaf rust of wheat. Phytopathology. 94:961-969.

David, S., Kirkby, R and Kasozi, S. 2000. Assessing the impact of bush bean varieties on poverty reduction in Sub-Saharan Africa: Evidence frown Uganda. Network on Bean Research in Africa. Occasional Publication Series, No. 31, Centro Internacional de Agricultura Tropical, Kampala, Uganda

Food and Agriculture Organization of the United Nation Organizations (FAO). 2004. The state of agriculture commodity and market www.fao.org/docrep/fao/007/y5419e/y5419e00.pdf Accessed on April 2, 2014.

Food and Agriculture Organization of the United Nation Organizations (FAO). 2008. Production data. http.fao.org/faostat. Accessed on July 28, 2014

Food and Agriculture Organization of the United Nation Organizations (FAO). 2010. Men and Women in Agriculture: Closing the gap (2010) retrieved from http://www.fao.org/sofa/gender/key-facts/en/, Accessed on March 6, 2014

.

Gan, Y. T., Siddique, K.H.M., W.J. M. MacLeod, W.J.M., Jayakumar, P. 2006. Management options for minimizing the damage by Ascochyta blight (*Aschochytarabei*) in chickpea (*Cicerarietinum* L.) Field Crops Research. 97: 121 – 134.

Hidalgo, O., Campilanand, D and Lama, T.  2001. Strengthening farmer capacity to grow a healthy potato crop in Nepal. In: CIP, Scientist and farmer, partners in research for the 21$^{st}$ Century. 1999-2000. CIP Programme Report. Lima, Peru

IBM Corporation Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.

Liebenberg, M.M and Z. A. Pretorius. 1997. A review of angular leaf spot of common bean (*Phaseolus vulgaris* L.).African Plant Protection. 3: 81–106.

Mamun, A and J. Adaikalam, J. 2011. Examining the levels of unsatisfied basic needs among low- income women farmers in Peninsular Malaysia. International Research Journal of Finance and Economics. 74: 42 - 50

Mwangombe, A.W., I.N. Wagara, J.W. Kimenju and R.A. Buruchara. 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: 235-241.

Nazareno, N. R., X. P. E. Lipps and L. V. Madden. 1993. Effect of levels of corn residue on epidemiology of gray leaf spot of corn in Ohio. Plant Disease. 77:67-70.

Nyomora, A. 1990.Screening of Peach Fruit Cultivars in the Southern Highlands of Tanzania. Retrieved from http://www.actahort.org/books/279/279 Accessed on February 3, 2014.

Sindhan, G. S. and S. K. Bose. 1979. Perpetuation of *Phaeoisariopsis griseola* causing angular leaf spot on French beans. Indian Phyopathology. 32(2):252 – 254.

Tanzania - FAO wheat database. Wheat production potential in Tanzania 2013. Retrieved http://www.fao.org/ag/Agp/AGPC/doc/field/Wheat/africa/tanzania/tanzaniaagec.htm Accessed on May 28, 2014. Uyole Agricultural Research Institute. Southern Highlands Zone. 2012.

http://www.erails.net/TZ/ari-uyle/ari-uyole/about-us/ Accessed on 4$^{th}$ August, 2013 Wagara, I. N., A. W. Mwango'mbe, J.W. Kimenyu and R.A. Buruchara. 2005. Virulence, variability and physiological races of angular leaf spot pathogen *Phaeoisariopsis griseola* in Kenya. African Plant Protect. 11: 23 – 31.

Wortmann, C. S., R.A. Kiluby, C.A. Eledu and D. T. Arron. 1998. Atlas of common bean. (*Phaseolus vulgaris* L).Production in Africa. CIAT, Cali- Columbia.

World Summit for Social Development.1995. Program of action of the world summit for social development. Copenhagen, Denmark

Xu, X. M., and P. Nicholson. 2009. Community ecology of fungal pathogens causing wheat head blight. Ann. Rev. Physiopathology .Doi: 10.1146/annual review of phytopathology

# 3 Agronomic performances and economics of yield losses associated with angular leaf spot disease of common bean in the Southern Highlands of Tanzania

**Abstract**

Angular leaf spot (ALS) caused by *Pseudocercospora griseola* is among the devastating diseases of common bean (*Phaseolus vulgaris* L.) in the Southern Highlands of Tanzania (SHT) with a significant negative effect on yield and seed quality of the crop. This study was conducted to examine the economics of yield losses associated with the disease on five selected bean varieties that are commonly grown by farmers, as an effort towards developing sustainable control measures against *P. griseola*. The varieties were evaluated with three different rates of fungicide treatments using a split plot design in a randomized complete block arrangement during the 2012/2013 and 2013/2014 bean cropping seasons. The main plots were fungicides and subplot consisted of varieties. Data were collected on disease severity, yield and yield components that were analyzed using Genstat software package. An economic analysis was done using partial budget where marginal rate of returns was determined. Results indicated significant decrease at P< 0.05 in yields, number of pods, seeds and, seed weight for plots that were not treated with fungicides at the recommended rates. Decrease in the yield and yield components were associated with ALS disease severity that in turn was influenced by the varieties and the rate of fungicide used. Higher grain yield losses as much as 61% with lowest marginal rate of returns were recorded for unsprayed plots during heavy rains compared to light rains. Since the varieties evaluated were susceptible, appropriate fungicides at the recommended rates should be considered as a short term while breeding for resistance be taken as an economical and sustainable strategy in managing the disease.

## 3.1 Introduction

Angular leaf spot (ALS) caused by *Pseudocercospora griseola* is one of the major diseases of common bean (*Phaseolus vulgaris* L.) reducing yields and seed quality. *P. griseola* is a seed borne pathogen that infects bean seedlings early in the season, while secondary infections occur rapidly under favorable conditions. The pathogen survives on infected seed, off season bean plants and saprophytically as mycelium and conidia in association with the host residues on or near the soil surface. Successful establishment of *P. griseola* inside the bean plant produces a toxin that causes necrosis of the plant guard cells and the adjoining mesophyll cells, damaging plasma and chloroplast membrane, followed by staining the chloroplast red contrasting with the normal green chloroplast (Cardona Alvarez and Walker, 1956). Additionally, the pathogen has the ability to colonize intercellular spaces of the palisade cells of the leaf and other aerial parts of a bean plant forcing these cells to disintegrate and form necrosis lesions (Cardona – Alvarez and Walker, 1956; Monda et al., 2001). These lead to a reduction in photosynthesis rate and subsequent yield and quality losses of the crop (Waggoner and Berger, 1987).

It has been reported that the photosynthetic rate in common bean increases from flowering to pod setting (Patterson et al., 1977), a period that coincides with the maximum infection of *P. griseola* on susceptible cultivars. Angular leaf spot disease at these stages of plant development causes high yield losses by reducing the net assimilation rate through a sink effect in which the pathogen diverts carbon fluxes from the growing seed for their own growth (Farrar, 1992). As a result, most of the plants bear pods with few seeds that are shriveled and often discolored. This poses negative effect on the yield, quality and marketability of the crop at farm level. Since the seed yield of common bean is usually influenced by the number of pods per plant, number of seed per pod and average seed weight (Sinha, 1977), reducing any of these yield components will lead to low yields. Yield losses as a result of reduction in photosynthesis have been documented on barley infected with *Rhychosporium secalis* and in peanut leaflets infected with *Cercosporium personatum* (Bourgeois and Boote, 1992; Walters et al., 2014). The same have been experienced in common bean where yield losses ranging from 50% to more than 80% have been reported in the bean growing countries of Uganda, Kenya, Mexico and Brazil (Sartato and Reva, 1992; Liebenberg and Pretorius, 1997; Stenglein et al., 2003; Borel et al., 2011). In the Southern Highlands of Tanzania (SHT), ALS is one of the diseases that results in the greatest negative

impact to common bean with up to 80% yield loss when susceptible cultivars are grown (Shao, 1987).

Considering the crop damage that is caused by the ALS disease it is necessary to establish yield loss and its relationship with disease severity in order to recommend proper control measures for the welfare of smallholder farmers. There are several methods that have been used to study the relationship between yield loss and disease severity. The most commonly used is the area under disease progress curve (AUDPC). The AUDPC model on host-pathogen and host pathogen – fungicide interaction have been used to directly link yield losses with diseases in potatoes, maize, rice, beans and pea (Filho et al., 1997; Godoy et al., 2003; Su et al., 2006; Simko and Piepho, 2011). The model takes into account the number of times the disease severity is evaluated and the duration of the epidemics which is defined as an increase in disease with time (Su et al., 2001). Additionally, AUDPC integrates host, pathogen and environmental effects occurring during the epidemics. This is important especially for facultative (hemi-biotrophic and nectrophic) pathogens like *P. griseola* that cause varying degrees of damage to plants throughout the growing period of the crop (Brower and Kinkel, 1997). Like any other foliar diseases of common bean, ALS on leaves and pods starts at low levels progressing gradually into high levels of severities overtime during pathogen epidemics. This is a typical sign of a quantitatively inherited trait conferred by the effect of multiple genes and has been observed in barley against powdery mildew and in common bean against angular leaf spot on pods and leaves (Aghnoum et al., 2010; Borel et al., 2011).

The economics of yield losses or gains on various crops has been evaluated by the yield levels that provide the highest or lowest financial returns to investments in producing or protecting the crop against diseases (Anders et al., 1996; Egbe and Idoko, 2010). Economic losses caused by the disease tend to occur when economic gains are otherwise depressed because of poor crop yields that result in low returns. The proportion of yield losses one might experience and economic gains/losses when control measures are undertaken to reduce damage to the crop are important as they influence the farmer's decision regarding the control technology to adopt. Estimation of farm income benefits through disease management using fungicides have been done for different crops including common bean against anthracnose disease (Lamessa et al., 2011; Sileshi et al., 2014). Although management of ALS disease under smallholder bean farming system is very limited and

in some cases does not exist at all, the use of fungicides in protecting the crop has been advocated by various stakeholders as a short term strategy in controlling ALS. However, no precise information is available on the impact of ALS disease on common bean yields and no experimental studies have been performed in the SHT that relates bean yield losses due to ALS and economic losses. Since no cultivars have been released in the zone that is highly resistant to *P. griseola,* the use of preventive fungicides to protect the plants against ALS is necessary to successfully establish the relationship between yield and the disease. Therefore, this study was conducted to examine the economics of yield losses associated with ALS disease using five selected bean varieties that are commonly grown by farmers, as an effort towards developing sustainable control measures against *P. griseola*.

## 3.2    Materials and Methods

### 3.2.1    Description of the study area

This study was conducted at the Uyole Agricultural Research Institute (ARI – Uyole) located in the Southern Highlands of Tanzania. The Institute is situated at $8^0 53$'S and $33^0 39$'E with altitude of 1798 meters above sea level (masl). The soils are loam, well drained and the temperature ranges from $18.0 – 23.7^0 C$ during the year with high relative humidity of 95% during the rainy season. The location is characterized by unimodal type of rainfall that occurs between the months of December and May, allowing for two plantings of common bean. This type of weather conditions favors ALS disease development and ARI – Uyole is one of the hot spots for the disease in the zone.   Additionally, the trial site had been planted with common bean in the previous years to ensure availability of primary inocula from infested plant debris for early infections.

### 3.2.2    Experimental material and cultural procedures

Three ALS susceptible varieties of common bean; Kablanketi (semi climber), Maini (bush type), Kigoma (bush type) that represented local varieties highly preferred by the farmers in the SHT and two susceptible commercial check varieties DRK and Kasukanywele (Uyole 94) both semi-climbers were used in the experiments. The seeds were obtained from the local market where they are sold as farm saved seed, which is one of the major sources of seed for the majority of smallholder farmers. About one kilogram of seed was purchased for each variety. Four trials were

conducted in 2012/203 and 2013/2014; two at the beginning of the rainfall during heavy rains (December – March) and two towards the end of the rainy season (March – July) also known as light rain season.

The trials were laid out in a split plot arrangement of a randomized complete block design with three replications. The main plots were assigned to fungicide sprays while the subplots were assigned to the five varieties. Each subplot measured 2 m x 3 m and consisted of five rows, each hand seeded with 32 seeds at 10 cm intra-row and 50 cm inter-row spacing. Border rows were not included as it has been established that they have no effect on yield (Parrella et al., 2013). All agronomic practices including fertilizer applications and weed management were as recommended. Plants were sprayed with insecticide (Actellic 50 EC) containing 500 g/l (49.02% w/w) pirimiphos-methyl at 10 days after planting and at pod setting to protect the plants against the bean fly and bean pod borers.

### 3.2.3   Fungicide application

Since there was no record of which bean varieties were highly resistant to ALS pathotypes for the SHT, a preventive and curative protection fungicide Othello Top containing Azoxystrobin 200 gm/ℓ + Difenoconazole 125 gm/ℓ SC was used. The fungicide was used as a foliar application, four times during the growth period of the bean plants at intervals of 10 days starting at 15 days after the beans had germinated. In all of the four trials, the main plots consisted of three treatments; i) zero application where no fungicide was applied, ii) farmers' practice in which the fungicide was applied once at a rate of 50 mℓ ha$^{-1}$ at late stages of crop development and disease infections, and iii) fungicide at the dosage of 400 mℓ ha$^{-1}$ applied four times at vegetative stage, flower initiation, late flowering and pod filling. The third treatment was per manufacturers' recommendation in protecting the bean crop against the ALS disease.

### 3.2.4   ALS disease evaluations

The trials were exposed to natural ALS disease infestation. Disease severity was scored at 10-day intervals starting from the first appearance of the tri-foliate leaf to physiological maturity of the bean plants. The CIAT 1 – 9 scale was used to assess the disease in each sub plot, where 1 – 3 scores were assigned to plants with nil or sparse lesions; 4 – 6 to plants with well-defined lesions

but sparse and 7 – 9 to plants with many and well defined lesions (Van Schoonhoven and Pastor – Corales, 1987). The disease severity ratings were used to calculate AUDPC for each subplot according to the formula used by Campbell and Madden (1990) as indicated below

$$\text{AUDPC} = \sum_{i=1}^{n-1} \left( \frac{y_i + y_{i+1}}{2} \right)(t_{i+1} - t_i) \quad \text{...................................................... (1)}$$

Where "$t_i$" is time in days of each reading, "$y_i$ is an assessment of the disease (proportion of infected leaves at a 0 – 9 CIAT scale)" and "$n$" is the total number of observations made

### 3.2.5 Yield and yield component evaluations

Data were collected for plant height, number of plants per plot, number of pods per plant and number of seeds per pod taking an average of 10 plants for each sub plot. Plant height was measured in centimeters from the base of a plant at ground level to the top of the canopy. At maturity all plots were harvested and the seeds were cleaned to remove all unwanted materials including plant debris and soil before weighing them. Hundred seed weight was taken for each plot and recorded in grams. Thereafter, the diseased seeds were sorted out and market valued seeds were expressed in metric tons per hectare.

### 3.2.6 Economics of yield losses

The economics of yield losses were determined by classifying the harvest into market valued and non-market valued seed. The classification was done by separating out diseased and discolored seed from the healthy seed for each plot, then weighed and recorded in kilograms per hectare. The market prices for a tin (20 kg) of beans were obtained from the local market where the five varieties are sold. These prices were used to estimate the total returns. Bean yields obtained in each plot were assumed to be the result of ALS occurrence, fungicide applied and the varieties used as all other agronomic practices were carried out as recommended. All the costs (labour, fertilizers, fungicide applications, fungicide, weeding and harvesting) were recorded per hectare basis.

The marginal rate of return (MRR) was calculated to determine the increase in net benefits (NB) for each treatment using the following formula:

MRR = (NB$_2$ – NB$_1$)/(TVC$_2$ – TV$_1$)......................................……………………………. (2)

Where MRR = marginal rate of return; NB$_1$ = net benefit for a treatment (Farmers' practice); NB$_2$ = net benefit for using fungicide at the recommended rates and TVC$_1$ (Total variable costs) for treatment one and TVC$_2$ is the total variable costs associated with the usage of treatment two.

### 3.2.7   Data analysis

The AUDPC were calculated for each variety using Microsoft excel programme. The analysis of variance was performed on AUDPC, yield and yield components using Genstat software version 14.1 (Payne et al., 2011). Least Significance Difference (LSD) was used to separate treatment means at $P \leq 0.05$. Fungicides x variety effects were not significant among the two heavy and two light rains. Therefore, the data were pooled and a combined analysis done. Percentage yield loss was determined for each variety using the following formula:

Yield loss = (YSP – YNF/YSP) x 100 …………………………………………………. (3i)

Yield loss = (YSP – YFP/YSP) x 100 …………………………………………………. (3ii)
Where YSP is yields obtained from fungicide sprayed plots; YFP = yields obtained from plots sprayed with fungicide at farmers rates and YNF = yields obtained from unsprayed plots. Correlation analysis was done for each variety to examine the relationship between the disease (AUDPC) and the yield data.
Cost benefit analysis was computed for market valued seed for all the three treatments using partial budget analysis method (Preez et al., 2005; Katungi et al., 2011). The prices used in the computation were obtained from the local market during the study. Labor costs for managing and harvesting the crop were calculated based on one hectare.

## 3.3   Results

### 3.3.1   ALS disease severity
Angular leaf spot occurred in non-sprayed plots for all trials, but more so during heavy rains than during light rains. The disease infections started on the primary leaves stage (VC growth stage) as circular lesions that progressed rapidly to the trifoliate where the symptoms were observed as angular shaped brown spots. The rate of expansion and the number of lesions were observed as

being slow initially, and then increased gradually to higher levels for untreated plots. The rate of disease progress started to decline at R3 - early podding stage in which at least one pod had reached maximum full length. Analysis of variance revealed significant differences between fungicide treatments on AUDPC (Table 3.1 and 3.2). Higher values were recorded during heavy rains compared to light rains (Tables 3.3 and 3.4). Plots treated with 400 mℓ ha$^{-1}$ of Othello top, had the lowest scores whereas, those sprayed once following farmers' practice (in which fungicides are applied late during the crop development and at a low rate of 50 mℓ ha$^{-1}$) and non-sprayed plots had the highest scores.

Varieties responded differently to fungicide treatments in all the seasons (Fig. 3.1a, b, c). Uyole 96 had the lowest AUDPC while Kigoma had the highest mean value when both were sprayed with the fungicide at the recommended rates. For example, the mean AUDPC value for variety Uyole 96 during the 2012/2013 and 2013/2014 heavy rains was 20% lower than that of Kigoma when both were sprayed with Othello top at a rate of 400 mℓ ha$^{-1}$ . Under farmers' practice and non-sprayed treatments, all the varieties showed high AUDPC that were accompanied by severe infections that resulted into premature defoliations of pods and leaves especially to variety Kigoma which has a bush type of growth habit. All the interactions were not significant regardless of the seasons indicating consistency in performance of the fungicide across the varieties and seasons. Consequently, the experimental precision as assessed by the coefficient of variation (CV) was good with values ranging from 5.2 − 10.4 during heavy rains and 7.1 − 13.4 during light rains.



(a)                  (b)                  (c)
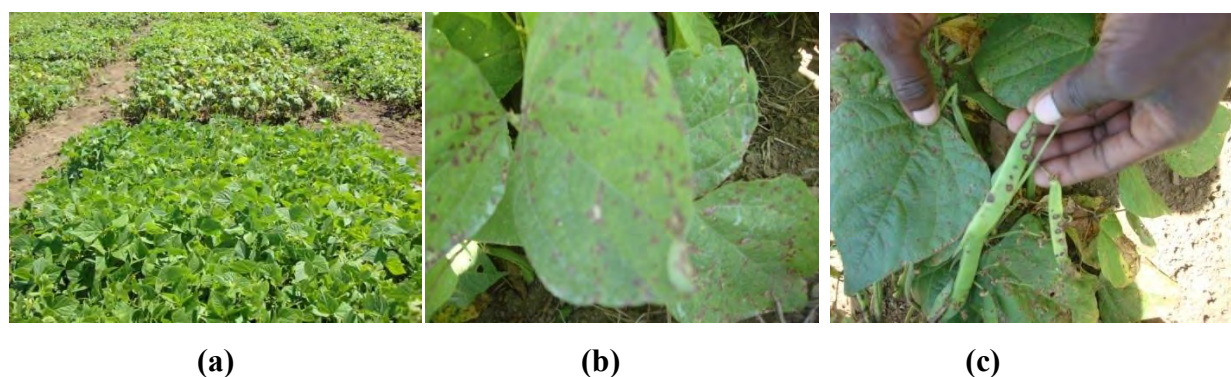
**Figure 3.1: (a) Trial on ALS disease at the Uyole Agricultural Research Institute (b) Plants from farmers' fields where fungicide was sprayed at a low rate and late after the onset of ALS disease (c) Non-sprayed plants with severe ALS symptoms on leaves and pods**

**Table 3.1: Analysis of variance for traits evaluated on ALS susceptible common bean varieties under different fungicide treatment during the 2012/2013 and 2013/2014 heavy rainy season**

| Source of variation | DF | AUDPC | Pods/P | Seed/P | 100 swt | GYD |
|---|---|---|---|---|---|---|
| Year (season) | 1 | 221..2 | 97.2 | 43.2 | 505.0 | 8411687 |
| Fungicides | 2 | 297765* | 226.3* | $0.1^{ns}$ | 1408* | 2002197* |
| Year x Fungicide | 2 | 3556.5 ns | 28.6 ns | 0.975 ns | 4.52 ns | 73282 ns |
| Variety | 4 | 6994.3* | $32.4^{ns}$ | 1.3* | 72.9** | 3.37* |
| Year x Variety | 4 | 1027.4ns | 32.59ns | 0.59ns | 167.1ns | 59512 ns |
| Fungicide x variety | 8 | $938.6^{ns}$ | $83.4^{ns}$ | $0.2^{ns}$ | $8.2^{ns}$ | $86^{ns}$ |
| Yr x Var.x fung. | 8 | 508.0 ns | 13.8 ns | 0.27ns | 9.67 ns | 55581 ns |
| Total | 119 | 5792.8 | 1421.1 | 0.6 | 41.8 | 5689.1 |

DF=degrees of freedom; AUDPC=area under disease progressive curve; Pods/P=pods per plant S/P=seed per pod; 100 swt=hundred seed weight; GYD=grain yield; ns = not significant at $P \leq 0.05$

**Table 3.2: Analysis of variance for traits evaluated on ALS susceptible common bean varieties under different levels of fungicide treatment during the 2012/2013 and 2013/2014 light rainy season**

| Source of variation | DF | AUDPC | Pods/P | Seed/P | 100 swt | GYD |
|---|---|---|---|---|---|---|
| Year | 1 | 3.3 | 40.8 | 5.2 | 122.2 | 3302.2 |
| Fungicides | 2 | 837.7** | 15.3* | $0.5^{ns}$ | $119.2^{ns}$ | $2042.9^{ns}$ |
| Year x Fungicide | 2 | 837.7 ns | 15.35 ns | 0.51 ns | 128.4 ns | 27891.7ns |
| Variety | 4 | $770.1^{ns}$ | $11.9^{ns}$ | 1.0* | 206.3** | 2795.2** |
| Year x Variety | 4 | 632.0 ns | 6.14 ns | 0.67ns | 3.14.6ns | 304809 ns |
| Fungicide x variety | 8 | $1000.1^{ns}$ | $2.8^{ns}$ | $0.1^{ns}$ | $18.6^{ns}$ | $702.1^{ns}$ |
| Yr x fung. x var. | 8 | 685.1 ns | 2.79 ns | 0.31ns | 9.02 ns | 8894.1 ns |
| Total | 119 | 1838.5 | 4.9 | 0.2 | 131.4 | 7988 |

DF=degrees of freedom; Yr x fung. x var. = year x fungicide x variety; AUDPC=area under disease progressive curve; Pods/P=pods per plant S /P=seed per pod; 100 swt=hundred seed weight; GYD=grain yield; ns = not significant at $P \leq 0.05$

**Table 3.3: AUDPC comparisons during heavy rains using five varieties susceptible to angular leaf spot disease under sprayed and unsprayed treatments in one of the disease hot spot environments at the Uyole Agricultural Research Institute Southern Highlands of Tanzania in the 2012/2013 and 2013/2014**

| Cultivars | Heavy rains 2012/2013 | | | Heavy rains 2013/2014 | | | Mean | | |
|---|---|---|---|---|---|---|---|---|---|
| | SP | FS | NS | SP | FP | NS | SP | FS | NS |
| Kigoma | 80.0 | 245.0 | 242.5 | 92.5 | 266.2 | 281.1 | *86.9* | 255.6 | 261.9 |
| Kablanketi | 82.5 | 206.2 | 237.5 | 60.0 | 212.5 | 241.2 | 71.2 | 209.4 | 239.4 |
| Maini | 77.5 | 217.5 | 208.8 | 65.0 | 215.5 | 261.2 | 71.9 | 216.3 | 235.0 |
| Uyole94 | 86.2 | 191.2 | 226.3 | 65.0 | 203.8 | 261.0 | 75.0 | 197.5 | 221.2 |
| Uyole96 | 76.2 | 182.5 | 183.8 | 62.5 | 205.0 | 226.1 | 69.4 | 193.8 | 205.0 |
| Mean | 80.5 | 208.5 | 219.8 | 69.0 | 220.5 | 254.1 | 74.9 | 214.5 | 232.5 |
| LSD(0.05)[1] | | 13.5 | | | 14.9 | | | 9.9 | |
| LSD(0.05)[2] | | 14.8 | | | 18.0 | | | 12.6 | |
| LSD(0.05)[3] | | 25.7 | | | 30.9 | | | 21.3 | |
| CV (%) | | 10.4 | | | 5.2 | | | 8.8 | |
| P<0.05[1] | | 0.001 | | | 0.001 | | | 0.001 | |
| P<0.05[2] | | 0.001 | | | 0.001 | | | 0.001 | |
| P<0.05[3] | | 0.059 | | | 0.45 | | | 0.072 | |
| P<0.05[4] | | | | | | | | 0.068 | |
| P<0.05[5] | | | | | | | | 0.059 | |
| P<0.05[6] | | | | | | | | 0.358 | |

SP = Fungicide sprayed plots at the recommended rates of 400 mℓ of Othello top ha$^{-1}$; FS = Farmers practice in which plots were sprayed once at the later stage of crop development (R 4) with 50 mℓ of Othello top; NS = non-spayed plots; P<0.05[1] = Fungicides; P<0.05[2] = Varieties; P<0.05[3] = Fungicide x varieties; P<0.05[4] = Seasons x Fungicide; P<0.05[5] = Season x Variety; P<0.05[6] = seasons x variety x year

**Table 3.4 : AUDPC comparisons during light rains using the five varieties susceptible to angular leaf spot under sprayed and unsprayed treatments in one of the disease hot spot environments at the Uyole Agricultural Research Institute Southern Highlands of Tanzania in the 2012/2013 and 2013/2014**

| Cultivars | Light rains 2012/2013 | | | Light rains 2013/2014 | | | Mean | | |
|---|---|---|---|---|---|---|---|---|---|
| | SP | FS | NS | SP | FP | NS | SP | FS | NS |
| Kigoma | 72.5 | 155.0 | 138.8 | 91.3 | 157.5 | 167.5 | 81.9 | 156.2 | 153.1 |
| Kablanketi | 77.5 | 126.3 | 161.2 | 86.3 | 138.8 | 130.0 | 81.9 | 132.5 | 145.6 |
| Maini | 70.0 | 138.8 | 156.2 | 90.0 | 108.8 | 142.5 | 80.0 | 123.8 | 149.4 |
| Uyole94 | 57.5 | 170.0 | 156.2 | 67.5 | 146.2 | 147.5 | 62.5 | 158.1 | 151.9 |
| Uyole96 | 67.5 | 143.8 | 132.5 | 62.5 | 146.2 | 146.2 | 65.0 | 145.0 | 139.4 |
| Mean | 69.0 | 112.8 | 148.9 | 79.5 | 139.5 | 146.7 | 74.3 | 143.1 | 147.8 |
| LSD(0.05)[1] | | 13.8 | | | 26.1 | | | 13.1 | |
| LSD(0.05)[2] | | 23.0 | | | 19.0 | | | 15.9 | |
| LSD(0.05)[3] | | 37.5 | | | 27.6 | | | 27.1 | |
| CV (%) | | 7.1 | | | 13.4 | | | 15.8 | |
| P<0.05[1] | | 0.001 | | | 0.001 | | | 0.001 | |
| P<0.05[2] | | 0.844 | | | 0.095 | | | 0.39 | |
| P<0.05[3] | | 0.391 | | | 0.161 | | | 0.25 | |
| P<0.05[4] | | | | | | | | 0.302 | |
| P<0.05[5] | | | | | | | | 0.459 | |
| P<0.05[6] | | | | | | | | 0.193 | |

SP = Fungicide sprayed plots at the recommended rates of 400 mℓ of Othello top ha $^{-1}$; FS = Farmers practice in which plots were sprayed once at the later stage of crop development (R 4) with 50 mℓ of Othello top; NS = Plots not sprayed with fungicide at all; P<0.05[1] = Fungicides; P<0.05[2] = Varieties;   P<0.05[3] = Fungicide x varieties; P<0.05[4] = Seasons x Fungicide; P<0.05[5] = Season x Variety; P<0.05[6] =  seasons x variety x year

### 3.3.2 Grain yield

Grain yield from each season varied significantly between fungicide treatments (Table 3.5, 3.6). High yields were obtained from plots sprayed with fungicide at the recommended rates regardless of the season in which they were planted. Low yields with little differences between treatments were observed on varieties subjected to farmers practice and non-sprayed plots for all of the four bean growing cycles, but was higher during heavy rains compared to light rains (Tables 3.5 and 3.6).

Significant varietal differences in yielding ability were observed during the 2012/2013 and 2013/2014 bean cropping seasons. Uyole 96 outperformed all other varieties evaluated during heavy rains with mean yields of 2575 kg ha$^{-1}$, while Kigoma outperformed all the varieties evaluated during light rains with yields of 2535 kg ha$^{-1}$. The same varieties were amongst the lowest yielders under farmers' practice and non-sprayed treatments

Considering the differences in weather conditions, data collected over the two seasons were analyzed according to heavy or light rains. The two way interaction effect was observed for the fungicide and the varieties indicating that yield performance depended on the applied fungicide during the heavy rains. During light rains, significant differences were also observed on fungicide applied but no differences were noted in all of the interactions. Consequently, non-significant three way interactions (Season x fungicide x variety) effect was observed. This indicates that the performance of Othello top did not depend on the type of varieties grown or in season in which they were planted.

When the harvested crop was classified into market valued seed, sprayed plots with the recommended rates during heavy rains, showed higher yields compared to the rest of the treatments (Fig. 3.2). Uyole 96 had the highest market valued seed while Kigoma had the lowest (Fig. 3.3). Non-significant differences were observed between treatments on the same varieties when market valued seed was considered.

**Table 3.5: Yield comparisons of the five varieties susceptible to angular leaf spot under sprayed and unsprayed treatments in one of the disease hot spot environments at the Uyole Agricultural Research Institute Southern Highlands of Tanzania in the 2012/2013 and 2013/2014 heavy rains**

| Cultivars | Heavy rains 2012/2013 | | | Heavy rains 2013/2014 | | | Mean data | | |
|---|---|---|---|---|---|---|---|---|---|
| | SP | FS | NS | SP | FP | NS | SP | FS | NS |
| Kigoma | 2229 | 1271 | 1329 | 2212 | 1292 | 925 | 2220 | 1281 | 1310 |
| Kablanketi | 2350 | 1626 | 1354 | 2387 | 1217 | 1050 | 2368 | 1421 | 1202 |
| Maini | 2375 | 1583 | 1292 | 2593 | 1304 | 1375 | 2484 | 1443 | 1333 |
| Uyole94 | 2312 | 1638 | 1505 | 2664 | 1275 | 1347 | 2488 | 1456 | 1426 |
| Uyole96 | 2525 | 1800 | 1100 | 2625 | 1342 | 1153 | 2575 | 1571 | 1362 |
| Means | 2358.2 | 1583.6 | 1316.0 | 2496.2 | 1286.0 | 1170.0 | 2427 | 1434 | 1326 |
| LSD(0.05)[1] | | 193.6 | | | 91.1 | | | 92.5 | |
| LSD(0.05)[2] | | 234.5 | | | 134.6 | | | 155.4 | |
| LSD(0.05)[3] | | 399.8 | | | 222.6 | | | 288.3 | |
| CV (%) | | 6.9 | | | 3.5 | | | 11.2 | |
| P<0.05[1] | | 0.001 | | | 0.001 | | | 0.001 | |
| P<0.05[2] | | 0.262 | | | 0.001 | | | 0.009 | |
| P<0.05[3] | | 0.001 | | | 0.08 | | | 0.057 | |
| P<0.05[4] | | | | | | | | 0.249 | |
| P<0.05[5] | | | | | | | | 0..34 | |
| P<0.05[6] | | | | | | | | 0.392 | |

SP = Fungicide sprayed plots at the recommended rates of 400 mℓ of Othello top ha$^{-1}$;

FS = Farmers practice in which plots were sprayed once at the later stage of crop development (R 4) with 50 mℓ of Othello top; NS = Plots not sprayed with fungicide at all; P<0.05[1] = Fungicides; P<0.05[2] = Varieties; P<0.05[3] = Fungicide x varieties; P<0.05[4] = Seasons x Fungicide; P<0.05[5] = Season x Variety; P<0.05[6] = seasons x variety x year

**Table 3.6: Yield comparisons of the five varieties susceptible to angular leaf spot under sprayed and unsprayed treatments in one of the disease hot spot environments at the Uyole Agricultural Research Institute Southern Highlands of Tanzania in the 2012/2013 and 2013/2014 light rains**

| Cultivars | Light rains 2012/2013 | | | Light rains 2013/2014 | | | Combined data for the two seasons | | |
|---|---|---|---|---|---|---|---|---|---|
| | SP | FS | NS | SP | FP | NS | SP | FS | NS |
| Kigoma | 2583 | 1375 | 1846 | 2488 | 1775 | 1850 | 2535 | 1575 | 1848 |
| Kablanketi | 2354 | 1921 | 1592 | 2500 | 2100 | 2038 | 2427 | 2010 | 1815 |
| Maini | 2292 | 1867 | 1779 | 2600 | 2175 | 2162 | 2446 | 2021 | 1971 |
| Uyole94 | 2250 | 1929 | 1858 | 2425 | 2162 | 2088 | 2338 | 2046 | 1973 |
| Uyole96 | 2167 | 2000 | 1950 | 2450 | 2238 | 2175 | 2308 | 2119 | 2062 |
| Mean | 2329.2 | 1814.4 | 1805 | 2492 | 2090 | 2062.6 | 2410.8 | 1954.2 | 1933 |
| LSD(0.05)[1] | | 104.1 | | | 188.0 | | | 105.4 | |
| LSD(0.05)[2] | | 153.0 | | | 255.0 | | | 154.3 | |
| LSD(0.05)[3] | | 253.1 | | | 426.7 | | | 354.4 | |
| CV (%) | | 9.3 | | | 13.9 | | | 8.8 | |
| P<0.05[1] | | 0.001 | | | 0.035 | | | 0.001 | |
| P<0.05[2] | | 0.262 | | | 0.001 | | | 0.001 | |
| P<0.05[3] | | 0.001 | | | 0.653 | | | 0.005 | |
| P<0.05[4] | | | | | | | | 0.063 | |
| P<0.05[5] | | | | | | | | 0.059 | |
| P<0.05[6] | | | | | | | | 0.057 | |

SP = Fungicide sprayed plots at the recommended rates of 400 mls of Othello top ha $^{-1}$;
FS = Farmers practice in which plots were sprayed once at the later stage of crop development (R 4) with 50 mls of Othello top; NS = Plots not sprayed with fungicide at all; P<0.05[1] = Fungicides; P<0.05[2] = Varieties;   P<0.05[3] = Fungicide x varieties;  P<0.05[4] = Seasons x Fungicide; P<0.05[5] = Season x Variety; P<0.05[6] = seasons x variety x year
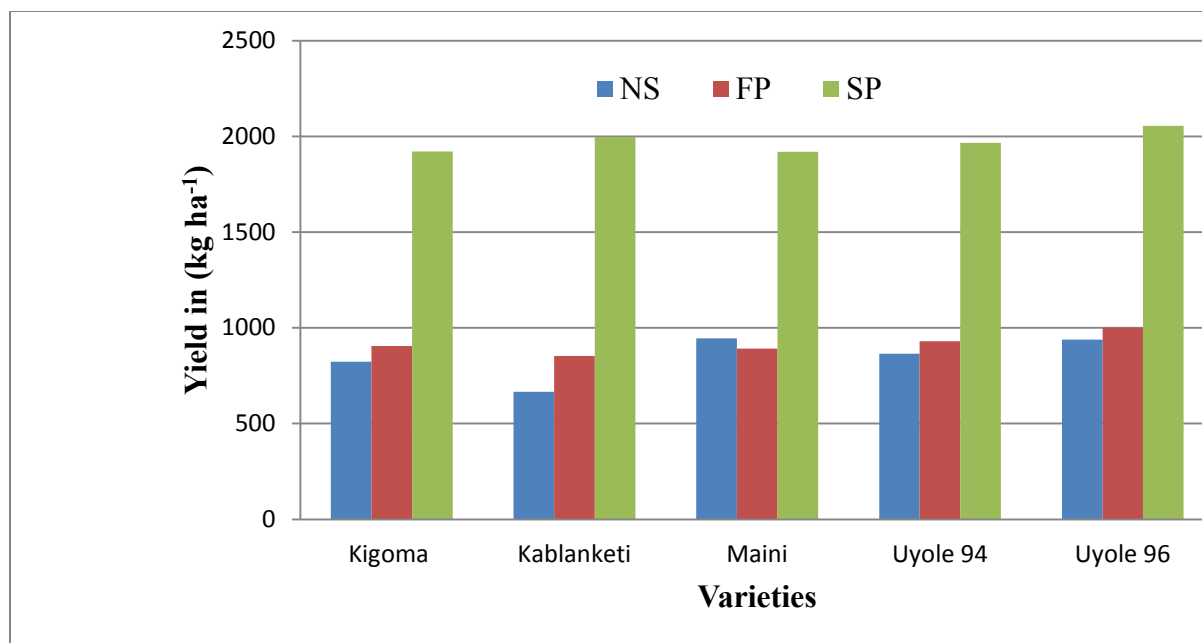
**Figure 3.2: Mean market valued seed obtained during the heavy rains 2012/2013 and 2013/2014**
NS=not sprayed; FP= Farmers practice in which plots were sprayed once at the later stage of crop development (R4) with 50 mℓ of Othello top; SP= Fungicide sprayed plots at the recommended rates of 400mℓ of Othello top ha$^{-1}$
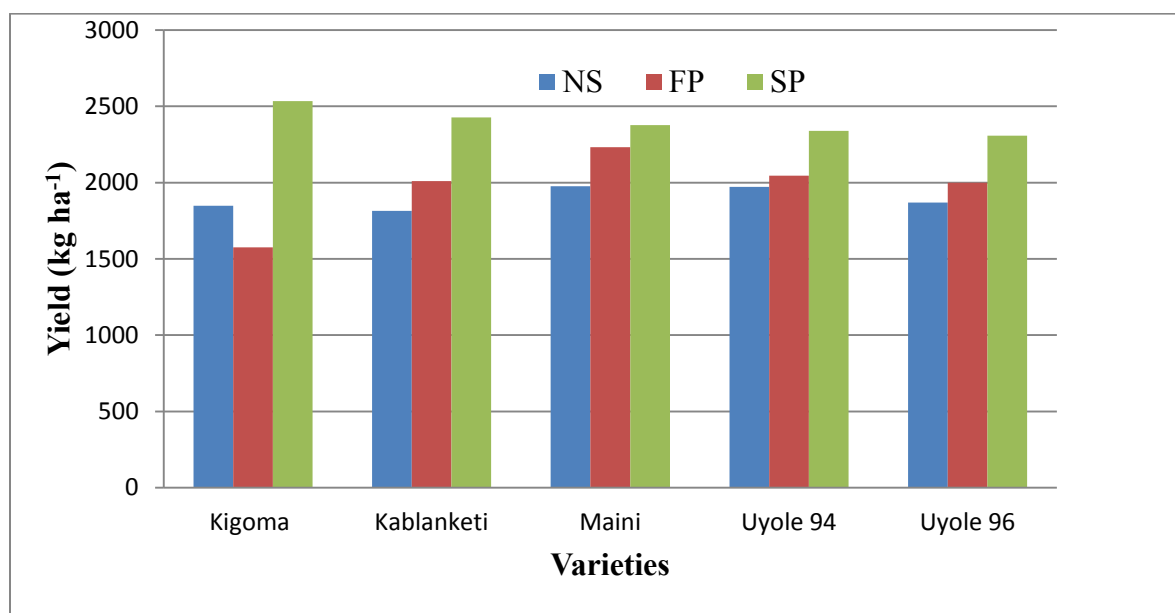


**Figure 3.3: Means of market valued seed obtained during the light rains 2012/2013 and 2013/2014**
NS=not sprayed; FP= Farmers practice in which plots were sprayed once at the later stage of crop development (R4) with 50 mℓ of Othello top; SP= Fungicide sprayed plots at the recommended rates of 400 mℓ of Othello top ha$^{-1}$

### 3.3.3 Yield losses

All of the three fungicide treatments were used to calculate percentage yield losses and the data is presented in Table 3.7. High yield losses were obtained during heavy rains ranging from 44.2% to 57% and 39% to 61% under farmers practice and no spray, respectively (Fig. 3.4). However, lower percentages were obtained from the experiments conducted under light rains that ranged from 6.1% to 37.8% and 6.0% to 25%, respectively under the same types of management.



**Figure 3.4: Pods and seeds obtained from control (unsprayed) (left), sprayed based on farmers' practice (centre) and sprayed as per manufacturer's recommendation (right).**

**Table 3.7: Percentage yield losses comparing data collected during the heavy and light rains in the SHT**

| Variety | Heavy rains | | Light rains | |
| --- | --- | --- | --- | --- |
| | % yield loss | | % yield loss | |
| | FP | NS | FP | NS |
| Kigoma | 48.6 | 49.5 | 37.8 | 27.1 |
| Kablanketi | 57.4 | 61.0 | 17.1 | 25.2 |
| Maini | 50.2 | 47.5 | 6.1 | 16.8 |
| Uyole 94 | 44.2 | 39.9 | 12.4 | 15.6 |
| Uyole 96 | 44.7 | 45.6 | 2.4 | 6.0 |

FS = Farmers practice in which plots were sprayed once at the later stage of crop development (R 4) with 50 mℓ of Othello top; NS = Plots not sprayed with fungicide at all.

### 3.3.4 Yield components

Significant differences between fungicide treatments were observed for the number of pods per plant during heavy rains and 100 seed weight for all the seasons in which the trials were conducted. High numbers of pods per plant were observed on fungicide sprayed plots at the recommended rates and the differences between treatments were significant (Tables 3.8 and 3.9). Uyole 94 had the highest number of pods per plant followed by Uyole 96. However, no significant differences on pods per plant were observed between fungicide treatments during light rains.

The number of seeds per pod varied with the varieties and the differences were significant at $P<0.05$. Heavier 100 seed weights were noted during the light rains and Uyole 96 had the heaviest seed compared to the rest of the varieties. The same observation was noted during the light rains (Table 3.9). The number of seeds per pod differed significantly between varieties and the differences were maintained across locations. However, no significant differences were observed between the interactions for all the four seasons as seasons with similar types of weather conditions were combined together during the analysis due to insignificant results of the interactions when individual season was analyzed.

**Table 3.8: Combined data analysis on yield components during heavy rains over the 2012/2013 and 2013/2014 bean cropping seasons**

| Cultivars | Pods/ plant | | | Seed/pod | | | 100 seed weight | | |
|---|---|---|---|---|---|---|---|---|---|
| | SP | FS | NS | SP | FS | NS | SP | FS | NS |
| Kigoma | 15.3 | 12.6 | 12.0 | 4.3 | 4.1 | 4.3 | 37.5 | 30.9 | 31.0 |
| Kablanketi | 15.5 | 14.0 | 12.0 | 4.1 | 3.9 | 3.7 | 37.6 | 33.7 | 31.7 |
| Maini | 15.8 | 13.4 | 15.0 | 4.3 | 4.6 | 4.9 | 32.9 | 32.9 | 31.2 |
| Uyole94 | 17.4 | 11.3 | 13.3 | 4.3 | 4.1 | 4.3 | 43.9 | 42.4 | 40.8 |
| Uyole96 | 16.1 | 14.1 | 13.8 | 4.4 | 4.3 | 4.4 | 43.6 | 42.9 | 41.6 |
| Mean | 16.0 | 13.1 | 13.2 | 4.2 | 4.2 | 4.3 | 39.1 | 36.6 | 35.3 |
| LSD(0.05)[1] | | 1.51 | | | 0.25 | | | 2.0 | |
| LSD(0.05)[2] | | 1.66 | | | 0.23 | | | 1.8 | |
| LSD(0.05)[3] | | 2.80 | | | 0.43 | | | 3.3 | |
| CV (%) | | 14.2 | | | 0.65 | | | 7.1 | |
| P<0.05[1] | | 0.001 | | | 0.56 | | | 0.001 | |
| P<0.05[2] | | 0.001 | | | 0.001 | | | 0.001 | |
| P<0.05[3] | | 0.067 | | | 0.227 | | | 0.212 | |
| P<0.05[4] | | 0.081 | | | 0.057 | | | 0.472 | |
| P<0.05[5] | | 0.068 | | | 0.07 | | | 0.119 | |
| P<0.05[6] | | 0.358 | | | 0.265 | | | 0.13 | |

SP = Fungicide sprayed plots at the recommended rates of 400 mℓ of Othello top ha $^{-1}$;

FS = Farmers practice in which plots were sprayed once at the later stage of crop development (R 4) with 50 mℓ of Othello top; NS = Plots not sprayed with fungicide at all; P<0.05[1] = Fungicides; P<0.05[2] = Varieties;   P<0.05[3] = Fungicide x varieties; P<0.05[4] = Seasons x Fungicide; P<0.05[5] = Season x Variety; P<0.05[6] = seasons x variety x year

**Table 3.9: Combined data analysis on yield components during light rains over 2012/2013 and 2013/2014 bean cropping seasons**

| Cultivars | Pods/ plant | | | Seed/pod | | | 100 seed weight (g/100 seed) | | |
|---|---|---|---|---|---|---|---|---|---|
| | SP | FP | NS | SP | FP | NS | SP | FS | NS |
| Kigoma | 12.5 | 11.5 | 12.4 | 4.6 | 4.7 | 4.7 | 41.2 | 39.2 | 37.3 |
| Kablanketi | 10.8 | 11.0 | 12.6 | 4.5 | 4.8 | 4.6 | 43.5 | 38.3 | 39.0 |
| Maini | 10.4 | 11.3 | 10.8 | 5.3 | 5.1 | 5.1 | 40.1 | 38.3 | 35.4 |
| Uyole94 | 11.3 | 11.3 | 11.4 | 4.9 | 4.6 | 5.0 | 44.2 | 46.3 | 43.4 |
| Uyole96 | 11.6 | 13.0 | 12.1 | 4.6 | 4.7 | 4.7 | 44.7 | 45.0 | 41.4 |
| Mean | 11.3 | 11.5 | 11.9 | 4.8 | 4.8 | 4.8 | 42.7 | 41.2 | 39.3 |
| LSD(0.05)[1] | | 1.34 | | | 0.19 | | | 1.63 | |
| LSD(0.05)[2] | | 1.21 | | | 0.24 | | | 1.46 | |
| LSD(0.05)[3] | | 0.72 | | | 0.41 | | | 2.68 | |
| CV (%) | | 14.2 | | | 6.7 | | | 4.7 | |
| P<0.05[1] | | 0.61 | | | 0.69 | | | 0.003 | |
| P<0.05[2] | | 0.04 | | | 0.001 | | | 0.001 | |
| P<0.05[3] | | 0.738 | | | 0.60 | | | 0.061 | |
| P<0.05[4] | | 0.134 | | | 0.082 | | | 0.061 | |
| P<0.05[5] | | 0.237 | | | 0.071 | | | 0.08 | |
| P<0.05[6] | | 0.741 | | | 0.878 | | | 0.25 | |

SP = Fungicide sprayed plots at the recommended rates of 400 mℓ of Othello top ha^{-1};
FS = Farmers practice in which plots were sprayed once at the later stage of crop development (R 4) with 50 mℓ of Othello top; NS = Plots not sprayed with fungicide at all; P<0.05[1] = Fungicides; P<0.05[2] = Varieties; P<0.05[3] = Fungicide x varieties; P<0.05[4] = Seasons x Fungicide; P<0.05[5] = Season x Variety; P<0.05[6] = seasons x variety x year

### 3.3.5 Correlations between AUDPC yield and yield components

Correlations among AUDPC yield and yield components were highly significant and negative during heavy rains with the exception of relationship between pods per plant and grain yield that showed low value that were highly significant. (Table 3.10). As the AUDPC increased the number of pods per plant, 100 seed weight, market valued seed and grain yield decreased as indicated by high and negative correlated values (Table 3.11). However, the market valued seed had a highly significant, strong and positive correlation with grain yield regardless of the season in which the varieties were evaluated. Non-significant correlations were observed for all the traits with grain yield except for market value seed with GY during the light rains (Table 3.11).

**Table 3.10: Correlations between AUDPC yield and yield components during the 2012/2013 and 2013/2014 heavy rains**

| Parameter | AUDPC | POP | HSW | MVS | GY |
|-----------|-------|-----|-----|-----|-----|
| AUDPC | 1 | | | | |
| POP | -0.3565** | 1 | | | |
| HSW | -0.7290** | 0.1890* | 1 | | |
| MVS | -0.8968** | 0.3889** | 0.7462** | 1 | |
| GY | -0.8730** | 0.3531** | 0.7181** | 0.9788** | 1 |

AUDPC = area under disease progressive curve; POP = Pods per plant; HSW = hundred seed weight; MVS = market valued seed; GY = Grain yield
*= Significant at P≤0.05; ** = significant at P≤0.01

**Table 3.11: Correlations between AUDPC yield and yield components during the 2012/2013 and 2013/2014 light rains**

| Parameter | AUDPC | POP | HSW | MVS | GY |
|-----------|-------|-----|-----|-----|-----|
| AUDPC | 1 | | | | |
| POP | -0.0457ns | 1 | | | |
| KWT | -0.0681ns | 0.2810* | 1 | | |
| MVS | -0.3967** | 0.1540ns | 0.5115** | 1 | |
| GY | -0.2475ns | 0.0671ns | 0.1748ns | 0.6606** | 1 |

AUDPC = area under disease progressive curve; POP = Pods per plant; HSW = hundred seed weight; MVS = market valued seed; GY = Grain yield; *= Significant at $P \leq 0.05$; ** = significant at $P \leq 0.01$

### 3.3.6  Economic analysis

The cost benefit analysis for the treatments used is presented in Table 3.12 and 3.13. Regardless of the varieties, the total variable costs and the total returns increased with the increased rates of fungicide treatments. Fungicide sprayed treatments at the recommended rates of 400 mℓ ha$^{-1}$ had higher costs and higher net benefits compared to farmers' practice and non-sprayed plots.

The trend of benefits produced when varieties were subjected to the three treatments of fungicide was consistent during heavy and light rains (Tables 3.12 and 3.13). However, during light rains there were higher yields in plots sprayed following farmers' practice and non-sprayed plots compared to those obtained during heavy rains. The average net benefit during heavy rains was USD 578 ha$^{-1}$ with a range of USD 286 – 1068 ha$^{-1}$, while during light rains the mean was USD 1106 ha$^{-1}$ and the range was USD 852 – 1427 ha$^{-1}$.

The marginal rate of return (MRR) that indicates investment and recovery of the money was high when recommended rates of managing ALS through fungicide usage were practiced. Differences were observed between heavy and light rain seasons. Variety Maini showed lowest MMR with a negative value under farmers practice of applying fungicide during heavy rains

whereas losses in investment was noticed on variety Kigoma when sprayed with fungicides following farmers practice during light rains (Table 3.13)

**Table 3.12: Economic analysis of market valuable seed from different fungicide treatment during the 2012/1013 and 2013/2014 heavy rains**

| Variety | Fungicide treatment | Grain yield (kg ha$^{-1)}$ | Costs that vary (USD) | Gross farm benefit | Net benefit (USD) | MRR |
|---|---|---|---|---|---|---|
| Kigoma | NS | 823 | 150.00 | 534.95 | 384.95 | - |
| Kigoma | FP | 906 | 178.12 | 588.90 | 410.78 | 0.91 |
| Kigoma | SP | 1921 | 237.50 | 1248.65 | 1011.15 | 10.11 |
| Kablanketi | NS | 667 | 150.00 | 433.55 | 283.55 | - |
| Kablanketi | FP | 854 | 178.12 | 555.10 | 376.98 | 3.32 |
| Kablanketi | SP | 1996 | 237.50 | 1297.40 | 1059.9 | 11.50 |
| Maini | NS | 945 | 150.00 | 614.25 | 464.25 | - |
| Maini | FP | 891 | 178.12 | 579.15 | 401.03 | -2.25 |
| Maini | SP | 1919 | 237.50 | 1247.35 | 1009.85 | 10.25 |
| Uyole 94 | NS | 865 | 150.00 | 562.25 | 412.25 | - |
| Uyole 94 | FP | 931 | 178.12 | 605.15 | 427.03 | 0.53 |
| Uyole 94 | SP | 1967 | 237.50 | 1278.55 | 1041.05 | 10.34 |
| Uyole 96 | NS | 938 | 150.00 | 609.70 | 459.70 | - |
| Uyole 96 | FP | 1000 | 178.12 | 650.00 | 471.88 | 0.43 |
| Uyole 96 | SP | 2054 | 237.50 | 1335.10 | 1097.6 | 10.53 |

FS = Farmers' practice in which plots were sprayed once at the later stage of crop development (R 4) with 50 mℓ of Othello top; NS = Plots not sprayed with fungicide at all

**Table 3.13: Economic analysis of market valuable seed from different fungicide treatment during the 2012/1013 and 2013/2014 light rains**

| Variety | Fungicide treatment | Grain yield Kg ha$^{-1}$ | Costs that vary (USD) | Gross farm benefit | Net benefit (USD) | MRR |
|---|---|---|---|---|---|---|
| Kigoma | NS | 1848 | 150.00 | 1201.20 | 1051.20 | - |
| Kigoma | FP | 1575 | 178.12 | 1023.75 | 845.63 | -12.6 |
| Kigoma | SP | 2535 | 237.50 | 1647.75 | 1410.25 | 9.51 |
| Kablanketi | NS | 1815 | 150.00 | 1179.75 | 1029.75 | - |
| Kablanketi | FP | 2010 | 178.12 | 1306.5 | 1128.38 | 3.51 |
| Kablanketi | SP | 2427 | 237.50 | 1577.55 | 1340.05 | 3.56 |
| Maini | NS | 1977 | 150.00 | 1285.05 | 1135.05 | - |
| Maini | FP | 2233 | 178.12 | 1451.45 | 1273.33 | 4.92 |
| Maini | SP | 2377 | 237.50 | 1545.05 | 1307.55 | 1.21 |
| Uyole 94 | NS | 1973 | 150.00 | 1282.45 | 1132.45 | - |
| Uyole 94 | FP | 2046 | 178.12 | 1329.9 | 1151.78 | 0.33 |
| Uyole 94 | SP | 2338 | 237.5 | 1519.7 | 1282.2 | 2.19 |
| Uyole 96 | NS | 1869 | 150.00 | 1214.85 | 1064.85 | - |
| Uyole 96 | FP | 2000 | 178.12 | 1300.00 | 1121.88 | 2.02 |
| Uyole 96 | SP | 2308 | 237.50 | 1500.20 | 1262.70 | 2.37 |

FS = Farmers practice in which plots were sprayed once at the later stage of crop development (R 4) with 50 mℓ of Othello top; NS = Plots not sprayed with fungicide at all

## 3.4. Discussion

This study revealed that *P. griseola* thrived well during heavy rains that were characterized by mild temperatures ($17^0 – 24^0$C) and high relative humidity (95%). The weather conditions experienced, supported the pathogen's ability to infect, germinate and subsequently sporulate on susceptible cultivar causing severe damage on all of the aerial parts of the bean plant including the leaf blades. The degree of damage differed between varieties, indicating differences in resistance levels against the pathogen, which might suggest presence of different genes conditioning the resistance. Environmental differences had an effect on the pathogen as lower infections were

recorded during light rains where higher temperatures and lower amounts of rainfall occurred. Higher and occasionally lower temperatures accompanied by limited free water on the surfaces of the bean plant might have contributed in lowering pathogens' ability to infect and colonize its hosts (Celetti, 2005)

The effect of fungicide in protecting the plants under recommended rates of 400 mℓ ha$^{-1}$, allowed the crop to attain physiological maturity with minimal infections. Fungicide applied following farmers' practice had a non-significant effect on the varieties in reducing ALS disease as the rates applied were too low and the time of application was past the critical stages of grain filling. This was supported by high AUDPC values and severe defoliations especially to the varieties Kigoma and Kablanketi. Application of a foliar fungicide such as Othello Top which was used in this study should be started slightly before the onset of the infection or at least before the disease signs have become evident. According to the manufacturer, Othello top is a systemic fungicide that inhibits spore germination and infection. In this regard, applying the fungicide at the start of *P. griseola* infection, or a little bit before the infection, will limit the earliest stages of the pathogen infection process (biotrophic) that is considered the most destructive stage (Monda et al., 2001).

High yields were obtained from varieties treated with fungicide at the recommended rates whereas varieties sprayed with fungicides based on farmers' practice rates, and those which were not sprayed had depressed yields. As indicated earlier, grain yield in common bean is determined by yield components that include pods per plant, seeds per pod and seed weight. However, for the grain to be well filled photosynthesis must occur and leaf blades are more responsible for the process compared to the other parts of the plant. The brown spots caused by *P. griseola* on the leaves of the susceptible plants led to the disappearance of chlorophyll and more likely interfered with other physiological activities of the plant such as respiration and ribulose 1, 5-bisphosphate (RuBP) carboxylase /oxygenase (RubisCO) activities (Bassinezi et al., 2000). Reductions in chlorophyll and consequently lowering of carbon assimilation have been reported on bean plants infected with angular leaf spot disease (Bassinezi et al., 2000). A 75% reduction in the rate of photosynthesis due to damaged leaf blades and abnormalities in form and function of the chloroplast have been reported on bean leaves susceptible to anthracnose caused by *Colletotrichum lindermuthianum* (Meyer et al., 2001). Consequently, decrease in the rate of transpiration, reduction in plant tissue air volume and clogging of the plant conducting tissue due

to hyphae growth have been associated with leaf defoliations and negative impact on the amount of assimilates manufactured for the grain (Bassanezi et al., 2002). Seeds resulting from infected plants are of poor quality, discolored and shriveled with low weight and low market value. A reduction in seed weight due to infections was observed in non-protected treatments and those under farmers practice particularly on variety Kablanketi.

The relationships between AUDPC, seed weight and grain yield were similar in that, all showed negative associations, suggesting that yield reduction was associated with decreased seed weight particularly during heavy rains and increased disease infection. Although the same trend was observed during light rains, the association was not significant probably because of lower disease pressure caused by weather conditions in that season. Significant differences were observed on number of pods per plant between sprayed and non-sprayed including farmers practice. It has been shown that heavy infections prior to flowering reduces pod number, while infections after flowering result in reduced number of seeds and weight (Garry et al., 1998). It is believed that *P. griseola* diverts carbon assimilation from the developing pods and seed forcing pre-mature defoliations of pods and reduces the number of seeds that a plant can bear (Sinha, 1977).

High yield losses were obtained from unsprayed plots in all seasons. However, minor differences in seed losses were recorded between farmers' practice and un-sprayed plots. This indicates that the fungicide applied late in the season and at reduced dosages has no significant effect on ALS disease. According to the results obtained in this study, fungicide performance did not depend on the type of varieties grown or in season in which they were planted.

Although common bean is one of the basic foods in the SHT, growing it during heavy rains without any control measures will result in losses of more than 50% especially to the Kablanketi variety which is highly preferred by farmers and the consumers. Yield losses of more than 50% due to ALS disease have also been reported by Hagedorn and Wade (1974) and Borel et al. (2011). Yield losses of the same magnitude have been reported on sesame infected with charcoal rot caused by M. *phaseolina* suggesting the importance of fungal diseases on food crops (Deepthi et al., 2014).

Economic analysis revealed high returns in plots sprayed with fungicide at the recommended rates of the fungicide of 400 mℓ ha$^{-1}$. Based on the net benefit, variety Kigoma had higher benefits

(USD 1421.77) followed by Kablanketi (USD 1351.08). High marginal rates of return (MRR) were obtained on Kablanketi (USD 11.58) followed by Maini (10.33). This implied that, for every USD invested, the dollar was recovered with the additional profit of USD 11.58 for Kablanketi and USD 10.33 for Maini by shifting from no fungicide spray and farmers' practice types of ALS disease management to applying recommended rates of 400 mℓ ha$^{-1}$ of the fungicide. It worth noting that the prices used were those existed during the period of study which in most cases are not stable.

Negative MRR were observed for the treatments applied following farmers' practice. This was observed on the variety Maini and Kigoma during heavy and light rains, respectively. In this regard, the dollar invested was lost with an additional loss of 2.25 USD and 7.35 USD, respectively. A treatment is considered a worthy investment by the farmer if the MRR is higher than the minimal acceptable rate of return of 100% (CIMMYT, 1998). Therefore, the use of fungicides for the variety Kigoma, Uyole 94, Uyole 96 and Maini following what farmers are practicing, is not economical. Consequently, a farmer will gain little if fungicides are sprayed on Kablanketi, Kigoma, Maini, Uyole 94 and Uyole 96 during light rains. It has been shown that, when disease severity is low and there is minimal yield loss, applying fungicides will not result in either yield or economic advantage when individual variety is considered (McGrath, 2004).

## 3.5. Conclusion

All the varieties evaluated were highly preferred by farmers but also susceptible to ALS disease. The severity of infection by *P. griseola* was associated with fungicide application rates and the prevailing whether condition during the crop season. Severe infections were noted during heavy rain season compared to the light rains with yield losses that ranged from 39 -61% and 6.1 – 37.8% during heavy and light rains respectively. The negative association between yield and AUDPC indicates the necessity of optimizing disease management practices to reduce infections. Fungicides applied at the recommended rates of 400 mℓ ha$^{-1}$ significantly reduced ALS disease severity and increased yields. Uyole 96 outperformed all other varieties during heavy rains with yields of above 2500 kg ha$^{-1}$. Economically, Kablanketi variety resulted in the highest MRR after all variable costs were taken into account. However, all the varieties evaluated proved superior under fungicide management at the recommended rates than non-sprayed treatments. In this regard, farmers should be trained on pesticide usage. On the other hand bean farmers are advised

to grow the five varieties during light rains where disease infections indicated to be low with minimum yield losses. However a more sustainable means of controlling ALS disease should be sought for as many farmers are poor with little or no knowledge on the types and usage of fungicides. Consequently, breeding for resistance against ALS disease should be considered as an economic and sustainable strategy in managing the disease.

# References

Aghnoum R., T. C. Marcel, A. Johrde, N. Pecchioni, P. Schweizer, and R. E. Niks . 2010. Basal host resistance of barley to powdery mildew: connecting quantitative trait Loci and candidate genes. *Molecular Plant-Microbe Interactions* 23: 91-102.

Bassanezi, R.B., L. Amorim, F. A. Bergamin and, R.D. Berger. 2002. Gas exchange and emissions. Of chlorophyll fluorescence during the monocycle of rust, angular leaf spot and anthracnose of bean leaves as a function of their trophic characteristics. Phytopathology 150: 37 - 47

Bassanezi, R.B., L. Amorim, F. Bergamin, A, R. D. Berger. 2002. Gas exchange analysis on common bean with rust, angular leaf spot and anthracnose. Phytopathology Brazil. 25: 643 - 650

Birachi, E. 2012. Value chain analysis of beans in Eastern and Southern Africa: Building partnerships for impact through research on sustainable intensification of farming systems. www.africarising.net

Bourgeois, G., K.J. Boote, and R.D. Berger. 1991. Growth, development, yield, and seed quality of Florunner peanut affected by late leafspot. Peanut Science. 18:137–143

Borel J. C., M. A. P. Ramalho, F.B. Abbreal and L.G.S. Maia. 2007. Genetic control of angular leaf spot production in common bean leaves and pods, Scientia Agricola.68:584 - 588

Browen, J.H and L.L. Kinkel. 1997. Interactive modeling of Disease Progress Curve. In: Excercises in Plant Disease Epidemiology. Francl, L.T and Neher, D.A (Eds). APS Press. St. Paul Minnesota, USA. Page 20 – 23

Campbell, C. L. and, L. V. Madden. 1990. Introduction to Plant Disease Epidemiology. John Wiley & Sons, New York City.

Cardona-Alvarez, C and J.C. Walker. 1956. Angular leaf spot of common bean. Phytopathology 46: 610 – 615.

Celetti, M. J., M. S. Melzer and G. J. Boland. 2005. Integrated management of Angular leaf spot (*Phaeoisariopsis griseola* (Sacc.) Ferr.) on snap beans in Ontario. Plant Health Progress doi:10.1094/PHP-2005-1129-0. Accessed December 12, 2015.

Deepthi, P., C. S. Shukla, K. P. Verma and E. Siva Sankar Reddy. 2014. Yield loss assessment and influence of temperature and relative humidity on charcoal rot development in sesame (Sesamum indicum L). The bioscan. 9:0

Egbe, O.M and A. J. Idoko. 2012. Evaluation of Pigeon pea genotypes for intercropping with maize and sorghum in southern Guinea savanna: Economic benefits. International Journal of Agricultural and Forestry. 2: 108-114

Farrar, J.F. 1992. Beyond photosynthesis: the translocation and respiration of diseased leaves. In: Ayres, P.G. (ed.) Pests and Pathogens- plant responses to foliar attack. BIOS Scientific Publishers Limited, Oxford OX1 1SJ, UK, pp 107-124.

Filho, A. B., S. M. T. P. G. Carneiro, C. V. Godoy, L. Amorim, R. D. Berger and B. Hau. 1997. Angular leaf spot of Phaseolus beans: Relationships between disease, health y leaf area, and yield. Phytopathology 87:506-515.

Garry, G., M.H. Jeuffroy and B. Tivoli. 1986. Effects of ascochyta blight (Mycosphaerella pinodes Berk. Box) on biomas production seed number and seed weight of dried pea (Pisum sativum L.) as affected by plant growth stages and disease intensity. Annals of Applied Biology. 132: 49 - 59

Godoy,C. V., L. Amorim, A. B. Filho, H. P. Silva, W. J. Silva and R. D. Berger. 2003. Temporal progress of southern rust in maize under different environmental conditions. Phytopathology 28: 273 – 278.

Hagedorn D and E. K. Wade.1974.Bean rust and angular leaf spot in Wisconsin. Plant Disease Reporter 58:30 -332

Hillocks, R. J, C.S. Madata, R. Chirwa, E.M. Minja and S. Msolla. 2006. *Phaseolus* bean improvement in Tanzania. Euphytica. 150:215-231. DOI:10.1007/s/10681–006-9112-9.

Jesus Junior, W. C, F.R. Vale, R.R. Coelho, L.C. Costa, P.A. Paul and L. Zabolim. 1999. Strategies of chemical control of angular leaf spot (*Phaeoisariopsis griseola*) on common beans In: XIVth International Plant Protection Congress (IPPC) Plant protection towards the third millennium - where chemistry meets Ecology. Page 122

Katungi, E., D. Karanja, D. Wozemba, T. Mutuoki and J.C. Rubyogo. 2011. A cost-benefit analysis of farmer based seed production for common bean in Kenya. African Crop Science Journal 19: 409 - 415

Lemessa, F., W. Sori and M. Wakjira. 2011. Association between angular leaf spot (*Phaeoisariopsis griseola* (Sacc.) Ferraris and common bean (*Phaseolus vulgaris* L.). yield loss at Jimma, Southwestern Ethiopia. Plant Pathology 10): 57- 65

Liebenberg, M. M and Z. A. Pretorius. 1997. A review of angular leaf spot of common bean (*Phaseolus vulgaris* L.). African Plant Protection 3: 81-106

Mathew, K. A, S. K, Gupta and K. R. Shyam. 1998. New strategies in fungicidal management of angular leaf spot (*Phaeoisariopsis griseola*) of French bean. Mycology Journal of Plant Pathology. 28:123-133.

Monda, E.O., F .E. Sanders and A. Hicks.2001 Infection and colonization of leaf by *Phaeoisariopsisgriseola*. Plant Pathology 50:103-110

Meyer, S., K. Saccardy – Adji, F. Rizza and B. Genty. 2001. Inhibition of photosynthesis by *Colletotrichum lindermuthianum* in bean leaves determined by chlorophyll imaging. Plant Cell Environment 24: 947 – 956

McGrath, M. 2004. What Are Fungicides? The Plant Health Instructor The American Phytopathology.

http://www.apsnet.org/edcenter/intropp/topics/Pages/Fungicides.aspx(accessed 02/February, 2015)

Mwalyego. F. 1987. Yield losses from bean diseases in the Southern Highlands of Tanzania. In: Bean Research, Volume 2. Benedictine Publishing, Ndanda. Page. 109 - 117.

Patterson, D. T., O.S. Duke and E. R. Hoagland. 1977. Effect of irradiance during growth on adaptive photosynthetic characteristics of velvet leaf and cotton. Plant physiology 61: 402 - 405

Payne, R.W., D. A. Murray, S. A. Harding, D. B. Baird and D. M. Soutar.2011. An Introduction to GenStat for Windows (14th Edition). VSN International, Hemel Hempstead, UK.

Preez, du E. D., N.C. van Rij and P.M. Caldwell. 2005. Cost-benefit analysis of fungicides for soybean rust control. South African Journal of Science; 101(7/8) 314- 324.

Patterson, D. T., O.S. Duke and E. R. Hoagland. 1977. Effect of irradiance during growth on adaptive photosynthetic characteristics of velvet leaf and cotton. Plant physiology 61: 402 - 405

Preez, du E. D., N.C. van Rij and P.M. Caldwell. 2005. Cost-benefit analysis of fungicides for soybean rust control. South African Journal of Science; 101(7/8) 314- 324.

Payne, R.W., D. A. Murray, S. A. Harding, D. B. Baird and D. M. Soutar.2011. An Introduction to GenStat for Windows (14th Edition). VSN International, Hemel Hempstead, UK.

Schoonhoven, S and M. A. Pastor − Corrales. 1994. Standard system for evaluation of bean germplasm. CIAT – Columbia.

Schoonhoven, V and O. Voysest. 1991. Common bean research for crop improvement. CAB International, Wallingford, UK.

Schoonhoven, van A. and M.A. Pastor-Corrales. 1987. Standard system for the evaluation of bean germplasm. CIAT, Cali. Colombia

Schoonhoven, van A. and M.A. Pastor-Corrales. 1987. Standard system for the evaluation of bean germplasm. CIAT, Cali. Colombia

Sileshi, F., A. Mohammed, T. Selvaraj and M. Negeri. 2014. Field management of Anthracnose (*Colletotrichum lindermuthianum*) in common bean through foliar spray fungicides and seed treatment bioagents. Science Technology and Art Research. 3(2): 19 - 25

Simko, I and H. Piepho 2011. The Area Under the Disease Progress Stairs: Calculation, advantage and application. http://dx.doi.org/10.1094/PHYTO-07-11-0216

Sinha, S. K. 1977. Yield, yield components and plant idiotypes in food legume. In: Food Legume Crops: Improvement and Production, FAO Plant Production and Protection Serials, Rome Italy. Page 102 - 103

Stenglein, S., L.D. Ploper, O. Vizgarra and P. Balatti. 2003. Angular leaf spot: A disease caused by the fungus *Phaeiosariopsis griseola* (Sacc.) Ferraris on *Phaseolus vulgaris* L. Advanced Application Microbiology 52:209-243.

Su, G., S. O. Suh, R. W. Schneider and J. S. Russin. 2001. Host specialization in the charcoal rot fungus, Macrophomina phaseolina. Phytopathology 91:120−126.

Waggoner P E and R D. Berger. 1987. Defoliation, disease, and growth. Phytopathology **77**:393-398.

Walters, D. R, N.D Havis and C. Sablon. 2014. Control of foliar pathogens using a combination of resistance elicitors. Frontiers in plant science. 5: 241 - 255

Wiik, L., H. Rosenqvist. 2010. The economics of fungicide use in winter wheat in southern Sweden. Crop Protection. 29: 11-19.

# 4 Virulence patterns and pathotype diversity of *Pseudcercospora griseola* isolates of the Southern Highlands of Tanzania

**Abstract**

Knowledge of the existence of races and their virulence patterns is important in designing a vibrant disease resistance breeding programme. *Pseudocercospora griseola* is the causal organism of angular leaf spot (ALS) disease of common bean that is associated with yield losses of up to 80% on susceptible varieties. The objective of this study was to assess virulence pattern, distribution and pathotype diversity within *P. griseola* populations of the Southern Highlands of Tanzania (SHT). Random samples of ALS diseased leaves were collected from seven bean growing agro-ecologies of the SHT. A total of 122 sample isolates were tested using a set of 12 differential cultivars. Disease reaction data were collected on the host differentials for each isolate based on a scale of 1 – 9, where 1 indicated resistance and 9 susceptible. The data on susceptible or resistance reactions were transformed into compatibility (virulent [+]) or incompatibility (avirulent [-]) patterns. Phenotypic diversity was estimated using the Shannon, Simpson and Gleason indices. Twenty races were identified with the most frequent being race 63/55 while the most virulent was 63/63. Of these races, eleven are new races which have not been reported in Tanzania before. A higher number of races were recorded in Mbeya Stepped Plains, whereas the lowest was found in Iringa and Mufindi Highlands. Moderate pathotype diversity was detected when individual agro-ecologies were considered, while the overall collection showed high diversity. The results suggest existence of virulence variability between *P. griseola* pathotypes of the Southern Highlands of Tanzania. This is a clue that bean cultivars with multiple resistance genes are required to combat angular leaf spot disease of the common bean. Additionally, scheduled race monitoring of the pathogen population and dynamics is important in the SHT through surveys.


**Key words**: Angular leaf spot, common bean, diversity, pathotypes, *Pseudocercospora griseola* virulence

## 4.1 Introduction:

Angular leaf spot disease of common bean (*Phaseolus vulgaris* L.) is caused by *Pseudocercospora griseola* (Sacc.) Crous and U. Brown (formerly *Phaeoisariopsis griseola* (Sacc.) Ferraris). *Pseudocercospora griseola* is a facultative (hemibiotrophic and necrotrophic) fungal pathogen that inflicts plant health, grain yield and seed quality in common bean. *P. griseola* has undergone parallel co-evolution with its host forming two formae speciales: *P. griseola* f.sp. *griseola*, virulent to large seeded Andean beans and *P. griesola* f.sp. *mesoamerican*, virulent to small seeded Mesoamerican beans (Crous et al., 2006; Stenglein and Balatti, 2006).

*P. griseola* has been described as a variable fungus pathogenically and in the degree of aggressiveness although the sexual form has not been found (Brock, 1951; Alvarez-Ayala and Schwartz, 1979; Buruchara, 1983; Mahuku et al., 2002; Stenglein et al., 2003; Gupta et al., 2007). Pathogenic variations in imperfect fungi have been associated with parasexuality, a nonsexual mechanism for transferring genetic materials without meiosis. The mechanism involves fusion of hyphae, followed by mitotic cross-over that leads to the exchange of genetic material between chromosomes. In *P. griseola*, parasexuallity has been observed between vicinal hyphae among isolates demonstrating exchange of genetic materials with high possibilities of altering levels of pathogenicity and aggressiveness within and between groups (Guzman et al 1995; Wagara et al., 2005).

Under favorable temperature conditions (6 to $24^0$C), high humidity and in the presence of a susceptible host, the pathogen has the ability to colonize different parts of the bean plants including leaves, pods and seed. Symptoms on leaves are brown spots that may appear on primary leaves as round shape, while on later foliage are angular brown spots limited by veins which normally do not become prevalent until late flowering or early podding. Fungal growths on the underside of the spot are observed as clusters of synnemata which bear spores (Mckenzie and Jackson, 1986). In the advanced stages the spots increase in size, coalesce and cause partial necrosis followed by leaf yellowing and defoliation. On pods and stems, oval to circular reddish brown sunken spots of varying sizes occur and are sometimes surrounded by darker colored borders. Infected pods bear shriveled and discolored seed causing huge losses if the disease is not controlled (Sohi and Sharma, 1974; Neergaard, 1977).

In the SHT, common bean is grown in all of the seven agro-ecological zones that are known to vary in weather conditions and soil types. These include Mbeya Stepped Plains, Rungwe, Iringa and Mufindi Highlands, Nkasi, Sumbawanga and Mbozi Plains representing the bean growing agro-ecologies of Tanzania in terms of growing environments. More than 80% of the beans in these agro-ecologies are under small scale production systems. Due to the high demand coupled with limited lands the crop is grown thrice in a year, twice during the long rains (December to February; March to July) and once during the dry season (September to November) mainly in wet lands and along the river banks. The overlapping seasons for bean production create 'green bridges' for *P.griseola* to survive throughout the year with inoculum transmitted between infected crops. The types of beans grown are a mixture of local varieties with different colors and sizes acquired informally within the country as well as across bordering countries of Zambia, Malawi, Rwanda, Burundi and Democratic Republic of Congo, mainly through seed exchange among friends and relatives as well as through gifts.

Angular leaf spot has attained economic importance not only in the SHT but also in other Eastern African countries of Kenya, Uganda and Rwanda and in more than sixty countries throughout the world (Guzman et al., 1993; Wagara et al., 2003). In the SHT, the disease is accelerated by the use of susceptible cultivars, recycling of infected seed, lack of awareness and the presence of environmental conditions favorable for the disease development as determine in the previous study which is part of this research. Considering the importance of *P. griseola*, identification of physiological races is necessary prior to developing control measures against the pathogen. Past studies have indicated that many races of *P. griseola* are variable in virulence such that a bean cultivar which is resistant in one location, season or year may be susceptible in another (Aggarwal et al., 2004).

Plant pathogenic microorganisms are often categorized below the species level into "physiological races" which are pathotypes that differ from one another solely on the basis of their visible disease reaction on a set of differential cultivars (Groth and Roelf, 1987). The degree of damage caused by the physiological races on a plant is termed virulence (Melotto and Kunkel, 2013). For a pathogen to be virulent, it must have the ability to invade and acquire nutrients and water from its host for it to successfully colonize and grow within the host tissue. However, successful infection relies to a great extent on the ability of the pathogen to modulate the

physiology of its host through the use of unique virulence strategies that facilitate plant – host pathogen interaction (Thrall and Burdon, 2003).

A set of 12 differential cultivars carrying different resistance genes to ALS disease have been identified and used in various places the world over to identify and characterize *P. griseola* races (CIAT, 1995).The set consists of cultivars from Andean and Mesoamerican gene pools. The Andean cultivars are large seeded beans that originated from wet and cool environments while Mesoamerican cultivars are medium to small seeded and originated mostly from lowlands (Gepts, 1998). Each cultivar in the differential set are assigned binary numbers that are used in the process of race identification. When a group of isolates infect two or more of the differential cultivars in the same way, the binary numbers are added together to form a race number that is separated by a slash or dash to distinguish Andean and Mesoamerican cultivars infected (Pastor-Corrales, 1995; Mahuku et al., 2002; Mahuku et al., 2004). Race characterization based on differential cultivars has been used successfully in different crops including stripe rust of wheat and barley caused by *Puccinia striiformis* f. sp. *tritici*, bacterial blight of cassava caused by *Xanthomonas exonopodis* pv *manihotis* and black spot disease of common bean caused by *Colletotrichum lindermuthianum* (Alves and Takatsu, 1984; Boher and Verdier 1994; Thomazella et al., 2000).

Determining the virulence and diversity of *P. griseola* is important for effective gene deployment. Pathotype diversity is defined as a rate of temporal and spatial change of a pathogen. A population is considered diverse by the number of distinct pathotypes or races characterized (Groth and Roelf, 1987; Kolmer, 2013). Indices of species diversity have been developed and used to study diversity of disease causing fungal pathogens on various crops including stem rust of wheat caused by *Puccinia graminins f. sp. tritici* and *Magnaporthe grisea* that causes blast disease in rice (Growth and Roelf, 1987; Kolmer, 1991; Silva et al., 2009). Among the commonly used indexes are the Shannon, Simpson and Gleason (Kolmer, 1990; Leonard et al., 1992). Shannon and Simpson indexes are based on relative frequency of different races while Gleason index takes into account the number of distinct pathotypes or races that exist in a given sample size (Growth and Roelf, 1987).

Previous studies on *P. griseola* races in the Southern highlands of Tanzania using a set of twelve ALS differentials cultivars (Don Timoteo, G11796, Bolon Bayo, Montcalm, Amendoin, G5686,

Pan 72, G2858, Flor de Mayo, Mexico 54, Bat 332 and Cornell 49 – 242)indicated the existence of 9 races most of which were of Andean type (Mwalyego, 1987; Ngulu, 1999). A large seeded cultivar G5686 and a small seeded Mesoamerican Mexico 54 were indicated to have good levels of resistance to nearly all races characterized in Tanzania. The same results were reported in Brazil (Pastor-Corrales et al., 1998; Nietsche et al., 2001; Aggarwal et al., 2004) .Cultivars G5686 carry a single dominant gene while Mexico 54 has three dominant genes. However, there are no recent studies available on the pathogen although there are reports of increasing levels of angular leaf spot disease in the bean producing agro-ecologies of the Southern Highlands of Tanzania. Therefore, it is worthwhile to use the same set of ALS differential cultivars used in Tanzania during the 1987 and 1999 to detect any new and confirmation of previously characterized *P. griseola* races.

The objectives of this study were:

1. To determine the distribution of *P. griseola* in the bean growing agro-ecologies of the SHT and study virulence patterns of the pathogen,
2. To identify pathotypes/races that exist in the Southern Highlands of Tanzania, and
3. To study the pathotype diversity within the pathogen.

## 4.2   Materials and Methods

An extensive field survey was carried out in the seven bean growing agro-ecologies of the Southern Highlands of Tanzania during the 2012/2013 and 2013/2014 bean cropping seasons to collect ALS disease samples from local bean cultivars grown by farmers. In each agro-ecology, one representative district was chosen. The districts and their villages were; Mbeya Rural district - Isangala; Mufindi district – Ihalimba and Vikula; Sumbawanga district - Malonje; Mbozi district - Nambinzo and Mbimba; Wanging'ombe district –Masaulwa; Njombe district - Igosi and Rungwe district - Kabate and Lukata.

### 4.2.1    Collection of ALS disease sample

Leaves showing symptoms of ALS disease were collected from farmers' fields and a distance of 15 – 20 km between sampled fields was maintained. Additional samples were collected at the Uyole Agricultural Research Institute (ARI –Uyole) bean experimental fields which are also known as hot spots for the ALS disease. The diseased samples were carefully placed in paper envelopes and labeled with the following information: district, village, date of collection and sample number. The samples were transported to ARI –Uyole where they were air dried. Dry samples were later kept in boxes at room temperature for future use.

### 4.2.2    Isolation of *P. griseola*

Individual diseased bean leaf samples were incubated under high humidity for 24 hours at room temperature. The high humidity was created by placing a moisturized filter paper in a 9 cm diameter glass petri dish. A piece of aluminum foil large enough to cover the petri dish was placed on top of the moistened filter paper followed by the diseased leaf to avoid direct contact with the free water in the petri dish. The plates were then placed in an incubator for 24 hours at $24^0$C. Lesions were thereafter examined under a dissecting microscope to view the synemata and assess the quality of the sporulation. Conidia of individual lesions were picked from the clean synemata by gently brushing the tips with a small piece of agar at the tip of an inoculating needle and transferred to a drop of sterile water placed on top of water agar as described by Mahuku et al. (2002) and Sartorato (2002). Inoculated petri dishes were swirled to distribute the spores evenly. The petri dishes were later incubated in darkness at $24^0$C for 24 hours in which the germinating spores were picked and immediately transferred to a fresh V8 or potato carrot agar. Each plate of V8 or potato carrot agar was inoculated with a single conidium to avoid mixtures of conidia during race identification. Each colony that rose from a single conidium was treated as an isolate. Generally single spore isolates are considered pure during race designation. The petri dishes were incubated in darkness for 14 to 18 days for the colonies to develop. Five petri dishes were sub-cultured from the colony to provide inocula for immediate virulence tests. Each colony was treated as an isolate (Fig 4.1 and 4.2).
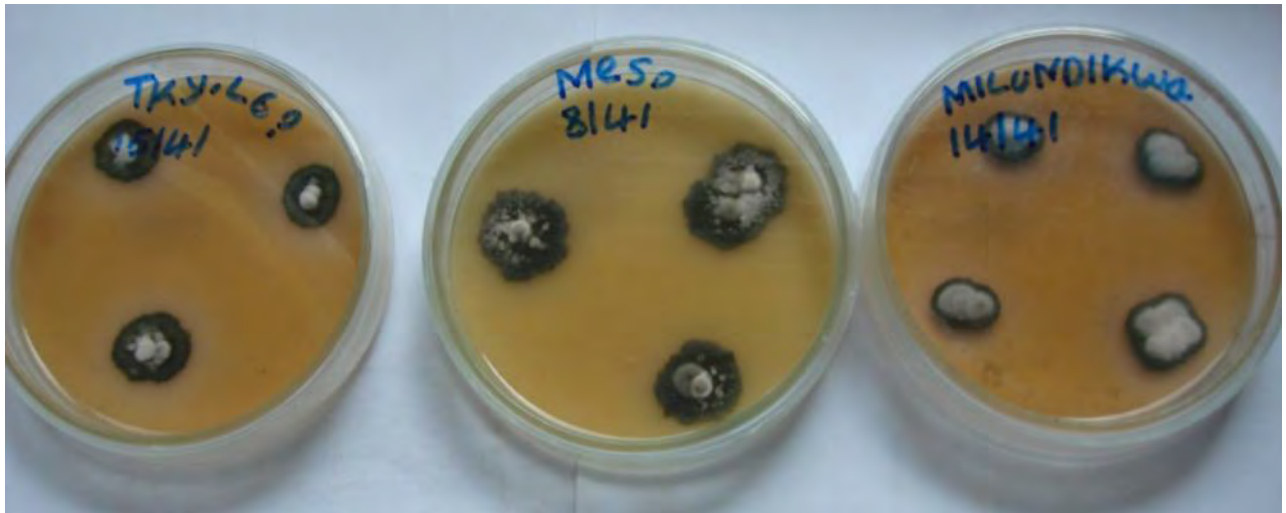
**Figure 4.1:** *Pseudocercospora. griseola* **single conidia colony isolate in plates collected from the bean growing agroecologies specifically Mbeya, Mbozi and Nkasi**



**Figure 4.2: Conidia of *P. griseola* as seen under the light microscope at x40 magnification**

### 4.2.3    Inocula preparation and resistance evaluations of ALS differential cultivars

The surfaces of the plates containing well developed isolates were gently scrapped while pouring sterile water. The conidia suspension was then filtered through a layer of cheesecloth to remove mycelia and any other clumps. Each single isolate was use to inoculate a complete set of 12 differential cultivars for the ALS disease (Table 4.1). Two-week old bean seedlings were sprayed with a spore suspension made from a single isolate. The suspension was calibrated to 3 x $10^4$ spores $ml^{-1}$ using a haemocytometer. The degree of virulence of the isolates was tested under controlled greenhouse conditions on the differential cultivars planted in pots of 12 cm diameter, containing 2 kg of forest soil mixed with organic manure. After inoculation, the plants were immediately placed in boxes well covered with clear polythene sheets for 72 hours to create high humidity required for spore germination and infection (Fig 4.3 and 4.4). The inoculated plants were later removed from the boxes to allow normal growth. The inoculated plants were arranged in a randomized complete block design with three replications using time differences of three weeks between plantings as a block factor (Fig. 4.4).



**Figure  4.3: Common bean seedling plants grown in polythene covered box creating high humidity to facilitate infection**

(a)



(b)

**Figure 4.4: (a) Differential cultivars arranged in four replications in the screen house with time as a blocking factor. (b) A closer look of resistant (clean plants) and susceptible (leaves with lesions) differentials inoculated with *P. griseola* isolates**

**Table 4.1: Characteristics of the common bean differential cultivars used in the study to characterize *Pseudocercospora griseola* pathotypes**

| Differential cultivar | [1]Seed size | Gene pool | [2]Race | Binary value | Resistant gene present |
|---|---|---|---|---|---|
| Don Timoteo | *G* | Andean | C | 1 | 1 dominant gene |
| G11796 | G | Andean | P | 2 | - |
| BolonBayo | G | Andean | P | 4 | - |
| Montcalm | G | Andean | NG | 8 | 2 recessive genes |
| Amendoin | G | Andean | NG | 16 | 2 recessive genes |
| G5686 | G | Andean | NG | 32 | 1 dominant gene |
| PAN72 | S | Mesoamerican | M | 1 | 1 dominant gene |
| G2858 | M | Mesoamerican | D | 2 | 1 dominant gene |
| Flor de Mayo | S | Mesoamerican | J | 4 | 2 duplicate genes |
| Mexico 54 | M | Mesoamerican | J | 8 | *Phg-2, Phg-5, Phg-6* |
| BAT 332 | S | Mesoamerican | M | 16 | *Phg - 6$^2$* |
| Cornell 49-242 | S | Mesoamerican | M | 32 | *Phg-3* |

Source: Caixeta et al., 2002; Mahuku et al., 2004; Sartorato et al., 2002.

[1]Seed size: G = large., M= medium., S = Small; [2]Races: C = Chile, P = Peru, NG= Nueva Granada, M = Mesoamerican, D = Durago, J = Jalisco.

## 4.3 Virulence frequency and race identification based on the differential cultivars

Virulence frequency of each race was calculated as a percentage of the total number of isolates analyzed. Disease development was assessed at 12 days after inoculation followed by measurements at 10 day intervals until the plants had attained physiological maturity. Disease assessments were carried out using a 1 to 9 scale described by the International Center for Tropical Agriculture (CIAT), where; 1 = plants with no ALS symptoms; 2 -3 = plants with sparse and small lesions; 4-6 = plants with well defined but sparse lesions; 7 -9 = plants with well defined, expanding and many lesions (van Schoonhoven and Pastor – Corrales, 1987). Plants that scored 1 – 3 were rated resistant (R) while those that ranged from 4 – 6 and 7 – 9 were rated moderately resistant (MR) and susceptible (S), respectively. Data on disease reaction of the host differentials to each of the isolates used that expressed susceptible or resistance reactions were transformed into compatibility (virulent [+] or incompatibility (avirulent [-] pattern).

Compatibility reaction showed the ability and incompatibility reaction was the inability of the pathogen to produce severe disease on its host.

The denomination of race was obtained by adding the binary values of the susceptible differential cultivars for Andean and Mesoamerican set but separated by a slash. All scores that rated MR and S were assigned the same binary number and considered susceptible.

## 4.4 Race distribution and diversity

Races identified through the use of ALS differential cultivars were assessed for pathotype diversity using the Shannon, Simpson and Gleason indices. The indices have been used to describe the intraspecific diversity of races in populations of stem rust of wheat (*P. graminis*) and can be used to describe the number of distinct pathotypes for a given number of sampled individuals (Groth and Roelf, 1987). Shannon-Weaver and Simpson indices measure between 0 and 1, where 1 represents great diversity and zero, no or minimum diversity. The Shannon and Simpson index was used to measure the number of distinct pathotypes and evenness of race frequency in the isolates using the following formula:

$$H_{SH} = -\sum_{i=1}^{S}(ni/N).\ln(ni/N) \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots (1)$$

Simpson index was calculated using the following formula;

$$S = 1 - \sum_{i=1}^{S}(ni-1)/N(N-1)\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots (2)$$

For both Simpson and Shannon, *ni* represents the number of isolates of the ith race; S = number of different races in the sample; N = total number of isolates. The value of 'S' in the Simpson index ranges between 0 and 1, where 0 represents no diversity and 1 represent high diversity.

Gleason index was used to detect the number of distinct races (richness of diversity) in a sample using the following formula;

$$H_G = (n-1)/\ln(N) \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots.(3)$$

Where *n* is the number of races and N is the number of isolates in a sample population.

The Gleason index is the most responsive to the number of pathotypes and represents a method of describing race/isolate ratio and in comparing populations that vary in number of pathotypes (Groth and Roelf, 1987)

## 4.5    Results

### 4.5.1    Reaction of differential hosts to *P. griseola* isolates

The 122 isolates caused lesions in some or all of the host plants. Symptoms on leaves were brown spots that appeared on primary leaves as rounded spots while on the later foliage, angular brown spots limited by veins were observed that prevailed until late flowering. Fungal growths on the underside of the spots were observed as clusters of synnemata which bared spores/conidia. Under the electronic microscope, conidia were observed as obclavate –cylindrical with two to four septae (Fig. 4.2).

### 4.5.2    Virulence analysis and race identification

Angular leaf spot disease was observed in all of the fields sampled regardless of the type of the varieties grown and in all of the twelve ALS differential cultivars inoculated with the isolates collected from the Southern Highlands of Tanzania. Variations in response of the ALS differential cultivars were observed regardless of the gene pools from which they originated (Table 4.2). Based on the virulence reaction, 20 races were characterized and all were virulent to Amendoin and G11796 but avirulent to Mexico 54, with the exception of race 63/63. Race 63/63 was the most virulent and aggressive, and overcame resistance genes in all of the cultivars evaluated with highest complexity value as determined by the number of differentials infected with that particular race. None of previously characterized races in the SHT have been indicated to overcome resistance in Mexico 54. Race 63/63 had a complexity value of 12 while 63/55, 63/53, 63/47 and 63/39 had complexity values of 10 each and none of the Andean differential cultivars had resistance genes that restricted the pathogens from infection. Of the twenty ALS races characterized in this study, seven and eleven races were able to overcome resistance in BATT 332 and Cornell 49-242 cultivars, respectively. Cultivars BATT 332 and Cornell 49-242 have more than one resistant genes characterized so far and are considered to have high levels of resistance compared to the rest of differential cultivars excluding Mexico 54. Race 29/00 was virulent only

to Andean cultivars and had the lowest complexity number. In general, Andean cultivars were more susceptible to the races of the SHT compared to the Mesoamerican cultivars. These results had identified 11 new races in addition to nine races previously reported in 1999 as indicated in Table 4.3

**Table 4.2: Differential cultivars and virulence patterns of *Pseudocercospora griseola* isolates collected in the major bean growing agro-ecological zones of the Southern Highlands of Tanzania during the 2012/2013 and 2013/2014 bean cropping seasons.**

| | | Differential cultivars | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Race | [1]NI | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | [2]CPX |
| 31/1 | 5 | + | + | + | + | + | - | + | - | - | - | - | - | 6 |
| 63/23 | 9 | + | + | + | + | + | + | + | + | + | - | + | - | 10 |
| 62/7 | 4 | - | + | + | + | + | + | + | + | + | - | - | - | 8 |
| 63/53 | 8 | + | + | + | + | + | + | - | + | + | - | + | + | 10 |
| 63/47 | 9 | + | + | + | + | + | + | + | + | + | - | - | + | 10 |
| 63/39 | 4 | + | + | + | + | + | + | + | + | + | - | - | + | 10 |
| 63/62 | 10 | + | + | + | + | + | + | + | + | + | - | + | + | 11 |
| 31/43 | 8 | + | + | + | + | + | + | + | + | - | - | - | + | 9 |
| 23/32 | 4 | + | + | + | - | + | - | - | - | - | - | - | + | 5 |
| 47/3 | 5 | + | + | + | + | + | - | + | + | - | - | - | - | 7 |
| 18/3 | 9 | - | + | - | - | + | - | + | + | - | - | - | - | 4 |
| 63/55 | 7 | + | + | + | + | + | + | + | + | + | - | + | - | 10 |
| 63/06 | 6 | + | + | + | + | + | + | - | + | + | - | - | - | 8 |
| 63/38 | 8 | + | + | + | + | + | - | + | + | - | - | - | + | 8 |
| 31/39 | 8 | + | + | + | + | + | - | + | + | + | - | - | + | 8 |
| 23/39 | 7 | + | + | + | - | + | - | + | + | + | - | - | + | 8 |
| 30/0 | 4 | + | + | + | + | + | - | - | - | - | - | - | - | 5 |
| 26/06 | 3 | - | + | + | + | + | - | - | + | + | - | - | - | 6 |
| 54/38 | 3 | - | + | - | + | + | + | - | + | + | - | - | + | 7 |
| 63/63 | 1 | + | + | + | + | + | + | + | + | + | + | + | + | 12 |

[1]NI – isolate number; 1 to 12 denote codes of differential cultivars: 1 = Don Timoteo; 2 = G11796; 3=BalonBayo; 4= Montcalm; 5 = Amendoin; 6 = G5686; 7 = Pan 72; 8= G2858; 9 = Flor de Mayo; 10 = Mexico 54; 11 = BAT 332; 12 = Cornell 49-242; [2]CPX=Complexity = number of infected differential cultivars regardless of the gene pool; + = compatible reaction; - = incompatible reaction

**Table 4.3. Pseudocercospora griseola races of the Southern Highlands of Tanzania characterized in1999**

| Race | Origin | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 31/0 | MBZ/NJ | + | + | + | + | + | - | - | - | - | - | - | - |
| 15/0 | MBZ/MB | + | + | + | + | - | - | - | - | - | - | - | - |
| 14/0 | MBY/SG | - | + | + | + | - | - | - | - | - | - | - | - |
| 30/0 | MBY/NJ | - | + | + | + | + | - | - | - | - | - | - | - |
| 63/39 | MBY/NJ | + | + | + | + | + | + | - | - | - | - | - | - |
| 18/3 | MBY | - | + | - | - | + | - | + | + | - | - | - | - |
| 47/3 | NJ | + | + | + | + | - | + | + | + | - | - | - | - |
| 23/32 | MBZ | + | + | + | - | + | - | - | - | - | - | - | + |
| 6/1 | MBY | - | + | + | - | - | - | + | - | - | - | - | - |
| 31/39 | MBZ | + | + | + | + | + | - | + | + | + | - | - | + |

*Source*: Ngulu, 1999.

MBZ =Mbozi; MBY/MB = Mbeya; NJ = Njombe.
1 to 12 denote codes of differential cultivars: 1 = Don Timoteo; 2 = G11796; 3=BalonBayo; 4= Montcalm; 5 = Amendoin; 6 = G5686; 7 = Pan 72; 8= G2858; 9 = Flor de Mayo; 10 = Mexico 54; 11 = BAT 332; 12 = Cornell 49-242. + = compatible reaction; - = incompatible reaction.

### 4.5.3 Virulence frequencies of *P. griseola* isolates of the Southern Highlands of Tanzania on ALS differential cultivars

Virulence frequencies of the races collected from the Southern Highlands of Tanzania were analyzed as percentages from the total number of isolates and the results are presented in Fig.4.5. A wide variation of 5- 100% was revealed, in which cultivar Mexico 54 had the lowest frequency while cultivar Amendoin scored the highest. However, Andean differentials had the highest values compared to the Mesoamerican differentials. Cultivars G5686 and Don Timoteo showed a frequency of 70% which was the lowest percentage among the Andean differentials.
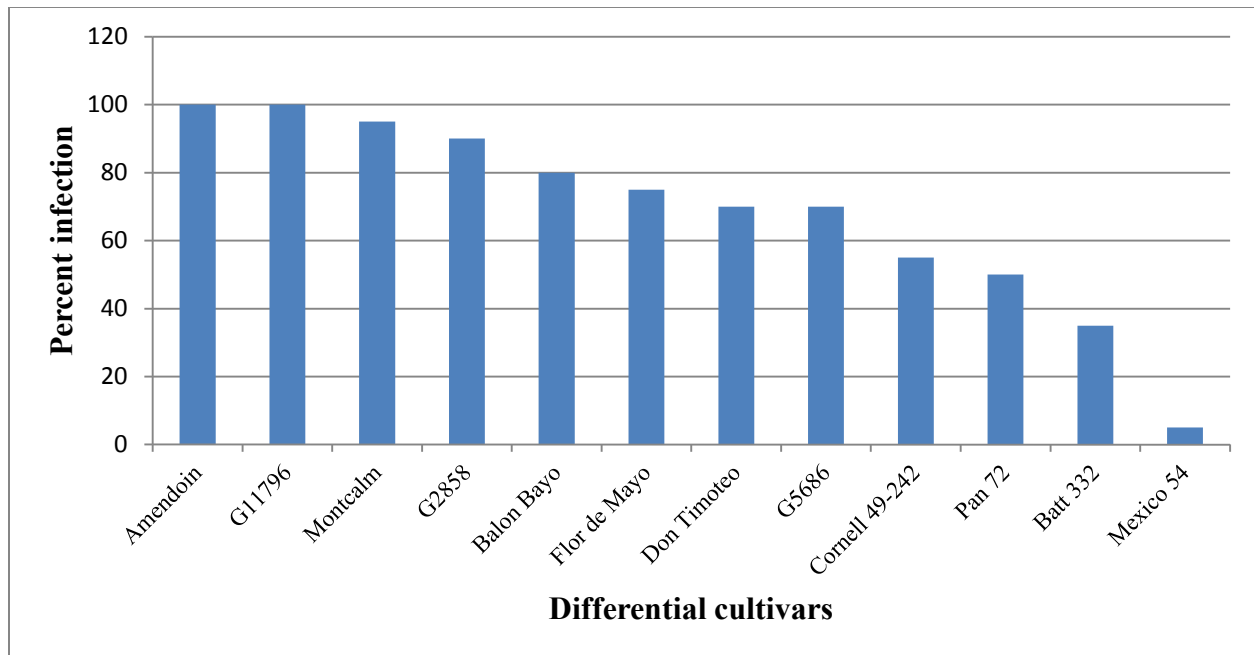
**Figure 4.5: Virulence frequencies on *Pseudocercospora griseola* races collected from the Southern Highlands of Tanzania that infected twelve ALS differential cultivars.**

### 4.5.4 Race distribution and frequency of occurrence in the Southern Highlands of Tanzania

Distribution of races and isolates of the Southern Highlands are presented in Table 4.3. Higher numbers of isolates and races were obtained from Mbeya Stepped Plains followed by Sumbawanga and Mbozi (Fig 4.6). Consequently, the majority of races were found in Mbeya Stepped Plains while Iringa Highlands had the lowest numbers. A few of the races in Mbeya Stepped Plains were also found in more than four agro-ecologies. Races 23/6 and 54/38 were found only in Rungwe whereas races 18/3, 31/1 and 62/15 were found only in Mbeya Stepped Plains. Race 63/55 was the most widely distributed and overcame the resistance genes in all of the differential cultivars with the exception of Mexico54. Races 63/62, 63/06, 63/38 were found in more than two different agro-ecological zones while races 63/63, 63/47, 63/39, 63/23 and 31/43 were recorded in two different agro-ecological zones. In 2014 the race was detected in Sumbawanga plains in addition to Mbeya Stepped Plains. Races that were recorded in Mbeya Stepped Plains were not found in Rungwe Highlands and those recorded in Nkasi were not found

in Rungwe and Mufindi Highlands. In this regard, only a few races occurred in more than three agro-ecological zones. The most virulent race was found in Nkasi and Mbeya, while the least virulent race was obtained from Rungwe Highlands. Of all the races, 45% were able to overcome resistance genes in the Andean differentials with varying virulence patterns on Mesoamerican differentials. The same patterns of virulence and avirulence to the 12 differentials were observed over the two years (2012/2013 and 2013/2014) in which the experiment was conducted.

Frequencies of occurrence of races ranged from 3.4 to 33.3% and race 63/55 had the highest frequencies in all of the bean growing agro-ecologies with the exception of Rungwe and Mufidi Highlands (Table 4.4). Occurrence of the most virulent race 63/63 was rare and confined to Mbeya Stepped Plains and Nkasi Hills. However, the reverse was true for race 63/62 which was found in five bean growing agro-ecologies. More than half of the races found in Rungwe Highlands and Mbeya Stepped Plains had low frequencies while Nkasi Hills, Sumbawanga and Mbozi Plains had races with frequencies of occurrence below 10%.
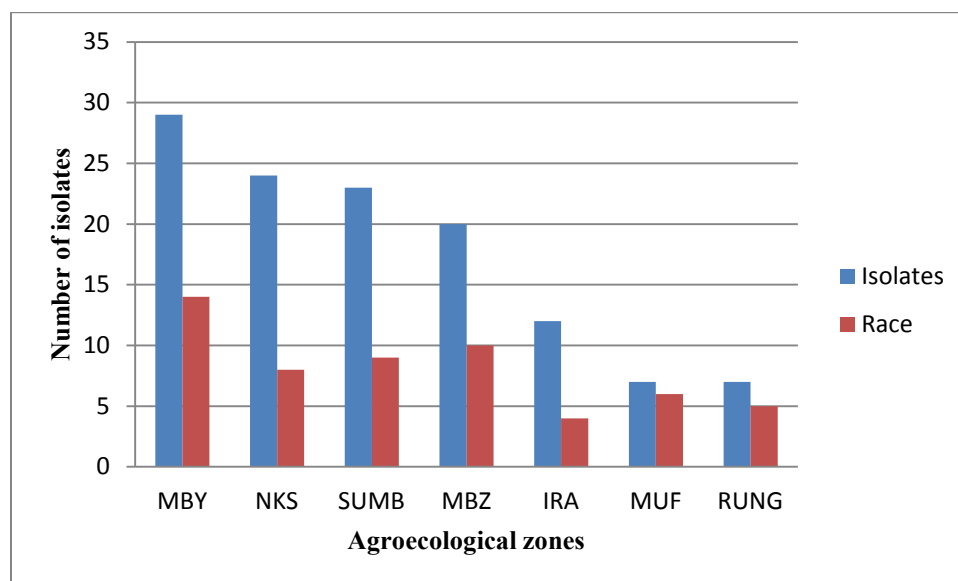


**Figure 4.6: Number of isolates collected and races identified in the bean growing agro-ecologies of the SHT during the 2012/2013 and 2013 /2014 bean cropping season**
SMB = Sumbawanga plains; RUNG= Rungwe Highlands; MBY = Mbeya stepped plains; IRA = Iringa; MBZ = Mbozi; NKasi plains; MUF = Mufindi

.

**Table 4.4: *Pseudocercospora griseola* race distribution and virulence frequency in the major bean growing agro ecologies of the Southern Highlands of Tanzania**

| Race | SMB | Fr(%) | RUNG | F (%) | MBY | Fr(%) | IRA | Fr(%) | MBZ | Fr(%) | NKS | Fr(%) | MUF | Fr(%) | TOTAL | Total Fr |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 63/55 | 5 | 23.8 | | | 4 | 13.8 | 4 | 33.3 | 3 | 14.3 | 6 | 25.0 | | | 22 | 18.0 |
| 63/62 | 2 | 4.7 | | | 3 | 10.3 | 2 | 16.7 | 3 | 14.3 | 7 | 29.2 | | | 17 | 13.9 |
| 63/6 | 4 | 19.0 | | | 3 | 10.3 | | | 3 | 14.3 | 3 | 12.5 | 2 | 28.6 | 15 | 12.3 |
| 31/39 | 4 | 19.0 | 2 | 28.5 | | | 3 | 25.0 | 2 | 9.5 | 2 | 8.3 | 1 | 14.3 | 14 | 11.5 |
| 23/39 | | | 2 | 28.5 | | | 3 | 25.0 | 2 | 9.5 | | | 1 | 14.3 | 8 | 6.6 |
| 63/47 | | | | | 4 | 13.8 | | | | | 1 | 4.1 | 1 | 14.3 | 7 | 5.7 |
| 63/53 | 3 | 14.3 | | | 2 | 6.9 | | | 2 | 9.5 | | | | | 7 | 5.7 |
| 63/38 | 2 | 9.5 | | | 1 | 3.4 | | | 2 | 9.5 | 1 | 4.1 | | | 6 | 4.9 |
| 31/43 | | | | | 2 | 6.9 | | | 1 | 4.7 | | | | | 3 | 2.5 |
| 63/23 | | | | | 1 | 3.4 | | | | | 1 | 4.1 | | | 3 | 2.5 |
| 63/39 | 1 | 4.7 | | | 2 | 6.9 | | | | | | | | | 3 | 2.5 |
| 30/0 | | | 1 | 14.3 | | | | | 1 | 4.7 | 1 | 4.1 | | | 3 | 2.5 |
| 63/63 | | | | | 3 | 10.3 | | | | | | | | | 3 | 2.5 |
| 23/32 | 1 | 4.7 | | | 1 | 3.4 | | | | | | | 1 | 14.3 | 3 | 2.5 |
| 47/03 | | | | | | | | | 1 | 4.7 | | | 1 | 14.3 | 2 | 1.6 |
| 31/1 | 1 | 4.7 | | | 1 | 3.4 | | | | | | | | | 2 | 1.6 |
| 18/3 | | | | | 1 | 3.4 | | | | | | | | | 1 | 0.8 |
| 62/7 | | | | | 1 | 3.4 | | | | | | | | | 1 | 0.8 |
| 23/6 | | | 1 | 14.3 | | | | | | | | | | | 1 | 0.8 |
| 54/38 | | | 1 | 14.3 | | | | | | | | | | | 1 | 0.8 |
| R/isolate | 9/23* | | 5/7 | | 14/29 | | 4/12 | | 10/20 | | 8/24 | | 6/7 | | 55/122 | 100 |

SMB = Sumbawanga plains; RUNG= Rungwe Highlands; MBY = Mbeya stepped plains; IRA = Iringa; MBZ = Mbozi; NKasi plains; MUF = Mufindi; % = percentage of total isolates collected per race; R/isolates = number of races per total number of isolates collected regardless of the type in each of the agro-ecological zone *a total number of isolates in each agro-ecological zone

### 4.5.5 Diversity of *P. griseola* races in 12 agro-ecologies of the Southern Highlands of Tanzania

Results derived from Shannon, Simpson and Gleason indices are presented in Table 4.5. Value trends from the three indices differed and higher values were obtained from Gleason index. The index values ranged from 0.23 to 0.75 for Shannon, 0.59 to 0.91 for Simpson and 2.65 – 4.94 for Gleason indices. However, all the indices showed Mbeya Stepped Plains and Sumbawanga Plains to have higher diversity compared to the other bean growing agro ecologies of the SHT. The lowest value of diversity was recorded by Shannon index in Mufindi Highlands while for the case of Simpson and Gleason indices, the lowest value was obtained in Iringa Highlands and Sumbawanga Plains. Mbozi Plains showed lower diversity with fewer distinct races identified. These sites achieved the second highest values of Shannon and Simpson indices indicating that the races exhibited a more homogenous diversity. Race diversity in Nkasi Hills, Mufindi and Rungwe Highlands was generally low. These areas are cool with heavy rains during the bean cropping season. Besides these differences, the overall total diversity for all the indices were in the range of 0.95 – 0.98.

**Table 4.5: Phenotypic diversity within *P. griseola* races of the Southern Highlands of Tanzania**

| Index | MUF | MBY | MBZ | NKS | RUNG | SUMB | IRA | Total |
|---|---|---|---|---|---|---|---|---|
| Shannon | 0.23±0.04 | 0.75±0.049 | 0.69±0.02 | 0.29±0.016 | 0.43±0.03 | 0.67±0.03 | 0.45±0.01 | 0.98±0.01 |
| Simpson | 0.66±0.04 | 0.91±0.02 | 0.89±0.07 | 0.6±0.07 | 0.62±0.09 | 0.88±0.05 | 0.59±0.07 | 0.95±0.02 |
| Gleason | 2.94±0.17 | 4.47±0.33 | 4.94+0.33 | 2.65±0.5 | 3.59± 0.02 | 2.49±0.23 | 3.13±0.04 | 3.95±0.4 |

MUF = Mufindi; MBY = Mbeya stepped plains; MBZ = Mbozi plateau; NKS = Nkasi plains; RUNG = Rungwe Highlands; SUMB = Sumbawanga plains

## 4.6  Discussion

The *P. griseola* isolates collected from the SHT were diverse in virulence patterns although no sexual reproduction has been reported. The diversity was high in such a way that even isolates

collected from the same location showed differences in their virulence patterns. This was not unusual as the existence of different virulence patterns that are specific to host genotypes within the pathogens has been reported in other countries including Mexico and Brazil (Busogoro et al., 1999; Sartorato, 2002). Varying virulence patterns observed revealed that the majority of the resistance genes in the differential cultivars were ineffective against most of the isolates. This suggests that these isolates probably carry different virulence genes which are not matched by resistance genes in the differential cultivars. In this study, new virulence patterns of *P. griseola* isolates were recorded resulting in the characterization of 11 new races which are herein reported for the first time in the SHT. Some 10 years back ten races were characterized in the zone based on a set of differential cultivars that are known to carry dominant and recessive resistance genes in different combinations (Ngulu, 1999; Sartorato, 2002). The same set was used for this study in which race 63/39, 30/0, 18/3, 47/3, 31/39 and 23/32 first reported in Mbeya Stepped Plains in 1999 were also found to be present during the cause of this study. The mechanism that facilitated the existence of these races without changes was not known. The two races have been reported in Brazil by Sanglard et al., 2013. Of the eleven new races, race 63/63 was the only one that overcame resistance genes to all the differential cultivars and was regarded as the most complex race so far recorded in the SHT. Consequently, race 63/55 overcame resistance in all the differential cultivars with an exception to cultivar Mexico 54 and was the most widely distributed in all the agroecological zones with the exception of Rungwe and Mufindi, agroecological zones characterized as having excess rainfall and cooler temperatures. It is well known that host cultivars with combinations of two or more resistance genes select complex pathogen pathotypes, whereas host cultivars with one gene select simpler pathotypes. The Andean cultivars, Amendoin and G11796 were susceptible to all races indicating lack of an effective resistance gene to *P. griseola*. The same races acted differently when infecting other cultivars of Andean and Mesoamerican backgrounds indicating the presence of resistant genes in the host plant that had varying ability to counteract the pathogenic infection processes. In this regard, there are high chances that these races might predominate if no selection factors are present.

During the past five years, the majority of bean cultivars grown in the SHT and probably elsewhere in Tanzania have shown severe infections of *P. griseola*, supporting the existence of new races. However, the sources of the new races are currently not known and it is likely that they might have been introduced in the zone through air movement and, informal seed exchange

systems that are currently practiced by the smallholder farmers, or the pathogen has undergone parasexuality that facilitates exchange of genetic material within and between isolates. Additionally, chromosomal inversion, deletions and the presence of transposons all have been documented to have the capability of increasing the diversity in *P. griseola* (Kristler and Miao, 1992; Kempken and Kuck, 1998)..

The SHT borders Malawi, Zambia and D. R. Congo where there are high seed exchanges between farmers extending up to Rwanda and Burundi through the border region of Kigoma. Angular leaf spot is a seed borne disease that can easily be transported from one area to another. The fact that the majority of the races were able to overcome resistance genes present in the Andean differential cultivars it is more likely that they will overcome or are overcoming the currently grown Andean bean cultivars. The bean breeding programme in the SHT has been recommending and promoting large seeded Andean varieties that are known to fetch higher prices in the market. Among them are Uyole 96 and Wanja that have shown severe infections of ALS disease, an indication of the presence of ineffective genes in overcoming virulence factors present in *P. griseola*.

From this study, it was evident that the Mesoamerican differentials reacted differently to the 20 races of *P. griseola* collected in the SHT compared to the Andean types. A higher number of resistant cultivars were observed in this group revealing a strong influence on host specialization of the ALS causing fungus. Traditionally, Mesoamerican cultivars have been extensively cultivated in the SHT for a long time and in environments that are known to be favorable for ALS development. It has been documented that the races attached to this gene pool have a higher genetic variability and have the ability to infect both Andean and Mesoamerican gene pools. In contrast, Andean *P. griseola* races have a narrower and more specific virulence range that attacks Andean large seeded cultivars (Mahuku et al., 2002). This might explain the differences observed between the two gene pools when infected with *P. griseola*.

Analysis of phenotypic diversity within *P. griseola* races revealed that the three indices used did not yield the same rank order between the isolates collected in the bean growing agro-ecologies of the SHT. This observation may have been due to the varying sensitivities of the indices on number of isolates, evenness of race frequencies and the samples size (Groth and Roelf, 1987). Although the results from individual agro-ecological zones indicated moderate diversity, the

overall collections from all the agro-ecologies showed high diversity. The low value of Shannon and Simpson indices obtained in Mufindi and Iringa may have been due to the low number of isolates collected. Sample sizes have been indicated to affect the diversity in wheat leaf rust populations (Kolmer et al., 2003).

## 4.7   Conclusion

Eleven new races were identified in addition to the 9 races previously reported about 10 years ago. This suggests that the new races might have been introduced or mutated from those reported earlier. The most resistant differential cultivar, Mexico 54 was susceptible to race 63/63 further support the existence of new races in the bean growing agro-ecologies of the Southern Highlands of Tanzania. Although characterization of isolates into races using differential cultivars was successful, the study was tedious because of the visual assessments of the disease symptoms that require only experienced personnel. In some cases, verifications of the ALS diseased lesions on leaves were transferred to humid chambers in this case petri dishes for approximately 24 hours to confirm sporulation before assessments were done. In this regard, the use of DNA – based molecular techniques should complement the studies conducted based on differential cultivars especially when dealing with a highly variable pathogen like *P. griseola*.

# References

Aggarwal V.D., M.A. Pastor-Corrales, R. M. Chirwa and R.A. Buruchara. 2004. Andean beans (*Phaseolus vulgaris*) with resistance to the angular leaf spot pathogen (*Phaeoisariopsis griseola)* in Southern and Eastern Africa. Euphytica. 136:201–210. doi: 10.1023/B:EUPH.0000030678.12073.a9

Alvarez-Ayala, G. and H.F. Schwartz. 1979. Preliminary investigations of pathogenic variability expressed by *Isariopsis griseola*. Annual report bean improvement cooperation. Fort Collins 22:86-88

Boher, B. and V. Verdier. 1994. Cassava bacterial blight in Africa: the state of knowledge and implications for designing control strategies. African Crop Science Journal 2: 505-509

Brock, R. D. 1951. Resistance to angular leaf spot among varieties of beans. Journal of the Australian Institute of Agriculture Science. Melbourne 17: 25 – 30.

Buruchara, R. 1983. Determination of pathogenic variation in *Isariopsis griseola* Sacc. and *Pseudomonas syringae* pv. *phaseolicola* (Burk) Young, Dye and Wilkie. PhD Thesis, University of Nairobi, Nairobi.

Busogoro J.P, M. H. Jijakli and P. Lepoivre. 1999. Virulence variation and RAPD polymorphism in African isolates of *Phaeoisariopsis griseola* (Sacc.) Ferr. The causal agent of angular leaf spot of common bean. European Journal of Plant Pathology105: 559–569

Caixeta, E. T., S. A. Borém, S. Fagundes, E. G. Niestche, S. Barros and M.A. Moreira. 2003. Inheritance of angular leaf spot resistance in common bean line BAT 332 and identification of RAPD markers linked to the resistance gene. Euphytica. 1343:297-303.

CIAT (Centro Internacional de Agricultura Tropical.1995. Annual report, Bean Program. 1995. Cali, Columbia.Crous, P. W., M.M. Liebenberg, W. Brawn and J. Groenewald. 2006. Re-evaluating the taxonomic status of *Phaeoisariopsisgriseola*, the causal agent of angular leaf spot of bean. Studies in Mycology 55:163-173

Gepts, P. 1996. Origin and evolution of cultivated Phaseolus species. *In*: B. Pickersgill and J. M. Lock (eds), Advances in Legume Systematics 8: Legumes of Economic Importance, 2nd edition. Royal Botanic Gardens, Kew, London, UK. Page 65-74

Groth, J. V and A. P. Roelfs. 1987. The concept and measurements of phenotypic diversity in *Puccinia graminis* on wheat. Phytopathology 77:1395 – 1399.

Gupta, R., A. Kalia and S. Kapoor. 2007. Bioinoculants: A Step towards Sustainable Agriculture. New India Publishing, New Delhi.

Guzman, P., R.L. Gilbertson, R. Nodari, W.C. Johson, S.R. Temple, D. Mandala, A.B.C. Mkandawire and P. Gupta. 1995. Characterization of variability in the fungus Phaeoisariopsisgriseola suggests co-evolution with the common bean (*Phaseolusvulgaris*). Phytopathology. 85:600 – 607.

Kempeken, F. and U. Kuck. 1998. Transposons in filamentous fungi – facts and perspectives. BioEssays 20 (8): 652 – 659.

Kolmer, J. A. 1990. Selection of virulence in a heterogeneous asexual population of *Puccinia recondite* f.sp. *tritici*. Phytopathology 80: 1377-1381

Kolmer, J. A. 1991. Evolution of distinct populations of *Puccinia recondita*f.sp.*tritici*. Phytopathology. 83:909 – 914.

Kolmer, J. A. 2013. Leaf rust of wheat: Pathogen biology variation and host resistance. Forest 4: 70-84; doi: 10.3390/f4010070

Kolmer, J. A., D. L. Long, E. Kosman and M. E. Hughes. 2003. Physiologic specialization of *Puccinia triticana* on wheat in the United States in 2001. Plant Disease 87: 859 – 866.

Kristler, H. C and V. P. W. Miao. 1992. New modes of genetic changes in filamentous fungi. Annual Reviews of Phytopathology. 30:131 – 152.

Leonard, K. J, Huerta-Espino and J. J. Salmeron. 2000. Virulence of oat grown rust in Mexico.Plant Disease 89:941-948

Leonard, K. J., A. P. Roelfs and D. L. Long. 1992. Diversity of virulence within and among populations of *Puccinia recondita* f. sp. *tritici* in different areas of the United States. Plant Disease 76:500-504.

Leonard, K.J*, Y.* Anikster and J. Manisterski. 2005. Virulence associations in oat crown rust. Phytopathology95*,* 53–61.

Mahuku, G., C. Jara, Teran, H. and S. Beebe. Inheritance of angular leaf spot resistance in selected common bean genotypes. 2003. Centro Internacional de agricultura tropical, Cali, Columbia.

Mahuku, G., C. Montoya, M.A. Henríquez, C. Jara, H. Teran and S. Beebe. 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.

Mahuku, G.S, C. Jara, J. B. Cuasquer and G. Castellanos. 2002. Genetic variability within *Phaeoisariopsis griseola* from Central America and its implications for resistance breeding of common bean. Plant Pathology. 51: 594-604.

Marotti, I., A. Bonetti., M. Minelli, P. Catizone and G. Dinelli. 2007. Characterization of someItalian common bean (*Phaseolus vulgaris* L.) landraces by RAPD semi-random and ISSR molecular markers. Genetic Resources and Crop Evolution. 54: 175 – 188**.**

McKenzie E. and G. Jackson. 1986. The fungi, bacteria and pathogenic algae of Solomon Islands. Strengthening Plant Protection and Root Crops Development in the South Pacific. FAO. RAS/83/001, Field Document 11. Suva, Fiji. Page 282.

Melotto, M and B. N. Kunkel. 2013. Virulence strategies of plant pathogenic bacteria. In E.Rosenberg (Ed). The Prokayotes – Prokaryotic physiology and Biochemistry. Sptnger-Verlag Berlin.

Mwalyego, F. 1987. Yield losses from bean diseases in the Southern Highlands of Tanzania. In Salema, M. P and Minjas A. N. (Eds). Bean Research Vol.2 page 109 – 117.

Namayanja A., R. Buruchara, G. Mahuku, P. Rubaihayo, P. Kimani, S. Mayanja and H. Eyedu. 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.

Neergaard, P. 1977. Seed Pathology. Vol. I and II. The Macmillan Press Ltd. London andAsingstoke. Page 1187.

Ngulu, F. S. 1999. Pathogenic variations within *Phaeosisariopsis griseola* in Tanzania. Annual bean report. Selman Agricultural Research Institute. Arisha – Tanzania.

Nietsche, S., A. Borem, G.A., Carvalho, T.J., Paula-Jr, C.F., Ferreira, E.G., Barros and M.AMoreira.2001.Genetic diversity of *Phaeoisariopsis griseola* in the state of Minas Gerais, Brazil. Euphytica 117:77- 84

Oblessuc, R. P., C. C. Martially, A.F. Chlorate, L. E. A. Cameron, L. L. Bench mol-Reis, M. Melotto. 2015. Common bean reaction to angular leaf spot comprises transcriptional modulation of gene in the ALS 10.1 QTL. Frontiers in Plant Science. 6:152 - 164

Papa, P. and P. Gepts. 2003. Asymmetry of genes flow and differential geographical structures of molecular diversity in wild and domesticated common bean (*Phaseolus vulgaris* L.) from Mesoamerica. Theoretical and Applied Genetics. 106: 239 – 250.

Pastor-Corrales, M. A., M. M. Toyota, A. Molina and S. P. Singh. 1995. Resistance to *Colletotrichum lindemuthianum* isolates from Middle America and Andean South America in different common bean races. Plant Disease79:63–67

Pastor-Corrales, M.A., C. Jar and S. P. Singh. 1998. Pathogenic variation in, sources of, and breeding for resistance to *Phaeoisariopsis griseola* causing angular leaf spot in common bean. Euphytica103:161-171

Sanglard, D. A., C. A. Ribera,  G. Balbo, B. P. Aruba, A. K. M, Barros, E. G and M. A. Moreira. 2013. Characterization of Angular leaf spot resistance gene present in common bean cultivar Ouro Negro. Journal of agricultural Science: 5(2): page 19 - 23

Sartorato, A.  2002. Identification of *Phaeoisariopsis griseola* pathotypes from five states in Brazil. Fitopatologia Brasileira 27:078-081

Schoonhoven, A. van and M. A. Pastor-Corrales. 1987. In standard System for the Evaluation of Bean of bean germplasm. Centro Internacional de agricultura tropical, Cali, Columbia

Silva, G.B., A. S. Prabhu, M.C.C. Filippi, M. G. Trindade, L.G. Araújo and L.Zambolim. 2009. Genetic and phenotypic diversity of *Magnaporthe oryzae* from leaves and panicles of rice in commercial fields in the State of Goiás, Brazil. Tropical Plant Pathology 34(2): 77–86

Stenglein S., L. D. Ploper, O.Vizgarra and P. Balatti. 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

Stenglein S.A and P. A. Balatti.  2006. Genetic diversity of *Phaeoisariopsis griseola* in Argentinaas revealed by pathogenic and molecular markers. Physiological and Molecular Plant Pathology. 68: 158 - 167

Thomazella, C., M.C.Gonçalves-Vidigal, J.B.Vida, P.S.Vidigal-Filho and F.Rimoldi. 2000. Identification of *Colletotrichum lindemuthianum* Races in *Phaseolus vulgaris* L. Annual Report Bean Improvement Cooperative 43:82-83.

Thrall, P. H. and J. J. Burdon. 2003. Evolution of virulence in a plant- host pathogen meta population. Science. 299: 1735 – 1737.

Wagara, I.N., A.W. Mwangombe, J.W. Kimenju and R.A. Buruchara. 2005. Virulence, variability and physiological races of the angular leaf spot pathogen *Phaeoisariopsisgriseola* in Kenya. African Plant Protection. 11: 23-31

Young, R.A and J. D. Kelly**.** 1996a. Characterization of genetic resistance to *Colletotrichum lindemuthianum* in common bean differential cultivars. Plant Disease. 80: 650-654.

Zhang, X., M.W. Blair and S.Wing. 2008. Genetic diversity of Chinese common bean (*Phaseolus vulgaris* L.) Landraces assessed with simple sequence repeats markers. Theoretical and Applied Genetics. 117: 629-640

# 5   Genetic diversity and relationships among isolates of *Pseudocercospora griseola* from the Southern Highlands of Tanzania

**Abstract**

*Pseudocercospora griseola* is a highly variable pathogen that requires both differential cultivars and molecular based techniques to discriminate between isolates and estimate genetic relationship between isolates to allow proper decisions to be made in combating the pathogen. The objective of the study was to determine genetic diversity and relationships among isolates of *Pseudocercospora griseola*, the fungal pathogen of angular leaf spot of common bean from the Southern Highlands of Tanzania. Forty two most aggressive isolates were genotyped using eight polymerase chain reaction (PCR) – based markers which included; random amplified microsatellite (RAMS), REP (repetitive estrogenic palindromic), enterobacterial repetitive intergenic consensus sequence (ERIC), and BOX. All primers used amplified and produced reproducible bands for all the isolates. The selected loci were polymorphic with mean polymorphic information content (PIC) of 0.622 and a mean genetic diversity of 0.635. The percentage of polymorphic loci had a mean of 68.92% and varied between populations. Analysis of molecular variance (AMOVA) allocated 95% of variation within isolates collected from the same district or population. The neighbor joining dendogram constructed using similarity matrix grouped the isolates into three clusters. Cluster I consisted of 17 isolates, while cluster II had 22 isolates. Only three isolates were allocated to cluster III. The markers used did not detect any association between the genetic diversity of the isolates and the locations where the isolates were collected. The presence of unique bands suggested the possibility of using these as molecular markers for further studies on the pathogen. Consequently, the information on the presence of genetic diversity among isolates of *P. griseola* in the Sothern Highlands of Tanzania may be used in designing effective resistance breeding strategies against the pathogen. This is the first study reporting the genetic diversity and differentiation of *P. griseola* isolates from Tanzania

**Key words**: angular leaf spot, common bean, genetic diversity, isolates, polymorphism, PCR-based primers, *Pseudocercospora griseola*.

## 5.1 Introduction

*Pseudocercospora griseola* (Sacc.) Crous and U. Brown is among the most destructive pathogens of common bean globally. The pathogen infects pods and leaves of the host plant causing leaf necrotic that are angular in shapes with sunken brownish spots on pods. The lesions significantly reduce the photosynthetic ability of the host plant followed by premature defoliations of leaves and pods while leaving shriveled seeds for the surviving pods (Correa – Victoria, 1988 and 1989; Shetty, 1992; Nietsche et al., 2001; Stenglein et al., 2003). Yield losses reaching up to 80% have been reported on susceptible bean varieties posing a threat on the livelihoods of the people who depend on the crop (Shao, 1987).

Of the control measures recommended, genetic resistance is considered to be the most economical and environmentally friendly. However, the pathogen is highly variable with the ability to form new and virulent races, posing challenges to resistance breeding (Guzman et al., 1995; Busogoro et al., 1997). Although the sources of variations are not well known, it is speculated that gene flow, asexual reproduction notably parasexuallity and selection pressure are among the determinants that cause substantial genetic changes of the pathogen, that leads to varying virulence patterns on the susceptible host plant (Burdon, 1992; Clay, 1995; Mahuku et al., 2002). For example, in Tanzania, a virulent isolate of *P. griseola* was described in 1967 and reported in 1970 with no significant economic yield losses. As the days went on, the pathogen reached epidemic proportions and in 1987, severe yield losses were reported in the Southern Highlands of Tanzania (Mwalyego, 1987). Although the pathogen has devastated the crop that much, there are no recent studies that documented the genetic diversity and virulence shift of *P. griseola* isolates from the Southern Highlands of Tanzania (SHT). The zone occupies the largest area of Tanzania where beans are produced but severe yield losses due to diseases are common (Hillocks et al., 2006).

*P. griseola* is reported to co-evolve with the Mesoamerican and Andean common bean gene pools and there are isolates/ pathotypes that attack both gene pools while some attack only Mesoamerican and others Andean types of bean varieties (Mahuku et al., 2002). Therefore, differentiating the isolates of *P. griseola* pathogenically and genetically have been an important step for strategic control of the disease through resistance breeding approaches (Nietsche et al., 2001; Crous., 2006; Mahuku et al., 2009; Abadio et al., 2012). The use of differential cultivars

provides valuable data on the degree of virulence of an isolate although the information is limited to phenotypic observations. It has been reported that, even the most virulent race of *P. griseola* so far characterized (race 63:63), differed significantly in the genetic make-up and on the degree of aggressiveness regardless of the place where the pathogen was collected (Pereira et al., 2015). In this regard, additional discriminating tools need to be employed to better understand *P. griseola* pathotypes that exist in an area before designing any control measures. DNA markers are reported to be valuable tools in the characterization and evaluation of genetic diversity within and between species and populations, and have been used extensively in crops like maize, rice and common bean against, fusarium root rot, rice blast and anthracnose caused by *Magnaporthe grisea* (Lavanya, and Gnanamanickam, 2000; Drori et al., 2012; Nagy et al., 2012;).

Several DNA markers have been used to assess diversity of fungal pathogens in various crops. Among them are restricted fragment length polymorphism (RFLP) and Random Amplified Polymorphic DNA (RAPD). These have been used to study genetic variability of fusarium head blight of wheat caused by *Fusarium graminearum* (Hayash et al., 2014), *Michrodochium nivale* of turf grass (Mahuku et al, 1998), red rot of sugar cane (*Colletotrichum falcatum*) (Saksena et al., 2013), brown spot of rice (*Bipolaris oryza*) and *Alternaria brassicae* that infects rapeseed mustard (Kumar et al., 2014). However, RAPD markers require a large number of primers to be tested before successful amplification is achieved (Overmeyer, 1996; Velásquez et al., 2007), while RFLP are known to be less powerful compared to other molecular techniques such as PCR-based techniques (Adachi et al., 1993; Hayden et al., 2003).

With advances in molecular technology, polymerase chain reaction (PCR) – based methods such as random amplified microsatellite (RAM), enterobacterial repetitive intergenic consensus (ERIC), repetitive extragenic palindromic (REP) and BOX – PCR are known to allow fast and accurate detection and quantification of plant pathogens and are widely being used to monitor the exposure of a crop to pathogen inocula, the information which is required in making decisions on disease control strategy (McCartney et al., 2003). Methods including REP, ERIC and BOX are collectively referred to as REP – PCR (Charan et al., 2011). The RAM-PCR and REP-PCR have been indicated to be comparatively cheaper, faster and easier to perform. These markers are reported to be reliable with strong discriminating power and have been used to distinguish closely related isolates of different pathogens including fungi and bacteria (Charan et al., 2011). Further,

the PCR-based techniques are feasible in small laboratories requiring less sophisticated equipment with reduced running costs. The techniques require no previous knowledge on the DNA sequences of the pathogen and do not use radioactive isotopes that are hazardous to human health and the environments (Hantula et al., 1996).

The RAM techniques involve the use of large primers (20 mers) that are bound to a repeated sequence of the genome (Meyer et al., 1993). The genomic DNA of all eukaryotes including fungi contains microsatellites that are known to evolve and mutate more rapidly than any other region of the genome (Kashi et al., 1997; Toth et al., 2000). The use of RAMS in detecting changes within fungal population is highly recommended as RAMS have a greater hybridization compared to the other molecular techniques described herein and have been used to measure genetic diversity in fungi, plants and animals (Zietkiewicz et al., 1994; Hantula et al., 1998; Weir et al. 1998; Gente et al., 2002; Peever et al., 2002; Zhou et al., 2001). On the other hand, REP, ERIC and BOX primers have been used to amplify fungal isolates of *Drechslera avenae* and *Stemphylium solani* associated with leaf blotch and leaf blight diseases of oats and cotton, respectively. Except BOX that constitutes three subunits (BOX A, BOX B and BOX C); other primers have a longer size (20 – 22 mers) and are highly reproducible. BOX – PCR creates distinct fingerprint patterns and does not share any sequence homology with either ERIC or REP – PCR (Olive and Bean, 1999). REP and ERIC – PCR techniques have been extensively used for characterization of phytopathogenic bacteria and for genetic diversity studies in fungi that successfully distinguished closely related isolates with fewer primers compared to the commonly used random amplified polymorphic DNA (Versalovic et al., 1991; Mehta et al., 2002;).

The polymerase chain reaction (PCR) based molecular markers described above have also been used successfully to determine genetic diversity and relatedness of *P. griseola* isolates (Abadio et al., 2012, Ddamulira et al., 2014). The markers elucidated genetic differences among isolates using genetic parameters such as polymorphism information content (PIC), effective alleles (Ne), Nei genetic diversity (H) and Shannon index (Brondani et al., 2000). The objective of the study was to determine genetic diversity and relationships among isolates of *Pseudocercospora griseola*, the fungal pathogen of angular leaf spot of bean from the Southern Highlands of Tanzania.

## 5.2 Materials and Methods

### 5.2.1 Collection of *P. griseola* isolates and mycelium production

Forty most aggressive *P. griseola* isolates were selected from 122 isolates collected previously from field grown bean plants showing ALS symptoms from three bean-growing regions of the southern highland of Tanzania. The degree of aggressiveness that was used to select the isolates based on compatible reactions in a set of 12 ALS differential cultivars. Two control isolates were acquired from Centro Internacional de Agricultura Tropical (CIAT) Uganda for this study (Table 5.1). Each isolate was cultured at the Uyole Agricultural Research Institute by transferring individual conidia from sporulating leaf lesions onto a drop of sterile water placed on top of water agar, that was evenly spread followed by incubating plates at $24^0$C for 24 hours. Single germinating spores of each isolate were collected and placed on V8 juice agar media and plates were incubated at 24°C for 10 days. Mycelium production was done in Erlenmeyer flasks containing 200 ml of liquid V8 media inoculated with three agar plugs from 10-day-old cultures at Mikocheni Agricultural Research Institute (MARI) in Tanzania. These liquid cultures were placed on a shaker (120 rpm), incubated for 14 days at 25°C. Total fungal genomic DNA was extracted from mycelium.

**Table 5.1: The origin of *P. griseola* isolates used in this study**

| Isolate name | Race* | Sampling sites and District | Region/country |
|---|---|---|---|
| 1. Meso 13 | 63/63 | Kilimo - Mbeya urban | Mbeya – Mbeya |
| 2. Meso 31 | 63/55 | Kilimo – Mbeya urban | Mbeya – Mbeya |
| 3. Meso 32 | 63/62 | Kilimo – Mbeya urban | Mbeya – Mbeya |
| 4. Meso 33 | 63/62 | Isangala – Mbeya rural | Mbeya – Mbeya |
| 5. Meso 34 | 63/55 | Isangala – Mbeya rural | Mbeya – Mbeya |
| 6. Meso 35 | 63/62 | Isangala – Mbeya rural | Mbeya – Mbeya |
| 7. Meso 37 | 63/63 | Kilimo – Mbeya urban | Mbeya – Mbeya |
| 8. Meso 38 | 63/55 | Mbimba – Mbozi | Mbeya – Mbeya |
| 9. Meso 40 | 63/62 | Mbimba - Mbozi | Mbeya – Mbeya |
| 10. Meso36 | 63/63 | Kilimo – Mbeya urban | Mbeya – Mbeya |
| 11. Meso39 | 63/55 | Kilimo – Mbeya urban | Mbeya – Mbeya |

| Isolate name | Race* | Sampling sites and District | Region/country |
|---|---|---|---|
| 12. Tky 22 | 63/55 | Utukuyu – Mbeya urban | Mbeya – Mbeya |
| 13. Tky 23 | 63/55 | Ibala – Mbeya urban | Mbeya – Mbeya |
| 14. Tky 24 | 63/55 | Utukuyu – Mbeya urban | Mbeya – Mbeya |
| 15. Tky 25 | 63/55 | Utukuyu – Mbeya urban | Mbeya – Mbeya |
| 16. Tky 26 | 63/62 | Utukuyu – Mbeya urban | Mbeya – Mbeya |
| 17. Tky 27 | 63/62 | Uyole – Mbeya urban | Mbeya – Mbeya |
| 18. Tky 28 | 63/55 | Ibala – Mbeya urban | Mbeya – Mbeya |
| 19. Tky 29 | 63/55 | Utukuyu – Mbeya urban | Mbeya – Mbeya |
| 20. Tky 30 | 63/55 | Utukuyu – Mbeya urban | Mbeya – Mbeya |
| 21.Milundikwa 10 | 63/55 | Milundikwa - Nkasi | Nkasi – Katavi |
| 22. Milundikwa 11 | 63/55 | Milundikwa - Nkasi | Nkasi – Katavi |
| 23. Milundikwa 12 | 63/62 | Milundikwa - Nkasi | Nkasi – Katavi |
| 24. Milundikwa 14 | 63/55 | Milundikwa - Nkasi | Nkasi – Katavi |
| 25. Milundikwa 19 | 63/55 | Milundikwa - Nkasi | Nkasi – Katavi |
| 26. Milundikwa 2 | 63/55 | Milundikwa - Nkasi | Nkasi – Katavi |
| 27. Milundikwa 20 | 63/55 | Milundikwa - Nkasi | Nkasi – Katavi |
| 28. Milundikwa 21 | 65/55 | Milundikwa- Nkasi | Nkasi – Katavi |
| 29. Milundikwa 3 | 63/62 | Milundikwa - Nkasi | Nkasi – Katavi |
| 30. Milundikwa 4 | 63/62 | Milundikwa - Nkasi | Nkasi – Katavi |
| 31. Milundikwa 6 | 63/55 | Milundikwa - Nkasi | Nkasi – Katavi |
| 32. Milundikwa 8 | 63/55 | Milundikwa - Nkasi | Nkasi – Katavi |
| 33. Milundikwa 16 | 63/62 | Milundikwa- Nkasi | Nkasi- Katavi |
| 34. Milundikwa 5 | 63/62 | Milundikwa- Nkasi | Nksai – Katavi |
| 35. Milundikwa 7 | 63/62 | Milundikwa - Nkasi | Nksai – Katavi |
| 36. Milundikwa 9 | 63/55 | Milundikwa – Nkasi | Nksai – Katavi |
| 37. Milundikwa 1 | 63/55 | Kantawa- Sumbawanga | Sumbawanga -RK |
| 38. Milundikwa 17 | 63/62 | Kantawa - Sumbawanga | Sumbawanga- RK |
| 39. Milundikwa 18 | 63/55 | Malonje - Sumbawanga | Sumbawanga-RK |
| 40. Milundikwa 15 | 63/62 | Malonje - Sumbawanga | Sumbawanga-RK |

| Isolate name | Race* | Sampling sites and District | Region/country |
|---|---|---|---|
| 41.Andean kax3(+) | 63/62 | Kawanda  ARI | Kampala - Uganda |
| 42 Meso 2A (+ve) | 63/62 | Kawanda  ARI | Kampala - Uganda |

RK = Rukwa region, Race* = races characterized during the 2012/2013 and 2013/2014 season; ARI = Agricultural Research Institute

## 5.2.2. DNA extraction

The fungal DNA extraction was done as described by Liu et al. (2000) and Mehta et al. (2002). Mycelium was harvested through filtration with a cheese-cloth and transferred into 1·5 ml eppendorf tubes. Eight hundred microliters of pre-warmed (65°C) extraction buffer (0·5 M NaCl, 10 mM Tris-HCl (pH 7·5), 10 mM EDTA, 0·2% mercapto-ethanol and 50 ng RNase A) was added to each tube and briefly mixed by inversion. Then 100 µl of 20% sodium dodecyl sulfate (SDS) was added to each tube. The tubes were incubated at 65°C for 30 min to lyse the cells and the supernatant harvested by centrifugation at 13,000 rpm for 20 min. Four hundred microliters of phenol: chloroform (1:1) was added to each tube, mixed carefully by inversion and the tubes incubated in ice for 7 min. The mixture was centrifuged at 13,000 rpm for 2 min and the aqueous phase containing the total nucleic acids was collected into new eppendorf tubes. Four hundred microliters of chloroform/iso-amyl alcohol (24:1) was added to each tube and mixed well by inversion to remove traces of phenol. The mixture was centrifuged for 2 min and the supernatant transferred into new eppendorf tubes. The total nucleic acids were recovered by the addition of two volumes of cold 95% ethanol and the tubes mixed again by inversion. Then 1/10 volume of 3 M sodium acetate was added to the tubes and the mixture mixed well. The tubes were placed at -20ºC overnight and the genomic DNA harvested by centrifugation at 13,000 rpm for 15 min. The DNA pellets were washed with 500 µl of 70% ethanol and pelleted at 13,000 rpm for 2 min. The genomic DNA pellet was air-dried, re suspended in 50 µl of sterile double distilled water and kept at 4ºC. The integrity and intactness of DNA was checked on 1·5% agarose gel, and the concentration and purity determined by the optical density method using a NanoDrop spectrophotometer 1000 at 230 nm, 260 nm and 280 nm.

### 5.2.2 PCR amplification and gel electrophoresis

In this study, eight random amplified microsatellite (RAMS), REP (repetitive extragenic palindromic) – PCR, enterobacterial repetitive intergenic consensus sequence (ERIC), and BOX – PCR markers were used (Table 5.2) to investigate the genetic diversity and relationship of P. *griseola* isolates. The RAMS and REP – PCR markers were used with DNA primers complimentary to naturally occuring, highly conserved DNA sequence present in multiple copies in the genome of fungi and bacteria (Charan et al., 2011). These methods have consistently directed the amplification of distinct polymorphic DNA fragments from *P. griseola* (Ddamulira et al., 2014).

The PCR was performed using Bioneer Pre mix PCR kit containing 25 - 50 ng genomic DNA, 0.25 μM of each primer and the volume adjusted to 10 μl with sterile double distilled water. Negative controls with no DNA were included to test for the presence of contaminations in the reagents and reaction mixtures. DNA samples from the isolates were loaded at 10 μl for each lane in the gel. The PCR programme was as follows: 5 min at 94°C; 35 cycles of 20 seconds at 94°C, 40 seconds at the optimized primer annealing temperature (Table 2), 8 min at 65°C, a final extension at 65°C for 16 min to allow complete extension of all the PCR products and the reaction was held at 10°C. The PCR reaction was performed in a Techne Prime thermal cycler (Bibby Scientific Limited, UK). The PCR products were size fractionated on 1·5% agarose gels, stained with ethidium bromide (0.5 μg/ml), in 0.5X TBE buffer at a constant voltage of 100 V for 3·5 hr. The bands were visualized on a UV transilluminator and digitized using the BioDoc-It@ 2010 Imaging System (Cambridge, UK) photo documentation system. To estimate the size of the amplified DNA fragments, a 1Kb+DNA ladder was used as molecular size marker, ranging in size from 12 kb down to 100 bp with two high intensity reference bands of 4 kb and 500 bp.

### 5.2.3 Data collection and analysis

The RAMS, ERIC, REPS and BOX – PCR amplicons were manually assessed and scored as present (1) or absent (0) and only reproducible bands were considered for analysis. Both polymorphic and monomorphic bands were incorporated into the analysis. The informativeness of the selected bands was calculated using Shannon's information index and polymorphic information content (PIC) using PowerMaker v.3.25 software based on the following formula:

$$PIC = 1 - \left( \sum_{i=1}^{n} p_i^2 \right) - \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2p_i^2 p_j^2$$

Where $n$ is the total number of fragments (bands) for primers and $p_i$ and $p_j$ are the frequencies of the *ith* and *jth* fragment in the isolates investigated (Botstein et al., 1980). A PIC value of 1 indicated that the marker can differentiate each line, and 0 indicated a monomorphic marker.

The genetic diversity of each group of isolates was determined using Shannon's information index using Genetic Analysis in Excel (GenAlex 6.5b3). The genetic distance and between isolates was calculated using Nei's genetic distance (Nei, 1973) in GenAIex 6.5b3 (Peakall et al., 2006). A dendogram was constructed using the unweighted pair-group method with arithmetic average (UPGMA) clustering algorithm in DARwin v.5.0.155 software (Perrier et al., 2006). Analysis of molecular variance (AMOVA) was performed using GenAlex 6.5b3 to partition the magnitude of variation attributable to differences within and among populations.

**Table 5.2: Molecular markers used for the PCR – based methods with sequences, GC content** and annealing temperatures to amplify *P. griseola* isolates collected from southern highlands of Tanzania

| Molecular marker sequence | Sequence (5' to 3') | $T_a$ (°C) | GC content% | Number of nucleotides |
|---|---|---|---|---|
| **Box A1R** | CTACGGCAAGGCGACGCTGACG | 50 | 68.2 | 22 |
| **REP 1R** | IIIICGICGICATCIGGC | 50 | 81.8 | 11 |
| **REP 2** | ICGICTTATCIGGCCTAC | 50 | 60.0 | 15 |
| **ERIC 2** | AAGTAAGTGACTGGGGGTGAGC | 50 | 54.5 | 22 |
| **ERIC 1R** | ATGTAAGCTCCTGGGGAT | 50 | 50.0 | 18 |
| **RAMS 2** | TGCCGAGCTG | 40 | 70.0 | 10 |
| **RAMS 5** | GGGTAACGCC | 40 | 70.0 | 10 |
| **RAMS 6** | GTGATCGCAG | 40 | 60.0 | 10 |

Markers obtained from Bioneer Inc, Korea

REP= repetitive extragenic palindromic; ERIC=; enterobacterial repetitive intergenic consensus;

RAMS=; random amplified microsatellite Ta=annealing temperature; GC base pair content

## 5.3 Results

### 5.3.1 Genetic analysis of *P. griseola* isolates

All the eight markers used produced clear and reproducible bands of the *P. griseola* DNA samples with variability in discrimination efficiency (Figs. 5.1 and 5.2, Table 5.3). Fifty eight (58) total bands were scored by adding the maximum number of bands per isolate. A maximum of 10, 9, 8, 7, 5, 5, 3, 9 and 5 bands were scored in the markers RAM 2, RAM 5, RAM 6, BOX AIR, ERIC 2, ERIC AIR, REP 2 and REP IR, respectively. Further, three unique bands that were found only in specific isolates but absent in others were identified, two from Mbeya (on isolates 10, 20) and one from Nkasi, isolates 31 (Fig.5.2). The sizes of the bands ranged from 1.5 to 12 Kb. Out of the 58, 37 bands were polymorphic. The highest percentages (100%) of polymorphic bands were scored from the markers BOXAIR, whereas the lowest (44%) was recorded with the marker REP 2 (Table 5.3). The number of bands and percentage polymorphism tended to be higher in isolates collected from Mbeya and Nkasi districts.

Differences in band intensities were observed between the isolates and markers. Markers RAM 2 and RAM 6 had a higher number of bands compared to the rest of the markers used (Fig. 5.2).

RAM 2 RAM5
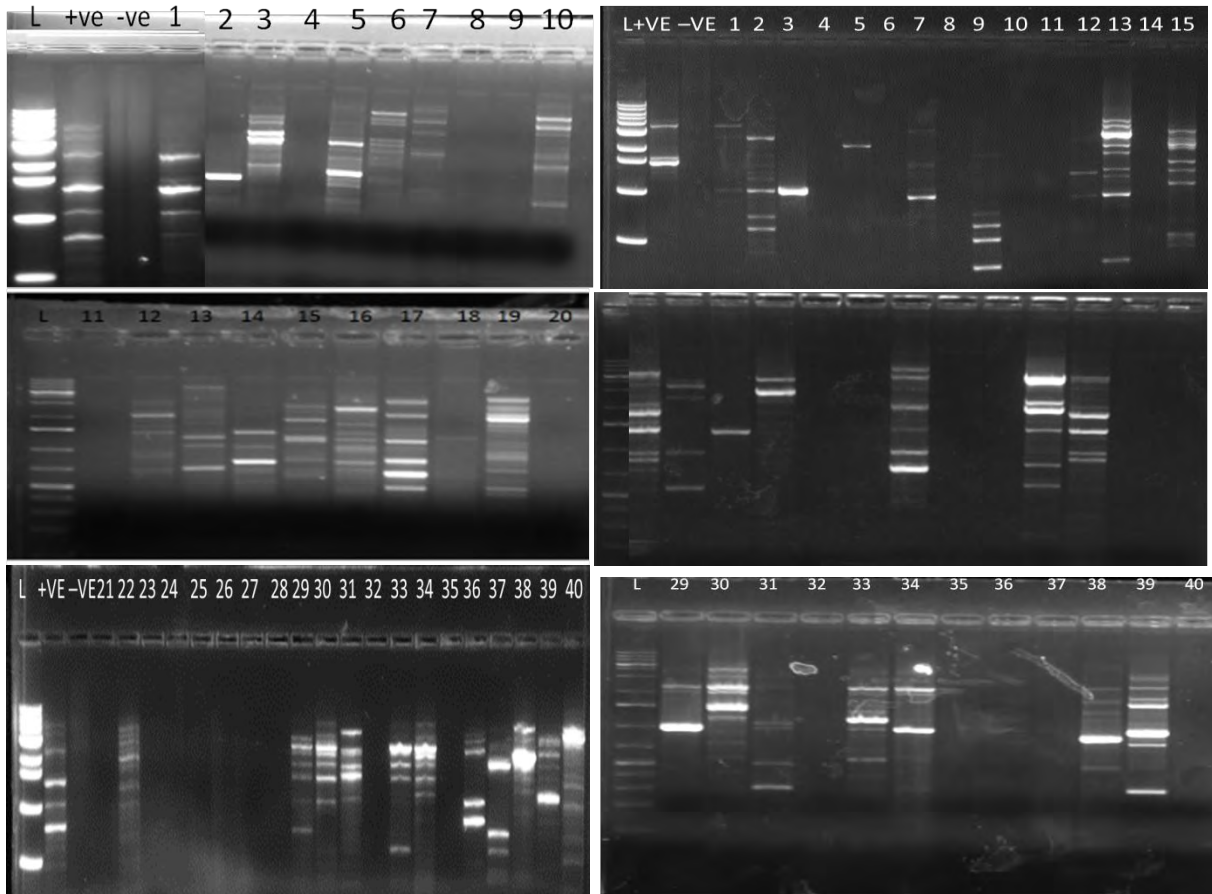


**Figure 5.1: PCR band patterns for *Pseudocercospora griseola* isolates from southern highlands of Tanzania. L=1 kb DNA ladder +ve=Andean kax3 and Meso2A; -ve=double distilled sterilized water, 1 to 40 represent lanes loaded with DNA from single isolates of the Southern Highlands**

RAM 6                                              BOX AIR
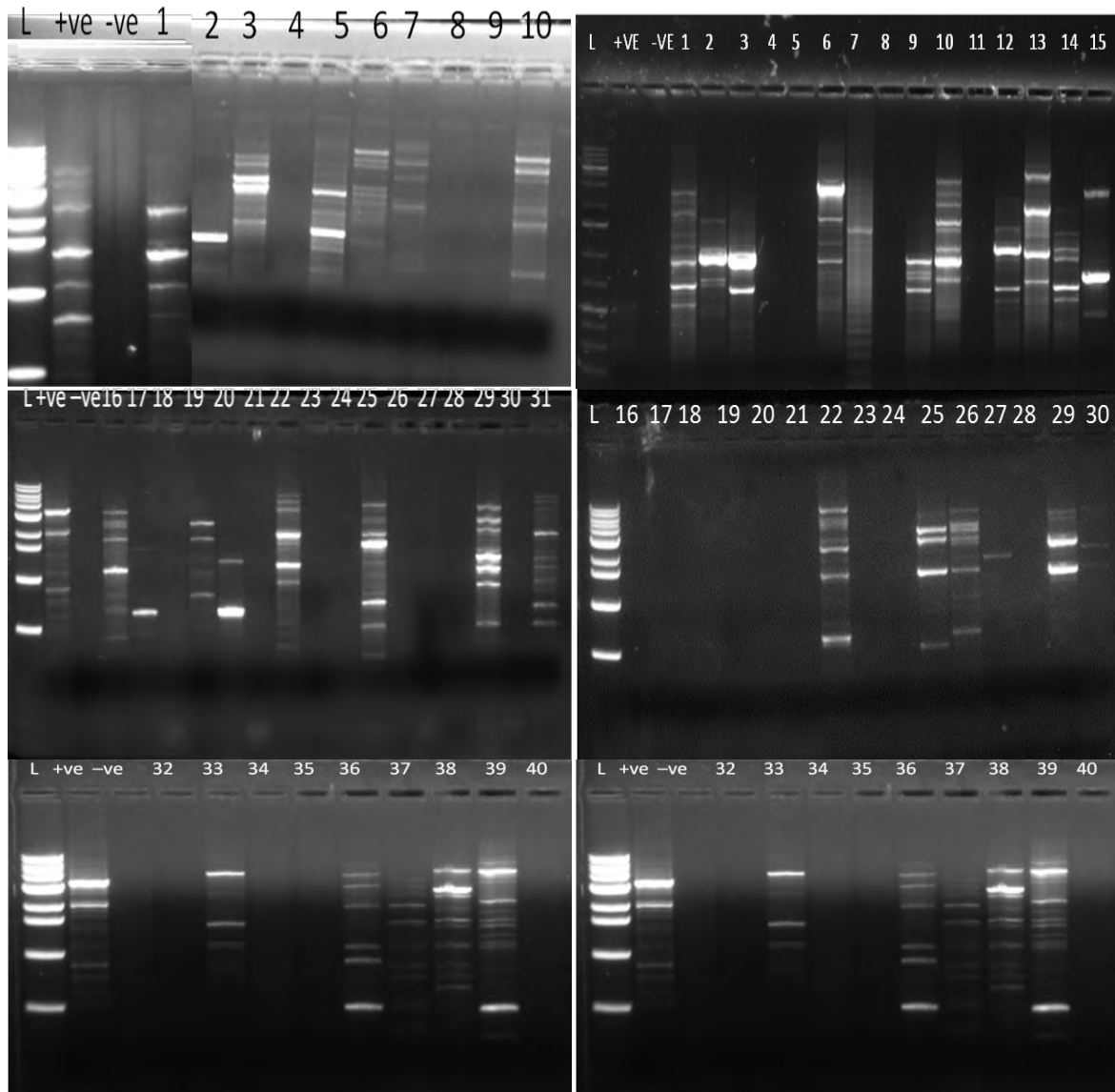


**Figure 5.2: PCR band patterns for *Pseudocercospora griseola* isolates from southern highlands of Tanzania using the most efficient primers; RAMS 2, 5, 6 and BoxA1R. Positive lanes (+ve) CIAT isolate Meso2A from CIAT**

**Table 5.3: Marker codes with their respective monomorphic and polymorphic bands reproduced in *P. griseola* isolates of the SHT**

| Marker code | Sequence : 5' to 3' | Unique bands | Monomorphic band | Polymorphic bands | Total bands | % polymorphism |
|---|---|---|---|---|---|---|
| RAM2 | TGCCGAGCTG | 3 | 1 | 0-6 | 10 | 90.0% |
| RAM5 | GGGTAACGCC | 1 | 3 | 0-7 | 11 | 72.7% |
| RAM6 | GTGATCGCAG | 1 | 2 | 0-6 | 9 | 77.7% |
| BOX AIR | CTACGGCAAGGCGACGCTGACG | 1 | - | 0-5 | 6 | 100.0% |
| ERIC 2 | AAGTAAGTGACTGGGGGTGAGC | 0 | 1 | 0-4 | 5 | 80.0% |
| ERIC IR | ATGTAAGCTCCTGGGGAT | 0 | 1 | 0-2 | 3 | 66.7% |
| REP 2 | ICGICTTATCIGGCCTAC | 0 | 5 | 0-4 | 9 | 44.4% |
| REP IR | IIIICGICGICATCIGGC | 0 | 2 | 0-3 | 5 | 60.0% |
| Total | | 6 | 15 | 37 | 58 | |

REP=; repetitive extragenic palindromic ERIC= enterobacterial repetitive intergenic consensus RAMS= random amplified microsatellite
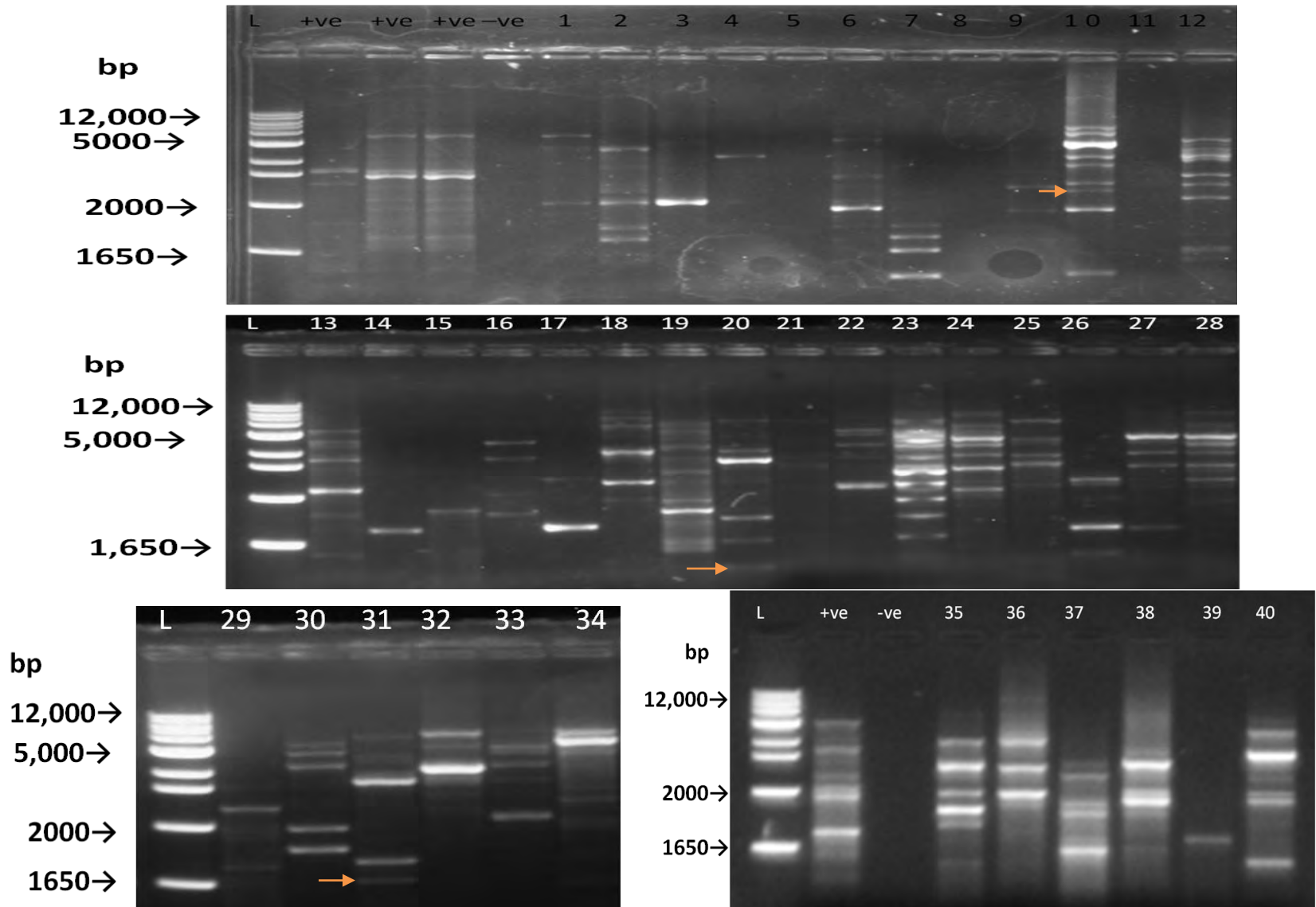
**Figure 5.3: Agar gel patterns of PCR products amplified with all the markers showing zoomorphic, polymorphic and unique bands. +ve are CIAT isolates while L = DNA plus ladder; unique bands**

Overall, the selected markers were polymorphic with a mean PIC of 0·622 and mean genetic diversity of 0·635 (Table 5.4). The percentage of polymorphic loci had a mean of 68·92% and varied between isolates. The most polymorphic locus with 91·89% was observed for isolates collected from Mbeya followed by from Nkasi (89·19%). The percentage of polymorphic loci for isolates collected from Sumbawanga had a mean of 56·76% and the lowest (37·84%) polymorphism was recorded for isolates from CIAT/Uganda.

Analysis of molecular variance (AMOVA) attributed 95% of the variation to be within isolates from the same district (Table 5.5). The neighbor joining dendogram using Unweighed Pair Group Method (UPGMA) based on Jaccard's similarity matrix grouped the isolates into three clusters according to similarity coefficients. Cluster I consisted of 17 isolates including the Andean KaX3 and Meso 2A from CIAT/Uganda that were included as checks. Cluster II Comprised 22 of the isolates, while Cluster III had only three isolates (Fig. 5.4).

**Table 5.4: Genetic parameters of 8 RAM and REP-PCR primers used for the analysis of 40 *P. griseola* isolates collected from Tanzanian and 2 from CIAT/Uganda**

| Marker | Gene diversity | PIC |
|---|---|---|
| BOX A1R | 0·9048 | 0·8974 |
| REP 1R | 0·4376 | 0·4183 |
| REP 2 | 0·6168 | 0·5959 |
| ERIC 2 | 0.5612 | 0·5397 |
| ERIC 1R | 0·1349 | 0·1300 |
| RAMS 2 | 0·8141 | 0·8011 |
| RAMS 5 | 0.8583 | 0·8507 |
| RAMS 6 | 0·7528 | 0·7432 |
| Mean | 0·6351 | 0·6220 |

**Table 5.5: Analysis of molecular variance (AMOVA) based on RAM and REP PCR based techniques**

| Source | Degrees of Freedom | Sum of Squares | Estimated Variance | % variation |
|---|---|---|---|---|
| Among Populations | 3 | 24·048 | 0·300 | 5% |
| Within Populations | 38 | 206·238 | 5·427 | 95% |
| Total | 41 | 230·286 | 5·727 | 100% |
| | **Value** | | | **Probability** |
| **PhiPT** | 0·052 | | | 0·010 |
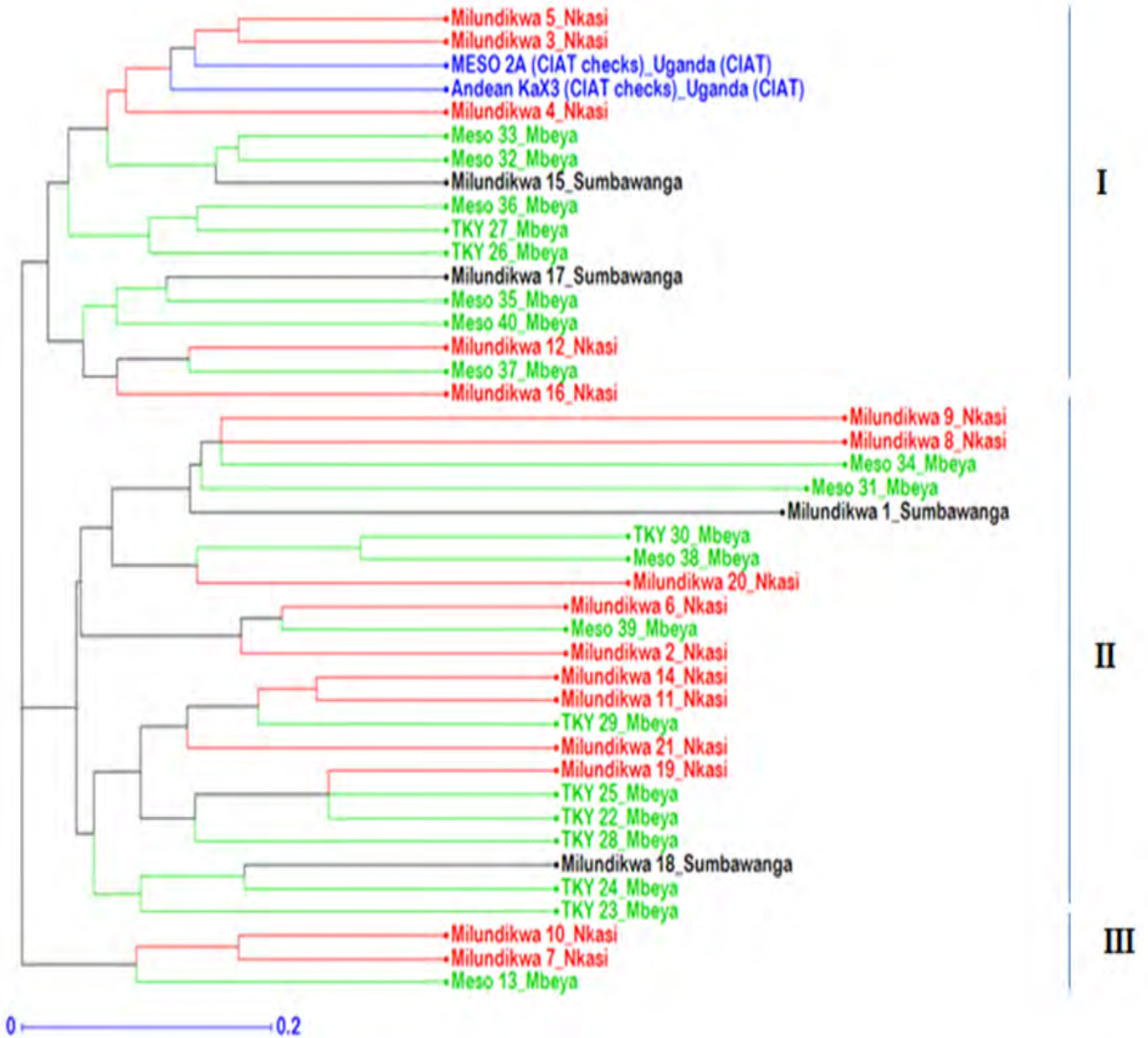
**Figure 5.4: UPGMA Dendogram based on RAM and REP-PCR markers showing the genetic relationship of 42 *P. griseola* isolates from southern highlands of Tanzania and CIAT/ Uganda.**

## 5.4 Discussion

The level of genetic differentiations of *P. griseola* isolates from the SHT used in this study was moderate. This was expected due to the varied virulence nature of the pathogen that was reported earlier. Genetic variations in some other fungal pathogens such as *Puccinia graminis* that cause stem rust in wheat and common smut of corn caused by *Ustilago maydis* have been associated with sexual reproduction of the pathogens (Pataky and Snetselaar, 2006; Zhao et al., 2013). However, this source of variation has not been described in *P. griseola*. The known biology of *P. griseola* indicates that asexual reproduction is a major mode of reproduction for this fungus (Hastie, 1981; Liebenberg and Pretorious, 1997; Busogoro et al., 1999; Mahuku et al., 2002 ;). It is well documented that parasexuality, somatic mutation and gene flow are among the mechanisms that generate variations in fungal pathogen and have been described *in Alternaria alternata* that cause stem canker of tomato (Morris et al., 2000; Peever et al., 2002). Given that *P. griseola* co-evolved with the Mesoamerican and Andean gene pools of common bean, the results obtained in this study may suggests the presence of both Mesoamerican and Andean virulence factors in a number of isolates studied. This hypothesis may be supported by several studies conducted on bean rust (*Uromyces appendiculatus*) and on bean anthracnose pathogen (*Colletotrichum lindemuthianum)*, where pathotypes with virulence factors to both host gene pools were described (Kelly, 1995; Sadlin et al., 1999). In the SHT common bean is grown as mixtures of seed with different sizes that are of Andean and Mesoamerican gene pools. This provides high chances of some levels of gene introgression between the two gene pools in which *P. griseola* might have evolved to infect and adapt to the new genotypes formed.

In this study, monomorphic, polymorphic and unique bands were obtained. Under normal circumstances, each band is considered as biallelic locus. Monomorphic bands were present in all isolates evaluated suggesting that they cannot be used to study the genetic diversity of the isolates. On the other hand, polymorphic bands reveal differences that can be used to examine and establish differences within and between isolates (Hardys et al., 1992). In this study, polymorphic bands were observed in some isolates and absent in others. Polymorphisms results from specific differences in DNA sequence of a fungal genome have been reported to be associated with genetic evolution (Schardl and Craven, 2003). The PCR – based markers used in this study detected a number of polymorphic bands per marker that ranged from 2 – 6 with a

mean polymorphism of 60.8%. The lowest polymorphism value (66.7%) was obtained with ERIC-IR marker that is known to amplify random regions in the genome of *P. griseola.* Since the PCR was performed at a restrictive annealing temperature of $50^0$C, mispriming might have occurred resulting into fewer bands. The marker BOX AIR was superior to all other primers in generating polymorphic bands, and it is known to have the ability to create distinct band patterns (Yang and Yen, 2012). The BOX AIR has been used successfully in cases where a detailed band pattern of the DNA is needed (Das et al., 2014). Variable efficiencies of different markers for detecting DNA polymorphism in *P. griseola* have been reported by Guzman et al. (1995); Sharma (2003), Busogoro et al. (1999), and Ddamulira et al. (2014). In general, the high levels of polymorphism observed for the described markers supports their application in *P. griseola* genetic studies that may supplement virulence and aggressiveness studies that are often conducted using differential cultivars. Unique bands were observed in some of the isolates analyzed and they were specific only to those particular isolates. The presence of unique bands reflects genetic differences and these can be used as positive markers for isolate identification and discrimination.

Differences in band intensity were noticed in the present study. The bands produced by the PCR – RAM and REP differed in the degree of intensity presumably due to hyperploids which is associated with gene dosage. Differences in band intensity as a result of gene dosage have been reported in *Bremia lactucae* (lettuce) where a band for two alleles in a locus differed in intensity with one band being approximately three times more intense like the other (Hubert and Michelmore, 1988).

The polymorphic information content (PIC) is another measure of genetic diversity within isolates. The PIC describes genotypic variations within the genome of the pathogen that may be caused by a change in a single base pair through duplication or deletions. The PIC value that is almost zero indicates no allelic variations and it can reach a maximum of 1 if a genotype has new allele (Botstein et al., 1980). Majority of markers showed PIC values that were above average while only one marker, ERIC IR indicated values that were low. A PIC value of $> 0.5$ is considered of high diversity; $0.2 – 0.5$ intermediate diversity and PIC $\leq 0.25$ indicates low diversity (Botstein et al., 1980). High PIC value is also associated with high degree of heterozygosity therefore high degree of polymorphism (Zimmer and Roalson, 2005). The high

values of PIC observed in this study could be attributed to the diverse nature of the pathogen or the high informative of the primers used. The PIC index has been used extensively in many genetic diversity studies of bacteria and fungi (Takikonda et al., 2009; Talebi et al., 2010). Among the markers used, BOX IR had the highest PIC values that were associated with genetic diversity while ERIC IR may not be useful in determining genetic variability within *P. griseola*.

The genotypic data for all the PCR- RAM and PCR – REP markers were used to calculate the distance matrix and similarity coefficient among the isolates of the same species through unweighed pair group method with arithmetic mean (UPGMA). The UPGMA classified the forty two isolates evaluated into three groups that clustered according to the genetic similarities but not according to the place of origin. The possible explanation of the results is that, there are free movements of common bean seed from one place to another allowing free movement of *P. griseola* infected seed. Therefore the possibility of moving pathogens from one area to the other is always there. As stated earlier there is a possibility of parasexuallity since more than one different isolates may co–exist in one plant increasing chances of mitotic chromosome exchange between isolates.

The results of this study are valuable in designing efficient resistance breeding programme against angular leaf spot (ALS) disease of bean. The variations in banding patterns observed between the most aggressive isolates indicated the presence of isolates in the same group that differ in the degree of aggressiveness. Considering that *P. griseola* is highly variable and that a cultivar may be resistant to a certain types of isolates and yet be susceptible to the other, designing resistance breeding programmes that target either gene pyramiding or durable resistance is important. Once the type of resistance to be introduced into the susceptible cultivars is known based on the information on genetic diversity of the pathogen, it is easier to solicit germplasm that possess resistance genes that will act against the pathogen that exists in a given area.

## 5.5  Conclusions

The present study showed that genetic variation exists in *P. griseola* isolates of the SHT and PCR – RAM and REP markers may be used as a quick and reliable tool in discriminating against the fungal isolates. The presence of unique bands that were specific to certain isolates suggests

the possibilities of using these as molecular markers for further studies on the pathogen. Considering the variability of the *P. griseola*, continuous disease survey and monitoring of the pathogen is essential for detecting new races and map the distribution. In this regard, specific resistance genes may be thought of and appropriate breeding method be employed to successfully develop resistance cultivars. *P. griseola* is one of the most destructive pathogen to the bean crop in the SHT and knowledge of the mechanism by which genetic variations arises in this asexually reproducing pathogen may allow the prediction of new isolates and types of genes required for the deployment in breeding for resistance. The isolates used in this study were previously characterized using differential cultivars that showed high levels of aggressiveness. However, the use of molecular techniques complimented the information that the isolates were not of the same type. Since the angular leaf spot disease is sporadic in the Southern Highlands of Tanzania and that the crop is grown by resource poor farmers with limited access to crop protection chemicals resistance cultivars against the pathogen is the best option in managing ALS.

# References

Abadio, K. R., S.S. Lima, M. F. Santana, T. M. T. Siloam, A. Saturator, E.S.G. Mizubuti and M.V. de Queiroz. 2012. Genetic diversity analysis of isolates of the fungal bean pathogen *Pseudocercospora griseola* from central and southern Brazil. Genetics and Molecular Research. 11(2): 1272–1279.

Adachi, Y., H. Watanabe, K. Tababe, N. Doke, S. Nishimura and T. Tsuge. 1993. Nuclear ribosomal DNA as a probe for genetic variability in the Japanese pear pathotype of *Alternaria alternate*. Applied and Environmental Microbiology 59: 3197-3205

Botstein, D., M, Skolnick and R. W. Davis. 1980. Construction of a genetic linkage map in manusing restriction fragment length polymorphisms. American Journal of Human Genetics. 32:314-331

Brondani, C., R. Pereira, V. Brondani, L. R. Garrido and M. E. Ferreira. 2000. Development of microsatellite markers for the genetic analysis of *Magnaporthe grisea.* Genetics and Molecular Biology, 23(4): 753-762

Burdon, J. J. 1992. Genetic variation in pathogen populations and its implication for adaptationto host resistance in: *Durability of Disease Resistance*. Th. Jacobs and J. E. Parlevliet, eds. Kluwer Academic Publishers, Amsterdam: Pages 41-56

Busogoro, J. P., M.H. Jijakli and P. Lepoivre. 1999. Virulence variation and RAPDpolymorphism in African isolates of *Phaeoisariopsis griseola* (Sacc.) Ferr., the causal agent of angular leaf spot of common bean. European Journal of Plant Pathology. 105:559-569.

Casimiro, S., M. Moura, L. Ze-Ze, R. Tenreiro and A.A. Monteiro. 2004. Internal transcribed spacer 2 amplicons as a molecular marker for identification of *Peronospora parasitica* (crucifer downy mildew). Journal of Applied Microbiology. 96: 579–587

Charan, A. R., V. Prathap Reddy, P. Narayana Reddy, S. S. Reddy and S. Sivaramakrishnan.2011. Assessment of Genetic Diversity in Pseudomonas fluorescens using PCR-based Methods. Bioremediation, Biodiversity and Bioavailability. 5(1):10-16.

Clay, K. 1995. Correlates of pathogen species richness in the grass family. Canadian Journal of Botany 73:42-49.

Correa –Victoria, F.J., M.A. Pastor-Corrales, A. W. Saettler. 1989. Angular leaf spot. In: Schwartz H.F, Pastor-Corrales M.A, editors. *Bean production problems in the tropics*. Centro Internacional de Agricultura Tropical (CIAT), Cali, Columbia. Page 59-75.

Correa-Victoria, F. J. 1988. Pathogenic variation, production of toxin metabolites and isozyme analysis in *Phaeoisariopsis griseola* (Sacc.) Ferr. Ph.D. thesis. Michigan State University, East Lansing

Crous, P. W. 2006. Taxonomy and phylogeny of the genus Mycosphaerella and its anamorphs. Fungal Diversity. 38: 1- 24

Das, S., H. R. Dash, N. Mwangwani, J. Chakraborty and S. Kumari. 2014. Understanding molecular identification and polyphasic taxonomic approaches for genetic relatedness and phylogenetic relationships of microorganisms. Microbiological Methods. 103: 80 - 100

Ddamulira G., C. Mukankusi., M. Ochwo-SSemakula., R. Edema, P. SSeruwagi and P. Gepts.2014. Distribution and variability of *Pseudocercospora griseola* in Uganda. Journal of Agricultural Science 6(6): 16 – 29

Drori, R., A. Sharon, D. Goldberg, O. Rabinovitz, M. Levy and O. Degani. 2013. Molecular diagnosis for *Harpophora maydis*, the cause of maize late wilt in Israel. Phytopathologia Mediterranea. 52: 16−29

Gente, S., N. Desmasures, C. Jacopi, G. Plessis, M. Beliard, J. P. Anoff, M. Gueguea. 2002. Intra- species chromosome length polymorphism in Geotrichum candidum revealed by pulsed field gel electrophoresis. International Journal of Food Microbiology. 76(1-2): 127 – 134.

Guzmán, P., R. L. Gilbertson, R. Nodari, W. C. Johnson, S. R. Temple, D. Mandala, A.B.C. Hadrys, H., M. Balick and B. Schierwater. 1992. Application of random amplified polymorphic DNA (RAPD) in molecular ecology. Molecular Ecology.1:55-63.

Hantula, J., E. M. Niemi, J. Kaitera, R. Jalkanen, and T. Kurkela 1998. Genetic variation of the resin top fungus in Finland as determined by random amplified microsatellites (RAMS). European Journal of Forest Pathology 28: 361-372

Hantula, J., M. Dusabenyagasani, R. C. Hamelin.1996. Random amplified microsatellites (RAMS) — novel method for characterizing genetic variation in fungi. European Journal of Forest Pathology, 26: 159–166.

Hastie, A.C. The genetics of conidial fungi. 1981. In: Cole, G.T. & Kendrick, B. (eds). Biology of conidial fungi. New York. Academic Press. page. 511-547.

Hayashi Y., T. Kozawa, D. Aiuchi, M. Koike, S. Akino and N. Kondo. 2014. Population genetic structure of *Microdochium majus* And Microdochium nivale associated with Fusarium head blight of wheat in Hokkaido, Japan. European Journal of Plant Pathology.140:787– 795

Hayden HL, J. Carlier, E. A. B. Aitken. 2003. Genetic structure of *Mycosphaerella fijiensis* populations from Australia, Papua New Guinea and the Pacific Islands. Plant Pathology 52, 703–712.

Hillocks, R. J., C.S. Madata, R. Chirwa, E.M. Minja and S. Msolla. 2006. Phaseolus bean improvement in Tanzania, 1959–2005. Euphytica: DOI: 10.1007/s10681-006-9112-9

Hulbert, S. H and R. W. Michelmore. 1988. DNA restriction fragment length polymorphism and somatic variation in the lettuce downy mildew fungus, bremia lactucae. Molecular plant – microbe interactions 1(1): 17 - 24

Kashi, Y., D. King and S. Soller. 1997. Simple sequence repeats as a source of quantitative genetic variation. Trends Genetics. 13: 74 – 78.

Kelly, J.D. 1995. Use of random-amplified polymorphic DNA markers inbreeding for major resistance to plant pathogens. HortScience 30:461 -465

Kumar, D., N. Maurya, Y. Bharati, A. Kumar, K. Kumar, K. Srivastava, G. Chand, C.Kushwaha, S. K. Singh, R. K. Mishra and A.Kumar. 2014. Alternaria blight of oilseed brassicas: A comprehensive review. African Journal of Microbiology. 8(30): 2816-2829, DOI: 10.5897/AJMR2013.6434

Lavanya, B and S. S. Gnanamanickam.2000. Molecular tools for characterization of rice blastpathogen Magnaporthe Grisea) population and molecular marker -assisted breeding for disease resistance. Current Science. 78: 3 - 10

Liebenberg, M. M and Z. A. Pretorius. 1997. A review of angular leaf spot of common bean (*Phaseolus vulgaris* L.). African Plant Protection 3: 81–106.

Liu, D., S. Coloe, R. Baird and J. Pederson. 2000. Rapid mini preparation of fungal DNA for PCR. Journal of Clinical Microbiology. 38: 471 – 479.

Mahuku, G. S., A. M. Iglesias and C. Jara, C. 2009. Genetics of angular leaf spot resistance inthe Andean common bean accession G5686 and identification of markers linked to the resistance genes. Euphytica, 167: 381-396.

Mahuku, G. S., C. Jara and J. B. Cuasquer. 2002. Genetic variability within *Phaeoisariopsisgriseola* from Central America and its implication for resistance breeding. Plant Pathology 51(5): 594 - 604

Mahuku. G. S., T. Hsiang and L. Yang. 1998. Genetic diversity of *Microdochium nivale* isolates from turfgrass. Mycology Research. 102(5): 559- 567

McCartney, H. A., S.J. Foster, B. A. Fraaije and E. Ward. 2003. Molecular diagnostics for fungal pathogen. Pest Management Science 59(2):129 -142

Meyer, W., T. G. Mitchell, E. Z. Freedman and R.Vilgalys. 1993. Hybridization probes for conventional DNA fingerprinting used as single primers in the polymerase chain reaction to distinguish strains of Cryptococcus neoformans. Journal of Clinical Microbiology 31: 2274-2280

Miklas P.N, J.D. Kelly, S.E. Beebe and M.W. Blair. 2006. Common bean breeding for resistance against biotic and abiotic stresses: from classical to MAS breeding. Euphytica 147:106-131

Morris, P. F., M. S. Connolly and D. A. S. Clair. 2000. Genetic diversity of *Alternaria alternate* isolated from tomato in California assessed using RAPDs. Mycological Research 104: 286-292.

Mwalyego, F. 1987. Yield losses from bean diseases in the Southern Highlands of Tanzania. *In* Salema, M. P and Mijas A. N. (Eds). Bean Research Vol.2 page 109 – 117.

Nei, M. 1973. Analysis of gene diversity in subdivided populations. Proc. Natl. Acad. Sci. USA *70*, 3321–3323.

Nietsche S., A. Borem, G.A. Carvalho, T. J. Paula Junior, C, Fortes-Ferreira and E. GonCalves. 2001. Genetic diversity of *Phaeoisariopsis griseola* in the State of Minas Gerais, Brazil. Euphytica. 117:77–84.

Overmeyer, C., S. Lunnemann, C. von Wallbrunn and F. Meinhardt. 1996. Genetic variability among isolates and sexual offspring of the plant pathogen fungus *Colonecteria morganii* on the basis of random amplification of polymorphic DNA (RAPD and Restriction fragment length polymorphism (RFLP). Current microbiology. 33: 249 – 255.

Pastor-Corrales, M.A., C. Jara and S. P. Singh. 1998. Pathogenic variation. In Sources of and breeding for resistance to *Phaeoisariopsis griseola* causing angular leafs pot in common bean. Euphytica 103:161-171.

Pataky, J. K., and K. M. Snetselaar. 2006. Common smut of corn. The Plant Health Instructor.DOI: 10.1094/PHI-I-2006-0927-01

Peever, T.L., A. Ibañez, K. Akimitsu, and L. W. Timmer. 2002. Worldwide phylogeography of the citrus brown spot pathogen, *Alternaria alternate*. Phytopathology 92: 794-802

Pereira R., E. A. Souza, Q. L. Barcelos, A. F. Abreu and S. S. Librelon. 2015. Aggressiveness of *Pseudocercospora griseola* strains in common bean genotypes and implications for genetic improvement.

Saksena, P., S. K. Vishwakarma, A. K. Tiwari, A. Singh and A. Kumar. 2013. Pathological and Molecular Variation in Colletotrichum Falcatum Went Isolates Causing Red Rot of Sugarcane in the Northwest Zone of India. Journal of Plant Protection Research. 3(1) Pages 37–41

Sandlin, C.M., J. R. Steadman, C. M. Araya and D. P. Coyne. 1999. Isolates of *Uromyces appendiculatus* with specific virulence to landraces of *Phaseolus vulgaris* of Andean origin. Plant Disease. 83:108-113.

Sartorato, A. 2000. Pathogenic variation in *Phaeoisariopsis griseola* from Brazil. Annual Report of Bean Improvement Cooperatives. 43: 180–181.

Schardl, C.L. and K.D. Craven, 2003. Interspecific hybridization in plant-associated fungi and oomycetes: A review. Mol. Ecol., 12: 2861-2873

Sharma, T. R. 2003. Molecular diagnosis and application of DNA markers in the management of fungal and bacterial plant diseases. Indian journal of biotechnology. 2:99-109

Shetty, H.S. 1992. Different types of damages in seeds caused by seed borne fungi. In: Mathur, S.B. and Jorgensen, J. (eds). Seed pathology. Proceedings of CTA Seminar held at Copenhagen, Denmark, on 20-25 June 1988, Technical Centre for Agricultural and Rural Cooperation, pp 53-62. Danish Government Institute of Seed pathology for developing countries, Meppel, Holland.

Singh, M. and Singh, R.P. 1997. Potato virus Y detection: Sensitivity of RT-PCR depends on the size of fragment amplified. Canadian Journal of Plant Pathology 19: 149-155

Stenglein S. A and P. A. Balatti. 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., L. D. Ploper, O. Vizgarra and P. Balatti. 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

Takikonda, L., S. P. Wani, S. Kannan, N. Beerelli, T. K. Sreedevi, D. A. Hoisington, P. Deviand R.A. Varshney. 2009. AFLP – based molecular characterization of an elite germplasm collection of Jatropha curcas L., a biofuel plant. Plant science. 176: 505 – 513.

Talebi R, A. Hanghnazari and I. Tabatabaci. 2010. Assessment of genetic diversity within international collections of *Brassica rapa* genotypes using inter simple sequence repeat DNA markers. Biharean Biology. 4:145 – 151.

Tóth, G., Z. Gaspari and J. Jurka. 2000. Microsatellites in Different Eukaryotic Genomes: Survey and Analysis. Genome Research. 10: 967-981.

Velásquez, V. B., M. P. Cárcamo, C. R. Meriño, A. F. Iglesias and J. F. Durán. 2007. Intraspecific differentiation of Chilean isolates of the entomopathogenic fungi *Metarhizium anisopliae* var. *anisopliae* as revealed by RAPD, SSR and ITS markers. Genetics and Molecular biology. http://dx.doi.org/10.1590/S1415-47572007000100017

Versalovic, J., T. Koueth, and J. R. Lupski.1991. Distribution of repetitive DNA sequences in eubacteria and application to fingerprinting of bacterial genomes. Nucleic Acid Research 19: 6823-6831

Wagara I.N., A.W. Mwang'ombe, J.W. Kimenju, R.A. Buruchara, R. Jamnadass and P.A. O. Majiwa. 2004. Genetic diversity of *Phaeoisariopsis griseola* in Kenya as revealed by AFLP and group-specific primers. Journal of Phytopathology. 152:235–242.

Weir, T. L., D. R. Huff, B. J. Christ and C. P. Romanie. 1998. RAPD – PCR analysis of genetic variation among isolates of *Alternania solani* and *alternaria* from potato and tomato. Mycologia 90: 813 – 821.

Williams J. G., A. R. Kubelik, K.J. Livak, J. A. Rafalsky and S. V. Tingey. 1990. DNApolymorphisms amplified by arbitrary primers are useful genetic markers. Nucleic Acids Research. 18:6531-6535

Yang, A and C. Yen, PCR optimization of Box – AIR for microbial source tracking of *Escherichia coli* in waterways. Journal of Experimental Microbiology and Immunology. 16: 85 – 89

Zhao, J., Z. Wang, X. Chen, H. Zhang, J. Yao, G. Zhan, W. Chen, L. Huang and Z. Kang. 2013. Identification of eighteen Berberis species as alternate hosts of Puccinia striiformis f. sp. tritici and virulence variation in the pathogen isolates from natural infection of barberry plants in China. Phytopathology. 103:927 - 934

Zhou, H., Z. Xie, Z and S. Ge. 2003. Microsatellite analysis of genetic structure of a wild rice (Oryza rufipogon Griff.) in China. Theory Applied Genetics. 107: 332 – 339.

Zietkiewicz, E., A. Rafalski and D. Labuda. 1994. Genome fingerprinting by simple sequence repeats (SSR) – Anchored polymerase chain reaction amplification. Genomics 20:176 – 183.

Zimmer E.A and E. H. Roalson. 2005. Molecular Evolution Producing the Biochemical Data. Academic Press, Elsevier, USA. 395(Part B):1-896.

# 6 Screening common bean landraces for resistanceto angular leaf spot disease

**Abstract**

Utilization of landraces as a source of resistance genes in genetic enhancement against biotic stresses has been reported in many crops. This study sought to determine the response of common bean landraces widely grown in the Southern Highlands of Tanzania to *Pseudocercospora griseola,* a causal pathogen of angular leaf spot disease and identify promising lines that can be used for resistance breeding. One hundred diverse landraces were collected from smallholder bean farmers, sorted according to seed size and color and evaluated in the field and screen house during the 2012/2013 and 2013/2014 cropping seasons. Two check varieties; Mexico 54 (resistant control) and Uyole 96 (susceptible control) were included for comparative assessment. In the screen house, genotypes were inoculated with 20 races of the pathogen at a concentration of $3 \times 10^4$ spores $ml^{-1}$. Field experiments were initiated under natural pathogen inoculums. Data were collected on disease severity which were later converted into area under disease progress curve (AUDPC) and subjected to the analysis of variance. Additional data on important agronomic traits were collected in the field trials only. Results indicated significant differences at $P \leq 0.05$ among the common bean landraces for all the traits evaluated. Most of the landraces grown were susceptible to ALS while three landraces designated as SHB002 SHB005 and SHB091 showed resistance to the majority of the pathogen races. These landraces can be used in the resistance breeding against *P. griseola* races of the SHT but further studies are required to elucidate the genetics of resistance.

**Key words**: Landraces, resistance genes, angular leaf spot, *Pseudocercospora griseol*a, common bean

## 6.1 Introduction

Landraces are heterogeneous plant varieties endowed with tremendous genetic variability that have been grown by farmers for many years. These genetic resources are known to be locally adapted to their natural agricultural environments after long periods of selection by generations of farmers. It is believed that, farmers based their selections on diverse cultural and local ecological needs (Camacho et al., 2005; Ray et al., 2013). The existence of a wide range of phenotypic traits within the landraces further supports its broader genetic base, which is important for crop improvement (Ray et al., 2013).

Utilization of landraces as a source of resistance genes in genetic enhancement against biotic and abiotic factors has been documented in pigeon pea, barley, wheat, rice, maize and common bean, among others (Arnason et al., 1994; Czembor, 2002; Jia et al., 2002; Gwata and Silim, 2009; Yuqiang et al., 2009; Goncalve et al., 2011; Lopes et al., 2015). For example; the mildew resistant gene *Mlo*, isolated from barley landraces in Ethiopia, Turkey and Libya has been a good source of durable resistance to powdery mildew, a devastating disease of barely (Czembor, 2001; Schwarzbach, 2001; Czembor, 2002). Likewise the powdery mildew resistant gene *Pm3* isolated from wheat landraces was incorporated into susceptible wheat varieties with promising results (Bhullar et al., 2009).

As in any other crops, common bean landraces have been used extensively as sources of resistance genes against fungal diseases including angular leaf spot (ALS) caused by *Pseudocercospora griseola* (Sacc.) Crous and Braun (Crous et al., 2006). Among them cultivars Mexico 54, BAT 332 and Oura Negro have been indicated as good sources of resistance genes to the pathogen designated as *Phg* (Sartorato et al., 2000; Caixeta et al., 2003). Currently there are six such genes that have been identified, most of them from common bean landraces. These are *Phg1, Phg2, Phg3, Phg4, Phg5* and *Phg6*, known as the major or race specific genes with monogenic type of inheritance (Oblessuc et al., 2015). The *Phg* genes confer qualitative or ephemeral type of resistance that can easily be overcome due to the emergence of new virulent races necessiting combination of genes from diverse sources to provide broad resistance to an array of races prevalent in the SHT. The presence of minor resistance genes in common bean landraces has also been reported and used in durable resistance breeding against the pathogen (Ng'ayu-Wanjau et al., 2015). However the *Phg* resistance genes so far identified are in cultivars

that may not be well adapted to the environments of the SHT or have inferior traits that may not be readily accepted by farmers. Therefore, resistance genes from locally adapted germplasm (landraces) are considered important because the genotypes are already acceptable by the local growers for their various traits such as seed color, seed size and taste (Marotti et al., 2007; Zhang et al., 2008). In addition, the landraces co-existed with populations of the pathogen in that area for a long time with high possibilities of obtaining some landraces with considerable levels of resistance to *P. griseola*.

Landraces have been used as sources of resistance to ALS in most of the existing improved bean cultivars in Africa (Young and Kelly, 1996a; Busogoro et al., 1999). Since *P. griseola,* is highly variable pathogenically, there is high possibilities of increased susceptibility of common bean cultivars currently grown, raising concerns about the future of the crop, in which majority of the populations depends on it for a livelihood. It has been indicated that common bean varieties previously described as resistant were later found to be highly susceptible suggesting the need for diverse sources of resistance against the prevailing races of the pathogen (Kelly et al., 1994; Mahuku et al., 2002). A case is in the Great Lakes Region of Africa, where common bean production were estimated at 3,961,679 tonnes of which 374,800 tonnes were losses due to ALS (Aggarwal et al., 2003; Wagara et al., 2003)

In the SHT, ALS is one of the devastating disease of common bean that cause significant yield losses estimated to be more than 50% (Mwalyego, 1991). Recent studies done in the SHT showed high yield losses as much as 61% suggesting increase in ALS infection on varieties currently grown (Mongi R., 2016 *unpublished*). Common bean is a highly valued crop in terms of nutrition and source of income, therefore strategic and cost effective measures against the disease should be thought for, to enhance productivity of the crop. Since common bean landraces has been used as sources for resistance elsewhere in the world, utilization of the sources that exists in the SHT in searching for resistance genes is important. However, limited studies have been conducted on bean landraces of the SHT (Fihawo and Msolla, 2012) and no current or exhaustive research that involved evaluation of large number of landraces against a large number of *P. griseola* races of the SHT. Therefore, the objective of this study was to determine the response of common bean landraces widely grown in the Southern Highlands of Tanzania against the *P. griseola* and to identify promising lines for use in resistance breeding.

## 6.2    Materials and Methods

### 6.2.1    Collection of bean germplasm and seed increase

Seeds of diverse landrace varieties grown by farmers were collected through designed field visits. Additional samples were collected from the local markets, where a large number of bean farmers obtain there seed. More than 90% of the seed samples collected were mixtures of seed colours and size (Fig. 6.1) which were well packed, labeled and transported to the Uyole Agricultural Research Institute (ARI-Uyole) where they were sorted out according to seed color and seed size. A 100 different seed types were obtained herein described as accessions. The seeds were planted in the field at ARI –Uyole for seed increase and to determine phenotypic variations that might exist within seed types before screening for ALS disease. Individual plants that showed similarities in all of the phenotypic traits (seedling stem color, seedling leaves, plant types, flower color, pod shape, seed size and seed color) were harvested and bulked for evaluations. Additional screening of the accessions was done in the screen house in which susceptible that might have resulted from infected seed were eliminated. Clean seed were bulked again for ALS evaluations



Figure 6.1: Seeds of landracevarieties of common bean collected from the Southern Highlands of Tanzania showing variable colors and sizes

## 6.2.2 Pathogen isolation and inoculum preparation

Twenty races of *P. griseola* previously characterized using a set of twelve differential cultivars and stored as single colonies at $4^0$C for a short term, were multiplied in petri dishes containing V8 media according to the method of Pastor – Corrales et al. (1998). Thereafter, the inoculum was increased through sub-culturing pure colonies by adding 100 µl of sterile water into each plate and then spreading the spore suspension onto fresh V8 agar media and incubating for 15 days at $24^0$C to allow more sporulation. Plates on which each race was grown were covered with 100 µl of sterile distilled water, and then the surface scraped with a glass rod to release the conidia. The dislodged spores in suspension were filtered through a sterile cheese cloth and conidial concentrations in the suspension were determined using a haemocytometer. The concentration was maintained at 3 x $10^4$ spores ml$^{-1}$ in a sterile distilled water (Pastor-Corrales et al., 1998; Mahuku et al., 2002).

## 6.2.3 Germplasm evaluation under screen house condition

The experiment was conducted between 2012 and 2013. The cultivars Uyole 96 (ALS susceptible) and Mexico 54 (resistant) were used as checks for comparison. One hundred uniform accessions were planted in 3 liters capacity plastic pots of 12 cm in diameter (Fig. 6.2). Seven pots were assigned to each accession and five seeds were planted per pot and later thinned to three plants. To increase inoculation efficiency; four sets of pots were constituted, each containing 350 pots. For each set there were 1050 plants available and each race was assigned to 10 plants per accession during the first planting that made a total of 40 plants by the end of the fourth round. Cross contamination was avoided by keeping the plants inoculated with the same race together. Planting was done at an interval of four days between sets to provide staggered dates of inoculation. The plants were inoculated with conidia suspension of the local *P. griseola* races identified from isolates collected from the SHT. Each race was inoculated separately on each accession. Pots were arranged in a completely randomized design with two replications.

**Figure 6.2: Screen house evaluations of common bean landraces against *P. griseola* races collected from the South Highlands of Tanzania**

After full expansion of the trifoliate leaves, plants were sprayed with *P. griseola* at a concentration of 3 x $10^4$ spores ml$^{-1}$ suspension till runoff. Inoculated plants were covered with clear plastic sheets for 48 hours to create high humidity for pathogen infection. Disease assessments were done every five days after inoculation using 1-9 rating scale described as follows: 1 was assigned to plants with no ALS symptoms; 2 -3 to plants with sparse and small lesions; 4-6 to well defined but sparse lesions; 7 -9 to well defined, expanding and many lesions (Schoonhoven van and Pastor – Corrales, 1987). Plants that scored 1 – 3 were rated as resistant (R) while those that ranged from 4 – 6 and 7 – 9 were rated moderately resistant (MR) and susceptible (S), respectively.

### 6.2.4   Germplasm evaluation under field conditions

Field evaluations were done on the same 100 bean accessions previously evaluated under screen house conditions to supplement the data obtained in the screenhouse. The trial site was at ARI – Uyole, which is amongst the hot spot areas for ALS disease in the SHT. Heavy rains and mild temperatures occurred in the first part of the season (December to mid-March),while the second part of the season (late March to early July) was characterized by lighter rains with mild temperatures, conditions that facilitate infection and development of *P. griseola*,.

The first planting was conducted during December while the second planting was done in late March. The experiment was laid out as a simple lattice design of 10 x 10 with plot size of 2.25 m$^2$, consisting of four rows spaced at 0.5 m apart. Intra row spacing was 0.1 m. The susceptible cultivar Kigoma was grown as spreader rows after every five plots. The seed rate, fertilizer

applications and all other agronomic practices were according to recommendations for bean production in the SHT. As in the screen house, disease scores were according to CIAT 1 – 9 scale after every twelve days from the first visible disease symptoms to physiological maturity. Additional data were collected on days to flowering, days to maturity 100 seed weight and grain yield.

### 6.2.5 Data analysis

Disease scores collected using the CIAT 1 -9 scale were converted into Area Under Disease Progress Curve (AUDPC) using the following formula (Forbes and Korva, 1994):

$$\text{AUDPC} = \sum_{i=1}^{n-1} \left( \frac{y_i + y_{i+1}}{2} \right) (t_{i+1} - t_i)$$

Analysis of variance was done for all the data collected from the field using the REML procedure and means separated by LSD at 5% level of probability using Genstat software version 14 (Payne et al., 2011).

Since there were no significant differences between 2012/2013 and 2013/2014 cropping season, the data was pooled and combined analysis of variance was done. The means were separated by LSD (least significant difference) at P≤0.05. The percentages of landraces with different reactions to the 20 *P. griseola* races of the SHT were calculated.

### 6.3 Results

### 6.3.1 Screen house evaluations

The infection patterns of the individual pathogen races to each genotype/landrace were consistent over the period of two years in which the trials were conducted. Differences in disease reactions were observed between genotypes where ratings of resistance (R), moderate resistance (MR) and susceptible (S) were scored (Table 6.1). Among the genotypes evaluated, the highest percentage of resistance (96.1%) was recorded against race 31/1(Table 6.2). Most landraces exhibited low infection rates indicating high levels of resistance against race 31/1. Only one genotype, SHB040 showed moderate resistance and three genotypes (SHB074, SHB087 and SHB088) were susceptible to the same race 31/1. Race 63/62 was the most aggressive, and 92.1% of the

landraces were susceptible, with only7.8% of the landraces moderately resistant. . Race 63/63 was equally aggressive with 92.4% of the landraces susceptible and only 6.8% moderately

High percentages of susceptible landraces were noted and none of the pathogen races completely overcame the genotypes evaluated while none of the genotypes showed high resistant reactions to all the twenty pathogen races used in this study (Tables 6.1 and 6.2). However, the most resistant genotypes against the majority of the races used in this study were: Mexico 54, SHB002, SHB005 and SHB091

**Table 6.1: Reactions of common bean landraces to 20 physiologic races of *P. griseola* collected from the Southern Highlands of Tanzania**

| Landrace | A | B | C | D | E | F | G | H | I | J | K | L | M | N | O | P | Q | R | S | T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SHB001 | + | - | + | + | + | - | MR | MR | - | - | + | + | - | + | + | + | MR | + | + | + |
| SHB002 | - | - | - | - | MR | - | MR | - | - | - | MR | - | - | MR | - | - | - | - | - | MR |
| SHB003 | + | + | + | + | + | + | + | - | - | - | - | + | - | - | + | - | + | - | + | + |
| SHB004 | + | + | + | + | + | + | + | + | - | - | - | + | - | - | + | - | + | + | + | + |
| SHB005 | - | - | - | - | - | MR | MR | - | - | - | - | MR | - | - | - | - | - | - | - | MR |
| SHB006 | + | + | + | + | + | - | + | + | + | - | - | + | - | - | + | - | + | - | + | + |
| SHB007 | - | + | + | + | + | - | + | + | - | - | - | + | - | + | + | - | + | - | + | + |
| SHB008 | + | MR | + | + | - | + | + | MR | - | + | - | + | - | + | - | - | + | + | + | + |
| SHB009 | - | - | + | + | + | - | + | + | - | - | - | + | - | + | + | - | + | - | + | + |
| SHB010 | + | + | - | + | + | + | + | + | + | - | - | + | - | + | - | - | + | - | + | + |
| SHB011 | + | - | MR | + | + | + | + | - | + | - | + | + | - | + | + | - | + | - | - | + |
| SHB012 | - | + | MR | + | + | + | + | + | - | - | + | + | - | MR | - | - | + | + | + | + |
| SHB013 | + | + | + | + | MR | - | + | + | + | MR | - | + | - | - | + | - | + | - | - | + |
| SHB014 | + | - | MR | + | - | + | + | + | - | - | + | + | - | - | - | + | + | - | - | + |
| SHB015 | - | + | + | + | + | - | + | + | MR | - | - | + | - | - | + | + | + | + | + | + |
| SHB016 | + | - | + | + | + | + | + | + | MR | - | + | + | - | + | - | - | MR | - | + | + |
| SHB017 | - | - | - | + | MR | - | + | - | - | - | + | MR | - | + | - | + | + | + | + | + |
| SHB018 | + | + | + | - | + | + | + | + | + | MR | + | + | - | + | + | MR | - | + | MR | + |
| SHB019 | - | - | + | + | + | + | + | + | - | + | - | + | - | + | - | - | + | - | - | + |
| SHB020 | + | + | + | + | + | - | + | MR | - | MR | + | + | - | + | - | - | + | + | + | + |
| SHB021 | - | + | + | + | + | + | + | + | - | MR | + | + | - | + | - | - | + | - | + | + |
| SHB022 | + | - | + | + | + | + | + | + | + | MR | + | + | - | MR | + | - | + | + | + | + |
| SHB023 | + | + | - | + | MR | + | + | + | - | - | + | + | - | + | + | - | + | - | + | + |
| SHB024 | + | - | + | + | + | + | + | - | + | + | + | + | - | + | - | - | + | - | + | + |
| SHB025 | - | + | + | + | + | + | + | + | - | - | + | + | - | + | + | - | + | - | - | + |
| SHB026 | + | + | - | MR | + | + | + | + | - | - | - | + | - | + | - | - | + | - | - | + |
| SHB027 | - | MR | + | MR | + | - | + | + | + | + | + | + | - | + | + | + | + | - | + | + |
| SHB028 | + | + | + | + | - | + | + | + | + | + | + | MR | - | + | - | + | + | - | + | + |
| SHB029 | - | - | + | + | + | + | + | + | - | - | + | + | - | MR | + | - | + | - | + | + |

| Landrace | A | B | C | D | E | F | G | H | I | J | K | L | M | N | O | P | Q | R | S | T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SHB030 | + | + | - | + | + | + | + | + | - | - | - | + | - | MR | - | - | + | - | + | + |
| SHB031 | - | + | + | + | + | + | + | + | - | - | + | + | - | MR | - | - | + | - | - | + |
| SHB032 | + | - | + | + | + | + | + | + | + | - | + | + | - | + | - | + | + | + | - | + |
| SHB033 | + | + | - | + | + | - | + | + | + | - | - | + | - | + | + | - | + | - | + | + |
| SHB034 | + | + | + | + | - | + | + | + | MR | - | - | + | - | + | + | - | + | - | + | + |
| SHB035 | + | - | + | + | + | + | + | + | + | - | - | + | - | + | + | - | + | - | - | + |
| SHB036 | - | + | + | + | + | + | + | + | + | + | - | + | - | + | + | - | + | - | - | + |
| SHB037 | + | - | + | + | + | + | + | + | MR | + | + | + | | + | - | - | + | - | + | + |
| SHB038 | - | + | + | + | + | + | + | + | + | + | + | + | - | + | - | - | + | - | + | + |
| SHB039 | + | + | - | + | - | - | + | + | - | + | + | + | - | + | - | + | + | - | + | + |
| SHB040 | + | - | + | + | + | - | + | + | - | + | + | + | MR | + | + | - | + | - | - | + |
| SHB041 | + | + | - | + | + | - | + | + | - | + | - | + | - | + | + | - | + | - | - | + |
| SHB042 | - | - | MR | + | MR | + | + | + | - | - | + | + | - | - | + | MR | + | - | + | MR |
| SHB043 | + | + | + | + | + | + | + | - | - | - | + | + | - | - | - | - | + | + | + | + |
| SHB044 | + | + | + | + | + | + | + | + | + | + | + | + | - | - | + | - | + | + | + | + |
| SHB045 | - | - | MR | MR | + | - | + | + | MR | MR | - | MR | - | - | MR | - | + | - | + | + |
| SHB046 | + | + | + | + | + | - | + | + | + | MR | - | + | - | + | - | - | + | + | + | + |
| SHB047 | MR | + | + | + | + | + | + | + | MR | MR | - | + | - | + | - | MR | + | + | + | + |
| SHB048 | - | + | - | + | + | - | + | + | + | - | - | + | - | + | - | + | + | MR | + | + |
| SHB049 | - | - | - | + | + | + | + | + | - | - | - | + | - | - | - | - | + | + | + | + |
| SHB050 | + | + | + | + | + | + | + | + | - | - | + | + | - | - | - | - | + | + | + | + |
| SHB051 | + | + | + | + | + | - | + | + | - | - | - | + | - | - | - | - | + | - | + | + |
| SHB052 | - | - | - | MR | + | - | + | + | - | + | + | + | - | - | - | - | + | - | + | + |
| SHB053 | + | + | + | + | + | - | + | + | - | - | + | + | - | - | - | + | + | + | + | + |
| SHB054 | MR | + | - | MR | + | MR | + | - | - | - | - | MR | - | - | + | - | + | - | + | + |
| SHB055 | + | + | + | + | + | + | + | + | + | - | + | + | - | MR | - | - | MR | - | - | + |
| SHB056 | - | - | + | + | + | + | + | + | + | + | - | + | - | - | - | - | + | - | + | + |
| SHB057 | - | + | + | + | + | + | + | + | - | - | - | + | - | - | - | + | + | - | + | + |
| SHB058 | + | + | - | + | + | - | + | + | - | + | - | + | - | - | - | + | + | - | + | + |
| SHB059 | - | + | + | + | + | - | + | + | - | - | - | + | - | - | MR | MR | + | - | + | + |
| SHB060 | + | - | - | + | + | - | + | + | - | - | + | + | - | - | - | + | + | + | + | + |
| SHB061 | - | + | + | + | + | + | + | + | - | - | - | + | - | - | - | - | + | + | + | + |

| Landrace | A | B | C | D | E | F | G | H | I | J | K | L | M | N | O | P | Q | R | S | T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SHB062 | + | + | + | + | + | + | + | + | - | - | - | + | - | + | - | - | + | - | + | + |
| SHB063 | + | MR | + | + | + | + | + | + | + | + | + | + | - | + | - | - | + | - | + | + |
| SHB064 | - | - | + | + | + | + | + | MR | MR | - | - | + | - | - | - | - | + | - | - | MR |
| SHB065 | + | + | - | + | + | + | + | + | + | + | - | + | - | + | - | + | + | + | + | + |
| SHB066 | + | - | + | + | + | + | + | + | - | + | - | + | - | + | - | - | + | + | - | + |
| SHB067 | - | + | - | + | + | + | + | + | + | - | - | MR | - | + | - | - | + | + | - | + |
| SHB068 | + | + | - | + | + | - | + | + | - | + | + | - | - | + | - | - | + | + | - | + |
| SHB069 | + | - | + | + | + | - | + | + | - | - | - | + | - | + | - | - | + | - | - | + |
| SHB070 | - | + | + | + | - | + | + | + | - | - | - | - | - | + | - | + | + | - | - | + |
| SHB071 | + | + | - | + | + | + | + | + | - | - | - | + | - | + | - | - | + | - | - | + |
| SHB072 | + | - | + | + | + | - | + | + | + | + | + | + | - | + | - | - | + | - | + | + |
| SHB073 | + | + | + | + | + | + | + | + | + | + | + | + | - | + | - | - | + | - | + | + |
| SHB074 | + | + | + | + | + | + | + | - | MR | - | + | - | + | + | + | + | + | - | + | + |
| SHB075 | - | + | - | + | + | - | + | + | - | + | + | + | - | + | - | - | + | - | + | + |
| SHB076 | + | + | + | + | + | - | + | + | - | - | + | + | - | + | - | - | + | + | + | + |
| SHB077 | - | - | - | MR | - | - | + | MR | - | - | - | MR | - | MR | - | MR | MR | - | - | + |
| SHB078 | + | + | + | + | + | + | + | + | - | - | MR | + | - | + | - | - | + | + | + | + |
| SHB079 | + | + | + | + | - | + | + | + | + | + | + | + | - | + | - | - | + | + | + | + |
| SHB080 | - | - | - | - | + | - | + | - | - | - | MR | - | - | MR | - | - | MR | - | - | MR |
| SHB081 | + | + | + | + | + | + | + | + | + | + | - | + | - | + | - | - | + | - | - | + |
| SHB082 | - | + | - | + | + | + | + | + | - | - | - | + | - | + | - | + | + | + | + | + |
| SHB083 | + | - | + | + | + | + | + | + | - | - | + | + | - | + | - | - | + | + | + | + |
| SHB084 | + | + | - | + | - | - | + | + | - | - | - | + | - | + | + | - | + | + | + | + |
| SHB085 | - | + | + | - | + | + | + | - | MR | - | + | + | - | MR | - | - | MR | - | - | + |
| SHB086 | + | + | - | + | + | + | + | + | - | - | - | MR | - | + | + | + | + | + | - | + |
| SHB087 | + | - | + | + | + | + | + | + | + | - | + | + | + | + | + | - | + | - | - | + |
| SHB088 | + | + | - | + | + | - | + | + | - | - | - | + | + | + | - | - | + | + | MR | + |
| SHB089 | MR | - | + | + | MR | - | + | + | + | - | + | + | - | MR | - | - | + | + | - | MR |
| SHB090 | + | + | + | + | - | + | + | + | - | - | + | + | - | + | - | - | + | - | + | + |
| SHB091 | - | - | - | - | - | - | MR | - | - | - | - | MR | - | - | - | - | - | - | - | MR |
| SHB092 | + | + | + | + | + | + | + | + | - | + | MR | + | - | + | - | - | + | - | + | + |
| SHB093 | - | + | + | + | + | + | + | + | - | + | - | + | - | + | + | - | + | + | + | + |

| Landrace | A | B | C | D | E | F | G | H | I | J | K | L | M | N | O | P | Q | R | S | T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SHB094 | + | - | - | + | + | + | + | + | - | - | - | + | - | + | - | - | + | - | + | + |
| SHB095 | - | + | + | + | + | + | + | + | - | - | - | MR | - | - | MR | - | + | - | - | + |
| SHB096 | + | - | + | + | + | + | + | + | + | + | + | + | - | + | - | - | + | + | - | + |
| SHB097 | - | + | + | - | + | - | + | + | + | + | + | + | - | + | + | - | + | + | - | + |
| SHB098 | - | - | - | + | - | - | MR | - | - | - | MR | - | - | MR | - | - | MR | MR | MR | + |
| SHB099 | + | + | + | + | + | + | + | + | + | + | + | + | - | - | + | + | + | + | + | + |
| SHB100 | - | + | + | MR | + | + | MR | + | - | - | MR | - | - | MR | - | - | + | - | - | + |
| Uyole 96 | + | + | + | + | + | + | + | + | MR | MR | + | + | - | + | + | + | + | + | + | + |
| Mexico 54 | - | - | - | - | - | - | MR | - | - | - | - | - | - | - | - | - | - | - | - | MR |

[a]**Races: A**= 63/6;  **B**=23/39;  **C**=23/32;  **D**= 63/53;  **E**= 63/47;  **F**= 63/39;  **G**= 63/62;  **H**=31/43;  **I**= 62/7;  **J**= 47/3;  **K**=18/3; **L** = 63/55; **M**= 31/1; **N**= 63/38; **O**= 31/39; **P**= 30/0; **Q**= 63/55; **R**=26/6; **S**=54/38; **T**= 63/63. - = INCOMPATIBLE; + = COMPATIBLE

NB: When a group of isolates infect two or more of the differential cultivars in the same way, the binary numbers are added together to form a race number that is separated by a slash or dash to distinguish Andean and Mesoamerican cultivars infected (Pastor-Corrales, 1995; Mahuku et al., 2002; Mahuku et al., 2004).

**Table 6.2. 1. Common bean landraces with resistance to the largest number of P. griseola isolates of the SHT**

| Landrace code number | Moderately susceptible (MR) | Resistance |
|---|---|---|
| SHB002 | 5 | 15 |
| SHB005 | 4 | 16 |
| SHB077 | 6 | 13 |
| SHB091 | 3 | 17 |
| SHB098 | 6 | 16 |
| **Mexico 54** | **2** | **18** |

**Table 6.2: Summary of the number of plants and corresponding percentages among 100 common bean landraces showing three levels of reactions against 20 *P. griseola* races collected from the Southern Highlands of Tanzania**

| Race | Resistant | Moderately resistant (intermediate) | Susceptible | Total |
|------|-----------|--------------------------------------|-------------|-------|
| 63/6 | 39 (38.2) | 3 (2.9) | 60 (58.8) | 102 (100) |
| 23/39 | 36 (35.3) | 3 (2.9) | 63 (61.7) | 102 (100) |
| 23/32 | 31 (30.3) | 5 (4.9) | 66 (64.7) | 102 (100) |
| 63/53 | 8 (7.8) | 7 (6.8) | 87 (85.3) | 102 (100) |
| 63/47 | 15 (14.7) | 6 (5.8) | 71 (69.6) | 102 (100) |
| 63/39 | 36 (35.3) | 2 (1.9) | 64 (62.7) | 102 (100) |
| 63/62 | 0 (0.0) | 8 (7.8) | 94 (92.1) | 102 (100) |
| 31/43 | 13 (12.7) | 5 (4.9) | 84 (82.3) | 102 (100) |
| 62/7 | 61 (59.8) | 10 (9.8) | 31 (30.3) | 102 (100) |
| 47/3 | 64 (67.7) | 8 (7.8) | 30 (29.4) | 102 (100) |
| 18/3 | 49 (48.0) | 6 (5.8) | 47 (46.1) | 102 (100) |
| 63/23 | 9 (8.8) | 10 (9.8) | 83 (81.3) | 102 (100) |
| 31/1 | 98 (96.1) | 1 (0.9) | 3 (2.9) | 102 (100) |
| 63/38 | 28 (27.4) | 13 (12.7) | 61 (59.8) | 102 (100) |
| 31/39 | 67 (65.6) | 3 (2.9) | 70 (68.0) | 102 (100) |
| 30/0 | 77 (75.4) | 5 (4.9) | 20 (19.6) | 102 (100) |
| 63/55 | 4 (3.9) | 7 (6.8) | 91 (89.2) | 102 (100) |
| 26/6 | 63 (61.7) | 2 (1.9) | 37 (36.2) | 102 (100) |
| 54/38 | 36 (35.3) | 3 (2.9) | 63 (61.7) | 102 (100) |
| 63/63 | 1 (0.9) | 7 (6.8) | 94 (92.1) | 102 (100) |

Values in parenthesis are percentages

## 6.3.2   Field evaluation

The environmental conditions in both 2012/2013 and 2013/2014 were conducive for disease development and ALS symptoms were first noted on primary leaves 10 – 12 days after planting. There were slight variations between the data collected during the 2012/2013 and 2013/2014 bean cropping season but the differences were not significant. Therefore, the data were pooled and a combined analysis of variance done.

Significant amounts of variation were observed amongst the germplasm for growth habit, days to flowering, days to maturity, grain yield and seed weight (Table 6.3). Days to flowering ranged from 35 – 45 with a mean of 40, whereas days to physiological maturity ranged from 76 – 93.5 with a mean of 84.9. Thirty five percent of landraces matured earlier compared to the local checks, Uyole 96 and Mexico 54.

Differences in flower colours were also observed. Most of landraces had pink flowers while a smaller number had white and purple flowers (data not shown). The duration of flowering depended on the growth habits (determinate bush – type 1; indeterminate bush –type II or semi-climbers; indeterminate climbing – type IV). The climbing types continued flowering for a longer period compared to bush and semi climbers.

The response of landraces to *P. griseola* infection was similar between seasons. However, variations in disease reaction were observed between genotypes and they were categorized into resistant, moderately resistant and susceptible as it was done in the screen house. The Area Under Disease Progress Curve (AUDPC) increased with the degree of susceptibility and the range were 17 – 47 for resistance; 48 – 80, moderate resistance and 81 – 118, susceptible. Of the 102 genotypes evaluated 5.8% were categorized as resistant, 32.4% moderately resistant and 63.0% susceptible. The landraces that showed resistance over two years were SHB002, SHB005, SHB077, SHB091 and Mexico 54 as majority of races were unable to infect the genotypes. The same trends observed during screen house evaluations were also noted in the field where individual pathogen races were used.

Most of the landraces were small to medium seeded Mesoamerican types while only 6% of the total germplasm evaluated accounted for large seeded Andean varieties. Grain yields were relatively low although all proper agronomic practices for bean production were observed. The

range was 270.5 to 1332.8 kg ha$^{-1}$ with a mean of 810.2 kg ha$^{-1}$. The highest yielding genotype was SHB005 (1332.8 kg ha$^{-1}$) followed by Mexico 54 (1310.2 kg ha$^{-1}$) which was one of the ALS resistant cultivars (Table 6.3).

**Table 6.3: Growth habit, seed color and mean days to flowering (DF), days to maturity (DM), area under disease progressive curve (AUDPC), grain yield (GY) and hundred seed weight (HSW) among 102 common bean genotypes filed evaluated in 2012/2013 and 2013/2014 bean cropping seasons**

| Landrace/ cultivar | Growth habit | Seed color | DF | DM | AUDPC | GY (kg ha$^{-1}$) | HSW (gms) |
|---|---|---|---|---|---|---|---|
| SHB001 | SC | Publish pink | 41.3 | 83.0 | 97.8 | 1063.5 | 22.6 |
| SHB002 | SC | yellow | 45.5 | 94.2 | 31.9 | 582.8 | 23.6 |
| SHB003 | Climber | cream | 42.8 | 90.5 | 69.4 | 426.6 | 22.1 |
| SHB004 | bush | white | 41.3 | 94.2 | 58.3 | 1020.3 | 32.9 |
| SHB005 | SC | Whitish striped | 39.3 | 81.5 | 31.3 | 1332.8 | 37.1 |
| SHB006 | bush | yellow | 41.3 | 84.5 | 106.3 | 1270.3 | 25.6 |
| SHB007 | SC | Yellowish | 42.6 | 85.5 | 101.3 | 1270.3 | 15.5 |
| SHB008 | SC | Purple mottled | 40.8 | 91.2 | 67.6 | 1030.7 | 20.6 |
| SHB009 | SC | Black | 42.3 | 86.5 | 95.7 | 999.5 | 13.7 |
| SHB010 | Bush | Brown | 42.0 | 82.0 | 104.7 | 614.1 | 16.5 |
| SHB011 | SC | Grayish cream | 42.8 | 93.5 | 103.8 | 697.4 | 16.3 |
| SHB012 | bush | white | 45.5 | 95.2 | 69.5 | 707.8 | 21.7 |
| SHB013 | SC | Creamy | 46.5 | 93.2 | 52.6 | 655.7 | 20.2 |
| SHB014 | SC | brown | 40.3 | 94.0 | 83.2 | 978.6 | 18.3 |
| SHB015 | bush | Yellowish brown | 39.8 | 76.0 | 98.8 | 947.4 | 21.3 |
| SHB016 | bush | Brown mottled | 44.3 | 85.7 | 93.2 | 593.2 | 17.5 |
| SHB017 | SC | brown | 39.3 | 82.5 | 88.2 | 1041.1 | 13.4 |
| SHB018 | SC | purplish | 40.3 | 88.0 | 56.9 | 707.8 | 18.8 |
| SHB019 | SC | Purplish brown | 48.0 | 91.7 | 52.6 | 1009.9 | 23.8 |
| SHB020 | bush | Brown mottled | 41.5 | 85.5 | 48.2 | 1270.3 | 16.1 |
| SHB021 | SC | black | 40.5 | 85.5 | 97.6 | 568.2 | 14.1 |
| SHB022 | SC | Creamy mottled | 39.3 | 82.7 | 86.3 | 1197.4 | 22.4 |
| SHB023 | bush | White black dots | 40.0 | 86.7 | 91.3 | 1166.1 | 22.2 |
| SHB024 | SC | Brownish yellow | 39.3 | 82.2 | 92.6 | 968.2 | 17.4 |
| SHB025 | SC | Cream black stripes | 41.7 | 88.5 | 67.6 | 478.6 | 24.8 |
| SHB026 | Climber | Light red | 40.3 | 84.5 | 88.2 | 489.1 | 21.3 |
| SHB027 | SC | Purplish yellow | 39.7 | 84.5 | 95.1 | 697.4 | 20.1 |
| SHB028 | bush | Cream purplish | 39.0 | 83.7 | 99.5 | 978.6 | 19.5 |

| Landrace/ cultivar | Growth habit | Seed color | DF | DM | AUDPC | GY (kg ha$^{-1}$) | HSW (gms) |
|---|---|---|---|---|---|---|---|
| SHB029 | bush | orange | 44.0 | 93.5 | 77.6 | 864.1 | 16.9 |
| SHB030 | SC | Pinkish black stripes | 41.2 | 86.2 | 101.3 | 370.3 | 17.7 |
| SHB031 | bush | brown | 40.5 | 85.2 | 80.1 | 512.0 | 19.1 |
| SHB032 | SC | Light yellow | 40.0 | 83.5 | 98.8 | 718.2 | 19.3 |
| SHB033 | Climber | Light orange | 39.2 | 81.8 | 108.2 | 832.8 | 21.3 |
| SHB034 | SC | white | 39.5 | 84.2 | 86.3 | 701.6 | 20.8 |
| SHB035 | bush | Purplish brown | 39.5 | 86.2 | 50.7 | 914.1 | 21.7 |
| SHB036 | bush | White striped | 39.5 | 83.3 | 72.6 | 1155.7 | 35.4 |
| SHB037 | bush | Purplish orange | 39.5 | 82.5 | 88.8 | 562.0 | 20.6 |
| SHB038 | bush | brownish | 38.7 | 82.5 | 61.3 | 937.0 | 30.6 |
| SHB039 | Bush | yellow | 40.7 | 82.7 | 71.3 | 905.7 | 30.5 |
| SHB040 | bush | Yellowish orange | 38.2 | 83.0 | 98.8 | 572.4 | 42.6 |
| SHB041 | bush | Light yellow | 38.0 | 83.5 | 89.5 | 791.1 | 30.3 |
| SHB042 | SC | Grayish purple | 39.3 | 84.7 | 101.9 | 780.7 | 30.5 |
| SHB043 | bush | Yellow (maini) | 38.5 | 84.7 | 91.6 | 989.1 | 38.2 |
| SHB044 | SC | Bright yellow | 39.8 | 84.2 | 87.6 | 707.8 | 25.6 |
| SHB045 | SC | white | 39.7 | 86.0 | 65.1 | 822.4 | 12.6 |
| SHB046 | climber | Light orange | 39.5 | 84.0 | 51.9 | 707.8 | 25.7 |
| SHB047 | SC | Creamy white | 39.3 | 85.0 | 73.8 | 1176.6 | 51.3 |
| SHB048 | SC | Purplish with dots | 38.7 | 84.3 | 60.7 | 853.6 | 32.8 |
| SHB049 | bush | brown | 39.0 | 80.2 | 118.2 | 853.6 | 17.6 |
| SHB050 | SC | Whitish purple | 40.5 | 84.7 | 91.9 | 464.1 | 20.5 |
| SHB051 | SC | purple | 39.3 | 84.0 | 91.9 | 447.4 | 35.9 |
| SHB052 | SC | orange | 37.7 | 80.2 | 105.1 | 624.5 | 37.5 |
| SHB053 | SC | Light brown | 38.7 | 80.3 | 102.6 | 624.9 | 21.3 |
| SHB054 | bush | White small | 39.5 | 83.3 | 93.2 | 582.8 | 20.6 |
| SHB055 | bush | Large white | 37.5 | 82.3 | 97.6 | 687.0 | 20.3 |
| SHB056 | bush | White medium | 40.8 | 85.3 | 69.5 | 905.9 | 21.7 |
| SHB057 | bush | Light orange | 38.2 | 81.3 | 86.9 | 937.0 | 31.9 |
| SHB058 | bush | Red mottled | 39.2 | 83.7 | 62.6 | 405.7 | 18.8 |
| SHB059 | SC | Pinkish mottled | 39.2 | 83.0 | 86.9 | 926.6 | 19.7 |
| SHB060 | bush | Orange large | 40.0 | 84.5 | 90.1 | 905.7 | 16.4 |
| SHB061 | bush | White round | 38.7 | 86.0 | 72.8 | 562.0 | 34.5 |
| SHB062 | bush | Whitish | 39.0 | 82.5 | 88.2 | 697.4 | 32.1 |
| SHB063 | SC | Cream with dots | 39.3 | 84.7 | 68.8 | 676.6 | 34.5 |

| Landrace/ cultivar | Growth habit | Seed color | DF | DM | AUDPC | GY (kg ha$^{-1}$) | HSW (gms) |
|---|---|---|---|---|---|---|---|
| SHB064 | bush | Purplish brown | 41.7 | 80.2 | 46.9 | 937.0 | 31.1 |
| SHB065 | bush | purple | 38.5 | 86.0 | 67.6 | 572.4 | 24.7 |
| SHB066 | SC | black | 41.5 | 83.3 | 65.1 | 1114.1 | 26.1 |
| SHB067 | bush | Light yellow | 37.3 | 80.0 | 95.1 | 791.1 | 35.1 |
| SHB068 | SC | black | 39.5 | 81.3 | 90.1 | 582.8 | 27.5 |
| SHB069 | SC | Purple mottled | 39.5 | 85.3 | 85.7 | 614.1 | 38.6 |
| SHB070 | SC | Purplish brown | 39.5 | 87.0 | 103.8 | 562.0 | 18.3 |
| SHB071 | SC | Light yellow | 39.5 | 82.0 | 96.3 | 509.9 | 33.3 |
| SHB072 | SC | Light purple mottled | 40.8 | 87.5 | 76.3 | 687.0 | 34.3 |
| SHB073 | SC | White brown stripes | 42.5 | 90.3 | 99.5 | 739.1 | 20.4 |
| SHB074 | SC | pink | 38.5 | 81.0 | 98.2 | 478.6 | 45.2 |
| SHB075 | SC | Purplish dots | 38.3 | 84.3 | 100.7 | 780.7 | 17.9 |
| SHB076 | SC | White brown stripes | 40.5 | 82.3 | 93.8 | 749.5 | 22.7 |
| SHB077 | Climber | Whitish striped | 42.3 | 91.3 | 31.9 | 874.5 | 26.5 |
| SHB078 | SC | Light brown | 39.0 | 84.7 | 87.6 | 1051.6 | 20.8 |
| SHB079 | bush | black | 39.0 | 83.8 | 91.3 | 895.3 | 23.8 |
| SHB080 | SC | purplish | 41.2 | 83.8 | 106.3 | 697.4 | 19.9 |
| SHB081 | SC | Cream with stripes | 40.8 | 84.3 | 88.3 | 822.4 | 15.9 |
| SHB082 | bush | brown | 38.7 | 92.5 | 86.9 | 812.0 | 34.9 |
| SHB083 | SC | Reddish brown | 38.7 | 82.3 | 99.5 | 1041.1 | 18.6 |
| SHB084 | SC | White purple | 39.0 | 82.3 | 88.2 | 999.5 | 37.5 |
| SHB085 | SC | Brown striped | 39.0 | 82.5 | 72.6 | 759.9 | 24.3 |
| SHB086 | bush | White brown stripes | 38.0 | 85.7 | 86.3 | 1103.6 | 37.9 |
| SHB087 | bush | Light brown | 37.7 | 84.5 | 89.5 | 853.6 | 40.6 |
| SHB088 | bush | Light yellow | 44.5 | 83.3 | 84.5 | 874.5 | 19.6 |
| SHB089 | SC | cream | 42.5 | 91.5 | 55.7 | 1312.0 | 20.1 |
| SHB090 | SC | red | 40.5 | 84.7 | 80.1 | 676.6 | 25.6 |
| SHB091 | SC | white | 38.3 | 81.8 | 44.5 | 864.1 | 26.1 |
| SHB092 | bush | Red mottled | 39.0 | 86.0 | 72.6 | 687.0 | 21.9 |
| SHB093 | SC | Pink | 39.5 | 82.0 | 78.2 | 749.5 | 54.9 |
| SHB094 | bush | White striped | 39.5 | 82.8 | 99.5 | 707.8 | 34.7 |
| SHB095 | bush | orange | 39.0 | 84.5 | 70.1 | 937.0 | 27.2 |
| SHB096 | bush | Greenish brown | 38.5 | 84.5 | 80.7 | 926.6 | 26.7 |
| SHB097 | SC | Cream dotted | 39.0 | 82.8 | 68.2 | 874.5 | 34.3 |
| SHB098 | SC | cream | 39.8 | 83.5 | 97.6 | 1041.1 | 16.5 |

| Landrace/ cultivar | Growth habit | Seed color | DF | DM | AUDPC | GY (kg ha$^{-1}$) | HSW (gms) |
|---|---|---|---|---|---|---|---|
| SHB099 | climber | Light red | 38.7 | 82.7 | 101.9 | 801.6 | 21.1 |
| SHB100 | SC | brown | 38.3 | 83.5 | 55.9 | 791.1 | 27.9 |
| Uyole 96 | SC | red | 35.0 | 83.1 | 110.0 | 450.5 | 42.0 |
| Mexico 54 | Climber | pink | 47.2 | 94.9 | 16.6 | 1310.2 | 32.2 |
| Mean | | | 40.15 | 84.9 | 81.4 | 810.2 | 25.2 |
| LSD (5%) | | | 3.58 | 2.08 | 20.2 | 119.5 | 2.7 |
| CV (%) | | | 6.4 | 10.8 | 17.9 | 10.6 | 7.8 |
| P≤0.05 | | | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 |

DF = days to flowering; DM = Days to maturity; AUDPC = area under disease progressive curves calculated from disease severity trends under natural inoculum from the field; GY = grain yield; HSW = hundred seed weight expressed in grams.

## 6.4   Discussion

Availability of suitable sources of resistance is a basic pre-requisite for a successful resistance breeding programme. Common bean landraces have long been indicated to harbor a number of useful resistance genes that could be exploited to develop varieties with multiple genes that are effective against a large number of *P. griseola* races (Arnason et al., 1994; Lopes et al., 2015). However, successful deployment of common bean cultivars with durable resistance depends partly on the sources of resistance genes and the type and number of pathogen used to screen the accessions (Pastor-Corrales et al., 1994)

Although the experiments were conducted under different environments; inoculated and natural infections, the results from screen house and field experiments revealed the variations that existed amongst common bean landraces of the SHT in response to *P. griseola* infections. Consequently, the use of the representative isolates of the races in screening for resistance under screen house environments, showed clearly which accessions are susceptible to what types of races compared to the results obtained in field experiments. The differences in response to ALS diseases shown by the landraces suggest the presence of candidate resistance genes against *P. griseola* races of the SHT within the genotypes evaluated. An example were accessions SHB002 and SHB091 that showed unique in disease resistance to both field and screen house conditions while others showed resistance either under field or screen house conditions. Similar variations

in response of the common bean landraces to *P. griseola* infections under different environmental conditions has been reported by Wagara et al., 2011, Goncalve – Vidigal et al., 2011 and Ddamulira et al., 2014.

Although the type and number of genes that caused the variable response within the landraces evaluated are not known yet, the results obtained in this study suggests the presence of a number of different genes that confer resistance against *P. griseola* races of the SHT. This was supported by the resistant and moderately resistant types of reactions recorded in this study that indicated the possible existence of both major and minor genes within the landraces collected.

Major genes are known to provide high levels of resistance conferred by single genes against crop pathogen and have been used to breed for resistance in various crops including maize, cowpea, rice, wheat and common bean (Wisser et al., 2006; Ellis et al., 2014; Oblessuc et al., 2015).They are known to occur in high number while providing complete resistance that are clear, easily detected and distinguished (Koch and Parlevliet, 1991; Burdon, 1994; Poland et al., 2008; Nemri et al., 2010). However, the primary limitations of major genes are the race specific in their action and lack of durability when acting singly with respect to pathogens that have high genetic variability such as *P. griseola* (Poland et al., 2008). In this study the majority of the landrace accessions evaluated were resistance to at least one of the races but none showed complete resistance to all of the twenty races used. Cultivar Mexico 54 posses three major genes *Phg-2*, *Phg-5* and *Phg-6* showed moderately resistance reactions when infected with *P. griseola* races 63/62 and 63/63, a good signs of defeat to some of the major genes carried in this cultivar. Mexico 54 is among the cultivars known to have high levels of resistance to *P. griseo*la races world over and have been used as a source of resistance genes to many bean breeding programmes. The same scenario was reported in wheat cultivars carrying stem rust resistance gene *Sr31* and *Sr34* that were recently overcomed by a new strain of stem rust caused by *Puccinia graminis* race *Ug99* (Pretorius et al., 2000; Stokstad, 2007). Breakdown of major genes have also been reported on potato (*Solanum tubersum*) against virulent populations of *Phytopthora infestans* that cause potato late blight.

Resistance conferred by minor genes is known to be qualitative, non – race specific with additive effect that results into moderate resistance reaction (Singh et al., 2005). In this regard, individual gene effect is small and adequate resistance is achieved by the contribution of many genes

(Singh et al., 2000). Breeding for resistance using minor genes have been reported in various breeding programmes including breeding for resistance against Mexican leaf and yellow rust done by CIMMYT and breeding against northern and southern blight in maize and to rice on bacterial blight (Valle et al., 2001; Singh et al., 2014)

The possibilities of existence of major and minor gene effects observed between landraces evaluated in this study can be manipulated in the bean breeding programme of the SHT to develop varieties with a lasting durability against *P. griseola*. The use of major and minor genes in breeding cultivar with resistance against diseases have been demonstrated in various plant breeding programmes including those dealing with rust diseases of wheat (Chen et al., 2009). It is worth noting that the seeds for each landrace used in this study were derived from single plants and the reactions to ALS disease observed for each landrace were uniform, suggesting that loci of the genes involved in the interactions with *P. griseola* has been fixed. Therefore, it is speculated that the varying responses observed between plants evaluated as representative of each accession were due to the presence of different resistance genes that can either have major or minor effects.

While efforts are required in developing resistant varieties of common bean against *P. griseola*, there are three options that are possible based on the results obtained in this study. These are; pyramiding major genes from landraces that indicated race specific resistance, breeding for durable resistance with those landraces that showed moderate resistance which under normal circumstances suggests presence of minor genes and pyramiding major resistant genes in the background of varieties carrying minor genes (Poland et al., 2008; Nisha et al., 2015). However a more detailed study is required to study the nature of inheritance of the genes present in the landraces that were rated resistant or moderately resistant.

## 6.5   Conclusion

This study indicated the importance of both screen house and field experiments in studying genetic resistance among common bean landraces and that the two methods complimented each other in deriving meaningful data. Although bean landraces are highly preferred by farmers in the SHT, the majority of them are susceptible to *P. griseola*. The resistant and moderately resistant reactions to the twenty races of the *P. griseola* observed in some of the accessions

evaluated suggest the presence of resistance genes that can be used in the resistance bean breeding programme against the pathogen. However genetic studies on the types of genes and mode of inheritance must be conducted. Of the 100 accessions evaluated; SHB002, SHB005 and SHB091 carried resistance genes that did not match those in Mexico 54. Considering the low yields of the accessions reported in this study, deliberate efforts must be made to develop varieties that are resistant to the pathogen.

# References

Aggarwal, V. D., M. A. Pastor – Corrales, R. M. A. Chirwa and R. A. Buruchara. 2004. Andean beans (*Phaseolus vulgaris* L.) with resistance to angular leaf spot pathogen (*Pseudocercospora griseola)* in the southern and Eastern Africa. Euphytica 136: 201 – 206**.**

Anarson, J. T., B. Braum, J. Gale, J. D. H. Lambert, D. Bergvinson, B.J. R. Philogen, J. A. Serratos, J. Mihm and D. C. Jewell. 1994. Variations in resistance of Mexican landraces of maize to maize weevil *Sitophilys zeamais* in reaction to taxonomic and biochemical parameters. Euphytica 74: 227 – 236

Busogoro, J. P., M. H. Jijakli and P. Lepoivre. 1999. Identification of novel sources of resistance to angular leaf spot disease of common bean within secondary gene pool. Plant breeding 118:417 - 423

Burdon, J. J. 1994. The distribution and origin of genes for race – specific resistance to *Melampsora lini* in *Linum marginale*. Evolution 48:1564 – 1575.

Bhullar N, K., K. Street, M. Mackay, N. Yahiaoui and B. Kelle. 2009. Unlocking wheat genetic resources for the molecular identification of previously undescribed functional alleles at the *Pm3* resistance locus. www.apnas.org. cgidoi10.1073 pnas.09041 (Accessed May 26, 2015)

Caixeta E.T., A. Borem, S. D. Fagundes, S. Niestche, E.G. de Barros and M.A. Moreira.2003. Inheritance of angular leaf spot resistance in common bean line BAT 332 and identification of RAPD markers linked to the resistance gene. Euphytica. 134(3):297–303.

Caixeta, E. T., A. Borem, A. L. Alzate – Marin, S. D. A. Fagundes, M. G. Silva, E. G. Barros. 2005. Allelic relationships for genes that confer resistance to angular leaf spot in common bean. Euphytica 145: 237 – 245

Camacho V., C. Taina, N. Maxted, M.  Scholten and B.  Ford-Lloyd. 2005. Defining and identifying crop landraces. Plant Genetic Resources 3(3): 373–384.

Chen Y H, R. M. Hunger, B. F. Carver, H. L. Zhang, L. L. Yan. 2009. Genetic characterization of powdery mildew resistance in U.S. hard winter wheat. Molecular Breeding. 24:141-152

Crous, P. W., M. M. Liebenberg, U. Braun and J. Z. Groenewald. 2006. Re-evaluating the taxonomic status of *Phaeoisariopsis griseola*, the causal agent of angular leaf spot of common bean. Studies in Mycology 55:163 – 173.

Czembor, J. H. 2002. Resistance to powdery mildew in selections from Moroccan barley landraces. Euphytica 125: 397- 409

Czembor, J. H. 2001. Sources of resistance to powdery mildew (*Blumeria graminis* f.sp. hordei) in Moroccan barley landraces. Canadian Journal of Plant Pathology 23: 260 – 269.

Ddamulira, G., C.Mukankusi, Ochwo-Ssemakula. R.Edema, P. Sseruwagi and P. Gepts. 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.

Ellis, J. G., E. S. Lagudah, W. Spielmeyer and P. N. Dodds. The past and future of breeding rust resistant wheat. 2014. Frontiers in Plant Science. 5: 1- 13

Fivawo, N.C and S.N. Msolla. 2012. The diversity of common bean landraces in Tanzania. Tanzania Journal of Natural and Applied Sciences 2: 337-351.

Forbes, G. A., O. Trillos, L. Turkensteen and O. Hidalgo. 1993. Field inoculation of potatoes with *Phytophthora infestans* and its effect on the efficiency of selection for quantitative resistance in the plants. Fitopatologia 28: 117-120.

Goncalve – Vidigal, M. C., A. S. Cruz, A. Garcia, J. Kami, P. S. V. Filho, L.L. Sousa, P. Mclean, P. Gepts and M. A. Pastor- Corrales. 2011. Linkage mapping of the *Phg-1* and *Co-1⁴*genes for resistance to angular leaf spot and anthracnose in the common bean cultivar AND 277. Theoretical and Applied Genetics 122 (5): 893 – 903.

Gwata, E. T and S. N. Silim. 2009. Utilization of landraces for genetic enhancement of pigeon pea in Eastern and Southern Africa. Journal of Food, Agriculture and Environment 7(2): 803 - 806

Jia, Y., Z. Wang and P. Singh. 2002. Development of dominant rice blast *Pi-ta* resistance gene markers. Crop Science 42:2145–2149.

Jones, E.R.L. and B. C. Clifford. 1997. Brown rust of wheat. UK Cereal Pathogen Virulence Survey 1996 Annual Report, pp. 18-28.

Koch, M.F. and J. E. Parlevliet. 1991. Genetic analysis of, and selection for, factors affecting quantitative resistance to *Xanthomonas campestris* pv *oryzae* in rice. Euphytica. 53:235- 245

Mahuku, G. S., C. E. Jara, C. C. Cajiao and S. Beebe. 2002. Sources of resistance to *Collectotrichum lindermuthianum* in the secondary gene pool of *Phaseolus vulgaris* and in crosses of primary and secondary gene pools. Plant Disease 86(12): 1383 – 1387.

Mahuku, G., C. Montoya, M. A. Henriquez, C. Jara, H. Teran and S. Beebe. 2004. Inheritance and characterization of angular leaf spot resistance genes present in common bean accession G10474 and identification of ALS marker linked to the resistant gene. Crop Science 44:1817 - 1824.

Marotti I, A. Bonetti, M. Minelli, P. Catizone, G. Dinelli. 2007. Characterization of some Italian common bean (*Phaseolus vulgaris*L.) landraces by RAPD, semi-random and ISSR molecular markers. Genetic Resource and Crop Evolution 54:175–88.

Mongi, R. 2016. Agronomic performances and economics of yield losses associated with angular leaf spot disease of common bean in the Southern Highlands of Tanzania. *Unpublished.*

Mwalyego, F. 1991. Effect of some bean mixtures on disease management and yield of beans. In:

Matagalpa, R.B. and N. Mole (ends) 1991. Bean research vol.6. The proceedings of the tenth bean research workshop, held at Soloing University of Agriculture, Morogoro, Tanzania. Pp 100-104.

Nemri, A., S. Atwell, A. Tarone, Y. Huang, K. Zhao, D. Studholme. 2010. Genome – wide survey of Arabidopsis natural variation in downy mildew resistance using combined association and linkage mapping. Proc. Natl. Academic Sci. U.S.A. 107: 10302 – 10307.

Nisha, R., M. Sivasamy, K. Gajalakshmi, P. Shajitha, V. K. Vikas, J. Peter, E. Punniakotti. 2015. Pyramyding stem rust resistance genes to develop durable and multiple disease resistant wheat varieties through marker aided selection. International Journal of Experimental Research. 5: 1–9

Ng'ayu-Wanjau, B. N., R. Melis, G. Mwangi, J. Sibiya and P. M. Kimani. 2015. Development of a breeding method for durable resistance to angular leaf spot in common bean. Euphytica DOI 10.1007/s10681-015-1582-1

Oblessuc, P.R., C.C. Matiolli, A. F. Chiorato, L. L. Benchimol-Reis and M. Melotto. 2015. Common bean reaction to angular leaf spot comprises transcriptional modulation of genes in the ALS10.1 QTL. Frontiers in Plant Science. doi:10.3389/fpls.2015.00152.

Pastor-Corrales, M.A., C. Jara and S. P. Singh. 1998. Pathogenic variation in, sources of, and breeding for resistance to *Phaeoisariopsis griseola* causing angular leaf spot in common bean.Euphytica103:161-171.

Payne, R. W., D.A. Murray, S.A. Harding, D.B. Baird and D. M. Soutar. 2011. An introduction to Genstat for Windows (14th Edition). VSN International, Heel Hempstead, UK

Pretorius Z.A., Singh R.P., Wagoire W.W., Payne T.S., 2000. Detection of virulence to wheat stem rust resistance gene Sr31 in *Puccinia graminis* f. sp. *tritici* in Uganda. Plant Disease 84: 203

Ray, A., D. Debal and B. Chattopadhayay. 2013. Phenotypic characteristic of rice landraces reveling independent lineages of short-grain aromatic indica rice. AoB Plants 5: plt032; doi: 10.1093/aobpla/plt032'

Sartorato, A. 2000. Pathogenic variation in *Phaeoisariopsis griseola* from Brazil. Annual Report of Bean Improvement Cooperatives. 43: 180–181

Singh, R.P., J.C. Nelson and M.E. Sorrells. 2000. Mapping Yr28 and other genes for resistance to stripe rust in wheat. Crop Science. 40:1148-1155

Singh, R.P., Huerta-Espino J., William H.M. 2005. Genetics and breeding for durable resistance to leaf and stripe rusts in wheat. Turkish Journal of Agriculture and Forestry. 29: 121–127

Singh, R., S. Herrera – Foessel, J. Huerta-Espino, S. Singh, S. Bhavani, C. Lan. 2014. Progress towards genetics and breeding for minor genes based resistance to Ug99 and other rust in CIMMYT high yielding spring wheats. Euphytica. 179: 175 - 186

Schwarzbach, E. 2001. Heat induced susceptibility of *mlo* – barley to powdery mildew (*Blumeria graminis* D. C. f.sp. *hordei* Marchal. Czech Journal of Plant Breeding and Genetics. 34: 3 – 10.

Schoonhoven, A. van and M. A. Pastor – Corrales. 1987. Standard system for evaluation of bean germplasm. Centro Internacional de Agricultura Tropical (CIAT), Cali Columbia.

Stokstad, E. 2007. Deadly wheat fungus threatens world' breadbaskets. Science 215:1786 – 1787.

Valle, F. X. R., J. E. Parlevliet, L. Zambolim. 2001. Concept in Plant disease resistance.Http://dx.doi.org/10.1590/50100 - 415822001 000300001. (Accessed May 26, 2015)

Payne, R.W., D. A. Murray, S. A. Harding, D. B. Baird and D. M. Soutar.2011. An Introduction to GenStat for Windows (14th Edition). VSN International, Hemel Hempstead, UK.

Wagara, I. N., A.W. Mwang'ombe, J.W. Kimenju, R.A. Buruchara and P.M. Kimani. 2011. Reaction of selected common bean genotypes to physiological races of *Phaeoisariopsis griseola* occurring in Kenya. African Crop Science. 19 (4):343 – 355.

Wisser, R. J., P. J. Balint-Kurti and R. J. Nelson. 2006. The genetic architecture of disease resistance in maize: a synthesis of published studies. Phytopathology. 96:120-129

Young, R. and J. D. Kelly. 1996a. RAPD markers flanking the *Are* gene for anthracnose resistance in common bean. Journal American Society Horticultural Science 121: 37-41.

Yuqiang l., S. Changchao, J. Ling, J. He, H. Wu, C. Pengand and J. Wan. 2009. The distribution and identification of brown plant hopper resistance genes in rice. Hereditas 146: 67-73.

Zhang X, M. W. Blair, S. Wang. 2008. Genetic diversity of Chinese common bean (*Phaseolus vulgaris* L.) landraces assessed with simple sequence repeat markers. Theoretical and Applied Genetics117:629–640.

# 7   Genetic and allelism analyses of resistance to *Pseudocercospora griseola* in common bean landraces of the Southern Highlands of Tanzania

**Abstract**

Breeding for angular leaf spot (ALS) disease resistance caused by *Pseudocercospora griseola* in common bean (*Phaseolus vulgaris* L.), is the most economical and sustainable way for controlling the disease because other measures are costly, ineffective or impractical to deploy. The objectives of this study were to determine combining ability, gene action, heritability, heterosis and allelism of the resistance genes present in selected common bean landraces widely grown in Tanzania. A total of eight parents that included seven common bean landraces and Mexico 54 were crossed in a half diallel mating design. An average of 21 $F_1$ seeds per cross were advanced to the $F_2$ in the screen house during 2014 and 2015. A total of 15,876 $F_2$ seeds were harvested and divided into two equal parts while maintaining individual crosses separately. An average of 441 $F_2$ seeds per cross was obtained and planted in two splits. The first split containing 204 seeds per cross was planted between February and July, 2015 and the second split between June and November. At full expansion of the first trifoliate leaf, artificial inoculations were done using spore suspension of the virulent and most prevalent race 63:55 at a concentration of 3 x $10^4$ml$^{-1}$. The experiments were laid out as a randomized complete block design with three replications. Disease assessments were visually recorded following the 1- 9 scale. Significant differences at P<0.05, P<0.01 and P<0.001 were observed between genotypes for general and specific combining ability indicating the importance of both additive and non-additive gene effects in the inheritance of ALS. However, the additive gene effect was predominant. The significant GCA and SCA suggested that parents and their progenies had sufficient genetic variation for effective selection within the segregating populations. A high broad sense heritability estimate at 0.97 was observed for the inheritance of ALS resistance. Significant heterosis and heterobeltiosis effects were detected among genotypes suggesting the existence of dominance gene effects. Allelism tests detected the presence of one and two non - allelic resistance genes as speculated by the ratios of 15:1 and 63:1 respectively that may be pyramided into desirable genotypes for durable resistance breeding. Further studies are required

to validate whether the genes identified in the test genotypes are associated with other minor or modifier genes in controlling the inheritance of ALS resistance.

**Key words**: *Pseudocercospora griseola*, common bean, combining ability, heritability, heterosis, heterobeltiosis, allelism

## 7.1   Introduction:

Breeding for disease resistance in common bean (*Phaseolus vulgaris* L.) is one of the major objectives in protecting the crop against various pathogens. *Pseudocercospora griseola* is among the most devastating and highly variable pathogen that causes angular leaf spot of common bean with significant negative impact on the livelihoods of people who depend on the crop. The disease colonizes leaves and progressively causes sunken brown spots on pods leading to premature defoliations followed by reduction in grain yield (Correa-Victoria et al., 1989; Saettler, 1991; Bassanezi et al., 2001). Yield losses due to ALS are estimated at 80% and have been reported in approximately 60 countries worldwide (Stenglein et al., 2003; Miklas et al., 2006). In Tanzania yield losses of 61% are recorded in the common bean growing agro – ecologies of the SHT (Mongi 2014 *unpublished*).

Although there are various methods available for the control of ALS disease such as the use of fungicides, phytosanitary and cultural practices, breeding for resistance is environmentally friendly, economically feasible and the best option especially to smallholder farmers who are the main producers of the crop. However, developing varieties with adequate levels of resistance against *P. griseola*, requires prior knowledge on the genetics of the parents to be used in the targeted cross combinations (Pastor – Corrales et al., 1994; Joshi et al., 2004)

Genetic resistance to *P. griseola* has been attributed by two general classes of genes. These are major genes known to exist as one or a group of more than one dominant genes and recessive genes known to complement each other in an additive manner (Singh and Saini, 1980; Mahuku et al., 2002). Major resistance genes also known as single or race specific have been used for decades in breeding for resistance in many crops including common bean, based on the classical gene for gene concept, where every resistance gene in the host has a corresponding avirulence gene in the pathogen (Flor, 1956; Flor, 1971). Major genes confer high levels of resistance with hypersensitive reactions but can be defeated by virulence genes present in specific races of the

174

pathogen when acting singly (Mahuku et al., 2004; Ogliari et al., 2005; Caixeta et al., 2008). The second categories are known as minor, recessive, polygenic or race nonspecific genes that act as multiple of genes ranging from two to more than ten (Young, 1996). Minor genes provide horizontal or partial resistance and have been described in common bean and other crops including maize and wheat. The inheritance of minor gene is complex, limiting its usage in breeding against diseases compared to the major genes (Skinner and Stuteville, 1989; Thakur, 2007).

The combinations of major and minor genes in a single genotype for durability of the resistance have been reported to be possible (Palloix et al., 2009), but the genetic analysis of resistance that exists within the chosen common bean parents must be done. Deployment of major and minor genes requires detailed study on the parents to be involved in a cross. One way of obtaining the genetic information and combining ability effects of parents and their crosses is the use of diallel genetic analysis developed by Griffing (Griffing, 1956). Diallel mating design has been used widely by bean breeders in obtaining information about a trait of interest from a set of fixed or random parental lines. Another important genetic parameter to be considered is heritability which allows comparison of the relative importance of the genes and environment that contribute to the variation of the trait within and across populations. Heritability studies have been used extensively in various crops to measure the magnitude of genetic variations between individuals in a population (Poehlman and Sleper, 1995; Visscher et al., 2008). The ability of the parental line to transfer important traits to its offsprings must be known. These are of two types; general combining ability (GCA) defined as the average performance of the parental line in producing hybrids. The GCA has been associated with additive gene effect and better segregants in later generation (Dhilon, 1975; Coyle and Smith, 1997; Pierozzi et al., 2008). It has been reported that, even crosses of divergent parents where at least one of them represents high GCA will have promising progenies (Aguiar et al., 2007). Specific combining ability (SCA) on the other hand is the relative performance of a hybrid, which is associated with non-additive gene action predominantly contributed by dominance and epistasis (Torres and Geraldi, 2007). The SCA reveals the best cross combinations and have been used in rice, wheat, beans and maize (Borghi and Perenzin, 1994; Machado et al., 2002; Torres and Geraldi, 2007). Therefore, choices of breeding parents based on both general and specific combining ability estimates is necessary and

have been successfully applied in breeding against fusarium root rot in common bean and in soybean rust resistance, among others (Machado et al., 2002; Maphosa et al., 2012).

Heterosis refers to a phenomenon in which the progenies exhibit phenotypic characteristics that are superior to their parents (Springer and Stupar, 2007). Heterosis has been used in the breeding and production of many crops and animals where better combinations of crosses were identified (Hansson and Westerberg, 2002). In self-pollinated crops such as common bean, estimation of heterosis is useful to understand the genetic contribution of parents towards their progenies for the traits of interest (Betran et al., 2003). Heterosis or hybrid vigor is a key indicator of the performance of the $F_1$ progenies over the mid or better parent (Radoevm et al., 2008). The mid parent heterosis also known as standard heterosis has been widely used in plant breeding (Virman, 1994), but several studies have indicated better parent heterosis also known as heterobeltiosis to be of higher economic importance (Meyer et al., 2004; Springer and Stupar, 2007). Heterosis may have positive or negative direction but both of them are useful in crop improvement programmes. The interpretation of positive or negative heterosis is dependent on the trait of interest (Alam et al., 2004).

Allelism tests of candidate resistance genes are important in determining whether the genes are alleles of the same locus or not, and to verify if the genes resemble those characterized previously. The information is important in the resistance breeding programmes especially when gene pyramiding methods are to be employed. An example is the allelism studies on common bean resistance genes against *P. griseola* that determined possible relationships among the genes before they were pyramided in the same background (Singland et al., 2013).

A considerable genetic diversity has been observed among local bean landraces grown by smallholder farmers in the Southern Highlands of Tanzania. These landraces showed promising resistance against the physiological races of *P. griseola* presently occurring in the zone. The effectiveness of these valuable germplasm should be determined using strategic crosses involving complementary lines to develop resistant varieties. It has been documented that the success of common bean breeding against diseases is related to the appropriate choice of parents which when hybridized will give a wide range of segregants that can be used to select for desirable genotypes. Therefore, the objectives of this study were to determine combining ability,

gene action, heritability, heterosis and allelism of angular leaf spot resistance genes present in selected common bean lines widely grown in Tanzania.

## 7.2 Materials and Methods

### 7.2.1 Genetic material and crosses

Seven common bean landraces with varying levels of resistance to *P. griseola* races were selected from 100 accessions collected from the bean growing agro-ecologies of the SHT (Table 7.1). Additionally, cultivar Mexico 54 known to have relatively high levels of resistance to *P. griseola* was included resulting in 8 parents. These were crossed in all possible combinations using half diallel technique developed by Griffing (1956). This generated 28 cross combinations. Five seeds of each parent were planted in the screen house in perforated plastic pots containing forest soil mixed with decomposed animal manure at a ratio of 3:1. The plants were later thinned to three plants per pot. At flowering stage, emasculation was done on the designated female plants by opening the flower buds manually with tweezers in which stamens were removed. The emasculated flower was immediately pollinated with pollen obtained from fully opened male flower. All the other parents which were not emasculated were selfed. On average 45 flower buds were emasculated and pollinated for each female cross. The $F_1$ seeds for each of the crosses made were advanced to $F_2$ generation through selfing at the Uyole Agricultural Research Institute screen houses during 2014 and 2015. Comparisons between parents and the $F_2$ population were done on seed color, shape, size and brightness to confirm success of the crosses.

### 7.2.2 Planting and experimental design

The 36 genotypes (28 crosses and eight parents) were planted during 2014 and 2015. An average of 441 $F_2$ seeds per cross was obtained and planted in two splits. The first split containing 204 seeds per cross was planted between February and July, 2015 under screen house condition. The weather during that time was characterized with high relative humidity (95 – 99%) and mild temperatures ($17^°C$ – $18^°C$), while the second split was planted between June and November 2015, and the weather was moderately dry (less than 90% humidity) and temperatures ranged from $15.5^°C$ to $21.1^°C$. For each split, three perforated plastic trays with the ability to hold 68 planted seeds were assigned to each cross. The soil to animal manure ratio was as indicated in

section 7.2.1 above. Three sequential plantings were done within seven day intervals. The trays were randomized while the planting time served as the blocking factor. Experiments were laid out in a randomized complete block design with three replications.

### 7.2.3 Inoculum preparation and inoculations

The study used a virulent race 63:55 of *P. griseola*. This race was selected from diverse isolates characterized in the previous study (Chapter 4). The race was identified as the most widely distributed and virulent in the bean growing agro ecologies of the SHT. Its virulence was maintained by frequent inoculations from cultured isolates on susceptible differential cultivar, Amendoin, in the screen house before its use in the current study. Re-isolation and culture preparations were performed according to Pastor – Corrales et al. (1998). Large quantities of inocula were prepared by inoculating V8 media in petri dishes with half a milliliter containing spores and mycelium fragments. The plates were incubated in darkness for $14 - 16$ days at $24^{\circ}$C to allow high levels of sporulation. The $F_2$ plants were inoculated at the V3 growth stage, i.e., a fully expanded first trifoliate (van Schoonhoven et al., 1987). Inocula were prepared with a suspension of conidia by scrapping the surface of the media with a spatula. The suspension obtained was filtered through cheese cloth where the number of conidia was adjusted with sterile water to $3 \times 10^4$ spores ml$^{-1}$ using a haemocytometer. Inoculations were done using a hand sprayer till run-off. The inoculated plants were covered with clear plastic sheets for 48 hours as a process of incubation. The plastic sheets were uncovered and the plants were allowed to undergo normal growth for the disease to develop.

### 7.2.4 Data collection and analysis

After 14 days twenty plants from each parent and $F_2$ progeny were selected randomly from each tray for disease assessments based on a $1 - 9$ scale, described by the International Center for Tropical Agriculture (CIAT), where; 1 = plants with no ALS symptoms; 2 -3 = plants with sparse and small lesions; 4-6 = plants with well defined but sparse lesions; 7 -9 = plants with well defined, expanding and many lesions (van Schoonhoven and Pastor – Corrales, 1987). Plants that scored $1 - 3$ were rated resistant (R), while those that ranged from $4 - 6$ and $7 - 9$ were rated moderately resistant (MR) and susceptible (S), respectively.

178

## 7.2.5 Allelism test

Seeds of the following four genotypes: SHB002, SHB005, SHB091 and Mexico 54 which possess resistance genes *Phg-2*, *Phg-5*, *Phg-6* and known for their resistance to *P. griseola* race 63:55 were used as differential cultivars. These genotypes were included in the crosses involving the diallel mating design excluding reciprocals as described earlier. All the crosses were performed in a screen house. The resistant x resistant crosses were maintained and harvested to produce $F_1$ seeds. The $F_2$ seeds were produced by selfing $F_1$ plants (200 – 204 plants per cross) and were used to study allelic relationships among these genotypes. Mendelian genetic ratios were used to determine segregation patterns of each cross at $F_2$ for the four genotypes that were resistant when inoculated with race 63:55. In this study, genotypes that scored 1- 3 were rated resistant and those with 4- 9 on the 1 – 9 scale were rated susceptible. Three phenotypic ratios (resistant: susceptible) commonly used in allelism studies of common bean were adopted to analyze the $F_2$ (Goncalves – Vidigal et al., 2007; Singlard et al., 2013). These ratios were as follows: 3:1 (monogenic ratio based on one dominant gene); 15:1 (digenic ratio based on two independent dominant genes) or 63:1 (trigenic ratio based on three dominant genes). To identify the ratio that best fits the segregation patterns of the $F_2$ mentioned above, the ratio of resistant and susceptible plants obtained in each cross was calculated. The data were later analyzed using the chi-square test for goodness of fit procedure (Pan et al., 1998; Caixeta et al., 2005; Srinivasan et al., 2008). The chi-square test was calculated using the following formula:

$$\chi^2_{\,(n-1)df} = \sum(\text{observed} - \text{expected})^2 / \text{expected} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \ldots\ldots\ldots.(1)$$

## 7.2.6 Combining ability analysis

The combining ability analysis was done using Griffing Model 1 (fixed), Method 2 (parents and crosses, no reciprocals) (Griffing, 1956), in which treatment sums of squares were portioned into GCA and SCA as follows;

$$Y_{ij} = \mu + g_i + g_j + s_{ij} + e_{ijlk} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \ (2)$$

Where; $Y_{ij}$ is the mean phenotypic value; $\mu$ is general mean; gi and gj are the GCA effects of the $i^{th}$ and $j^{th}$ parents, respectively; $s_{ij}$ is SCA effects of the $ij^{th}$ cross and $e_{ijlk}$ is the residual effect.

General Predicted Ratio (GPR) (Baker, 1978), was used to determine the preponderant gene action as follows:

$2\sigma^2_g / (2\sigma^2_g + \sigma^2_s)$ ................................................................................................ (3)

Additive and non additive gene action was calculated using variance components calculated from the analysis of variance (ANOVA) tables as follows:

$\sigma^2_A = (MS_G - MS_S) / (P+2)$ ................................................................................ (4)

$\sigma^2_D = MS_S - MS_E$

$\sigma^2_E = MS_E$

Where; $MS_G$ and $MS_S$ are mean square for GCA and SCA respectively

$MS_E$ = Error Mean square

P = number of parents

Broad ($H^2$) and narrow ($h^2$) sense heritability were computed using the following formulae; with the assumption that segregation of chromosomes were random, no epitasis, no maternal effects, presence of homozygous parents and independent assortment.

$H^2 = (\sigma^2_{A} + \sigma^2_{D}) / \sigma^2_{A} + \sigma^2_{D} + \sigma^2_{E}$ ................................................................. (4)

$h^2 = (\sigma^2_{A}) / \sigma^2_{A} + \sigma^2_{D} + \sigma^2_{E}$ ................................................................. (5)

Where $\sigma^2_A$ is the variance due to additive gene effects; $\sigma^2_D$ is the variance due to dominance gene effects and $\sigma^2_E$ is the error variance

The performances of the progenies for each environment were estimated by relative heterosis (heterosis over the mid-parental value) and heterobeltiosis (heterosis over the better parent) according to the following formulae;

$[(F_1 - MP)/MP*100]$ ........................................................................................... (6)

$[(F_1 - BP)/BP*100]$ ........................................................................................... (7)

Where MP = Mid parent ([P1+P2]2)); BP = Better parent in a cross.

## 7.3   Results

### 7.3.1   Performance of parents and progenies

The eight parents used in this study showed variation in ALS disease reaction when infected with *P. griseola* race 63:55. Low mean severity values that indicated high levels of resistance were

displayed by the cultivar Mexico 54 and the landrace genotype SHB091 showing disease severity of 1.1 and 1.8, in that order (Table 7.2). High ALS disease severity was recorded in the landrace SHB042 showing susceptibility to ALS infection. This landrace is commonly known as Kablanketi which is highly preferred in Tanzania. Overall, there were adequate levels of infection to discriminate the genotypes for ALS susceptibility. The typical ALS disease symptoms on this landrace were noticed as early as 10 days after inoculation followed by severe infections that resulted in premature defoliation of the infected leaves. The following landraces: SHB001, SHB080 and SHB098 also had susceptible disease reactions (Table 7.1).

**Table 7.1: Characteristics of common bean landraces used as parents in the present study.**

| Name/code of genotypes | Hundred Seed weight (gms) | Seed size | Seed color | Reaction to race 63:55 |
|---|---|---|---|---|
| SHB002 | 23.6 | small | Yellow | Resistant |
| SHB005 | 37.1 | Medium | Whitish striped | Resistant |
| SHB091 | 26.1 | Small | White | Resistant |
| Mexico 54 | 32.2 | Medium | Pink | Resistant |
| SHB001 | 22.6 | Small | Purplish pink | Susceptible |
| SHB042 (KBT) | 30.5 | Medium | Grayish purple | Susceptible |
| SHB080 | 19.9 | Small | Purplish | susceptible |
| SHB098 | 16.5 | small | Cream | Susceptible |

KBT = Kablanketi

The expression of ALS disease varied considerably between crosses and between plants within a cross during the $F_1$ and $F_2$ generations (Appendix A1, Figure 7.1). Disease severity varied from 1.1 – 6.8 during the $F_1$ and 1.1 – 7.7 during the $F_2$ generations. Crosses between parents with lower scores resulted in progenies with lower scores, while the reverse was true for crosses made between parents with higher scores. In general, crosses that resulted from susceptible parents (with high scores) showed higher levels of resistance (lower values) compared to the parents. For instance, crosses involving SHB001 x SHB080 and SHB080 x SHB042 all had lower values of 6.6 (Table 7.2). These cross had the following parents that were susceptible and disease ratings of 7.6 (SHB001), 8.5 (SHB042) and 7.6 (SHB080). Overall, the crosses generated from SHB002

x Mexico 54 and SHB091 x Mexico 54 were exceptional showing the highest levels of ALS resistance against race 63:55.

**Table 7.2: Mean scores of angular leaf spot reaction of common bean parents tested during season 1 (above diagonal) and season II (below diagonal) evaluated in the Southern Highlands of Tanzania.**

| Name/code of parents | SHB002 | SHB005 | SHB091 | Mexico 54 | SHB001 | SHB042 | SHB080 | SHB098 |
|---|---|---|---|---|---|---|---|---|
| SHB002 | | 1.9 | 2.3 | 1.9 | 7.0 | 7.4 | 6.8 | 7.0 |
| SHB005 | 2.1 | | 2.9 | 2.8 | 7.0 | 7.9 | 6.5 | 4.7 |
| SHB091 | 2.5 | 2.0 | | 1.2 | 7.3 | 6.8 | 6.3 | 6.4 |
| Mexico 54 | 1.1 | 1.5 | 1.2 | | 5.5 | 5.7 | 6.1 | 4.9 |
| SHB001 | 4.1 | 6.0 | 5.7 | 4.5 | | 4.9 | 7.9 | 7.8 |
| SHB042 | 5.8 | 6.3 | 5.5 | 4.5 | 6.0 | | 7.4 | 8.0 |
| SHB080 | *5.8* | 5.8 | 6.6 | 5.0 | 6.6 | 6.6 | | 7.5 |
| SHB098 | 6.0 | 6.5 | 5.5 | 4.3 | 6.1 | 6.0 | 6.5 | |

### 7.3.2   Analysis of variance and estimates of combining ability across environments

To study the breeding value of the parental lines and their progenies, analysis of variance for ALS disease scores was done. Significant differences were observed between genotypes and the environments in which they were evaluated. However, the interaction was not significant (Table 7.3). The mean squares due to general combing ability (GCA) and specific combining ability (SCA) were highly significant indicating the presence of considerable genetic variation useful for selection of resistant genotypes against *P. griseola*. The GCA effects for resistant parents ranged from -1.01 to -2.02 in a desirable direction, while the GCA for the susceptible parents ranged from +1.06 to +1.42. The Bakers ratio was close to a unity (0.85) indicating the predominance of additive over non-additive gene action in the inheritance of ALS resistance. Mexico 54, SHB002 and SHB002 were the best combiners based on the low mean score for ALS disease and high negative values. According to Viana and Matta (2003), parents with negative GCA effects are considered as good combiners with high contribution towards resistance while positive values stand for the opposite based on the rating scale used where 1 is resistant and 9 is susceptible.

**Table 7.3: Analysis of variance and combining ability of 36 common bean genotypes (eight parents and 28 crosses) for ALS reaction when evaluated across two environments using three replications in the Southern Highlands of Tanzania**

| Source of variation | df | Mean square | F. value | P. value |
|---|---|---|---|---|
| Environment | 1 | 2.66 | 7.06 | 0.008* |
| Replication (Environment) | 4 | 0.44 | 1.18 | 0.323 ns |
| Genotypes | 35 | 28.23 | 74.74 | 0.0001** |
| Genotypes x Environment | 35 | 0.238 | 0.63 | 0.94 |
| Error | 140 | 0.377 | | |
| GCA | 7 | 10.99 | 29.1 | 0.0000*** |
| SCA | 28 | 3.96 | 10.48 | 0.0000*** |
| GCA x Environment | 7 | 0.148 | 0.392 | 0.906 ns |
| SCA x Environment | 28 | 0.268 | 0.709 | 0.855 ns |

$2\sigma^2_{g} / (2\sigma^2_{g} + \sigma^2_{s}) = 0.85$

*, **, *** Significant at P< 0.05; P< 0.01; P< 0.001 respectively.
df=degree of freedom; GCA=General combining ability; SCA=specific combining ability

The SCA effect has been indicated to represent the dominance and epistatic interactions that can be related to heterosis in hybrid production (Singh, 2000). In this study, there existed variation for SCA effects in which two crosses showed significantly large negative values suggesting the contribution of non-additive gene effects to ALS resistance. The range of ALS means scores were 1.2 – 6.6 on a scale of 1 – 9. The SHB091 x Mexico 54 had the lowest mean scores of the disease and the largest negative SCA value. SHB091 and Mexico 54 are characterized as resistant to race 63:55. On the other hand, cross SHB080 x SHB098 had a lower negative value compared to the two parents that were characterized as susceptible to ALS disease (Table 7.4).

**Table 7.4: : Mean ALS scores of common bean parents and corresponding crosses with combining ability effects when evaluated across two environments in the Southern Highlands of Tanzania**

| Name/code of parents | ALS score | GCA effects | Cross | ALS score | SCA effects |
|---|---|---|---|---|---|
| SHB002 | *2.3* | -1.11** | SHB002 x SHB005 | 2.1 | -0.48* |
| SHB005 | 2.1 | -0.99* | SHB002 x SHB091 | 2.5 | -0.77*** |
| SHB091 | 1.8 | -1.01** | SHB002x Mexico54 | 1.6 | -0.42* |
| Mexico 54 | 1.1 | -2.02** | SHB002 x SHB001 | 4.1 | -1.09*** |
| SHB001 | 7.6 | 1.29** | SHB002 x SHB042 | 5.8 | -0.067 ns |
| SHB042 (KBT) | 8.5 | 1.35** | SHB002 x SHB080 | 5.8 | 0.53** |
| SHB080 | *7.6* | 1.45** | SHB002 x SHB098 | 6.0 | 0.47** |
| SHB098 | 7.0 | 1.06** | SHB005 x SHB091 | 2.0 | -0.90*** |
| | | | SHB005xMexico 54 | 1.5 | -1.04*** |
| | | | SHB005 x SHB001 | 6.0 | -0.84** |
| | | | SHB005 x SHB042 | 6.3 | 0.64*** |
| | | | SHB005 x SHB080 | 5.8 | 0.911*** |
| | | | SHB005 x SHB098 | 6.5 | 0.34 ns |
| | | | SHB091xMexico 54 | 1.2 | -1.19 *** |
| | | | SHB091 x SHB001 | 5.6 | -0.35ns |
| | | | SHB091 x SHB042 | 5.5 | 1.09** |
| | | | SHB091 x SHB080 | 6.6 | 1.2** |
| | | | SHB091 x SHB098 | 5.5 | 0.14ns |
| | | | SHB001xMexico 54 | 4.5 | 0.16 ns |
| | | | SHB042xMexico 54 | 4.5 | 0.09 ns |
| | | | SHB080 x Mexico 54 | 5.0 | 0.53* |
| | | | SHB098xMexico 54 | 4.3 | 0.01 ns |
| | | | SHB001 x SHB042 | 6.0 | -0.71*** |
| | | | SHB001 x SHB080 | 6.6 | -0.11ns |
| | | | SHB001 x SHB098 | 6.1 | 0.72*** |
| | | | SHB042 x SHB080 | 6.6 | -1.17*** |
| | | | SHB042 x SHB098 | 6.0 | -0.23ns |
| | | | SHB080 x SHB098 | 6.5 | -0.81* |

*. **, *** Significant at P< 0.05; P< 0.01; P< 0.001 respectively

ALS=angular leaf spot; GCA=General combing ability; SCA=specific combining ability

### 7.3.3    Gene action and heritability estimates

Additive gene action was higher when compared to the non–additive effects and accounted for 73.8% and 23.6% of the total variance respectively. This suggests presence of minor genes that contribute additively to the expression of ALS resistance (Table 7.5). Data on broad sense heritability ($H^2$) estimates and narrow sense heritability estimates on ALS disease reaction are also shown in Table 5. The broad sense heritability was higher than the narrow sense heritability ($h^2$). The high $\sigma^2_A$: $\sigma^2_D$ suggested the predominance of additive gene action.

**Table 7.5: Estimates of heritability, additive and non-additive gene action among 36 common bean genotypes for the angular leaf spot disease resistance**

| Genetic parameter | $F_2$ population |
|---|---|
| $\sigma^2_A$ (additive) | 10.59 |
| $\sigma^2_D$ (non additive) | 3.39 |
| $\sigma^2_E$ (Error variance) | 0.37 |
| $H^2$ (broad sense) | 0.97 |
| $h^2$ (narrow sense) | 0.73 |
| $\sigma^2_A$: $\sigma^2_D$ | 3.12 |

### 7.3.4    Heterosis of common bean lines evaluated in the Southern Highlands of Tanzania

In this study, heterosis was determined as the deviation from mid and better parent. Analysis of variance was done and the results are presented in Table 7.6. Mean square for entries, parents, overall heterosis, GCA and SCA were significant. However, the average heterosis was not significant. Although the mean squares for combining ability (GCA and SCA) were all significant, the mean square value for GCA effect were greater than that of SCA as observed earlier.

**Table 7.6: Analysis of variance for ALS disease reaction caused by Pseudocercospora *griseola* in the diallel crosses made between eight selected common bean lines**

| Sources of variation | df | Mean square | F value | P value |
|---|---|---|---|---|
| Entries | 35 | 28.23 | 74.74 | 0.0001*** |
| Parents | 7 | 13.34 | 35.33 | 0.001** |
| Heterosis | 28 | 3.96 | 14.78 | 0.000*** |
| Average | 1 | 3.4 | 11.56 | 0.18 ns |
| Parents | 7 | 4.5 | 20.48 | 0.000*** |
| GCA | 7 | 10.99 | 29.10 | 0.0000*** |
| SCA | 20 | 4.56 | 20.48 | 0.0000*** |
| Error | 140 | 0.37 | | |

*. **, *** Significant at P< 0.05; P< 0.01; P< 0.001 respectively.
df=degrees of freedom; GCA=GCA=General combing ability; SCA=specific combining ability

Means for ALS disease reaction, heterosis and heterobeltiosis effects are presented in Table 7.7. Both positive (+) and negative (-) directions of the genetic effect were observed. The disease resistant plants had lower severity ratings that resulted into negative values. The range for heterosis effects were -22.7 to +32.5 when mid-parent values were used whereas a range of -29 to +37 values were determined when the better parent was used in the analysis. Crosses made from resistant x resistant parents (SHB002 x Mexico 54) showed lowest value (-25.0) while highest value was recorded on cross SHB042 x SHB98, in which both parents were susceptible to race 63:55. The crosses that were resistant to ALS with low severity scores on the 1 – 9 scale had SHB002, SHB005, SHB091 and Mexico 54 as one of the parents.

**Table 7.7:  Mean mid-parent values, mid-parent and better-parent heterosis and and heterobeltiosis for angular leaf spot disease resistance among 28 common bean families derived from half-diallel crosses of eight parents**

| Family/cross | Mid parent value | Mid-parent heterosis | Better-parent heterosis | Heterobeltiosis |
|---|---|---|---|---|
| SHB002 x SHB005 | 2.9 | -11.2 | 2.8 | -27.2 |
| SHB002 x SHB091 | 2.3 | -10.9 | 2.8 | -12.1 |
| SHB002 x Mexico54 | 1.9 | -25.0 | 1.3 | -41.1 |
| SHB002 x SHB001 | 5.2 | -1.4 | 2.8 | -3.9 |
| SHB002 x SHB042 | 5.7 | -7.3 | 2.8 | -14.4 |
| SHB002 x SHB080 | 5.2 | -6.3 | 2.8 | -9.3 |
| SHB002 x SHB098 | 4.9 | 8.0 | 2.8 | 6.8 |
| SHB005 x SHB091 | 2.2 | 15.9 | 1.8 | 2.9 |
| SHB005 x Mexico54 | 2.1 | -23.5 | 1.3 | -27.5 |
| SHB005 x SHB001 | 5.3 | -2.0 | 3.0 | -6.3 |
| SHB005 x SHB042 | 5.8 | -2.7 | 3.0 | -9.3 |
| SHB005 x SHB080 | 5.3 | 12.3 | 3.0 | 9.1 |
| SHB005 x SHB098 | 5.0 | -21.7 | 3.0 | -29.1 |
| SHB091 x Mexico 54 | 1.5 | -22.5 | 1.3 | -25.0 |
| SHB091 x SHB001 | 4.7 | 16.5 | 1.8 | 12.6 |
| SHB042 x SHB091 | 5.2 | -3.7 | 1.8 | -13.3 |
| SHB080 x SHB091 | 4.7 | -2.0 | 1.8 | -4.0 |
| SHB098 x SHB091 | 4.4 | 10.2 | 1.8 | 7.9 |
| SHB001 x Mexico 54 | 4.4 | 5.0 | 1.3 | 3.3 |
| SHB042 x Mexico 54 | 4.8 | -18.4 | 1.3 | -21.1 |
| SHB080 x Mexico 54 | 4.4 | -8.8 | 1.3 | -15.0 |
| SHB098 x Mexico 54 | 4.1 | -16.4 | 1.3 | -17.6 |
| SHB001 x SHB042 | 8.1 | -14.6 | 7.6 | -21.1 |
| SHB001 x SHB080 | 7.6 | 11.2 | 7.6 | 21.1 |
| SHB001 x SHB098 | 7.3 | -4.0 | 7.6 | -12.8 |
| SHB042 x SHB080 | 8.1 | -15.2 | 7.0 | 21.4 |
| SHB042 x SHB098 | 7.8 | 19.3 | 7.0 | 26.1 |
| SHB080 x SHB098 | 7.3 | -16.6 | 7.0 | 18.6 |

### 7.3.5 Allelism tests

Allelism tests were conducted to determine whether the resistance genes in landraces SHB002, SHB005, SHB091 and cultivar Mexico 54 were allelic or not. All the $F_1$ genotypes that resulted from the crosses of these genotypes were resistant to the *P. griseola* race 63:55. However, the levels of resistance varied between plants and between crosses. According to the ALS disease assessment scale, no susceptible plants were observed in the $F_1$ population. When the $F_2$ plants were evaluated, susceptible plants were noticed in all of the six progenies indicating that the parents had resistance genes that were probably non–allelelic. Allelism test showed segregations that fitted ratios of 15R:1S and 63R:1S (Table 7.8). The SHB002 x SHB005; SHB002 x SHB091 and SHB005x SHB091 fitted the 15R: 1S ratio indicating the presence of two independent complementary genes. The SHB091 x Mexico 54; SHB005 x Mexico 54 and SHB002 x Mexico 54 had a ratio of 63R: 1S that explained the presence of three non-allelic genes. For all cases, the Chi square test indicated that the data fits the ratios in which they were tested.

**Table 7.8: Allelism test for resistance to *P. griseola* against race 63:55 in common bean landraces of the SHT**

| Families | TNP | Observed Resist | Observed Susp. | Expected Resit. | Expected Susp | Ratio | $\chi2$ | P value |
|---|---|---|---|---|---|---|---|---|
| SHB002 x SHB005 | 185 | 172 | 13 | 173.4 | 11.5 | 15:1 | 0.21 | 0.85 |
| SHB002 x SHB091 | 195 | 186 | 9 | 182.8 | 12 | 15:1 | 0.81 | 0.89 |
| SHB002 x Mexico 54 | 200 | 195 | 4 | 196.8 | 3.1 | 63:1 | 0.27 | 0.33 |
| SHB005 x SHB091 | 167 | 160 | 7 | 156.6 | 10.4 | 15:1 | 1.18 | 0.05 |
| SHB005 x Mexico54 | 200 | 195 | 5 | 187.5 | 12.5 | 63:1 | 0.51 | 0.31 |
| SHB0091 x Mexico 54 | 189 | 187 | 4 | 186.0 | 2.9 | 63:1 | 0.42 | 0.78 |

TNP=total number of plants Resist = resistance; Susp. = susceptible

### 7.4 Discussion

Breeding for angular leaf spot disease resistance in common bean remains the most economical and sustainable way of protecting the crop against *P. griseola*. However, the success of the breeding programme is related to the appropriate choice of parents and good mating design,

which will provide progenies with wide genetic variability for selections and improved genetic gain for the trait of interest.

The eight parents used in this study were diverse in ALS disease reactions when infected with the virulent and widely distributed race 63:55. The significant differences observed between the genotypes further supported the existence of genetic variability among the genotypes evaluated. Similarly, the different scores of ALS disease for the parents, $F_1$ and $F_2$ revealed the existence of genetic variability within the materials.

The value of the seven landraces plus cultivar Mexico 54 in hybrid formation was quantified by the combining ability effects. In this study, both GCA and SCA were highly significant validating the importance of materials in developing crosses. It has been indicated that highly significant GCA and SCA suggests the presence of both additive and non-additive gene effects in the inheritance of ALS disease resistance. Additive gene action is measured by the extent to which phenotypic individual differences are predictable from the additive effect of the alleles (Vakili et al., 2010). In this study, parents with low and negative GCA were considered better combiners as negative values indicated contribution towards resistance whereas positive values contribution towards susceptibility based on the rating scale used where 1 represents resistant and 9 susceptible (Ramezanpour et al., 2010). Parents SHB002, SHB005, SHB091 and Mexico 54 exhibited significantly higher negatives values, and therefore may be exploited in the ALS disease resistance breeding programme as it is more likely that they will produce the best hybrid combinations that can easily be identified through pedigree selection method (Kalwar et al., 1999). Additionally, high additive gene effect increases the possibility of conducting progeny selections in early generation.

Significances of GCA and SCA observed in this study were an indication that parents and their progenies had sufficient genetic variation for effective selection within the segregating populations. It is expected that, parents with high GCA will produce transgressive segregants as a result of accumulation of additive genetic component (Kimani and Derera, 2000), thereby facilitating pedigree selection methods (Ragsdale and Smith, 2007).

Since the ratio of GCA and SCA was greater than unity, it was clear that additive gene effects were more important than non-additive gene effects. The results obtained in this study are in

agreement with those reported by Mendonca et al. (2003), that resistance genes against *P. griseola* in common bean acts additively. The importance of additive and non-additive gene action in resistance breeding against crop pathogens has been reported in common bean, sunflower, wheat and cotton, among others (Li et al., 1995; Machado et al., 2002; 2004; Joshi, 2004; Iiyas et al., 2007).

The performances of the progenies were compared by their respective SCA values obtained from the diallel mating design. This identified three types of progenies based on the type of parents involved in a cross. These were; progenies that resulted from good combiners with low negative value; two poor combiners with better progeny in terms of the SCA values; good and poor combiners with better progeny. The differences observed on parents and progenies may have been due to various factors including over dominance and epistatic interactions. However, this was not tested in this study. The hypothesis of over dominance, dominance and epistatis as related to the gene action within progenies has been reported by Goodnight (1999) and Springer (2007).

The efficacy of the selection of superior progenies depended on the genetic variation and the percentage of heritability. It has been indicated that populations that are genetically uniform normally showed low heritability than genetically diverse populations (Singh and Prasad, 1999). In this study broad sense heritability was higher than the narrow sense heritability. This was a good indication that selection for ALS disease resistance will be possible with significantly high progress. Narrow sense heritability indicates high additive variance. Narrow sense heritability estimates have been classified into three groups; values of >50% were classified as high, 30 – 50%, medium and bellow 30% as low (Bhateria et al., 2006). The high narrow sense heritability observed in this study (73%) suggests that simple selection within the segregating populations could be effective in improving ALS disease resistance in common bean.

Crosses that showed high negative heterosis values for ALS disease resistance had either R x R or R x S parents in the combination. Negative heterosis is desirable as it indicates the superiority of the progenies to either mid- or better-parents as a result of combined gene interactions. Crosses that showed high negative values for ALS disease resistance had either R x R or R x S in the combination. An example was the SHB002 x Mexico 54 (R x R) and SHB042 x Mexico 54 (S x R). High negative values were also observed in crosses that involved S x S with an example

of SHB042 x SHB098. However, the mechanisms underlying the better performance of the progenies over the susceptible parents were not evident in this study. High heterosis in self-pollinated crops has been reported in experiments conducted under controlled environments (Meyer et al., 2004).

Under normal circumstances, a cross between two resistant parents would have resistant progenies in the $F_1$ and $F_2$ if the genes involved for resistance are allelic and dominant in nature. The $F_1$ plants for the entire crosses involved in this study were resistant but the $F_2$ plants segregated into two types of ratios as indicated earlier. The crosses SHB002 x SHB005 and SHB005 x SHB091 had a ratio of 15:1, while crosses SHB002 x SHB091 fitted into 15:1, revealing the existence of two non-allelic possibly contributed to the offspring from each parent. Therefore, parents SHB002, SHB005, SHB091 are speculated to carry one resistant gene each. Further studies need to be conducted to understand more of these genes. It is well known that Mexico 54 carries three dominant genes to *P. griseola* but according to the data obtained in the crosses that involved the cultivar, there is a possibility that the variety contributed two of its genes in the crosses with the ratio of 63R:1S, as these were crosses made between resistant parents. This type of ratio was reported when cultivar BAT332 (R) was crossed with Oura Negro (R) (Sanglard et al., 2013). Cultivar BAT 332 is known to have allelic form of *Phg $6^2$* while Oura Negro harbors at least one resistance locus. For the SHB002 x SHB091 and SHB005 x Mexico 54, the observed resistant to susceptible ratio fitted 15R:1S, a typical segregation pattern of two genes that suggested the contribution of different single gene from each parent to the resistance. These can be pyramided into single genotype of interest to obtain durable resistance (Caixeta, et al., 2005). It has been indicated that accumulation of resistant genes with major effects as those obtained in this study, have the ability to delay the appearance of disease and therefore counteract with frequent outbreaks of the disease that is often experienced in the bean growing agro-ecologies of Tanzania.

It has been reported by various scientists that most of the genes that are against *P. griseola* are in the Mesoamerican genotypes that are classified in Mesoamerican gene pool. Studies conducted on Cornell 49-242, Mexico 54 and BATT 332 have indicated the presence of major genes that confer resistance to the pathogen (Ferreira et al., 2000; Sartato et al., 2000). The present study involved Mesoamerican type of landraces and revealed similar results. Since resistance genes

originating from the Mesoamerican gene pool have been indicated to be effective against Mesoamerican and Andean types of *P. griseola* races, considering gene pyramiding using the available information in this study, may contribute in alleviating the problem associated with ALS disease in Tanzania.

## 7.5   Conclusion

The study demonstrated the existence of genetic variability for ALS disease resistance in common bean landraces evaluated. Significant GCA and SCA effects suggested the importance of both additive and non-additive gene effects for the expression of ALS disease resistance. The predominant additive gene effects coupled with high heritability and high negative heterosis suggested that, resistant progenies from crosses made between SHB002, SHB005 and SHB091 could be identified and selected from the segregating populations. The diverse genes for resistance against the virulent and prevalent race 63:55 identified can be used in improving resistant levels of common bean varieties of the Tanzania. The allelism test carried out in the four resistance cultivars revealed the presence of three genes within the parents that were readily transferred to the offspring as indicated into the $F_2$ segregations ratios. These can be pyramided in the same background for development of varieties with durable resistance against *P. griseola*.

# References

Aguiar P. A., J. C. V. Penna, E. C. Freire and L. C. Melo. 2007. Diallel analysis of upland cotton cultivars. Crop Breeding and Applied Biotechnology 7: 353-359.

Alam, M. F., M. M. R. Khan, M. Nuruzzaman, S. Parvez, A. M. Swaraz, I. Alam, and N. Ashan.2004. Genetic basis of heterosis and inbreeding depression in rice (*Oryza sativa* L.). Journal of Zhejiang University (Science) 20: 406- 411.

Baker, R. J. 1978. Issues in diallel analysis. Crop Science. 18:533 - 536

Barelli, M.A.A., M. C. Gonçalves-Vidigal, A. T. Amaral Júnior, P. S. VidigaL Filho, and C. A.Scapim. 2000a. Combining ability among common bean cultivars adapted to the Northwest region of Paraná State, Brazil. Bragantia 59:159-164.

Barelli, M.A.A., M. C. Gonçalves-Vidigal, A. T. Amaral Júnior, P. S. VidigaL Filho and C. A.Scapim. 2000b. Diallel analysis of the combining ability of common bean (*Phaseolus vulgaris* L.) cultivars. Brazilian Archives of Biology and Technology. 43: 409-414.

Bassanezi, R. B., L. Amorim, A. B. Filho, B. Hau and R. D. Berger. 2001. Accounting for photosynthetic efficiency of bean leaves with rust, angular leaf spot and anthracnose to assess crop damage. Plant Pathology 50: 443-452

Betran F.J., J. M. Ribaut, D. Beck and D. G. Leon. 2003. Genetic diversity, specific combining ability, and heterosis in tropical maize under stress and non stress environments. Crop Science. 43**:** 797–806.

Bhateria, S., S. P. Sood, A. Pathania. 2006. Genetic analysis of quantitative traits across environments in Linseed (*Linumusitatisimum* L.). Euphytica 150: 185-194

Borghi, B., and M. Perenzin. 1994. Diallel analysis to predict heterosis and combining ability for grain yield, yield components and bread-making quality in bread wheat (*T. aestivum*). Theoretical and Applied Genetics 89:975–981.

Caixeta E.T., A. Borém, A. L. Alzate-Marin, S. Fagundes, S. M. G. Morais, E. G. Barros and. A. Moreira. 2005. Allelic relationships for genes that confer resistance to angular leaf spot in common bean. Euphytica. 145:237–245.

Caixeta, E. T., A. Borém, S. D. A. Fagundes, S. Niestche, E. G. Barros, and M. A. Moreira.2003. Inheritance of angular leaf spot resistance in common bean line BAT 332 and identification of RAPD markers linked to the resistance gene. Euphytica.134: 297-303

Correa-Victoria, F.J., M. A. Pastor-Corrales and A. W. Saettler. 1989. Angular leaf spot. In: Bean Production Problems in the Tropics (Schwarts HF, Pastor-Corrales MA, eds). Centro Internacional de Agricultura Tropical, Cali, Colombia: 59–75.

Coyle G. G and C. W. Smith. 1997. Combining ability for within-boll yield component in cotton, *Gossypium hirsutum* L. Crop Science 37: 1118-1122.

Dhillon, B. S. 1975. The applications of partial diallel cross in plant breeding- A Review of Crop Improvement. 2:1-7.

Estakhr, A and B. Heidari. 2012. Combining ability and gene action for maturity and agronomic traits in different heterotic groups of maize inbred lines and their diallel crosses. Journal of Crop Science and Biotechnology. 15: 219 - 229

Falconer, D.S. Introduction to Quantitative Genetics. 1989. John Wiley and Sons; New York. page 340.

Fanseco, S. F. L. and Peterson. 1968. Hybrid vigor in a seven parent diallel cross in common wheat (*T. aestivum* L.). Crop Science 8:85-88.

Ferreira, C. F., A. Borém, G. A. Carvalho, S. Nietsche, T. J. Paula, E. G. Barros and M. A. Moreira. 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.

Flor H. H. 1956. The complementary genic systems in flax and flax rust. Advances in Genetics 8:29–54

Flor, H. 1971. Current status of gene for gene concept. Annual Reviews of Phytopathology. 28:275 – 276.

Goodnight, C. J. 1999. Epistasis and heterosis. In The Genetics and Exploitation of Heterosis in Crops, (J. G. Coors and S. Pandey, eds). American Society of Agronomy, Madison, WI. page. 59– 68.

Hansson, B. and Westerberg, L. 2002. On the correlation between heterozygosity and fitness in natural populations. Molecular Ecolog*y* 11**:** 2467–2474.

Hayman, B.I., 1954. The theory and analysis of diallel cross-I. Genetics 32: 789-809.

Ilyas, M., M. Naveed, T. M. Khan and I. A. Khan. 2007. Combining ability studies in some quantitative and qualitative traits of *Gossypium hirsutum* L. Journal of Agriculture and Social sciences. 3: 39 - 42

Jinks, J.L., 1954. The analysis of continuous variation of a diallel cross of *Nicotiana rustica* L. Genetics. 39: 767-788

Joshi, S. K., S. N. Sharma, D. L. Singhania and R. S. Sain. 2004. Combining ability in the F1 and F2 generations of diallel cross in hexaploid wheat (*Triticum aestivum* L. em. Thell). Hereditas 141: 115- 121.

Kalwar, M. S. and S.B. Babar .1999. Estimates of combining ability in upland cotton (*Gossypium hirsutum* L.). The Pakistan Cotton. 43: 25 – 30.

Kimani, J. M and J. Derera. 2009. Combining ability analysis across environments for sometraits in dry bean (*Phaseolus vulgaris* L.) under low and high soil phosphorous conditions. Euphytica. 166: 1 - 13

Li, Y. M., R. L. Chaney, A. A. Schneiter and J. F. Miller. 1995. Combining ability and heterosis estimates for kernel cadmium levels in sunflower. Crop Science 35:1015 - 1019

Machado, C. F., J. B. Santos, G. H. S. Nunes and M. A. P. Ramalho. 2002. Choices of common bean parents based on combining ability estimates. Genetic and Molecular Biology. 25(2): 179 – 183

Mahuku G., C. Montoya, M. A. Henriquez, C. Jara, H. Teran and S. Beebe. 2004. Inheritance and Characterization of angular leaf spot resistance genes present in common bean accession G10474 and identification of an AFLP marker linked to the resistance gene. Crop Science. 44: 1817 – 1824.

Mahuku, G.S., C. Jara, J. B. Cuasquer and G. Castellanos. 2002. Genetic variability within *Phaeoisariopsis griseola* from Central America and its implications for resistance breeding of common bean. Plant Pathology. 51: 594-604

Maphosa, M., H. Talwana, P. Gibson and P. Tukamuhabwa. 2012. Combining ability for resistance to soybean population. Field Crops Research. 130:1 - 7

Mendonnca, H. A., J. B. Santos and M. A. P. Ramalho. 2003. Genetic control of common bean reaction to angular leaf spot. Crop Breeding and Applied Biotechnology. 3: 209 -216

Miklas, P. N., J. D. Kelly, S. E. Beebe and, M. W. Blair. 2006. Common bean breeding for resistance against biotic and abiotic stresses: from classical to MAS breeding. Euphytica 147, 105–131

Ogliari, J. B., M. A. Guimarães, I. O. Geraldi, L. E. A. Camargo. 2005. New resistance genes in the *Zea mays* - *Exserohilum turcicum* pathosystem. Genetics and molecular biology. 28: 435-439

Palloix, A., V. Ayme and B. Moury. 2009. Durability of plant major resistance genes to pathogens depends on genetic background, experimental evidence and consequences forbreeding. New Phytologists. 183: 190 – 199.

Pan, Q., L. Wang, T. Tanisaka and H. Ikehashi. 1998. Allelism of rice blast resistance genes in two Chinese rice cultivar and identification of two new resistance genes. Plant breeding. 47: 165 - 170

Pastor-Corrales, M. A., O. A. Erazo, E. I. Estrada, and S. P. Singh .1994. Inheritance of anthracnose resistance in common bean accession G 2333. Plant Disease 78: 959 - 962.

Pierozzi, P. H. B., A. S. Ribeiro, J.U.V. Moreira, L. D. C. Laperuta, B. F. Rachid, W. F. Lima,C. A. A. Arias, M. F. Oliveira and J. F. F. Toledo. 2008. New soybean (*Glycine* max *Fabales, Fabaceae*) sources of qualitative genetic resistance to Asian soybean rust caused by *Phakopsoraf pachyrhizi* (Uredinales, Phakopsoraceae). Genetics and Molecular Biology. 31:505-511

Poehlman, J.M. and D.A. Sleper, 1995. Breeding Field Crops. 4th Edition., Panima Publishing Corporation, New Delhi.

Radoevm, M., C. H. Becker and E. Wolfgang. 2008. Genetic Analysis of Heterosis for Yield and yield Components in Rapeseed (*Brassica napus* L.) by Quantitative Trait Locus Mapping. Genetics 179: 1547–1558

Ragsdale, P. I and C. W. Smith. 2007. Germplasm potential for trait improvement in upland Cotton: diallel analysis of within-boll seed yield components. Crop Science. 47: 1013-1017

Ramezanpour, S. S., S. V. Bastam, H. S. S. Kia and M. K. Arabi. 2010. Estimation of combining abilities and heterosis of *Septoria tritici* blotch resistance in wheat genotypes. Australian Journal of Crop Science. 4:480-484

Saettler, A.W. 1991. Angular Leaf Spot. In: Compendium of Bean Diseases (Hall R, ed.). APS Press, St. Paul, U.S.A.: 15–16

Sanglard D. A., C. A.G. Ribeiro, B. P. Balbi, K. M. A. Arruda, E. G. Barros and M. A. Moreira. 2013. Characterization of the angular leaf spot resistance gene present in common bean cultivar Oura Negro. Journal of Agricultural Science. 5:1 – 23.

Sartorato, A., S. Niestche, E. G.  Barros and M. A. Moreira. 2000. RAPD and SCAR markers linked to resistance gene to angular leaf spot in common beans. Fitopatologia Brasileira. 25: 637-642

Schoonhoven A and M. A. Pastor-Corrales .1987. Standard system for the evaluation of bean germplasm. CIAT, Cali

Silva, M.P., A. T. Amaral Júnior, R. Rodrigues, M. G. Pereira and A. P. Viana. 2004b. Genetic control on morphoagronomic traits in snap bean. Brazilian Archives of Biology and Technology. 47: 855-862

Singh, A. K and S. S. Saini. 1980. Inheritance of resistance to angular leaf spot (*Isariopsis griseola* Sacc.) In French beans (*Phaseolus vulgaris* L.). Euphytica. 29:175 - 176

Singh, B. D., P. K. Majumdar and K. K. Prasad. 1999. Heritability studies in late sown irrigated wheat (*Triticum aestivum* L.). Journal of Applied Biology. 9: 105 – 107

Singh, R. 2000. Heterosis studies in rice using WA based CMS system for developing hybrids for Eastern Uttar Pradesh. Annals Agricultural Research. 21: 79-83.

Skinner, D. Z and D. L. Stuteville.1989. Accumulation of minor gene resistance to *Peronospora trifoliorum* in diploid alfalfa. The American Phytopathological Society. 79:721 – 724

Sprague, G.F and L. A. Tatum. 1942. General vs specific combining ability in single crosses of corn. American Society of Agronomy. 34: 923-932.

Springer, N. M and R. M. Stupar. 2007. Allelic variations and heterosis in maize: How do two halves make more than a whole? Genome Research 17: 264 – 275.

Srinivasan S., P. M Gaur and B. V. Rao. 2008. Allelic relationship between spontaneous and induced mutant gene for stem fasciation in chickpea. 2008. Plant breeding 127:319 – 321

Stenglein S., L. D. Ploper, O. Vizgarra and 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.Torres, E.A and I. O. Geraldi. 2007. Partial diallel analysis of agronomic characters in rice (*Oryza sativa* L.). Genetics and Molecular Biology. 30: 605-613.

Thakur, R. P. 2007. Host plant resistance to diseases: Potential and limitations. Indian Journal of Plant Protection. 35: 17 – 21.

Vakili Bastam S.H., S. S. Ramezanpour, H. Soltanloo, S. H. Kia, M. Kalate and M. H. Pahlevani. 2010. Inheritance of resistance to septoria tritici blotch (STB) in some Iranian genotypes of wheat (*Triticum aestivum* L.). International Journal of Genetics and Molecular Biology. 2:34-42

Viana, J. M. S and F. Matta. 2003. Analysis of general and specific combining abilities of popcorn populations, including selfed parents. Genetics and Molecular Biology. 26: 465 – 471

Virmani, S.S., 1994. Heterosis and Hybrid Rice Breeding. Springer-Verlag, Berlin Heidelberg, Germany

Visscher, P. M., W, G. Hill and N. R. Wray, 2008. Heritability in the genomics era- Concepts and misconceptions. Nature Reviews Genetics. 9: 255 – 256

Young N. D. 1996. QTL mapping and quantitative disease resistance in plants. Annual Review of Phytopathology. 34:479–501

Zuk O. E., E. Hechter, S. R. Sunyaev and E. S. Lander. 2011. The mystery of missing heritability: Genetic interactions create phantom heritability. www.pnas.org/cgi/doi/10.1073/pnas.1119675109. Accessed on 5th December, 2015

# Appendix A1



(a)                                                              (b)



(c)

**Figure. 7.1.** Different planting dates of the $F_2$ progenies in the screen house. (c) an arrow pointing to a plant susceptible to ALS disease

# 8   Overview and way forward

## 8.1   Introduction

Common bean (*Phaseolus vulgaris* L.) has the potential of improving livelihoods of many Tanzanians. It is estimated that more than 75% of the households in the country depend on the crop as a source of protein, carbohydrates, vitamins and minerals, while 45% obtain their income from bean sales (Xavery et al., 2005). Common bean provides the largest portion of the protein served in a diet as it is consumed with starchy foods made from maize, rice, cassava and green banana. However, the average yield of the crop remains low (<500 kg ha$^{-1}$) in most of the bean producing agro ecologies of Tanzania. Although the crop occupies the largest area planted to pulse crops with annual production of 944, 000 metric tons (FAO, 2011), the national average yield remains low at 741 kg ha$^{-1}$. In this regard, bean production has not kept pace with the high demand of the crop. This  has been reflected in the poor records of children's health assessments in the bean growing agro-ecologies showing that 44% children had stunted growth and 17% were underweight  (UNICEF, 2006). According to the World Bank, 33% of the populations in Tanzania live below the poverty line suggesting low purchasing power of alternative sources of proteins that might be available (World Bank, 2012).

Despite the importance of the crop to the majority of the population, the average production per unit area remains constant or is declining due to a number of production constraints that are grouped into biotic and abiotic factors. Among the biotic factors, angular leaf spot caused by *Pseudocercospora griseola*, is the most devastating disease of common bean in Tanzania and was the main focus of this study. This section presents the study objectives with subsequent summary of major findings and their implications to angular leaf spot management in common bean and way forward.

The objectives of the study were:

- to assess the common bean farming systems, farmers' awareness of angular leaf spot disease, and examine poverty levels within smallholder bean farmers, and relate all these to the management of ALS.

- to examine the economics of yield losses associated with the disease on five selected bean varieties that are commonly grown by farmers, as an effort towards developing sustainable control measures against *P. griseola*.

- to determine the response of common bean landraces widely grown in the Southern Highlands of Tanzania against the pathogen and to identify promising lines that can be used for resistance breeding.

- to determine genetic diversity and relationships among isolates of *Pseudocercospora griseola,* the fungal pathogen of angular leaf spot of bean from the Southern Highlands of Tanzania.

- to determine combining ability, gene action, heritability, heterosis and allelism of the resistance genes present in selected common bean lines widely grown in Tanzania.

## 8.2   The results of the study are outlined below:

1. Angular leaf spot disease is a problem in the bean farming communities of the Southern Highlands of Tanzania but farmers are unaware of the disease and sources of inoculums.  The majority of smallholder bean farmers were poor (not satisfied by the basic needs available at the household), growing mainly landraces that exists as mixtures of seed with varying sizes and colors. The average yield ranged from 200 – 400 kg ha$^{-1}$ way below the national average of 741kg ha $^{-1}$. Farmers' main source of seed was farm-saved seed acquired from the local market, neighbors, friends and family, with high chances of propagating and spreading the disease further to new places. Fields planted to beans were small ranging from 0.5 – 1 hectare lower than the average landholding in Africa which is 1.6. Such small fields were an indicator of high population density that prohibits crop rotation as a method of controlling ALS disease. This forced farmers into intensive bean production practices that provided green bridge for the disease. Fungicides used and application regimes practiced by a few farmers were not those recommended for controlling ALS.

2. The five varieties used in the yield loss experiment were susceptible to ALS although they were under production and highly preferred by farmers. Severe infections were noted during the heavy rainy season compared to the light rains with yield losses that ranged from 39 - 61% and 6.1 – 37.8% respectively. The variety Kablanketi which was

highly susceptible had the highest yields of all varieties when sprayed with fungicides at the recommended rates and times. The negative association between yield and AUDPC indicates the necessity of optimizing disease management practices to reduce infections. Fungicide application at the recommended rates and times reduced ALS disease infections significantly. All the varieties evaluated proved superior under fungicide management at the recommended rates than non-sprayed treatments. It was suggested that farmers should be trained on fungicide usage as a short term strategy in managing ALS disease.

3. Eleven new races were identified in addition to the 9 races previously reported about 10 years ago. High numbers of races were recorded in Mbeya Stepped Plains, the agro-ecological zone that borders Malawi and Zambia, where bean germplasm is exchanged informally between farmers. In this regard, the new races observed might have been introduced or mutated from those reported earlier. The most resistant differential cultivar, Mexico 54 was susceptible to race 63/63 which was not reported before supporting the existence of new races in the bean growing agro-ecologies of the Southern Highlands of Tanzania. Although characterization of isolates into races using differential cultivars was successful, the study was tedious because of the visual assessments of the disease symptoms that require only experienced personnel. In some cases, there was a need to verify sporulations on ALS diseased lesions on leaves by transferring the sample into humid chambers, in this case petri dishes, for approximately 24 hours to confirm sporulation before assessments were done.

4. The use of molecular techniques particularly PCR–RAM (random amplified microsatellite) and REP (Repetitive Extragenic Palindromic) markers revealed genetic variations that existed in *P. griseola*. Given that *P. griseola* co-evolved with the Mesoamerican and Andean gene pools of common bean, the results obtained in this study suggests the presence of both Mesoamerican and Andean virulence factors in a number of isolates studied. Moderate genetic diversity was observed on the most virulent group of isolates with a mean of 60.92% polymorphism. The identification of unique bands that were specific to certain races could be used as molecular markers for further studies that involve *P. griseola*.

5. Although bean landraces are highly preferred by farmers in the SHT, majority of them are susceptible to *P. griseola.* The resistant and moderately resistant reactions observed when the landraces were infected with *P. griseola* races suggested presence of resistance genes that can be used in the bean breeding programme against the pathogen. The following three accessions were identified being resistant to *P. griseola*: SHB002, SHB005 and SHB091.

6. Considerable genetic variability was observed when crosses were made between selected landraces (SHB002, SHB005 and SHB091). These were confirmed based on the varying degree of reaction of the crosses t*o P. griseola* infections. It was observed that, the mode of gene action for the expression of ALS disease resistance were both additive and non-additive with additive gene effects being more important for the expression of ALS disease resistance. This coupled with high heritability and high negative heterosis suggested possibilities of making selections within segregating populations in early generations. Genetic ratios of 15:1 and 63:1 resistant and susceptible respectively obtained in the allelism study suggested the presence of two and three no-allelic genes respectively in the ALS resistant landraces. Based on these results it was concluded that there is potential to breed for ALS disease resistance using common bean landraces that have been kept by farmers for a long time indicating presence of desirable traits.

## 8.3. The implications of the results and the way forward

1. The results of this study implied a great need to create awareness of the ALS disease and train farmers on the disease management practices. Strengthening formal seed system to ensure availability of improved bean seed to smallholder farmers will be an added advantage in controlling the ALS disease.

2. Since majority of farmers were resource limited growing beans during heavy rains will be a great loss. Instead, farmers are advised to grow the crop during light rains where ALS disease severity was indicated to be low.

3. The use of DNA–based molecular techniques should complement virulent studies conducted based on differential cultivars especially when dealing with a highly variable pathogen like *P. griseola*.

4. Although three landraces were identified as carrying resistance genes to *P. griseola*, prior genetic studies on number and mode of inheritance of these genes must be conducted before being used to develop varieties that are resistant to the pathogen.

5. The selected families from the cross combinations between resistance genotypes should be advanced to develop pure line cultivars of common bean with ALS resistance and high yields for release in the SHT or similar environments in Tanzania.

## 8.3   References

FAO. 2010. Food Agriculture Organization Statistics available at [www.fao.org](www.fao.org) . Visited on 24th December, 2015

UNICEF, 2009. Tracking the progress on child and maternal nutrition: A survival and development priority. New York

World Bank trade Press. 2012a. Population density map of Tanzania.

Xavery P., R. Kanyebara, S. Kasambala and F. Ngulu. 2006. The impact of improved bean production technologies in the northern and north western Tanzania. Pan African Bean Research. CIAT African region.