

**LEVELS OF ANGULAR LEAF SPOT (ALS) RESISTANCE IN COMMON
BEAN (*Phaseolus vulgaris*. L) POPULATIONS DEVELOPED FOR ALS
RESISTANCE**

BY

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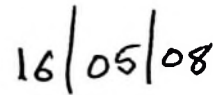
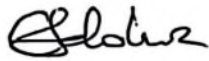
ABSTRACT

Angular leaf spot (ALS) incited by *Phaeoisariopsis griseola* is wide spread in all bean growing areas in Tanzania. It is of great concern in wet bean growing areas of the country, yet little information is available in Tanzania on cultivars resistant to the disease. The objective of this work was to incorporate angular leaf spot (ALS) resistance in adapted common bean lines through backcrossing procedure, determine inheritance of ALS in the crosses and screen advanced backcross lines for ALS resistance. Backcrossing was done in the screen house at SUA where G5686 x C/wonder, Amendoim x Rojo and Mexico54 x Soya were crossed to their respective parents. The backcrossed lines obtained together with the advanced bean lines from Prosser USDA-ARS Washington were evaluated for resistance to ALS. Completely randomized design (CRD) was used in screen house and Randomized Completely Block Design (RCBD) was used for field trials. For F_2 , F_3 , BC_{1-1} and BC_{1-2} there was no randomization nor replication as this has no effect in early generations of bean improvement. For the advanced lines there were three replications in each of the two different locations i.e. SUA-Morogoro and Madira, Arusha. An appreciable amount of genetic variation ($P \leq 0.05$) for ALS resistance, pods per plant and seed yield was observed. The following advanced lines were found resistant to ALS in both locations PSO4-336Ab-7, PSO4-126Aa-8, PSO4-335B-1, PSO4-139Ba-1, PSO4-139Bb-5, PSO4-139Bb-1, PSO4-106Ba-2, PSO4-139Bb-4, PSO4-126Ab-8, PSO4-139Ba-7, PSO4-139Ab-10 and PSO4-126Aa-6. Both lines showed intermediate or resistant reaction to ALS disease, moreover, some of these did not develop any disease symptoms and thus could be immune to the disease or have escaped the disease. Significant and positive correlation between number of pods per plant and

yield was observed in advanced lines where $r = 0.751$, ($P \leq 0.001$) for Morogoro trial where $r = 0.509$ and ($P \leq 0.001$) for Arusha trial. Heritability estimates were low 0.190 to medium 0.228 for disease resistance and moderate 0.30 to high 0.73 for number of pods per plant. It is recommended that further studies be conducted among the lines evaluated and the best performing lines to be selected then advanced and later be evaluated for other characteristics such as size, cooking time and protein concentration.

DECLARATION

I, Eninka John Mndolwa, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work, and has not been submitted for a higher degree in any other University.

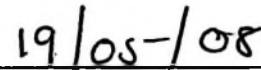
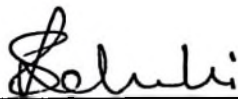


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DEDICATION

This work is dedicated to my beloved parents Mr. and Mrs. Mndolwa, for their inspiration, encouragement and support throughout my studies. They have been instrumental in all my academic achievements for which I am greatly indebted.

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LIST OF ABBREVIATIONS AND SYMBOLS

ALS	Angular leaf spot
Amend	Amendoim
ANOVA	Analysis of Variance
Ar	Arusha
ARI	Agriculture Research Institute
ARS	Agriculture Research Station
<i>b</i>	Regression coefficient
BC ₁₋₁	Backcross one to parent one
BC ₁₋₂	Backcross one to parent two
BCMV	Bean Common Mosaic Virus
C.V	Coefficient of Variation
C/w	Canadian wonder
CAN	Calcium Ammonium Nitrate
CIAT	Centro Internacional de Agricultura Tropical
cm	centimeter
CRD	Completely Randomized Design
CRSP	Collaborative Research Support Project
cv	Cultivar
DMRT	Duncan Multiple Range Test
e.g.	for example
EC	Emulsified Concentrate
F ₁	Filial one generation
F ₂	Filial two generation
F ₃	Filial three generation

g	Gram
h^2	Heritability
HB	Halo blight
i.e.	that is
IRRISTAT	Statistical-computer program for data analysis.
Kg/ha	Kilogram per hectare
LSD	Least Significant Difference
Lt	liters
m	meter
Mex54	Mexico 54
Mor	Morogoro
MSTAT-C	Statistical-computer program for data analysis
N	Nitrogen
ns	Not significant
°C	Degree-Celsius
P	Probability
P_1	First parent
P_2	Second parent
P_2O_5	Phosphorus pentoxide
PhD	Doctor of Philosophy
r	Regression coefficient
RAPD	Random Amplified Polymorphic DNA
S.E	Standard error
SADC	Southern African Development Countries
SCAR	Sequence Characterized Amplified Region

SUA	Sokoine University of Agriculture
TSP	Triple Super Phosphate
USDA	United States Department of Agriculture
μm	micrometer

CHAPTER ONE

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is one of the most important food legumes in the world because of its commercial value, extensive production, consumer use, and nutrient value (being a source of carbohydrate, protein, minerals, and vitamins) (Liebenberg and Pretorius, 1997; Pastor-Corrales *et al.*, 1998; Singh and Munoz 1999). It is an important source of dietary protein and starch in Africa and a primary staple in parts of the Great Lakes Region (Kagera and Kigoma)

Beans are the main grain legume crop grown in Tanzania, where they are often intercropped with maize. The main areas of production are the mid to high altitude areas of the country. The most suitable areas for bean cultivation in Tanzania are in the northern zone particularly Arusha Region, the Lakes region in the west and in the Southern Highlands. Tanzania is among the top twenty largest producers of dry beans in the world and the second largest producer in sub-Saharan Africa, after Kenya (FAO, 2005). Average bean yields in Tanzania are around 500 kg/ha although the potential yield under reliable rain-fed conditions is 1500–3000 kg/ha, using improved varieties and proper crop and land husbandry. The main reasons for the low yield obtained by most smallholders are; poor seed quality, poor performance of the local landraces, mainly due to their susceptibility to pests and diseases, low soil fertility, drought and poor crop management, such as late weeding (Hillocks *et al.*, 2006).

Despite the common bean being an important food legume in developing world including Tanzania, it is however hampered by a number of problems. Major constraints

include lack of suitable varieties, insect pests, diseases and nematodes (Liebenberg and Pretorius, 1997; Wortmann *et al.*, 1998). Diseases are the most important single factor limiting bean production in most bean growing areas, among the diseases is Angular leaf spot (ALS) caused by *Phaeoisariopsis griseola* (Cordona and Walker 1956). Other important bean diseases in Tanzania include halo blight (HB) (*Pseudomonas phaseolicola*), rust (*Uromyces phaseoli*, *U. appendiculatus*), and *Bean common mosaic virus* (BCMV) (Allen, 1983, 1995; Allen and Lenne, 1998).

Angular leaf spot (ALS) is one of the most devastating diseases of common bean (*Phaseolus vulgaris* L.) in the tropical and subtropical countries (Mahuku *et al.*, 2003). It is regarded as the leading constraint to increased common bean (*P. vulgaris* L.) production worldwide (Kelly and Miklas, 1998). The causal fungus, *Phaeoisariopsis griseola*(Sacc.) Ferr. is highly variable (Guzmán *et al.*, 1995; Sartorato, 2002; Pastor Corrales *et al.*, 1998) and a diverse source of resistance genes is required to manage this disease. Angular Leaf Spot induces yield losses, which can reach up to 80% under severe conditions of infection (Schwartz *et al.*, 1981).

The disease is favored by a warm and humid climate (Saettler, 1991). The optimum temperature for *P. griseola* development is about 24 °C (Cardona & Walker, 1956; Bassanezi *et al.*, 1998). Moisture is required for infection and sporulation of *P. griseola* (Cardona & Walker, 1956; Sindhan and Bose, 1980). The ALS lesions on leaves can cause severe defoliation (Cardona & Walker, 1956; Ferraz, 1980; Saettler, 1991) which has strong implications on bean growth and yield, as it causes a decrease in green leaf area index (LAI) (Bergamin *et al.*, 1997; De Jesus *et al.*, 2001).

Larger losses due to angular leaf spot are experienced year after year, especially in regions where warm temperatures prevail during the growing season (Inglis and Hagedorn, 1984). Angular leaf spot affects yield largely due to damage caused by intense spotting and consequent loss of photosynthetic area (Inglis and Hagedorn, 1988). The fungus infects most aerial parts of the plant; especially pods, seeds, leaf petioles, and lower surface of leaflets, causing premature leaf drop, foliar and stem necrosis that culminate in poorly filled seeds and reduce seed quality (Liebenberg and Pretorius, 1997). In addition, the quality, market value, and suitability of seed for transport and use across bean-producing regions and national borders may be severely affected.

Several control strategies exist these include chemical applications (Bhardwaj *et al.*, 1994) for example, use of fungicides (Busogoro *et al.*, 1999; Liebenberg and Pretorius 1997) cultural practices (Correa *et al.*, 1989) and genetic resistance (Schwartz *et al.*, 1981).

Studies have revealed that the ALS pathogen is mainly transmitted through contaminated seed and that *P. griseola* can survive for 10-20 months in infected debris. The pathogen can also survive between seasons on infested seed. Spores from lesions on stems, leaves and pods, as well as on crop debris, only develop after continuous high humidity or wet conditions for 24-48 hours. Spores produced on infested debris or seed are rain-splashed and/or wind-blown onto healthy tissue after planting. When spores land on susceptible bean tissue, they germinate and infect through natural pores (stomata). Disease develops rapidly during periods of warm temperatures (24°C) but can occur over a range of moderate to warm temperatures (16°C-28°C) when accompanied

by wet weather or high humidity alternating with dry, windy conditions. However, significant disease development and yield loss can also occur on earlier seeded crops if moderate temperatures coincide with prolonged periods of wet weather (Celetti, *et al.*, 2006). Thus the use of clean seed and resistance cultivars are pivotal to any effective, economical and environmentally friendly strategy to the control of ALS. Genetic resistance appears to be the most appropriate method for the subsistence agriculture prevailing in developing countries because of the limited finances of the farmers' mainly medium and small size farmers. Selection of beans resistance to ALS is important for improving beans quality and quantity. Evidence of co-evolution of *P. griseola* and bean suggests that combining resistance from the Andean and Mesoamerican gene pools is a good strategy for disease control (CIAT, 2001).

Therefore the main objective of this study was to incorporate ALS resistance in adapted common bean lines through backcross procedure, determine inheritance of ALS in the crosses and screen advanced backcross lines for ALS resistance.

The specific objectives were:-

- i. To produce backcross lines by crossing the F₂ common bean lines with their parents.
- ii. To determine the inheritance of ALS from the three sets of crosses G5686 x Canadian wonder, Amendoim x Rojo, and Mexico54 x Rojo.
- iii. To evaluate advanced backcross lines for ALS resistance.

CHAPTER TWO

LITERATURE REVIEW

2.1 Distribution

Angular Leaf Spot (ALS) disease has been reported previously in over 70 countries, in tropical and sub tropical regions such as Brazil, Colombia, Costa Rica, Mexico, and Africa (Barros *et al.*, 1957; Mahuku *et al.*, 2003), it is known to exist also in other regions such as in Europe and United States. (Saettler and Correa 1983; Inglis and Hagedon 1984). The disease was considered as a minor nuisance at first but is now one of the most economically important diseases of dry beans (Mahuku *et al.*, 2003). The pathogen can infect common bean (*Phaseolus vulgaris*. L), Lima bean (*P. lanatus*. L), Scarlet runner bean (*P. coccineus*. L), Lerd bean (*Vigna mungo* (L) Hepper), Tepary bean (*P. auctifolius* A, *V. angularis*, *V. umbellate*), Pea (*Pisium sativum*. L) Cowpea (*V. unguiculata* (L) Walp), Soybean (*Glycine max* (L) Merrill) (Diaz-Polanco *et al.*, 1965).

2.2 Etiology

In nature, the fungus produces groups of 8-40 conidiophores, which join loosely to form the dark columnar synnemata that bear conidiospores (Barnet and Hunter, 1972). A synnemata may have a diameter of 20-40µm and be 80-500µm in length (Golata and Meossi, 1972). The conidiophores tend to separate near maturity and fruitification. Conidia are gray, cylindrical to fusiform, slightly curved and measure 3-8 by 43-68 µm with 1-6 septations (Golata and Meossi, 1972). The conidial length of 10 isolates from Colombia varied between 18 and 76 µm with means of 38.5 µm (Buruchara, 1983). The

width varied between 3.8 and 8.8 μm . With an average of 6.4 μm , whereas the number of septa varied between 0-7 with a mean of 3. These parameters varied significantly both within and between isolates.

Phaeoisariopsis griseola grows slowly on artificial culture media over a range of temperatures between 8-28 °C with an optimum of 24 °C. Optimum pH is between 5 and 6. Adequate growth media include potato dextrose agar plus bean leaf extract, honey peptone agar, baby food, calcium carbonate agar, and potato yeast dextrose agar. Abundant sporulation occurred in 10-15 days when the fungus was grown at 19 °C in darkness on V-8 vegetable juice agar (200ml V-8 vegetable juice, 3g calcium carbonate, and 18g Bacto-agar added to sufficient distilled water to make 1 liter) (CIAT, 1979).

2.3 Symptomatology

All aerial plant parts, including leaves, petioles, stems and pods can be infected, but symptoms are most recognizable on leaves. Lesions on leaves usually appear as brown spots with a tan or silvery centre that are initially confined to tissue between major veins, which gives it an angular appearance. On primary leaves lesions are round and usually larger than the spots on trifoliate leaves and may develop concentric rings initially gray. On some varieties a yellow halo occasionally surrounds lesions and eventually the entire leaflet becomes yellow before senescing. Lesions can be observed on the underside of the leaf and appear slightly more pale than those on the upper surface of the leaflet. A close look at lesions on the underside of a leaflet with a magnifying lens will reveal tiny dark tufts (synnemata) protruding from lesions. These dark tufts are a collection of stalks (conidiophores) that produce spores, which are wind-blown or rain-splashed to other

healthy tissue. Lesions on stems and petioles appear dark brown and elongated. Lesions on pods are circular, black and sunken, and look similar to anthracnose (*Colletotrichum lindemuthianum*). Yield reduction caused by ALS is mainly due to a reduction in photosynthetic area; however, the fungal pathogen can also reduce quality by causing lesions on pods. Yield losses of 10%-50% have been reported in the northern U.S. and up to 80% in tropical and subtropical countries (Celetti, *et al.*, 2006).

2.4 Factors that favour Angular leaf spot development

It has been observed that disease reaction from ALS is highly dependent on such factors as pathogen isolate, inoculum concentration, host cultivar and its age, temperature, and humidity. Alvarez and Schwartz (1979) noted that disease reactions are very dependent on inoculum concentration.

The infection and disease development occurs over a wide range of temperature 16 °C - 28 °C with the optimum temperature of 24 °C. It has been reported that, when infection occurred at 16 °C, 20 °C and 24 °C leaf chlorosis was greater and days to defoliation were reduced.

High relative humidity favours the growth of the fungi, which cause ALS. Longer periods of moisture increases disease intensity. Cardona and Walker (1956) found that Relative Humidity was necessary for the initiation of the disease. Optimum humidity ranges from 90%-100%, below 85% infection does not occur.

Age of plant is known to affect the development of ALS. When plants at the age between 30-75 days were artificially inoculated and incubated, the disease was most intense on 30-45 days old plants and yield was similarly decreased drastically.

Wind, water, insects and man are important agents for the dissemination of pathogen. Water stream, surface water, rain, overhead irrigation or during spraying plays a major role in the spread of inoculum. Wind may spread the disease within an area and even over long distances. Insects' pests can spread the pathogen during feeding a host plant thus transmit pathogens from one plant to another.

2.5 Disease control

Cultural practices that include crop rotation, use of good quality seeds, field sanitation measures and planting time have been used. Other control measures include chemical control and use of resistant varieties.

2.5.1 Cultural control

Crop rotation with non-host crops for at least two years and deep ploughing infested crop residue significantly reduces survival of the fungal pathogen.

Field sanitation is very important, studies indicate *P. griseola* can survive in infested bean residues left on the soil surface; however, the pathogen does not survive very long when infested bean debris is buried in soil and decomposes.

2.5.2 Chemical control

Application of an effective fungicide during early bloom (10%-30% flowering) when environmental conditions are conducive for disease, is important. Application of a second fungicide seven days later at late bloom (50%-70% flowering) is useful, if environmental conditions favoring infection and disease development occur between early bloom and late bloom, or are predicted after late bloom. It is necessary to always read and follow the fungicide label before applying a fungicide. (Celetti, *et al.*, 2006).

2.5.3 Use of Resistant varieties

The use of least susceptible varieties, particularly in fields with a history of ALS will reduce disease impact on the quantity and quality of yield. The use of resistant cultivars currently offers the only economical means of control. Genetic resistance appear to be the most appropriate method for controlling the disease in the subsistence agriculture prevailing in developing countries because of the limited finances of the farmers mainly medium and small size farmers, who are the major bean producers in developing countries like Tanzania. The use of resistant cultivars is preferable to other methods of control, being cheaper and more permanent as the farmers can keep seeds (Edje *et al.*, 1990).

2.5.3.1 Inheritance of ALS resistance

Heritability is the proportion of a population's phenotypic variation that is attributable to genetic factors and may be transmitted (Poehlman and Borthakur, 1977; Falconer and Mackay, 1998; Russel, 1998). Heritability is used to examine the relative contributions

of genes and environment to variation in a specific trait as continuous traits are frequently influenced by multiple of genes and by environmental factors.

One of the difficult problems of the breeder is to determine to what extent a characteristic is heritable and is the result of gene action, and to what extent it may be the result of favorable or unfavorable influence in the environment. Variation among individuals can be partitioned into genetic and environmental components (Russel, 1998). Selection of plants for a character of interest would be ineffective if the environmental variation is so great that it masks the genetic variation. The degree to which the variability of a quantitative character may be transmitted to the progeny is referred to as heritability.

If in a progeny, variation due to environment is large in relation to the heritable variations, the heritability will be low. If variation due to environment is small in relation to the heritable variation, then heritability will be high (Poehlman and Borthakur, 1977). Herald (1994) categorized heritability estimates as low or weak (0-0.2), moderate or medium (0.21 – 0.39) and high or strong (0.4 – 1).

Inheritance of resistance is controlled by a single recessive gene (Santos-Filho *et al.*, 1976), but in an earlier study, Cardona (1958) reported resistance to ALS to be controlled by a single dominant gene. This shows that inheritance of ALS resistance is complex, involving both dominant and recessive genes that may be or may not be independent. Resistance to specific isolates of *P. griseola* has been reported to be simply inherited and molecular markers have been identified for some of these resistance genes

(Mahuku *et al.*, 2004; Ferreira *et al.*, 2000; Carvalho *et al.*, 1998; Miklas *et al.*, 2005). Ragagnin *et al.* (2005) found the ALS resistance in AND 277 to race 63:23 to be conferred by a single dominant gene (*Pgh-1*). Cornell 49-242 has *Pgh-2* which confers resistance to *P. griseola* pathotype 31:17 (Nietsche *et al.*, 2000). G10474 has a single dominant gene that confers resistance to pathotype 63:63 (Mahuku *et al.*, 2004). A SCAR marker at 5 cM from the resistance gene was identified but the marker was found only to be effective in the Andean gene pool of common bean. CIAT (2003) reports that the RAPD marker OPE04 has been used in Uganda to select for an ALS resistance gene derived from Mexico 54. SCAR markers are also available for ALS resistance genes from G 10474 and G 10909. In Honduras, the Andean lines G 05668 and G 06727 have been used as sources of resistance to ALS. G 06727 has resistance to pathotype 63:59. Sartorato (2005) reported that 'Ouro Negro' had resistance to 8 pathotypes, including *P. griseola*. race 63:63 from Brazil.

Line G 5686 (a large seeded Andean line) and Mexico 54 display fairly good levels of resistance to nearly all races. These cultivars are not only good sources of resistance to *P. griseola* but could also serve as reliable indicators of new races of the pathogen in the future (Ngulu, 1999). Mexico 54 is a medium seeded, pink in colour and resistant to all *P. griseola* isolates so far characterized in Africa (CIAT, 1986). Resistance in G 5686 is conditioned by two dominant epistatic genes; and Amendoim by two recessive genes (CIAT, 2001).

Several resistance genes against *P. griseola* that are controlled by major genes, that are either dominant or recessive, acting singly or duplicated and which may interact in an

additive manner with or without epistasis were identified Mexico 54 is one of them and is an important source of resistance in Africa, that appears to have more (recessive and dominant) than one resistant gene (Mahuku *et al.*, 2003).

2.6 Pathogenic variability

Most pathogens exhibit high race variation, fungus that causes ALS has many strains that are genetically different. That is why a bean variety that is resistance in one region or season may not necessarily be effective in another; resistance thus breaks down (Aggrawal *et al.*, 2004).

Sets of six differential cultivars have been identified for race typing (Allen *et al.*, 1998). Fifty six resistant genotypes were selected from a collection of 13,000 accessions screened at CIAT (Schwartz *et al.*, 1981). These formed the basis of an international nursery containing several sources of broad-based resistance to the disease. CAL 143 was one of the first Andean beans to be identified with resistance to ALS and has proved to be resistant in Malawi, South Africa and Tanzania (Aggrawal *et al.*, 2004). Resistance to specific isolates of *P. griseola* has been reported to be simply inherited and molecular markers have been identified for some of these resistance genes (Mahuku *et al.*, 2004; Ferreira *et al.*, 2000; Carvalho *et al.*, 1998; Miklas *et al.*, 2005).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

3.1.1 Germplasm

The bean lines used were collected from CIAT-Colombia through Selian Agricultural Research Institute-Arusha, Prosser USDA-ARS, and from Sokoine University of Agriculture (SUA)-Morogoro. Resistant cultivars used in this study were Mexico 54, G5686, and Amendoim while adapted cultivars used were Soya, Canadian wonder, and Rojo. Mexico 54 is a Mesoamerican type while G 5686 and Amendoim are Andean types. Andean Beans have predominantly large seeds, although some medium sized beans are found in this group (Gepts, 1988a; 1988b: Singh *et al.*, 1991), while Mesoamerican beans have small or medium sized seeds (Gepts. 1988a; 1988b: Singh *et al.*, 1991).

Table 1: Bean cultivars used in production of backcrosses and their characteristics

Cultivar	Collected from	Seed size	Seed colour
1. Rojo	SUA (Morogoro)	Large	Red
2. Soya	SUA (Morogoro)	Medium	Cream
3. Mexico54	SELIAN A.R.I. (Arusha)	Medium	Pink
4. G5686	SELIAN A.R.I. (Arusha)	Large	Cream
5. Amendoim	SELIAN A.R.I. (Arusha)	Medium	Pink mottled
6. C/Wonder	SELIAN A.R.I. (Arusha)	Large	Red
7. Advanced lines	Prosser USDA-ARS Washington	Refer Table 2	Refer Table 2

A.R.I - Agricultural Research Institute
 USDA - United States Department of Agriculture
 ARS - Agriculture Research Station

Table 2: Advanced backcrossed common bean lines screened for ALS under field conditions at Moro and Arusha and their characteristics

Cultivar/line	Background	Resistant Gene	Seed size	Seed colour
1. PSO4-107Ca-7	SELIAN97/PSO3-550	AND 277	Medium	Red
2. PSO4-126Aa-8	LY90/PSO3-565	MAR-2	Large	Cream stripped
3. PSO4-333Aa-19	ROJO/CAL 143	Phg-1	Large	Cream
4. PSO4-333Aa-21	ROJO/CAL 143	Phg-1	Medium	Dark red mottled
5. PSO4-207Ab-6	ROJO/PSO4-083D	MAR-2	Medium	Red
6. PSO4-335B-1	ROJO/CAL 143	Phg-1	Large	Pink mottled
7. PSO4-139Bb-7	ROJO/PSO1-576	MAR-2	Medium	Red stripped
8. PSO4-330a-1	ROJO/CAL 143	Phg-1	Medium	Cream
9. PSO4-139Ba-1	ROJO/PSO1-576	MAR-2	Medium	Light red
10. PSO4-333Aa-3	ROJO/CAL 143	Phg-1	Medium	Light red
11. PSO4-139Bb-5	ROJO/PSO1-576	MAR-2	Small	Gray stripped
12. PSO4-333Aa-18	ROJO/CAL 143	Phg-1	Medium	Deep red
13. PSO4-139Bb-1	ROJO/PSO1-576	MAR-2	Small	Red stripped
14. PSO4-112D	LY90/PSO3-550	AND 277	Large	Purple mottled
15. PSO4-106Ba-2	SELIAN97/PSO3-547	MAR-2	Medium	Purple reddish
16. PSO4-139Aa-10	ROJO/PSO1-576	MAR-2	Medium	Grey stripped
17. PSO4-139Bb-4	ROJO/PSO1-576	MAR-2	Small	Cream stripped
18. PSO4-126Ab-8	LY90/PSO3-565	MAR-2	Large	Cream mottled
19. PSO4-335B-12	ROJO/CAL 143	Phg-1	Large	Purple mottled
20. PSO4-139Ba-7	ROJO/PSO1-576	MAR-2	Small	Gray stripped
21. PSO4-139Aa-6	ROJO/PSO1-576	MAR-2	Small	Cream stripped
22. PSO4-336Aa-7	ROJO/CAL 143	Phg-1	Medium	Red
23. PSO4-139Ab-10	ROJO/PSO1-576	MAR-2	Medium	Cream stripped
24. PSO4-126Aa-6	LY90/PSO3-565	MAR-2	Large	Light red mottled
25. PSO4-106Bb-3	SELIAN97/PSO3-547	MAR-2	Medium	Deep red
26. SUA90 (Check)		None	Medium	Deep gray
27. ROJO (Check)		None	Large	Red
28. C/wonder (Check)		None	Large	Red

Note: SUA90, ROJO and C/wonder are adapted cultivars used as checks

3.1.2 Inoculum

Inoculum used was collected in May 2006 during rainy season in Morogoro Region (Kilosa and Mvomero districts) from naturally infected field grown beans showing typical ALS disease symptoms. The collected leaf samples were placed in between sheets of newspapers in clipboards and air dried for 48hours. Thereafter, samples were placed in envelopes and kept in boxes at room temperature until spore isolation stage.

3.2 Methods

3.2.1 Production of backcrossed bean lines

The F_2 bean lines of crosses between G5686 x Canadian wonder, Amendoim x Rojo, Mexico54 x Soya, were crossed to their respective parents (G5686, Canadian wonder, Amendoim, Rojo, Mexico54 and Soya) where the first backcross to resistant parent, i.e. parent 1 (BC_{1-1}) and the first backcross to susceptible parent i.e. parent 2 (BC_{1-2}) of crosses between (G5686 x Canadian wonder, Amendoim x Rojo, Mexico54 x Soya) were obtained. The crosses were made in the screen house at Sokoine University of Agriculture (SUA). The crossing procedure involved emasculation of female flowers and transfer of pollen from just opened flowers to the stigma of emasculated plants. The crossing was done between 8.00 a.m. - 10.00 a.m. and 5.00 p.m. – 6.30 p.m. when the temperatures were optimum i.e. between 18 °C and 27°C. This is because at higher temperatures, the pollen and stigma desiccate (CIAT, 1986). The mean monthly Temperature were 25 °C and 28 °C with daily mean minimum of 26 °C and mean maximum of 30 °C. Actual mean monthly Temperature when conducting experiment range between 25 °C and 30 °C with daily mean minimum 25 °C and mean maximum 34 °C. Seeds were harvested and stored until next planting during evaluation stage.

3.2.2 Screening of the backcrossed lines from the crosses in section 3.2.1 for ALS resistance

The experiment was conducted in the screen house at Sokoine University of Agriculture. BC_{1-1} and BC_{1-2} of G5686 x Canadian wonder, Amendoim x Rojo, Mexico54 x Soya were involved. The F_2 and F_3 lines of crosses between Amendoim x Rojo, and Mexico54

x Rojo and only F₃ line of G5686 x Canadian wonder were used as checks. This makes a total of 11-treatments. Each treatment was planted separately in a block and a total of 30 seeds were planted per treatment. There were no randomization nor replication as normally this has no effect in early generations of bean improvement. The inoculum for the *P.griseola* was distributed equally to all treatments.

Planting was done in pots previously filled with sterilized loam soil where one seed was planted at the center of each pot. Gap-filling was done one week after germination for some of the seeds which failed to germinate. Irrigation was done daily so as to provide the required moisture. Triple Super Phosphate (TSP) fertilizer was applied at a rate of 60kg (P₂O₅) per hectare (i.e. 0.12g P₂O₅ per pot) at sowing and at flowering, Calcium Ammonium Nitrate (CAN) fertilizer was top dressed at a rate of 20kg (N) per hectare (i.e. 0.04g N per pot). Control of insect pests (Spider mites and white flies) was done by spraying Thionex 35 EC (40mls/20litres of water).

3.2.3 Dry Inoculum preparation and inoculation

Dry inoculum was prepared according to the method described by Inglis *et al.* (1988), as previously described in section 3.1.2. The dried leaves were grinded, packed in polythene bags and stored in refrigerator at 7°C in the laboratory. The concentration of conidia per gram of dried leaves was estimated by diluting the inoculum 1:100 with water and counting the number of conidia per milliliter with a hemacytometer. The final concentration of field collected dry leaf inoculum was approximately 1.2×10^4 conidia per gram.

Plants were inoculated 24 days from sowing at this time they had 2-3 sets of fully expanded trifoliate leaves. Plants receiving dry inoculum were first wetted with water. Dry inoculum was shaken gently over the leaves so the distribution would be as uniform as possible. Plants were inoculated early in the evening following irrigation to ensure that moist conditions prevailed throughout the infection period. There after the plants were covered by the white polythene bag to create micro-climate for disease development. The plants remained covered for 4 days while maintaining high levels of moisture to raise the RH necessary for fungal sporulation have taken place.

3.2.4 Evaluation of advanced backcrossed bean lines for ALS resistance

The experimental design used was Randomized Completely Block Design (RCBD). The experiment was done in the field, and there were three replications in two different locations, one at Morogoro (Sokoine University of Agriculture) and another one at Arusha (Selian Agriculture Research Institute). Each location involved 25 advanced backcrossed bean lines plus three check lines (Table 2). Sowing was done at a spacing of 50cm between rows and 20cm within rows. A plot had three rows per treatment and 15 plants per row which gave a plot size of $2\text{m} \times 3.2\text{m} = 6.4\text{m}^2$. Each treatment made a single plot, so there were 28 plots. Treatments were randomly assigned to plots.



Plate 1: Field plot layout

Planting was done by dibbling one seed per hill. No gap filling done due to limited number of seeds. There was no irrigation as the trial was conducted during rain season. Fertilizers were applied, DAP was applied during planting time at a rate of 25kg per hectare (16g per plot) and top dressed with nitrogenous fertilizer (UREA) at a rate of 20kg (N) per hectare (12.8g (N) per plot) to facilitate vegetative growth. Weeding and insecticide application to control insects were also done as per requirement.

3.3 Data collection

3.3.1 Disease evaluation

Data were collected at 7, 14, 21, and 28 days after inoculation for the disease reaction on trifoliate leaves. The disease rating was done based on assessment of the severity of disease on trifoliate leaves and general appearance of the diseased plant using CIAT rating scale of 1-9 described as follows: 1 represented plants with no visible symptoms; 3 = plants with 4 – 10% of the leaf area with lesions; 5 = plant with 20% of the leaf area with lesions and sporulation; 7 = plants with up to 60% of the leaf area with lesions and sporulation associated with chlorosis and necrosis; 9 = plant with up to 90% of the leaf area with lesions associated with loss of the leaves and plant death. Genotypes that had a score of 1 - 3 were considered resistant. 4 – 6 intermediate and scores greater than 6 were considered susceptible (Mahuku *et al.*, 2003; Appendix 3). Because the scores recorded on the 22 day were the highest, these are the ones that have been reported here.



Plate 2: Symptoms of angular leaf spot on trifoliate leaf

3.3.2 Agronomic and crop yield data

Yield and yield components data were collected, including 50% flowering, 85% maturity, number of pods per plant, number of seeds per pod, 100 seed weight per plot and seed yield per plant.

3.3.2.1 Number of pods per plant

Counting of pods was done on each individual plant harvested, and total number of pods per plant were recorded.

3.3.2.2 Number of seeds per pod

Counting of seeds was done from all pods, and then average number of seeds per pod was determined by the following relationship:

Total number of seeds per plant/Number of pods per plant = Number of seeds per pod

3.3.2.3 100 seed weight (g) and seed yield per plant (g/plant)

Weight of 100 seeds obtained from each plot was recorded. Then the seeds harvested in each plot were weighed and the seed yield per plant was calculated as follows:

Seed yield / plant = Seed weight of harvested plants/Number of harvested plants.

3.4 Data analysis

Data were analyzed by using the statistical package MSTATI-C for the screen house experiment and IRRISTAT for field experiment. Analysis of variance (ANOVA) and mean separation were done using Duncan Multiple Range Test (DMRT) and correlation analysis were performed among variables (disease score and yield data). For the inheritance study, frequency analysis was performed and distribution graph were plotted.

Heritability values can be estimated based on the resemblance between parent-offspring. The regression coefficient of offspring on one parent (b) can be calculated and heritability can be estimated as $h^2 = 2b$. When both parents are involved regression coefficient of offspring on mid-parent is clearly related to the procedure for estimating realized heritability where $h^2 = b$. In the regression (x values) are the mean values of parent and (y values) are the mean values of offspring. In this study heritability was estimated using $h^2 = b$ as both parents were involved.

Statistical models

- a) For screen house experiment the following model was used:

$$X_{ij} = \mu + T_i + \epsilon_{ij}$$

Where: X_{ij} = Response

μ = overall mean

T_i = treatment effect for treatment i , $i = 1, 2 \dots t$,

ϵ_{ij} = error term for each observation

- b) For field experiment the following model was used:

$$Y = \gamma + R_i + S_j + \epsilon_A + V_i + (VS)_{ij} + \epsilon$$

Where: Y = Response

γ = Constant

R_i = Replication effect

S_j = Site effect

ϵ_{ij} = Error due to replication and Site

V_i = Variety effect

$(VS)_{ij}$ = Interaction effect due to site and variety

ϵ = Residue error

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Production of backcrossed lines

Results for number of crosses made, number of pods harvested, %successful crosses and total number of seeds harvested are presented in Table 3. For every parent many successful crosses were made.

Table 3: Number of crosses, pods and F₁ seeds

F ₂ bean lines	Parents	No. of crosses made	No. of pods harvested	% successful crosses	Total No. of F ₁ seeds harvested
G5686 x C/w	G5686 (P ₁)	23	17	73.9	63
G5686 x C/w	C/w (P ₂)	22	16	72.7	54
Amend x Rojo	Amend (P ₁)	19	17	89.5	59
Amend x Rojo	Rojo (P ₂)	29	22	75.8	74
Mex54 x Soya	Mex54 (P ₁)	18	12	66.6	39
Mex54 x Soya	Soya (P ₂)	19	11	57.9	35
Total		150	95	63.3	324

4.2 Screening of backcrossed lines

4.2.1 General observations

Symptoms were observed in the second week after inoculation these were in agreement with observations reported by (Inglis *et al.*, 1988; Mlabwa and Lyimo 1989; Pastor Corrales *et al.*, 1998; Mahuku *et al.*, 2003) that angular leaf spot developed on inoculated plants within two to three weeks after inoculation.

The effect of ALS on common bean depends on susceptibility of bean genotype, stage of bean growth at which the disease was severe and initial inoculum concentration. These

factors could have contributed to the result observed in this experiment for disease expression. The results presented in Table 4 shows that bean lines Amendoim x Rojo x Amendoim, Amendoim x Rojo (F_2), Amendoim x Rojo (F_3), G5686 x Canadian wonder x G5686, G5686 x Canadian wonder (F_2) expressed resistance where as the lines Mexico54 x Soya (F_2), Mexico54 x Soya (F_3), Mexico54 x Soya x Soya, Mexico54 x Soya x Mexico54, Amendoim x Rojo x Rojo, G5686 x Canadian wonder x Canadian wonder, showed intermediate resistance.

Table 4: Result summary for backcrossed common bean lines

Bean line/variety	Days to 50%	Days to 85%	Disease data				No of pods per plant	No of seeds per pod	100 seed weight (g)	Seed yield per Plant (g)	
			Mean disease score	Variance	Standard deviation	Standard Error					
MEX54×SOYA	F ₂	37	58	3.9	0.487	0.698	0.134	6	2	39.5	3.8
	F ₃	39	55	3.6	0.704	0.839	0.161	9	2	44.4	7.2
	BC ₁₋₁	42	60	4.2	0.397	0.630	0.119	7	2	35.9	4.5
	BC ₁₋₂	42	58	3.8	0.387	0.622	0.120	10	3	41.1	9.2
AMEND×ROJO	F ₂	42	55	2.8	0.362	0.602	0.112	10	3	39.5	6.3
	F ₃	42	56	2.6	0.317	0.563	0.103	16	3	38.9	9.2
	BC ₁₋₁	39	58	2.2	0.254	0.504	0.092	13	3	41.1	9.0
	BC ₁₋₂	36	58	3.6	0.731	0.855	0.156	8	3	37.3	8.8
G5686×C/W	F ₃	39	53	3.2	0.841	0.917	0.187	7	4	51.1	11.7
	BC ₁₋₁	43	56	2.4	0.312	0.558	0.100	7	4	48.1	11.5
	BC ₁₋₂	36	53	3.5	1.593	1.262	0.252	11	4	47.4	18.6

Table 5: Mean performance of parents

Gene pool	Parent	Disease score	Number of Pods per plant	Seed yield per plant (g)
Donor	G 5685	1.0	3	6.4
	Amendoim	1.0	7	7.5
	Mexico 54	1.0	7	13.6
Recipients	C/Wonder	5.0	8	10.9
	Rojo	2.8	9	11.1
	Soya	4.5	7	9.9

Kweka, (2005)

The overall disease reaction of lines to *Phaeoisariopsis griseola* indicates that there was variation among lines in disease reaction ($P \leq 0.05$). This could be contributed to differences in genotypes since the environmental factors, method of inoculation, inoculum concentration, and the management of the crop were similar.

The minimum score was 2.2 obtained in the backcrossed population Amendoim x Rojo x Amendoim while the highest mean score for disease reaction observed was 4.2 obtained in backcrossed population Mexico54 x Soya x Mexico54. Such scores are low as the disease reaction is resistant to intermediate resistant. This implies that the varieties Soya, Rojo and Canadian wonder have been improved for angular leaf spot resistance to a certain level. When this improved varieties for Angular leaf spot were crossed to the donor parents (Mexico 54, Amendoim and G5686), their level of resistance tend to increase to a certain level, if the character of interest (resistant to angular leaf spot disease) is still deficient the backcrossing to their respective parents is expected to improve that character. Ngulu, (1999) reported that G5686, Mexico 54 and Amendoim displayed good levels of resistance to angular leaf spot, could be

used as sources for resistance and also serve as reliable indicators of new races of ALS pathogens.

Results shows that there was variation among lines for yield and yield components data which includes; number of pods per plant, number of seeds per pod, 100 seed weight and seed yield per plant (Table 4). The significant variation ($P \leq 0.05$) observed amongst lines evaluated in the screen house could be explained by the fact that the populations were genetically variable. Similar results were reported by Adams, (1967), Chung and Goulden, (1971), Denis and Adams, (1978), Coyne, (1978), Mduruma, (1996), Kweka, (2005) that the common bean varieties and their crosses vary significantly for each yield component.

4.2.2 Performance of F_2 , F_3 , BC_{1-1} and BC_{1-2} of Mexico54 $\times$ Soya

4.2.2.1 Disease reaction

There was significant difference ($P \leq 0.05$) for disease reaction between BC_{1-1} (Mexico54 $\times$ Soya $\times$ Mexico54) and F_2 (Mexico54 $\times$ Soya) populations (Table 4). But there were no significant differences between BC_{1-2} (Mexico54 $\times$ Soya $\times$ Soya) and F_3 (Mexico54 $\times$ Soya), to *P. griseola*. The crosses showed intermediate resistance to the disease (Figure 1). The mean disease score for F_2 , F_3 , BC_{1-1} and BC_{1-2} populations were 3.9, 3.6, 4.2 and 3.8 respectively. This indicates that there are one or few genes controlling ALS disease. These results support earlier studies by Cardona-Alvarez (1958), Mahuku *et al.* (2003), which showed that inheritance of ALS resistance is complex, involving both dominant and recessive genes that may be or may not be independent.

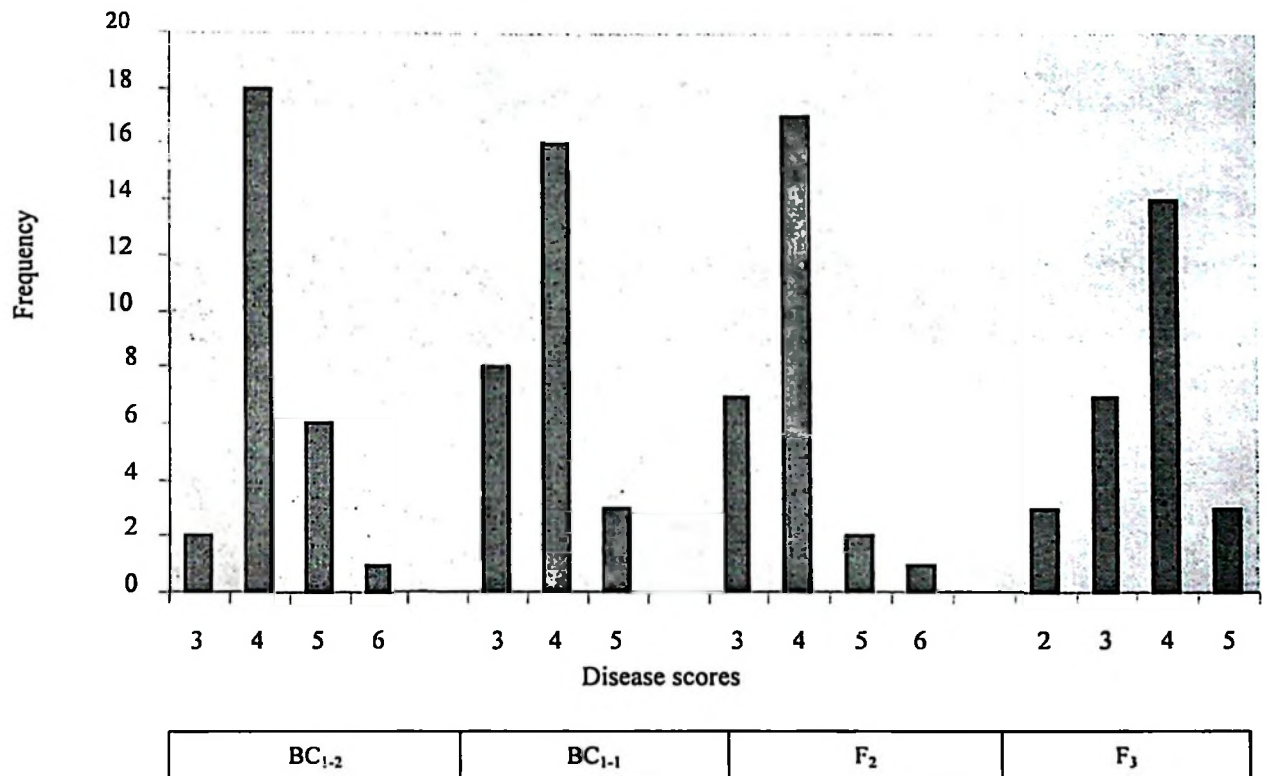


Figure 1: Distribution for (ALS) disease scores among the populations of Mexico54 × Soya

4.2.2.2 Number of pods per plant

There was variation in number of pods per plant ($P \leq 0.05$) among the F₂, F₃, BC₁₋₁ and BC₁₋₂ of Mexico54 × Soya (Table 4); The means for F₂, F₃, and BC₁₋₁, BC₁₋₂ were 6, 9, 7 and 10 respectively while the mean for resistance parent 1 (Mexico 54) was 7 and that of susceptible parent 2 (Soya) was also 7 (Table 5). The highest mean was observed in BC₁₋₂ population indicating that the yield potential of the variety Mexico54 × Soya has been improved by crossing it with Soya. Thus Soya has higher yield potential.

4.2.2.3 Number of seeds per pod

Results for the F_2 , F_3 , BC_{1-1} and BC_{1-2} of Mexico54 $\times$ Soya show that the mean value for seeds per pod were 2, 2, 2 and 3 respectively (Table 4). These results indicate that there has been some improvement in increasing number of seeds per pod as the backcrosses performed better than the F_2 and F_3 .

4.2.2.4 100 seed weight and seed yield per plant (g)

The 100 seeds weight for the F_2 , F_3 , BC_{1-1} and BC_{1-2} of Mexico54 $\times$ Soya were 39.5, 44.4, 35.9 and 41.1. Seed yield per plant in F_2 , F_3 , BC_{1-1} and BC_{1-2} of Mexico54 $\times$ Soya were good; The F_2 and BC_{1-1} yields were low compared to those of F_3 and BC_{1-2} (Table 4). This shows that although there is an improvement in yield in F_2 generation but backcrossing to the recipient parent Soya which is good in yield potential than donor parent (Mexico 54) has contributed largely to the improvement of seed yields per plant. In this case the highest yield per plant was observed in BC_{1-2} which yielded 9.2g.

4.2.3 Performance of F_2 , F_3 , and BC_{1-1} , BC_{1-2} of Amendoim $\times$ Rojo

4.2.3.1 Disease reaction

There was variation among F_2 , F_3 , and BC_{1-1} and BC_{1-2} in disease reaction in all populations from this cross (Table 4), where the mean scores for disease reaction were 2.8, 2.6, 2.2 and 3.6 respectively. Except for BC_{1-2} (Amendoim $\times$ Rojo $\times$ Rojo) which had intermediate resistance to the disease, all other crosses showed resistance to the disease (Figure 2).

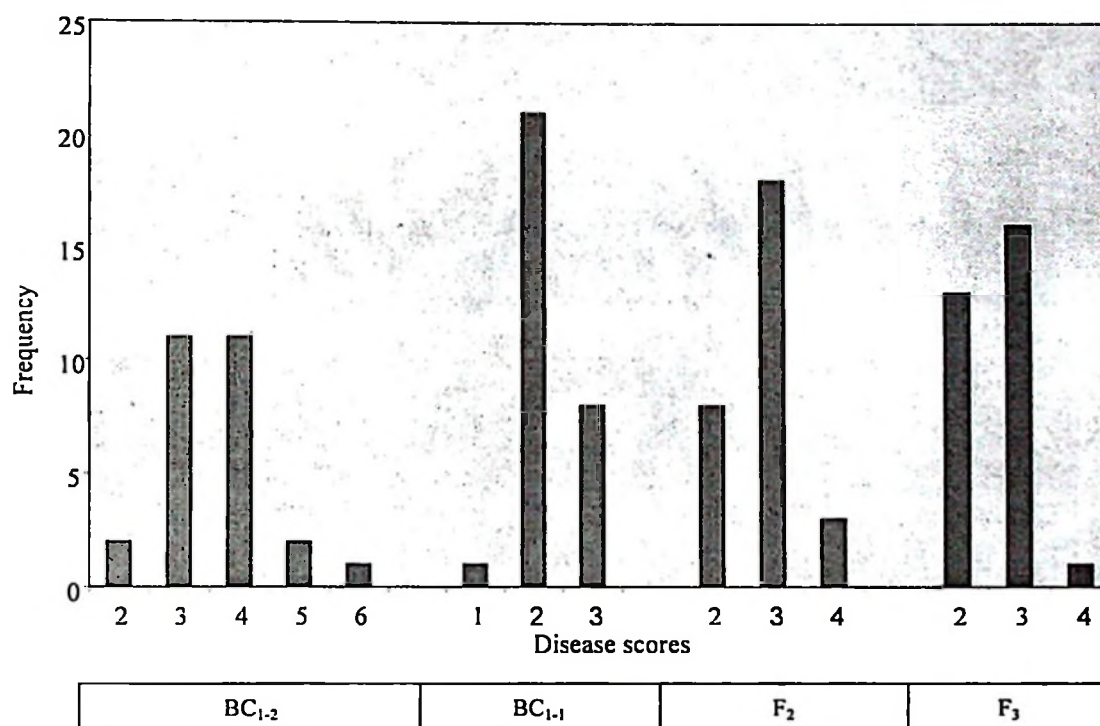


Figure 2: Distribution for (ALS) disease scores among the populations of Amendoim × Rojo

4.2.3.2 Number of pods per plant

There was significant ($P \leq 0.05$) variation in number of pods per plant among the F₂, F₃, and BC₁₋₁, BC₁₋₂ of Amendoim × Rojo (Table 4). The means for F₂, F₃, and BC₁₋₁, BC₁₋₂ were 10, 16, 13 and 8 respectively; The mean for resistant parent 1 (Amendoim) was 7 and that of susceptible parent 2 (Rojo) was 9 (Table 5). The highest mean was observed in F₃ lines. This shows that the yield potential of variety Rojo has been improved by crossing it with Amendoim. Thus Amendoim is good in ALS disease resistance and also in yield potential.

4.2.3.3 Number of seeds per pod

Results for F₂, F₃, and BC₁₋₁, BC₁₋₂ of Amendoim × Rojo show that the mean value of seeds per pods in all populations was 3 (Table 4). This indicates that there is no variation in seeds per pod among the F₂, F₃, and the backcrosses. Results may imply that both varieties Amendoim and Rojo are good in yield potential.

4.2.3.4 100 seed weight and seed yield per plant (g)

100 seeds weight for F₂, F₃, and BC₁₋₁, BC₁₋₂ of Amendoim × Rojo were 39.5, 38.9, 37.3 and 41.1 respectively. Seed yield per plant in F₂, F₃, and BC₁₋₁, BC₁₋₂ of Amendoim × Rojo were also good, and the backcrossed lines yielded higher than the F₂ lines (Table 4). This indicates that Amendoim has medium seeds and Rojo has bigger seeds that contributed to high seed yield per plant. The highest seed yield per plant was observed in F₃ with 9.2g per plant.

4.2.4 Performance of F₃, and BC₁₋₁, BC₁₋₂ of G5686 × Canadian wonder

4.2.4.1 Disease reaction

The F₃ and BC₁₋₁ (G5686 x Canadian wonder x G5686) showed good level of resistance to the disease (Table 4) with mean disease scores of 3.2 and 2.4 respectively, while BC₁₋₂ (G5686 x Canadian wonder x Canadian wonder) showed intermediate resistance to the disease with a mean disease score of 3.5 (Figure 3). This shows that in addition to major genes, minor genes have been found to condition resistance of common bean to *P. griseola*. On backcrossing to susceptible parent there is segregation of resistant and susceptible genes which gave an intermediate resistance, but in F₃ population and on backcrossing to resistant parent

there is high segregation to resistance compared to susceptible genes thus demonstrate a resistant reaction to ALS disease.

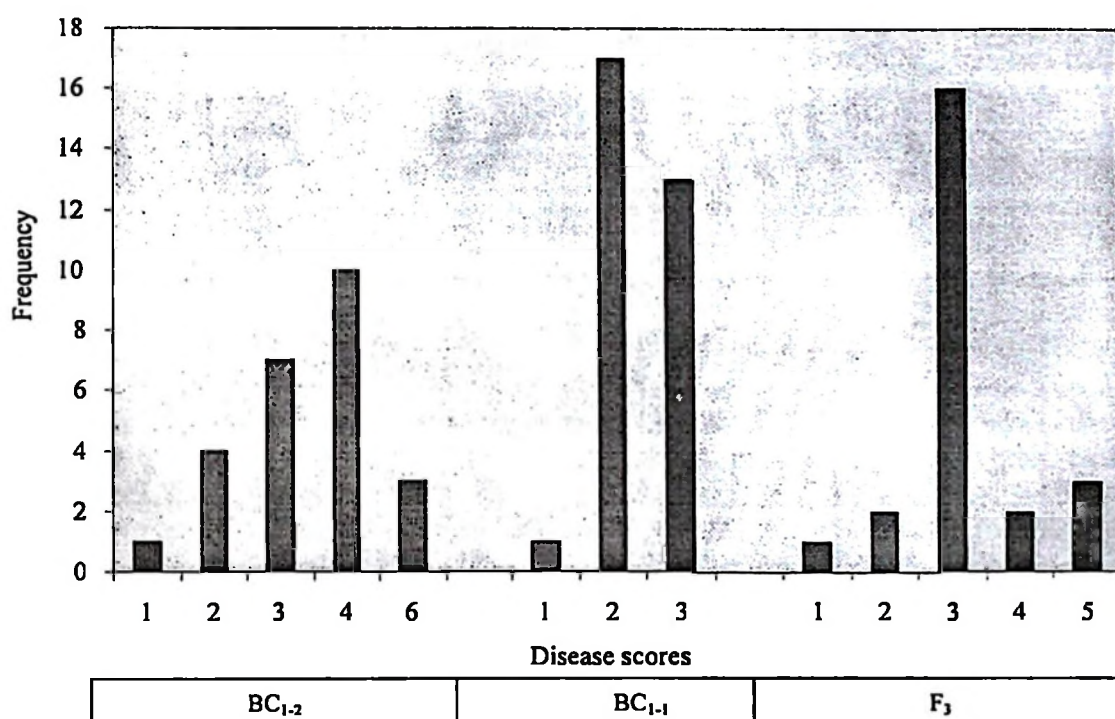


Figure 3: Distribution for (ALS) disease scores among the populations of G5686 × C/wonder

4.2.4.2 Number of pods per plant

There was variation ($P \leq 0.05$) in number of pods per plant among the F₃, BC₁₋₁ and BC₁₋₂ of G5686 × Canadian wonder (Table 4). The means for F₃, BC₁₋₁ and BC₁₋₂ population were 7, 7 and 11 respectively. The highest mean was observed in BC₁₋₂. This higher number of pods per plant for this backcrossed line might have been influenced by the high potential ability showed by Canadian wonder in producing many flowers as a result more pods per plant.

4.2.4.3 Number of seeds per pod

Results for F_3 , and BC_{1-1} , BC_{1-2} of $G5686 \times$ Canadian wonder gave the mean value of 4 for the number of seeds per pod (Table 4). This shows that there is no variation in seeds per pods in the F_3 , and the backcrosses.

4.2.4.4 100 seed weight and seed yield per plant (g)

The 100 seeds weights (g) of F_3 , and BC_{1-1} , BC_{1-2} of $G5686 \times$ Canadian wonder were 51.1, 48.1 and 47.4 respectively (Table 4). In comparison with other populations of $Mex54 \times$ Soya and $Amend \times$ Rojo, this population gave the highest means of 100 seeds weight (Table 4). This is due to larger seed sizes in this population. Seed yield per plant in F_3 , BC_{1-1} and BC_{1-2} of $G5686 \times$ C/wonder were 11.7, 11.5, and 18.6. The highest yield was observed in the BC_{1-2} . This is justified by the high potential of Canadian wonder to produce many flowers, and this has contributed largely to the high yield potential of BC_{1-2} .

4.3 Inheritance studies on angular leaf spot (ALS) and yield components

High number of pods per plant is an indication of high yield so number of pods per plant have been used in estimating heritability for yield component. In this study heritability estimates for ALS and number of pods per plant were done for two populations of $Mexico54 \times$ Soya and $Amendoim \times$ Rojo. The study was not possible in $G5686 \times$ Canadian wonder population as F_2 population were not planted due to lack of seeds.

4.3.1 Inheritance studies on ALS

Results obtained on heritability estimates for ALS disease for Mexico54 x Soya and Amendoim × Rojo were 0.228 and 0.190 respectively. According to Herald (1994) classification, these results shows that heritability for disease resistance in Mexico54 x Soya was Moderate or medium and low or weak in Amendoim × Rojo. Kweka (2005) also reported similar observations that heritability estimate for disease resistance was 0.173 in Amendoim group which is low/weak. This shows that non-genetic factors such as weather soils and concentration of initial inoculum play a greater role in the overall performance. This indicates that selection in early generation would not be effective as it is difficult to select genetically superior individuals so it is suggested that selection for disease resistant should be done in later generations.

4.3.2 Inheritance studies on number of pods per plant

Estimates of heritability for number of pods per plant were 0.3 in Amendoim × Rojo and 0.73 in Mexico54 x Soya respectively. According to Herald (1994) classification the value for Amendoim x Rojo is moderate or medium and for Mexico54 x Soya is high or strong this implies that, this character has not been influenced by environment. These results are in agreement with the results reported by (Kweka 2005) Traits with relatively high heritability or additive gene variance have been reported to respond highly to selection and cross breeding (Falconer and Mackay, 1998). Thus early selection for this trait will be effective in crop improvement (Singh and Sharma 1982; Mduruma, 1996; Falconer and Mackay, 1998; as cited by Kweka 2005) also reported similar observations.

4.4 Performance of advanced bean lines

Results are presented in Table 6. The ALS symptoms developed mostly at flowering stage. Of the 25 advanced lines screened all were identified as intermediate or resistant to ALS in the field in both Morogoro and Arusha sites. Moreover, few of these lines did not develop any disease symptoms and these might be immune or have escaped the disease (i.e. had a score of 1).

High levels of resistance to *P. griseola* were found (scores of 2 to 3) in the lines screened on field in Morogoro. This is due to the field history for this site the field used was new so the initial inoculum concentration might be low. For those screened in Arusha 12 lines showed intermediate levels of resistance that is had scores of 4 to 6 (Table 6). The field history for this site is good, which gave good levels of initial inoculum, none of the lines were highly susceptible (score 7 to 9) as all lines demonstrated high and moderate levels of resistance in ALS pathogen.

Results for yield and yield component of advanced bean lines shows that there was significant variation among 25 advanced lines within location and between locations. In general Arusha trial out yielded the trial in Morogoro (Table 6).

Table 6: Performance of advanced backcrossed lines on disease and yield components in two sites

Bean line/Variety	Days to 50% flowering		Days to 85% maturity		Disease score		No. of pods per plant		No. of seeds per pod		100 seed weight (g)		Seed yield per plant (g)	
	Mor	Ar	Mor	Ar	Mor	Ar	Mor	Ar	Mor	Ar	Mor	Ar	Mor	Ar
PSO4-336Ab-7	47	50	66	65	1.7	2.3	9	15	3	4	35.9	44.3	8.4	21.5
PSO4-126Aa-8	44	49	62	66	2.4	2.0	8	14	3	3	39.0	36.6	8.8	16.7
PSO4-333Aa-19	47	46	65	67	2.2	4.0	9	16	4	3	49.5	43.4	7.7	17.6
PSO4-333Aa-21	49	49	64	72	2.1	4.0	18	16	4	3	40.0	63.3	16.6	20.0
PSO4-207Ab-6	51	48	65	72	1.4	4.2	13	11	3	3	38.1	33.3	8.6	7.9
PSO4-335B-1	47	48	67	72	1.9	3.3	16	18	2	3	40.3	51.2	9.6	25.9
PSO4-139Bb-7	47	52	65	70	2.3	4.4	11	17	3	3	33.0	27.6	6.8	14.7
PSO4-330a-1	49	51	66	68	1.5	3.9	18	18	3	3	38.3	36.4	16.1	19.3
PSO4-139Ba-1	44	50	63	68	2.2	1.1	13	22	3	2	29.4	33.9	10.5	16.7
PSO4-333Aa-3	49	53	64	71	2.0	3.5	13	18	4	3	40.8	37.3	10.2	16.3
PSO4-139Bb-5	45	50	61	70	2.3	3.1	11	16	4	3	33.0	29.3	10.4	16.5
PSO4-333Aa-18	46	54	64	70	2.8	4.4	10	16	3	3	41.4	35.7	10.9	13.9
PSO4-139Bb-1	46	46	65	66	2.8	2.3	11	18	3	3	33.9	29.1	11.2	16.2
PSO4-112D	50	54	66	72	2.7	4.8	6	19	3	4	46.3	45.8	6.3	32.2
PSO4-106Ba-2	51	53	67	71	1.7	2.5	10	14	4	3	39.7	40.1	7.7	13.0
PSO4-139Aa-10	45	50	63	67	3.5	6.0	6	16	3	2	38.8	36.4	5.6	11.8
PSO4-139Bb-4	45	48	65	70	1.6	2.1	8	24	4	3	30.8	30.0	6.3	23.9
PSO4-126Ab-8	45	46	64	68	1.6	3.2	12	15	4	3	48.0	49.2	10.7	16.7
PSO4-335B-12	47	47	63	68	1.5	3.5	13	20	3	3	48.6	45.7	15.6	22.9
PSO4-139Ba-7	49	51	65	67	2.2	1.1	16	18	4	3	37.9	29.6	8.9	16.2
PSO4-139Aa-6	45	53	64	72	3.9	2.9	15	30	3	3	26.5	22.1	12.0	21.7
PSO4-336Aa-7	45	48	66	66	3.3	5.0	9	16	3	3	29.0	33.6	7.1	14.8
PSO4-139Ab-10	46	51	64	70	3.3	1.7	9	20	3	3	33.0	38.1	6.5	17.0
PSO4-126Aa-6	45	48	65	70	2.0	2.9	9	13	4	3	43.3	54.7	10.3	16.4
PSO4-106Bb-3	45	49	63	66	2.4	4.6	11	14	4	3	31.0	30.9	6.0	11.1
SUA 90	46	52	63	70	2.4	3.0	7	15	4	4	28.2	29.2	2.6	13.5
ROJO	46	52	62	69	3.0	5.2	6	14	3	3	44.7	47.0	6.7	14.5
C/WONDER	47	52	63	68	2.7	3.2	7	14	4	3	41.1	43.2	9.3	15.2
Mean	47	50	64	69	2.4	3.3	11	17	3	3	37.7	38.5	9.2	17.3
C.V	3.8	4.8	3.9	2.9	36.3	27.1	53.5	48.3	40.6	32	8.4	8.0	0.0	0.0
S.E	1.03	1.39	1.45	1.45	0.18	0.15	1.17	1.42	0.28	0.17	1.83	1.77	0.00	0.10
LSD (0.05)	2.91	3.93	4.11	3.26	0.49	0.43	3.26	3.94	0.78	0.47	5.19	5.01	0.00	0.28

Key: Mor Artusha
Ar Morogoro

4.4.1 Performance of advanced bean lines in Morogoro

4.4.1.1 Disease reaction

In Morogoro there was significant variation among the lines ($P \leq 0.05$) it was revealed that the line PSO4-207Ab-6 was the only one that might be immune to the disease (i.e. it has a score of 1.4). With the exception of line PSO4-139Aa-6 which showed intermediate resistant to the disease all other lines were resistant to ALS because they had disease scores of 2 to 3 (Table 6). Contrary to what was expected the three adapted cultivars used as checks (SUA90, Rojo, and Canadian wonder) were also resistant to the disease with the mean scores of 2.4, 3.0 and 2.7 respectively (Table 6), this has been contributed by the level of initial inoculum as the field used was new, with no history of ALS disease.

4.4.1.2 Number of pods per plant

There was significant variation in number of pods per plant ($P \leq 0.05$). The overall mean for number of pods per plant was 11. Bean line PSO4-139Aa-10 with mean number of pods per plant of 6 had the lowest value while the line PSO4-330a-1 had a mean number of 18 which was the highest value obtained in Morogoro. This indicates that the lines are good as the general performance is good. Results are in agreement with result for the disease reaction as plants are resistance to the disease or have escaped the disease (like the susceptible checks) and produce more pods per plant.

4.4.1.3 Number of seeds per pod

There was significant variation in number of seeds per pod ($P \leq 0.05$). The overall mean number of seeds per pod was 3. The minimum mean number of seeds per pod was 2 obtained in the line PSO4-335B-1 and the highest mean number of seeds per pods was 4 obtained in the line PSO4-139Bb-4. Except for line PSO4-335B-1 with the minimum value all other lines had scores between 3 and 4 (Table 6). The results for the three checks used did not differ from those of 25 advanced lines. This shows that the lines perform better as they produce many seeds per pod.

4.4.1.4 100 seed weight and seed yield per plant (g)

Results are presented in Table 6. There was significant variation in 100 seeds weight between lines ($P \leq 0.05$). The difference observed between lines was mainly due to differences in seed size. Larger seeds have more weight compared to small seeds. But the difference obtained within line between locations was mainly due to performance of a line in different location. For example in the line PSO4-333Aa-21 100 seeds weight in Morogoro was 40g while 100 seeds weight in Arusha was 63.3g, in the line PSO4-333Aa-18 the 100 seeds weight in Morogoro was 41.4g, while in Arusha was 35.7g. This shows that the production situation were not homogeneous with regard to factors influencing yield, such as soil, microclimate, cultural practices, and cultivar. Results for seed yield per plant shows that yield per plant in Morogoro were generally low compared to yield in Arusha trial as the average yield per plant in Morogoro were 9.2g and that of Arusha were 17.3g (Table 6).

4.4.2 Performance of advanced bean lines in Arusha

4.4.2.1 Disease reaction

Evaluation of the same lines in Arusha, results presented in Table 6 revealed a significant variation in disease score ($P \leq 0.05$). Two lines (PSO4-139Ba-1 and PSO4-139Ba-7) might be immune to ALS disease (score of 1), 13 lines were found to be resistant, and 12 lines were intermediate resistant to ALS. Of the three checks used SUA90 and Canadian wonder were found resistant to the disease while Rojo showed intermediate resistance to the disease contrary to what was expected. This support the early findings that the two varieties released at SUA (SUA 90 and Rojo) are adapted to low and mid-altitude (300–1500 m) bean agro-ecologies, and are high yielding under smallholder conditions (up to 2000 kg/ha) and are moderately resistant to rust, ALS, BCMV and BCMNV. Both varieties show some tolerance to drought, and bean fly [observations in farmers' fields in northern Tanzania], are early-maturing (65–74 days) and cook more quickly than most local varieties (CRSP, 2005)

4.4.2.2 Number of pods per plant

There was variation in number of pods per plant ($P \leq 0.05$). The overall mean was 17. The lowest value for number of pods per plant was 11 obtained in the line PSO4-207Ab-6 and the highest value was 30 obtained in the line PSO4-139Aa-6. The results shows that apart from improving disease resistance, yield of the crosses has been improved thus increased pod numbers.

The results shows that the checks produce few pods per plant compared to the 25 advanced lines used although the values do not differ much among all the lines tested and the checks used (Table 6).

4.4.2.3 Number of seeds per pod

The results indicate that there was variation in number of seeds per pod ($P \leq 0.05$). The overall mean for number of seeds per pods was 3, the minimum mean number of seeds per pod was 2 obtained in the line PSO4-139Ba-1 and the highest mean number of seeds per pod was 4 obtained in the line PSO4-112D except three lines which had a mean of 4 all other 25 lines had a mean ranging between 2-3 (Table 6) which is lower compared to Morogoro. This can be justified by the fact that trial in Arusha produced many seeds per pod (Table 6) so this balances the distribution of biomass. But the general performance is good.

4.4.2.4 100 seed weight and seed yield per plant (g)

There was significant variation in 100 seeds weight among bean lines between locations ($P \leq 0.05$) for example the line PSO4-336Ab-7 100 seeds weight in Morogoro was 35.9g, while in Arusha was 43.3. This proves that the production situation were not homogeneous with regard to factors influencing yield, such as soil, microclimate, cultural practices, and cultivar. The significant differences which were observed between lines within location were mainly due to seed sizes; always larger seeds are heavier than small sizes seeds. Results for seed yield per plant shows that

Arusha trial performed better than the trial in Morogoro the mean yield per plant in Arusha was 17.3g while that of Morogoro was 9.2g (Table 6). Although some of the seeds seem to be well developed but it seems that these lines prefer highland conditions as the trial in low altitudes (Morogoro) seeds are lighter in weight than those from high altitudes (Arusha).

4.5 Correlation between variables

Correlation helps in selecting the desired traits in any breeding programme. Genotypic correlations are important in connection with the genetic causes of correlation, and also in changes brought about by selection (Mduruma, 1996).

The relationship between the severity of ALS and yield data was also investigated by comparing the data for disease scores and yield components. Conversely, the disease and yield data showed coherence in all trials, higher yield and lower disease were always characteristic (Table 6). Although there were few exceptions for example in Arusha site PSO4-126Aa-8 a large seeded, with a disease score of 2.0 and low yield of 16.7g per plant, PSO4-139Ba-1 with a disease score of 1.1 and low yield of 16.7g or PSO4-139Ba-7 with a disease score of 1.1 with low yield of 16.2g per plant, this is based on their background (Table 2), crossing a high yielding and low yielding varieties may result into an intermediate progeny. The parameters were correlated to determine their influence on each other and results are presented in Tables 6 and 7.

4.5.1 Correlation coefficients of variables in Morogoro

Significant and positive correlations ($P \leq 0.01$) were observed in number of pods per plant and seed yield, 50% flowering and 85% maturity. The results give more evidence that more pods per plant indicate more yields (Saraji, 1978; Malley *et al.*, 1987; Mduruma and Nchimbi, 1992; as cited in Kweka, 2005). This means that as number of pods per plant increases, seed yield per plant also increases. The highly significant and positive correlations between seed yield and number of pods per plant indicates that the number of pods per plant could also be used as selection criterion in breeding for high yielding cultivars.

Significant and negative correlation were observed in disease score and 100 seeds weight, this implies that highly diseased plants give seeds which are light in weight. Correa, (1984) reported that susceptibility to ALS is associated with large and medium sized seed types, this is in agreement with the findings by Guzman *et al.* (1995) that *Phaeoisariopsis griseola* was the first bean pathogen in which coevolution was clearly demonstrated between pathogen and host gene pool. Molecular data on pathogen variability continues to support the separation into two major groups Andean and Middle American, (Guzman *et al.*, 1999). The Andean types that are large seeded are more susceptible to ALS compared to the Middle American type.

Table 7: Advanced lines Morogoro

Parameters	1	2	3	4	5	6	7
1. Disease (ALS)	1.00						
2. Pods per plant	-0.319 ns	1.00					
3. Seeds per pod	-0.134 ns	-0.071 ns	1.00				
4. 100 seed weight	-0.417*	-0.046 ns	0.072 ns	1.00			
5. Seed yield	-0.298 ns	0.751**	0.039 ns	0.215 ns	1.00		
6. 50% flowering	-0.247 ns	0.311 ns	-0.034 ns	0.157 ns	0.045 ns	1.00	
7. 85% maturity	-0.182 ns	0.151 ns	-0.138 ns	0.283 ns	-0.019 ns	0.045**	1.00

** Significant at 1% level

* Significant at 5% level

ns Not significant

ALS Angular Leaf Spot

4.5.2 Correlation coefficients of variables in Arusha

Significant and positive correlation ($P \leq 0.01$) were observed in pods per plant and seed yield, seeds per pod and seed yield, 50% flowering and 85% maturity, The results proves the above evidence that more pods per plants means more yield, this implies that as number of pods per plant increases, seed yield per plant tended to increase. Similar observations were reported by (Mhina, 1985). Results also shows that more seeds per pod means more yields, and that early flowering means early maturity.

Table 8: Advanced lines Arusha

Parameters	1	2	3	4	5	6	7
1. Disease (ALS)	1.00						
2. Pods per plant	-0.314 ns	1.00					
3. Seeds per pod	0.058 ns	0.014 ns	1.00				
4. 100 seed weight	0.120 ns	-0.380 ns	-0.039 ns	1.00			
5. Seed yield	-0.125 ns	0.509**	0.463**	0.326 ns	1.00		
6. 50% flowering	0.165 ns	0.102 ns	0.143 ns	-0.109 ns	0.092 ns	1.00	
7. 85% maturity	0.101 ns	0.254 ns	0.010 ns	-0.017 ns	0.162 ns	0.761**	1.00

** significant at 1% level

ns Not significant

ALS Angular Leaf Spot

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

- a) The lines used in this study were intermediate or resistant to ALS disease; moreover, some of these did not develop any disease symptoms and thus might be immune or escaped the disease. (i.e. had a score of 1).
- b) The three donor parents used in backcrossed lines (that is G 5686, Mexico54, and Amendoim) still displayed good levels of resistance to ALS; The finding implies that these donor parents can also be used as indicators of new races of pathogen in nature because *Phaeoisariopsis griseola* is highly variable.
- c) There was optimum environmental condition in the screen house at SUA during the crossing process that led to fewer abortions, and during screening for disease resistance the environmental condition was also optimum for the disease initiation and disease development.
- d) Moderate to high heritability estimates obtained in this study for number of pods per plant indicates that this trait could be improved through selection in the early generations.

- a) Heritability for disease resistance was moderate or medium and low or weak. This shows that selection in early generation is not effective. It is difficult to select genetically superior individuals, therefore the results suggests that selection for disease resistant should be done in later generations.
- b) Number of pods per plant was significant and positively correlated with seed yield. This indicates that increased number of pods per plant will contribute to increase in yield.

5.2 Recommendations

- a) Since this study was the initial stage in hybridization programme, further studies are still needed in the advanced generations of the backcrossed lines, and breeders may use this hybridization programme for improving other genotypes by incorporating the desired traits from donor parents.
- b) For advanced lines it is important to consider gene pyramiding for durable resistance. The use of molecular markers, therefore, can be a useful tool for the selection of resistant plant genotypes. Breeding for durable resistance to ALS will be easier provided ALS-resistant parents are thoroughly tested against *P.griseola* populations, and appropriate recombination and selection methods are employed for field and greenhouse screenings of segregating populations, families, and advanced generation lines against the broadest

range of *P.griseola* populations and under conditions that prevent disease escapes.

- c) The experiment should be repeated to give clearer conclusion. The field plots to be used must have a clear record of ALS disease pathogen to confirm the results obtained in this trial. Weather data recorded in all locations (Appendix 3 and 4) justify the variability observed for disease scores, pods per plant and seed yield per plant, but as weather is unpredictable so the experiment should be repeated to give clearer conclusion.
- d) The backcrossed lines should be advanced for further evaluation to ascertain ALS resistance and yield potential.

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APPENDICES

Appendix 1: A CIAT scale of 1-9 used to assess disease severity

Rating	Category	Description	Comments
1. 2. 3.	Resistant	No visible symptoms or very light symptoms. (Lesion covering 2 - 4.1 %)	Germplasm useful as parents or commercial varieties
4. 5. 6.	Intermediate	Visible and conspicuous symptoms resulting only in limited economic damage. (Lesion covering 4.1 – 9 %%)	Germplasm can be used as commercial varieties or as sources of resistance to certain diseases
7. 8. 9.	Susceptible	Severe to very severe symptoms causing considerable yield losses or plant death.(Lesion covering 10 % and more)	Germplasm in most cases not useful as parents or commercial varieties

Appendix 2: Combined means for Advanced backcrossed lines screened at Morogoro and Arusha

Bean line/variety	Days to 50% flowering	Days to 85% maturity	Disease score	No. of pods per plant	No. of seeds per pod	100 seed weight (g)	Seed yield per Plant (g)
PSO4-336Ab-7	49	65	2.1	12	3	40.10	15.0
PSO4-126Aa-8	47	64	2.2	12	3	37.82	12.8
PSO4-333Aa-19	47	66	3.4	13	3	46.43	12.7
PSO4-333Aa-21	49	68	3.2	17	4	51.50	18.3
PSO4-207Ab-6	49	69	1.9	12	3	35.65	8.3
PSO4-335B-1	48	70	2.8	17	3	45.31	17.8
PSO4-139Bb-7	49	67	3.0	15	3	29.60	10.8
PSO4-330a-1	50	67	2.5	18	3	37.22	17.7
PSO4-139Ba-1	47	66	1.6	18	3	31.87	13.6
PSO4-333Aa-3	51	68	2.8	15	3	38.75	13.3
PSO4-139Bb-5	48	65	2.7	14	4	31.38	13.5
PSO4-333Aa-18	50	67	3.9	14	3	38.47	12.4
PSO4-139Bb-1	46	65	2.5	16	3	31.55	13.7
PSO4-112D	52	69	2.9	8	4	45.95	19.3
PSO4-106Ba-2	52	69	2.2	13	3	39.90	10.4
PSO4-139Aa-10	48	65	4.9	12	3	37.23	8.7
PSO4-139Bb-4	47	67	2.0	18	4	30.41	15.1
PSO4-126Ab-8	46	66	2.7	14	3	38.77	13.7
PSO4-335B-12	47	65	2.7	17	3	47.12	19.3
PSO4-139Ba-7	50	66	1.6	17	4	33.78	12.6
PSO4-139Aa-6	49	68	3.4	23	3	24.30	16.9
PSO4-336Aa-7	47	66	4.2	13	3	31.47	11.0
PSO4-139Ab-10	49	67	2.3	15	3	35.55	11.8
PSO4-126Aa-6	47	68	2.6	12	3	48.97	13.4
PSO4-106Bb-3	47	64	3.8	13	3	31.07	8.6
SUA 90	50	67	2.8	12	4	28.67	8.1
ROJO	49	66	4.4	11	3	45.85	10.6
C/wonder	50	66	3.1	12	3	42.18	12.3
Mean	49	67	2.9	14	3	38.10	13.2
C.V	4.4	3.4	30.3	50.7	36.4	8.2	0.0
S.E	0.86	0.92	0.15	1.07	0.16	1.27	0.00
LSD (0.05)	2.42	2.59	0.41	2.97	0.44	3.57	0.00

Appendix 3: Weather data for Morogoro (2007)

Month	Rainfall (mm)	Maximum Temperature (°C)	Minimum Temperature(°C)
April	192.4	30.8	21.0
May	81.0	29.2	20.4
June	18.1	28.2	16.7
July	12.2	27.9	16.8

Appendix 4: Weather data for Tengeru-Arusha (2007)

Month	Rainfall (mm)	Maximum Temperature (°C)	Minimum Temperature(°C)
April	261.7	28.4	18.5
May	124.8	25.8	18.3
June	15.8	23.4	16.6
July	7.1	23.6	15.2