Breeding for durable resistance to angular leaf spot (*Pseudocercospora griseola*) in common bean (*Phaseolus vulgaris*) in Kenya

By

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Thesis abstract

Common bean (*Phaseolus vulgaris* L.) is an important legume crop in Kenya and is a cheap source of proteins. The small scale farmers in Kenya produce common bean under low agricultural input systems and this predisposes the crop to pests and diseases. Among the diseases, angular leaf spot (ALS) is a major constraint to common bean production and contributes to yield losses as high as 80%. The causative pathogen *Pseudocercospora griseola* (Sacc.) Crous & Braun is highly variable and several races have been reported. There are few common bean genotypes with resistance to this disease. Therefore breeding for resistance to ALS is important for the country. This study was carried out to; i) evaluate the common bean production systems, constraints and farmer varietal preferences in Kenya, ii) evaluate local landraces and selected introductions of common bean for yield performance and reaction to ALS, iii) study the genetics of resistance to ALS in common bean and iv) develop a breeding method for durable resistance to ALS in common bean.

To determine the common bean production systems, farmers' preferred traits and their knowledge on common bean constraints including ALS, a survey was conducted in Kiambu county using a semi-structured questionnaire, interviews, and focus group discussions. The study revealed that farmers cultivate common beans during the short and long rain seasons. However, they experience better yields in the short rains due to reduced disease incidence. The majority of the farmers (71%) intercrop common bean and this ensures maximum utilisation of space. A high percentage (70%) of the farmers utilise their retained seed for production. The farmers identified ALS as one of the most important constraints to production. The only preventative measure they undertake to control the disease is weeding. The farmers reported that they would prefer improved varieties that were resistant to ALS. Farmers have a preference for particular common bean traits that include high yield (80%), resistance to insect-pests and diseases (72%), type I growth habit (52%), early maturity (68%), seed size and colour (21%) and cooking time (20%). These should be incorporated in breeding programmes.

Two hundred common bean landraces and market class varieties were evaluated for ALS resistance in a nethouse at University of Nairobi, Kabete Field Station and for ALS resistance and yield in the field in KARI-Tigoni. The results showed that disease severity scores for the genotypes were similar in the two locations, with the top three resistant genotypes being Minoire, GBK 028123 and Murangazi with disease severity scores of 2.9, 2.9 and 3.2 in Kabete

and 2.6, 2.8, and 2.9 in Thika respectively. These resistant genotypes can be used as sources of resistance in a breeding programme or they can be used as resistant varieties. All the market class varieties were susceptible to ALS (disease severity score 6.7-8.0). There was a non-significant correlation between disease and yield most likely because most of the resistant genotypes were exotic and hence not adapted to the local conditions. There was also a non-significant correlation between disease and seed size.

The two hundred common bean genotypes were evaluated for yield at University of Nairobi, Kabete Field Station and KARI-Thika. The results indicated that the 2011 and 2012 seasons had similar mean yields and that yields at Kabete were higher than at KARI-Thika. The highest yielding genotypes across the two locations were; GLP 2 (766 kg ha^{-1}), Nyirakanyobure (660 kg ha^{-1}), GBK 028110 (654 kg ha^{-1}), GLP 585 (630 kg ha^{-1}) and Mukwararaye (630 kg ha^{-1}). There was a significant genotype x environment interaction and hence it is important for breeders to carry out stability analysis, so as to recommend varieties for a wide range of environments.

To study the genetics of ALS resistance in common bean, three inter-gene pool crosses: Super-rosecoco x Mexico 54, Wairimu x G10909 and Wairimu x Mexico 54 were made. The resistant genotypes were Mexico 54 and G10909, while Super-rosecoco and Wairimu were susceptible. The generations F_1, F_2, BC_1P_1 and BC_1P_2 for each of the crosses were developed. The parents P_1, P_2 and the five generations of each cross were evaluated for resistance to ALS in Kabete Field Station. Results showed that both dominance and additive gene action were important in the expression of resistance to ALS. However, additive gene action was predominant over dominance gene action. There was a moderately high narrow sense heritability estimate (52.9-71.7%). The minimum number of genes controlling resistance to ALS was between 2 and 3. The predominance of additive gene effects and the moderately high narrow sense heritability estimates recorded imply that progress in resistance to ALS could be made through selection in the early segregating generations.

A double cross followed by selection against resistant genotypes was used to develop a method to breed for durable resistance to ALS in common bean. The method was used to accumulate minor genes of ALS resistance into single genotypes. Four intermediate resistant landraces were used to develop a double cross population that was screened using a mixture of ALS races. Selection in F_1 and F_2 population was done on the basis of intermediate resistance (disease severity score 4.0-6.0), while selection from F_3 population was based on resistance

(disease severity score 1.0-3.0). Ten advanced F_4 lines along with their parents were evaluated for ALS resistance. The F_4 advanced lines had a significantly improved resistance to ALS compared to their parents. Hence the method was successful in accumulating minor genes for resistance thus showing significant breeding progress in breeding for durable resistance.

Declaration

I, **Beatrice Njoki Ng'ayu-Wanjau,** declare that

1. The research reported in this thesis, except where otherwise indicated, is my original research.
2. This thesis has not been submitted for any degree or examination at any other university.
3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.
4. This thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:
    a) Their words have been re-written but the general information attributed to them has been referenced
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Signed ……Date……..

Beatrice Njoki Ng'ayu-Wanjau

As the candidate's supervisors, we agree to the submission of this thesis:

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Prof. Rob Melis (Principal supervisor)

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Prof. Githiri Mwangi (Co-Supervisor)

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Dr Julia Sibiya (Co-Supervisor)

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Dedication

I dedicate this thesis to my dearest and beloved husband Dr Kenneth Lawrence Wanjau and to the Ng'ayu's family; my parents John Ng'ayu and Alice Nyawira, my brothers and sisters, James Wang'ombe, Isaiah Mukundi, Fred Alan Muya, Elizabeth Wangechi and Rosemary Wanjiku, and to my nephew Shawn Alan Mukiri and my niece Samara Wairimu.

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Introduction to Thesis

1. Importance of the common bean

Common bean (*Phaseolus vulgaris L.*) is the third most important food legume crop worldwide after soybean and peanut in production. It is the most utilized legume for direct consumption in the world (CIAT, 2001). In Africa, it is the second most important source of proteins and the third most important source of calories for over 100 million people in the rural and poor communities (Buruchara, 2006). The annual per capita consumption of dry beans in the United States averaged 3.1 kg from 1998 to 2007, while in developing countries the figure exceeds 50 kg. For example it is highest in Africa, reaching 55 kg yr^{-1} in Rwanda and 66 kg yr^{-1} in western Kenya (Buruchara, 2006). Common bean forms a significant part of the diet in Africa and hence plays a critical role in human nutrition, providing as much as 45% or more of the total proteins consumed (Allen et al., 1996; Wachenje, 2002). In addition to being an important source of protein and carbohydrates, common bean also supplies essential vitamins and micronutrients such as Zn and Fe (Welch et al., 2000). The vitamins and minerals in the crop lower cholesterol levels and have preventive and curative faculties to terminal diseases such as cancer (Hangen and Bennink, 2003). Antifungal peptides have been isolated from several *Phaseolus* species and are able to inhibit the activity of reverse transcriptase, an enzyme for virus replication. This may help to slow down the onset of symptoms in patients infected with HIV (Wong et al., 2006). The parts of the common bean that are cooked to provide the nutrients are the green pods, mature soft seeds and the dry grain. The importance of the common bean and its nutritional benefits makes it an important legume in most parts of the world.

2. Common bean production

The common bean is cultivated in all the continents of the world. World production of the crop in 2011 was approximately 23.3 million tons harvested from 29.2 million ha. The land area under common bean production in Africa and eastern Africa was 6.3 million ha and 4.3 million ha, respectively (FAO, 2013). The main common bean producing regions in Africa are also the most densely populated and these include Burundi, Rwanda, south western Uganda, eastern Congo, slopes of Mt Elgon in Uganda, western Kenya, slopes of Mt Kenya, the aberdares, eastern province of Kenya, south western and northern highlands of Tanzania, and the Hararghe highlands of Ethiopia. The rapid urbanization of Africa is increasing consumer demand in local and regional markets for common bean, thus providing small scale bean producers an

opportunity to generate income (Buruchara, 2006). In Africa, the common bean is produced under different cropping systems: either as sole crops or mixtures. The commonly found mixtures include: relay or row intercropping of bush or indeterminate beans with maize, or intercropping beans with other cereals or with bananas, fruit crops or cassava (Wooley et al., 1991).

In Kenya, common bean is ranked as the most important legume crop in both production and utilisation (Table 1), with an annual production averaging 461 734 metric tons (MT). However, production has been fluctuating over the years due to several constraints such as erratic rainfall patterns, drought, low soil fertility, insect-pests and diseases. A major contributor to low yields in Kenya and in eastern Africa as a whole is the angular leaf spot disease (Wortmann et al., 1998).

Table 1: Production (MT) of major legume crops in Kenya

Crop	2008	2009	2010	2011	2012	Mean
Common bean	261 137	465 363	390 598	577 674	613 902	461 734
Pigeon pea	84 168	46 474	103 233	84 313	89 390	81 515
Cowpeas	47 958	60 152	72 274	81 534	113 961	75 175
Green gram	26 713	42 333	61 125	70 225	91 824	58 444

Source: Food and Agriculture Organisation (MOA, 2013).

3. Angular leaf spot and its control

Angular leaf spot (ALS) caused by the fungus *Pseudocercospora griseola* (Sacc.) Crous & Braun, (syn. *Phaeoisariopsis griseola* (Sacc.) Ferraris), is one of the most damaging and widely distributed diseases of common bean in Africa causing losses as high as 80% (Shwartz et al., 1981; Liebenberg and Pretorius, 1997; Wortmann et al., 1998; Stenglein et al., 2003). Its incidence and severity has recently increased in many areas under common bean cultivation (Stenglein et al., 2003). The crop losses due to ALS result from premature defoliation of the plant that occurs during the flowering and pod-filling stages. The leaf area of diseased plants is greatly reduced, thus affecting the photosynthetic process resulting in reduced crop yields (Waggoner and Berger, 1987).

In Kenya, ALS has been reported in all common bean growing areas, with a prevalence of 65-80% in Embu, Kakamega, Machakos, Taita taveta and Kiambu districts, spanning across all the agro-ecological zones and altitudes where common bean is grown (Mwang'ombe et al., 2007). The ALS pathogen, *Pseudocercospora griseola* (*P. griseola*), is highly variable and several races have been shown to occur. For example, in Kenya, *P. griseola* has been shown to have a wide pathogenic variability, whereby 100 isolates collected from common bean growing areas were characterised into 44 different physiological races (Wagara et al., 2005) belonging to the Andean, Mesoamerican and the Afro-Andean groups. Virulence variability of *P. griseola* has also been reported in Central America (Mahuku et al., 2002a) and its variability shown to occur using polymerase chain reaction, group specific primers and random amplified polymorphic DNA markers (Pastor-Corrales et al., 1998; Guzman et al., 1999). Therefore, there is a need to develop common bean genotypes that have a stable and durable resistance across the different races present.

The control of ALS through various methods such as; chemical, cultural, biological or a combination of the three as integrated pest management, is not adequate. Moreover, for the smallholder farmers in Kenya, chemical control is expensive in addition to being harmful to the farmers and the environment. Cultural practices, although effective in reducing the amount of initial infection, are vulnerable to environmental conditions such that when these are favourable, disease increases at a high rate (Mmbaga et al., 1996). Biological control on the other hand, has the disadvantage of insufficient control agents available that can be released in quantities which are adequate to reduce the pathogen populations. Hence the use of resistant genotypes is essential as it is effective and affordable to the farmers, without an extra expense on disease management.

4. Breeding for angular leaf spot resistance

Studies on screening common bean genotypes for resistance to ALS have been conducted and some sources of resistance identified. Some of these sources include MAR-1, MAR-2, MAR-3, Mexico 54 and BAT 332 (Pastor-Corrales et al., 1998; Buruchara and Bua, 1999; Caixeta et al., 2003; Mahuku et al., 2003; Namayanja et al., 2006), G10909 (Mahuku et al., 2011), G5686 (Mahuku et al., 2009) and G10474 (Mahuku et al., 2004). Previous studies showed that Mexico 54 was resistant to most African isolates that have so far been characterised (Namayanja et al., 2006). Out of 163 African isolates, Mexico 54 was resistant to 158 of them hence it is an

excellent source of resistance to ALS in Africa (Namayanja et al., 2006). The sources of resistance vary in the number of genes that condition resistance to ALS.

Studies on inheritance have shown that resistance to *P. griseola* is conditioned by a few genes that can either be recessive or dominant, depending on the cultivar used as the susceptible parent (Sartorato et al., 1999; Ferreira et al., 2000). The pathotype or race used for inoculation also affects the nature of inheritance (Pastor-Corrales et al., 1994). For example, Sartorato et al. (1999) showed that a single dominant resistance gene conferred the resistance of cultivar Mexico 54 to pathotype 63-19 using a Mesoamerican cultivar Ruda, while Mahuku et al. (2002b), showed that Mexico 54's resistance to pathotype 31-55 was due to a single recessive gene when using a snap bean cultivar as the susceptible parent. Therefore, it is important to ascertain the nature of the resistance in different common bean cultivars.

5. Durable resistance

Developing resistant genotypes is the best way for managing ALS. Taking into account that the ALS pathogen is highly variable, the use of resistance that is conditioned by few major genes may not be effective for a long time. It would be important to utilise new strategies of breeding common bean varieties to ensure durability of resistance. One such strategy would be to use sources with resistance to a wide range of *P. griseola* races. The most effective durable resistance would be achieved through the use of minor genes. Gamete selection is one of the methods of breeding that could be used to combine the minor genes and favourable alleles contributing to resistance in a single genotype. Gamete selection was proposed by Singh (1994) as a method to simultaneously improve multiple traits in common bean, by crossing multiple parents followed by early generation testing and selection. It was used by Teran and Singh (2009) to improve resistance to white mould disease in common bean and by Asensio et al. (2006) to improve resistance to common and halo bacterial blights in common bean inter-gene pool populations. Gamete selection has also been used to combine resistance to different bacterial, fungal and viral diseases into one cultivar (Teran et al., 2013). However there is no research reported on the use of gamete selection to breed for resistance to ALS.

6. Importance of landraces in breeding

Since the introduction of common bean to the eastern African coast by the Portuguese, farmers have used the crop to develop farming practices that are adapted to local conditions. Hence they have exploited useful alleles in the crop, which has resulted in a wide range of

morphologically diverse landraces (Singh et al., 1991a; Wortmann et al., 1998). The genetic diversity helps to broaden the genetic base of new cultivars and hence maximises the available germplasm resources (Escribano et al., 1998). Different regions have specific temperatures, humidity and other production requirements, and hence each landrace may not be grown successfully in regions where they are not traditionally cultivated (Piergiovanni and Lioi, 2010). Hence landraces were evaluated in the study for their resistance to ALS, and used in the breeding process.

7. Farmer participation in breeding

Plant breeding should be carried out with the participation of farmers, which ensures that released varieties meet their demands. Sperling et al. (2001) discussed participatory plant breeding as involving all the stakeholders including scientists, farmers, agriculture organisations, the industries and consumers. Farmers can participate in the breeding process when they are consulted, and also when trials are conducted on their farms (Biggs, 1989). Ceccarelli and Grando (2007) showed that decentralized and demand driven research was essential, especially for the small scale farmers in low input farming systems. They reported that this would help farmers choose the varieties that do well in their environmental conditions and hence adopt new released varieties. Participatory plant breeding has been shown to have the potential to develop crop varieties that are better adapted to farmers' local environmental conditions and with farmer preferred traits (Sthapit et al., 1996; Ceccarelli et al., 2003). In this study, farmer perceptions on common bean production and their preferred common bean traits were studied.

8. Problem Statement

The bean improvement programme in Kenya has had three major common bean variety releases during the period 1984-2006. The first variety release in 1984 was under the Grain Legume Project (GLP), which resulted in the GLP series of varieties currently under production in the country. The second releases were in 1987 and 1989 as the Katumani series, which are adapted for the drylands, while the third was in 2006 where the first climbing common bean varieties and new bush varieties were released to farmers. Resistance to ALS in all these varieties was not a major breeding objective (Kimani, P.M., *personal communication*[1]). Therefore with the current knowledge on the variability of ALS pathogen in Kenya, this

[1] Kimani, P.M. Department of Crop Science, University of Nairobi, P.O Box 30197-00100, Nairobi, Kenya

resistance could easily have broken down. New sources of resistance have since been identified, but are conditioned by major genes. These major genes have been used to breed for ALS resistance as an interim strategy. The use of minor genes that are race non-specific has been shown to be durable (Van der Plank, 1968; Parlevliet, 2002). No research has been reported on breeding for common bean resistance to ALS using minor genes, hence the study.

9. Goal and objectives of the study

The study aimed to contribute to enhanced food security by improving resistance to angular leaf spot in preferred Kenyan bean varieties, hence contributing to increased yields and thus increased income to the small holder farmers.

The specific objectives were to:

1. Evaluate common bean production systems, constraints, and farmer varietal preferences in selected regions of Kenya.
2. Evaluate local landraces and selected introductions of common bean for yield performance and resistance to angular leaf spot.
3. Analyse the genetics of resistance to angular leaf spot in common bean.
4. Develop a breeding method for durable resistance to angular leaf spot in common bean.

10. Outline of Thesis

The thesis is in the form of discrete research chapters, each following the format of a stand-alone research paper. This is the dominant thesis format adopted by the University of KwaZulu-Natal, because it facilitates the publishing of research out of theses far more than the older monograph form of thesis. As such, there is some unavoidable repetition of references and some introductory information between chapters. The referencing system used in the chapters of this thesis is based on the “Crop Science Society of America (CSSA), referencing style, and follows the specific style used in “Crop Science Journal”.

Therefore the outline of the thesis is as follows:

1. Introduction to Thesis
2. Chapter One: Literature review
3. Chapter Two: Evaluation of common bean production systems, constraints, and the farmer varietal preferences.

4. Chapter Three: Evaluation of local landraces and selected introductions of common bean for yield performance and resistance to angular leaf spot.
5. Chapter Four: Genetic analysis of resistance to angular leaf spot in common bean.
6. Chapter Five: Development of a breeding method for durable resistance to angular leaf spot in common bean.
7. Chapter Six: General overview of the study and implications to plant breeding.

References

Allen, D.J., J.K.O. Ampofo, and C.S. Wortmann. 1996. Pests, diseases, and nutritional disorders of the common bean in Africa: A field guide. CIAT, Cali, Colombia.

Asensio, S.M.M.C., C. Asensio, and S.P. Singh. 2006. Gamete selection for resistance to common and halo bacterial blights in dry bean intergene pool populations. Crop Science 46:131-135.

Biggs, S.D. 1989. Resource-poor farmer participation in research: A synthesis of experiences from nine national agricultural research systems. OFCOR Comparative Study Paper No. 3, The Hague: International Service for National Agricultural Research, The Netherlands.

Buruchara, R.A. 2006. Background information on common beans. Biotechnology, breeding and seed systems for African crops. [Online]. Available by Rockefeller foundation, Nairobi, Kenya. http://www.africancrops.net/rockefeller/crops/beans/index.htm (verified 21st July 2009).

Buruchara, R.A., and B. Bua. 1999. Identification of sources of resistance to angular leaf spot. In: Annual Report 1999, Project IP-2: Meeting demands for beans in sub Saharan Africa in sustainable ways. CIAT, Cali, Colombia.

Caixeta, E.T., A. Bor´em, S. de Azevedo 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 134:297-303.

Ceccarelli, S., and S. Grando. 2007. Decentralized-participatory plant breeding: An example of demand driven research. Euphytica 155:349-360.

Ceccarelli, S., S. Grando, M. Singh, M. Michael, A. Shikho, M. Al Issa, A. Al Saleh, G. Kaleonjy, S.M. Al Ghanem, A.L. Al Hasan, H. Dalla, S. Basha, and T. Basha. 2003. A methodological study on participatory barley breeding II. Response to selection. Euphytica 133:185-200.

CIAT. 2001. Annual Bean report, Bean Program. CIAT, Cali, Colombia.

Escribano, M.R., M. Santalla, P.A. Casquero, and A.M. Ron. 1998. Patterns of genetic diversity in landraces of common bean (*Phaseolus vulgaris* L.) from Galicia. Plant Breeding 117:49-56.

FAO. 2013. Common bean production statistics. Available by Food Agricullture Organisation, Statistics division. [Online] http://www.faostat.fao.org/site/567 (verified 6th June 2013).

Ferreira, C.F., A. Borém, G.A. Carvalho, S. Nietsche, T.J. Paula-Jr, 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.

Guzman, P., P. Gepts, S. Temple, A.B. Mkandawire, and R.L. Gilbertson. 1999. Detection and differentiation of *Phaeoisariopsis griseola* isolates with the polymerase chain reaction and group specific primers. Plant Disease 83:37-42.

Hangen, L., and M.R. Bennink. 2003. Consumption of black beans and navy beans (*Phaseolus vulgaris* L.) reduced azoxymethane-induced colon cancer in rats. Nutrition Cancer 44:60-65.

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.

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. 2002a. Genetic variability within *Phaeoisariopsis griseola* from central America and its implications for resistance breeding. Plant Pathology 51:594-604.

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. Montoya, Y. Mantilla, M. Contreras, C. Jara, and S. Beebe. 2002b. RAPD, SSR, and AFLP markers linked to genes conferring resistance to angular leaf spot in common bean, *In* J. DeVries, F.M. Mwaura, P.L Woomer, ed. Proceedings, Biotechniology, Breeding and Seed systems for African Crops. Research and product development that reaches farmers. Rockefeller Foundation, Entebbe, Uganda.

Mahuku, G.S., C. Montoya, M.A. Henrı´quez, C. Jara, H. Teran, and S. Beebe. 2004. Inheritance and characterization of the angular leaf spot resistance gene in the common bean accession, G 10474 and identification of an AFLP marker linked to the resistance gene. Crop Science 44:1817-1824.

Mahuku, G.S., M.A. Henrı´quez, C. Montoya, C. Jara, H. Teran, and S. Beebe. 2011. Inheritance and development of molecular markers linked to angular leaf spot resistance genes in the common bean accession G10909. Molecular Breeding 28:57-71.

Mmbaga, M.T., J.R. Steadman, and J.R. Stavely. 1996. The use of host resistance in disease management of rust in common bean. Integrated Pest Management Reviews 1:191-200.

MOA. 2013. Economic Review of Agriculture. CPPMU, Nairobi, Kenya.

Mwang'ombe, A.W., I.N. Wagara, J.W. Kimenju, and R.A. Buruchara. 2007. Occurence and severity of angular leaf spot of common bean in Kenya as influenced by geographical location, altitude and agroecological zones. Plant Pathology 6:235-241.

Namayanja, A., R. Buruchara, G.S. 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.

Parlevliet, J.E. 2002. Durability of resistance against fungal, bacterial, and viral pathogens; present situation. Euphytica 124:147-156.

Pastor-Corrales, M.A., C. Jara, and S.P. 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.

Pastor-Corrales, M.A., O.A. Erazo, E.L. Estrada, and S.P. Singh. 1994. Inheritance of anthracnose resistance in common bean accession G 2333. Plant Disease 78:959-962.

Piergiovanni, A.R., and L. Lioi. 2010. Italian common bean landraces: History, genetic diversity and seed quality. Diversity 2:837-862.

Sartorato, A., S. Nietsche, E.G. Barros, and M.A. Moreira. 1999. SCAR marker linked to angular leaf spot resistance gene in common bean. Annual Report of the Bean Improvement Cooperative 42:23-24.

Shwartz, H.F., V.F. Correa, D.D.A. Pineda, M.M. Otoya, and M.J. Katherman. 1981. Aschochyta, angular and whitefly leaf spots in Colombia. Plant Disease 65:494-496.

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

Singh, S.P. 1994. Gamete selection for simultaneous improvement of multiple traits in common bean. Crop Science 34:352-355.

Sperling, L., J.A. Ashby, M.E. Smith, E. Weltzien, and S.M. Guire. 2001. A framework for analysing participatory plant breeding approaches and results. Euphytica 122:106-119.

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. Advanced Applied Microbiology 52:209-243.

Sthapit, B.R., K.D. Joshi, and J.R. Witcombe. 1996. Farmers participatory crop improvement. III. Participatory plant breeding, a case study for rice in Nepal. Experimental Agriculture 32:479-496.

Teran, H., and S.P. Singh. 2009. Gamete selection for improving physiological resistance to white mold in common bean. Euphytica 167:271-289.

Teran, H., C. Jara, G. Mahuku, S. Beebe, and S.P. Singh. 2013. Similtaneous selection for resistance to five bacterial, fungal and viral diseases in three Andean x Middle American inter-gene pool common bean populations. Euphytica 189:283-292.

Van der Plank, J.E. 1968. Disease resistance in plants. Academic Press, New York.

Wachenje, C.W. 2002. Bean production constraints, bean seed quality and effect of intercropping on floury leaf spot disease and yields in Taita Taveta district, Kenya. M.Sc. Thesis, University of Nairobi.

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

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

Welch, R.M., W.A. House, S. Beebe, and Z. Cheng. 2000. Genetic selection for enhanced bioavailable levels of iron in bean (*Phaseolus vulgaris* L.) seeds. Journal of Agriculture Food Chemistry 48:3576-3580.

Wong, J.H., X.Q. Zhang, H.X. Wang, and T.B. Ng. 2006. A mitogenic defensin from white cloud beans (*Phaseolus vulgaris* L.). Peptides 27:2075-2081.

Wooley, J., R. Lepiz, Y. Aquinas-Portes, T. Castro, and L. Voss. 1991. Bean cropping systems in the tropics and subtropics and their determinants, p. 679-706, *In* A. Van Schoonhoven and O. Voyest, eds. Common beans: Research for crop improvement. CIAT, Cali, Colombia.

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

CHAPTER 1

Literature Review

1.1 Introduction

This chapter gives an overview on breeding common bean for angular leaf spot resistance and other agronomic traits in Kenya. The origin and distribution of the common bean, its taxonomy and genetic diversity are described. Common bean production constraints are also discussed. Angular leaf spot (ALS), caused by *Pseudocercospora griseola* (Sacc.) Crous & Braun, an important common bean disease causing high yield losses in Kenya, is discussed. Its taxonomy, epidemiology and symptoms are described. The pathogenic variability of *Pseudocercospora griseola* (*P. griseola*), sources of resistance to ALS, and breeding common bean for durable resistance, will be reviewed. In addition, gamete selection will be discussed as a method to accumulate favourable alleles into single genotypes. The importance of landraces and their genetic diversity is reviewed. Finally this review highlights the importance of participatory plant breeding in common bean breeding programmes.

1.2 Origin and distribution of common bean

Domestication of common bean took place in two regions distributed from northern Mexico to Colombia (Mesoamerican gene pool) and from southern Peru to northwestern Argentina (Andean gene pool) (Koenig and Gepts, 1989; Koinange and Gepts, 1992; Freyre et al., 1996). Once domesticated, the common bean was introduced to other regions of the world, whereby both the Mesoamerican and the Andean cultivars were dispersed to lowland south America and Africa (Gepts and Debouck, 1991). Gepts and Debouck (1991) showed that the Mesoamerican cultivars became predominant in the south western United States, while the Andean cultivars in Africa, Europe and north eastern United States. Domestication in the two regions led to two distinct gene pools (Singh et al., 1991b; Becerra Vela´squez and Gepts, 1994) because they arose from two already diverged gene pools and selection under domestication (Kwak and Gepts, 2009). The domestication of the common bean has altered the form, morphology, and phenology of the plant, especially the growth habit, seed size, seed retention, and maturity. During domestication, selection was inclined towards smaller, denser plants with short internodes, suppressed climbing ability, fewer and thicker stems and larger leaves (Debouck, 1991). The end result of the selection was a compact growth habit of determinate and

indeterminate common bean cultivars. However, the most distinct difference between the wild ancestors and the cultivated common bean, are the changes in pod size and the seed size, hence the diversity. The cultivated common beans are also quite diverse in seed size and edible parts such as the green immature pod and dry seed (Debouck, 1991).

1.3 Taxonomy and morphology of common bean

The common bean (*Phaseolus vulgaris* L.*)* belongs to the family Fabaceae (Leguminosae) and the genus *Phaseolus.* The genus *Phaseolus* comprises 30 species (Debouck, 1991) which have been grouped into sections according to the plant morphology and molecular genetics that show the different lines of evolution and speciation. Four sections were classified as Chiapasana, Phaseolus, Minkelersia, and Xanthotricha (Debouck, 1991). The *Phaseolus* section includes four of the cultivated *Phaseolus* species: *P. vulgaris* L. (common bean); *P. coccineus* L. (runner bean); *P. lanatus* L. var. *lanatus* L. (lima bean); and *P. acutifolius* A. gray var. *acutifolius* (tepary bean). Of the four *Phaseolus* species, the common bean is the most widely grown occupying more than 85% of the production area sown to all *Phaseolus* species worldwide (Singh, 2001). Common beans are classified in the sub-phylum dicotyledons (embryo with two cotyledons, parallel veined leaves and the stem with the vascular bundles arranged irregularly and cambium usually present), division Magnoliophyta, class Magnoliopsida, family Leguminosae, sub-family Papilionoideae or Fabaceae or Lotoideae (pulse family characterized by edible seeds and pods) and order Leguminales. Common beans are diploid (2n = 2x = 22) and are self-pollinated (Rutger and Beckham, 1970; Stoetzer, 1984).

1.4 The inflorescence and pod formation of common bean

After germination, the plant forms a taproot, after which adventitious roots emerge and develop, while the tap root maintains a length of 10-15 cm (Duke, 1981). Morphologically, common bean has primary leaves that are unifoliate and the subsequent leaves are trifoliate. The flowers are borne in the axillary and terminal racemes which may be one or many flowered. The flowers of the common bean are zygomorphic having a bi-petalled keel, two lateral wing petals, and a large standard petal. The colour of the flower may be white, pink or purple, and is genetically independent of the seed colour, but there is an association of particular seed colours with flower colours. The flowers contain ten stamens and a single multi-ovuled ovary which is predominantly self-fertilized. Once fertilized it develops into a pod which could be straight or slightly curved. The seeds borne in the pod may be round, elliptical, flattened, rounded elongate

in shape with an assortment of seed coat colours and patterns that are used to differentiate the cultivars (Graham and Ranalli, 1997).

1.5 Growth habit of common bean

The cultivated forms of the common beans are herbaceous annuals which have a determinate or indeterminate growth habit. This variation in the growth characteristics of common bean is used to separate germplasm into four classifications that are based on the plant architecture. The classifications include type I (determinate, bush) which may have 3-7 trifoliate leaves on the main stem before the terminal double raceme, or may be many noded with 7-15 (Mesoamerican) or 12-25 (Andean) trifoliate leaves on the main stem. There are three indeterminate types classified as, type II (indeterminate, upright), type III (indeterminate, semi-vine), and type IV (indeterminate, climbing vine) and described as shown in Table 1.1 (Shwartz et al., 2005).

Table 1.1: Growth habit classification and description of common bean

Growth habit	Description
Type I	*Habit determinate
	Terminal bud reproductive
	Stem and branches erect
	Terminal guide absent or small
	Pods distributed along the length of the stem
Type II	*Habit indeterminate
	Terminal bud vegetative
	*Stem and branches erect
	Terminal guide absent or medium
	Pods distributed along the length of the stem
Type III	Habit indeterminate
	Terminal bud vegetative
	*Stem and branches prostrate with little or no climbing ability
	Terminal guide small or long
	*Pods distributed mainly in the basal portion
Type IV	Habit indeterminate
	Terminal bud vegetative
	Stem and branches twining with strong climbing ability
	Terminal guide long or very long
	*Pods distributed along the length of the stem or mainly on the upper portion

Key characteristics marked with an asterisk. Source: Shwartz et al. (2005)

1.6 Genetic diversity of common bean

The genetic diversity of common bean is mainly in the seed size, which is divided into three groups. The groups include large seeded Andean (>40 g 100-seed weight^{-1}), small seeded Mesoamerican (<25 g 100-seed weight^{-1}), and medium seeded/Middle American (25 to 40 g 100-seed weight^{-1}) gene pools (Evans, 1980). The cultivated gene pools of Andean and Mesoamerican origin were further divided into six races: the Andean (all large seeded) have the races Chile, Nueva Granada, and Peru; Middle American has the races Durango, Jalisco (medium seeded); and Mesoamerican (all small seeded), each of which has its distinguishing characteristics and agronomic traits (Singh et al., 1991a)

Common bean is also divided into two groups based on their edible parts: snap beans (French beans or Haricot beans) are consumed as immature pods, and; dry beans are usually consumed as the mature dry seed after rehydration. The snap bean cultivars have a thick succulent mesocarp and have reduced or no fibre in the green pods and sutures (Myers, 2000). The green pods are used as fresh pods, or frozen or canned. There are different market classes of the snap bean cultivars determined by the pod shape (flat, oval or cylindrical), colour (dark green, light green, yellow or purple), and the length of the pod. Among the snap bean cultivars, there is a large variation in their growth habits and their adaptation traits (Singh, 2001). Common bean cultivars have also shown large variations in growth habit, phenological traits, seed colour, seed size and shape, as well as canning and cooking qualities (Voysest and Dessert, 1991). The largest production however is as dry beans, followed by a lower production of the snap beans, hence the importance of the study.

1.7 Common bean production constraints

Literature has been reviewed by several researchers on common bean production constraints. The constraints include abiotic and biotic factors that reduce the yields of the common bean hence result in low income and possible food shortage. The major abiotic constraints in the tropics and in Africa include drought, high temperatures, excessive and erratic rainfall, nutritional disorders such as nitrogen (N), phosphorous (P), potassium (K), magnesium (Mg), zinc (Zn), and calcium (Ca) deficiencies; and manganese (Mn), aluminium (Al) and salt (NaCl) toxicities (Shwartz and Pastor-Corrales, 1989; Allen et al., 1996; Wortmann et al., 1998).

The biotic constraints that cause reduced yield are the insect-pests and diseases. The insect-pests include foliage pests such as the bean stem maggot (beanfly) (*Ophiomyia phaseoli*,

O. spencerella, and *O. centrosematis*), cutworms (larvae of various moths mostly in the genera Agrotis and Spodoptera), striped bean weevil (*Alcidodes leucogrammus*), foliage beetles (*Ootheca mutabilis and O. bennigseni*), black bean aphid (*Aphis fabae*, and *A. craccivora*), common whitefly (*Bemicia tabaci*), leafhoppers (*Empoasca dolichi* and *E. lybica*), flower thrips (*Megalurothrips sjostedti*), red spider mites (*Tetranychus spp*.), pod and seed feeders, legume pod borer (*Maruca vitrata*), cotton bollworm (*Helicoverpa*(=*Heliothis*) *armigera),* and bruchids (*Acanthoscelides obtectus* and *Zabrotes subfasciatus*) (Karel and Antrique, 1989; Allen et al., 1996).

The common bean diseases include, common bacterial blight (*Xanthomonas axonopodis* pv*. phaseoli*) (Saettler, 1989), anthracnose (*Colletotrichum lindemuthianum*) (Pastor-Corrales and Tu, 1989), rust (*Uromyces appendiculatus*) (Stavely, 1989), angular leaf spot (*Pseudocercospora griseola*) (Pastor-Corrales and Saettler, 1989), ascochyta blight (*Phoma exigua* var. *diversispora*) (Allen et al., 1996), powdery mildew (*Erysiphe polygoni*) (Allen et al., 1996), white mould (*Sclerotinia sclerotiorum*) (Shwartz and Steadman, 1989), halo blight (*Pseudomonas syringae* pv. *phaseolicola*) (Shwartz, 1989). Others include root rots, such as rhizoctonia root rot (*Rhizoctonia solani*), dry root rot (*Fusarium solani* f. sp. *phaseoli*) and fusarium wilt (yellows) (*Fusarium oxysporum* f. sp. *phaseoli*) (Abawi, 1989). Viral diseases include bean common mosaic virus and bean common necrosis virus (Galvez and Morales, 1989). Common bean is also affected by parasitic nematodes such as the root knot nematodes (*Meloidogyne incognita*, *M. hapla*, *M. javanica*, *meloidogyne spp*) (Abawi and Agudelo, 1989). Of these production constraints, angular leaf spot disease is the most important in Kenya.

1.8 Angular leaf spot of common bean

Angular leaf spot caused by the fungus *Pseudocercospora griseola*, is one of the most damaging and widely distributed diseases of common bean in Africa, causing yield losses as high as 80% (Shwartz et al., 1981). Angular leaf spot incidence and severity has increased in many areas under common bean cultivation (Stenglein et al., 2003). In Africa, especially in Kenya, Malawi, Ethiopia, Uganda and Tanzania, ALS is ranked as the most important constraint to common bean production (Pastor-Corrales et al., 1998; Wortmann et al., 1998). Crop losses due to the disease are a result of premature defoliation, shrivelled pods, and shrunken seeds. A survey conducted in Kenya on ALS showed a prevalence of between 65-80% in the districts surveyed namely Embu, Kakamega, Machakos, Taita Taveta and Kiambu (Mwang'ombe et al., 2007). The disease was found prevalent at an altitude ranging from 963 to 2322 m above sea

level. Hence ALS is severe and highly prevalent in Kenya, spanning across all the agro-ecological zones and altitudes where common bean is grown.

1.9 Taxonomy and epidemiology of angular leaf spot

Pseudocercospora griseola, the causative pathogen of ALS is an imperfect fungus belonging to the class Hyphomycete, order Moniliales, and family *Stibaceae*. *Pseudocercospora griseola* produces synnemata, which are 20-40 µm wide, and consist of joined conidiophores that are as long as 500 µm. The conidia (Figure 1.1) are formed singly at the tips of the conidiophores and they are smooth, obclavate, 2-6 septate, and pale olive to olivaceous brown, measuring 30 to 70 µm in length, 5 to 8 µm wide, and thinning to 1.5 to 2.0 µm at the base. Different variations in length and width of the synnemata have been reported among isolates (Liebenberg and Pretorius, 1997).

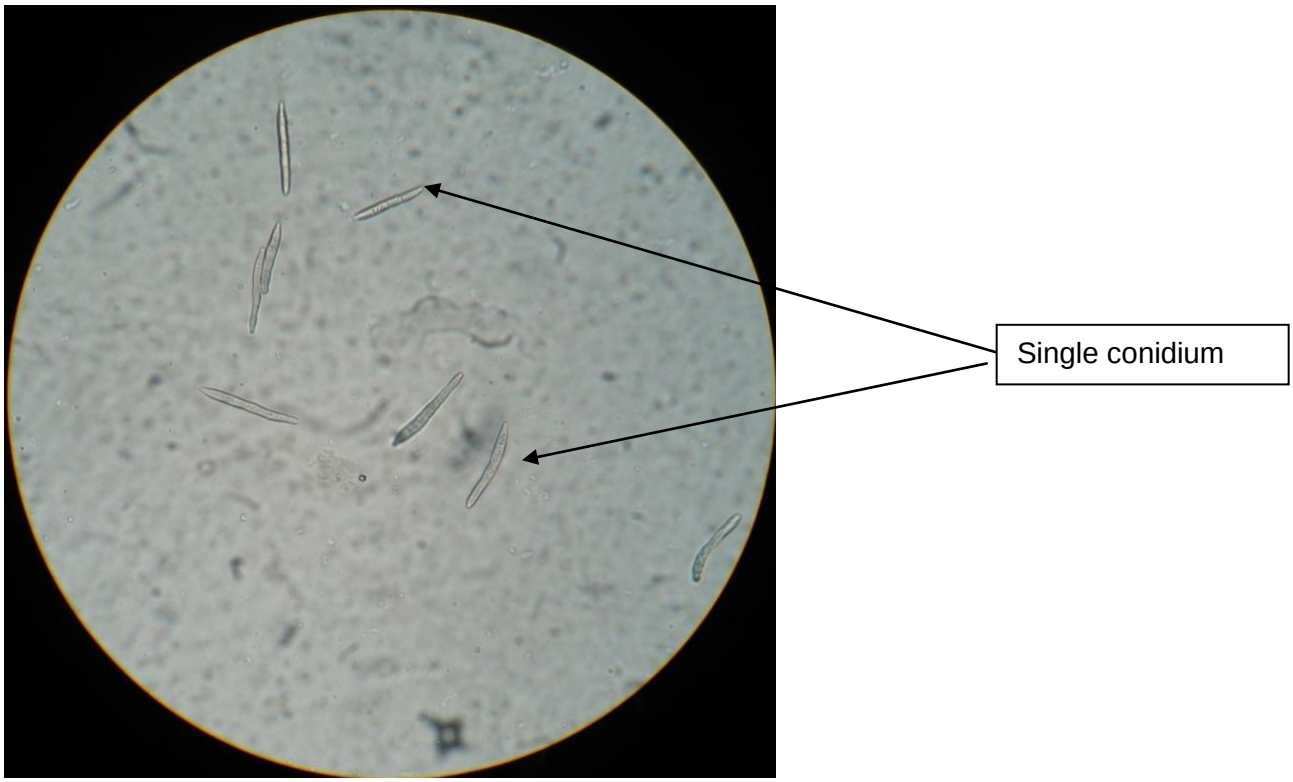

Figure 1.1: Conidia of *Pseudocercospora griseola* observed under an electron microscope

When grown on V8 media, *Pseudocercospora griseola* produces conidiophores in groups, and at their tips they bear pale grey conidia that are cylindrical to spindle shaped and they sporulate at a temperature of between 16-26^{o}C (Liebenberg and Pretorius, 1997) (Figure 1.2).

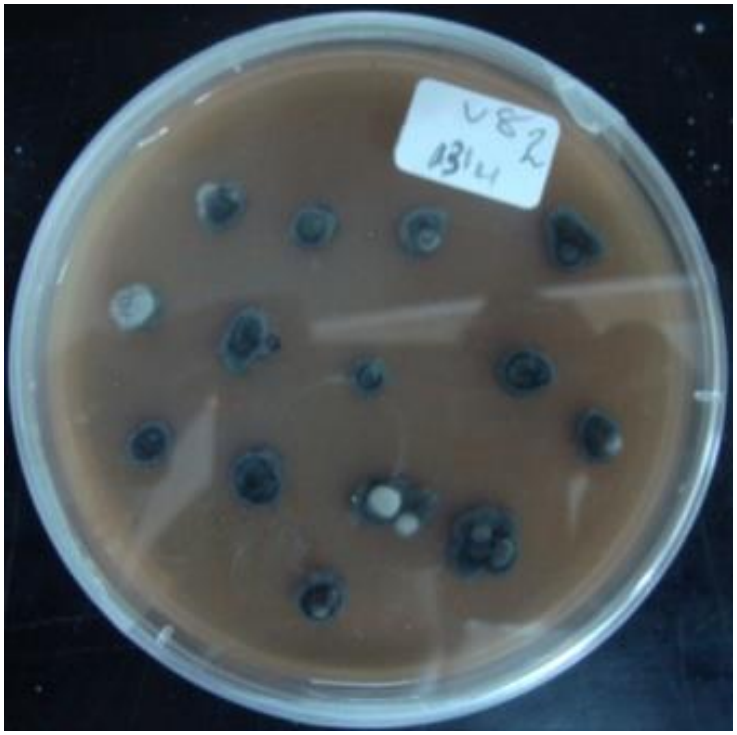

Figure1.2: *Pseudocercospora griseola* (twelve days old) growing on V8 media

The primary sources of the ALS inoculum are the off-season crops and contaminated seed. The mode of dissemination is by wind, rain or water splash. The conidia germinate in the presence of water or high humidity and enter the host through the stomata growing intercellularly in the mesophyll and palisade layers. This leads to tissue disintegration extending to the upper epidermis. The fungus then grows intercellularly in the necrotic tissues, delimited by the vascular bundles in the veins (hence the characteristic angular shape of symptoms). The stomata then develop in the substomatal cavities 9-12 days after infection, synnemata form, and sporulation occurs during periods of high humidity causing secondary spread of the disease. Infection and disease development require temperatures of 16-28^{o}C, with optimum temperature of 24^{o}C. Infections stop above 36^{o}C and below 5^{o}C. Once the infection has occurred even if the conditions become unfavourable the pathogen still develops successfully (Stenglein et al., 2003; Shwartz et al., 2005). Angular leaf spot is most destructive during and after flowering and thrives

in conditions that are moist and warm, and when there is a lot of inoculum from infested plant residues and from seeds that are contaminated (Shwartz et al., 2005). An epidemic is rapid under conditions of high relative humidity and moderate temperature alternating with periods of wind and low humidity.

1.10 Symptoms of angular leaf spot in common bean

Angular leaf spot lesions become visible 8-12 days after infection. The lesions on leaves first appear as grey or brown irregular spots that may be bordered by a chlorotic halo. About 9 days later, these lesions become necrotic and assume the angular shape that is characteristic of the disease (Figure 1.3 and 1.4). The lesions then coalesce as the disease progresses leading to necrosis and the affected leaves ultimately abscise. The symptoms on the primary leaves show circular lesions with or without concentric rings rather than the angular shape. On the pods of the common bean, the lesions are large, oval to circular reddish brown spots, surrounded by a darker coloured border (Figure1.5) (Liebenberg and Pretorius, 1997; Stenglein et al., 2003)

Figure 1.3: Angular shaped symptoms on upper side of common bean trifoliate

Figure 1.4: Angular shaped symptoms on the lower side of the leaf showing black synnemata protruding from lesions

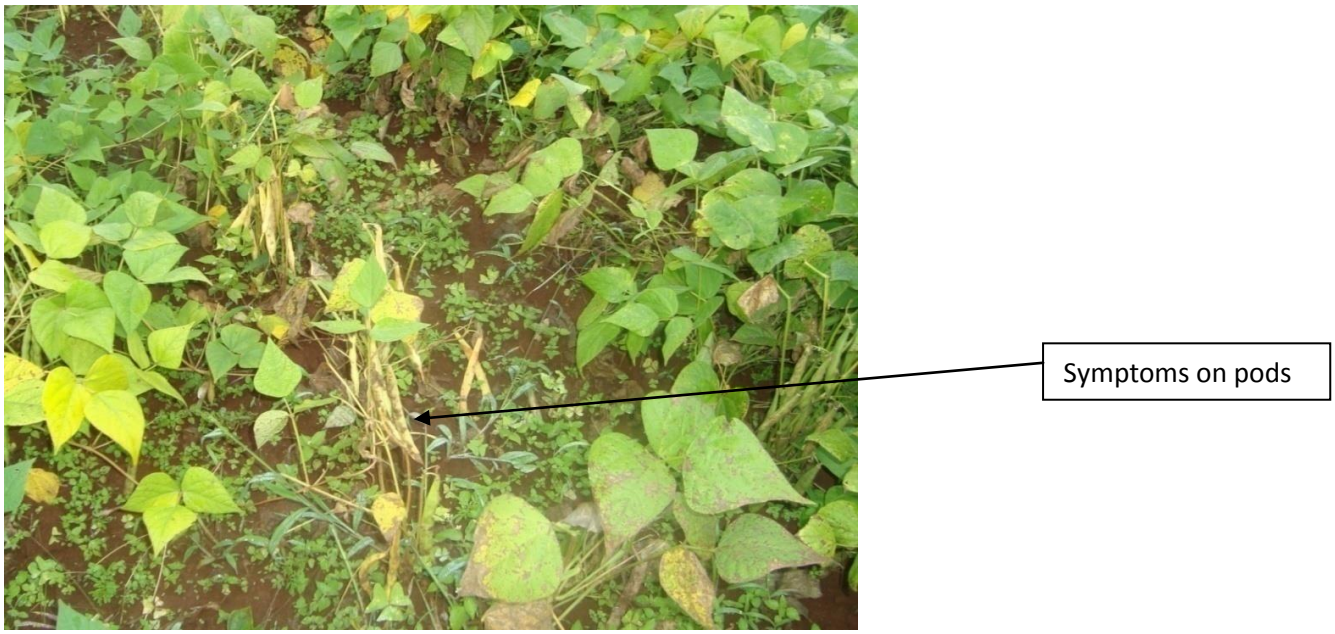

Figure1.5: Angular leaf spot symptoms on common bean pods and leaves at Tigoni field trial

1.11 Variability of the angular leaf spot pathogen *Pseudocercospora griseola*

The variability of *P. griseola* has been reported in several parts of the world where common beans are produced. For example, virulence variability of *P. griseola* has been reported in Central America (Mahuku et al., 2002a) and in Brazil (Nietsche et al., 2001; Damasceno e Silva et al., 2008). Pastor-Corrales et al. (1998) showed the variation in one hundred and forty three isolates collected from eleven Latin American and ten African countries and tested them on common bean accessions to source for resistance against the isolates. The high pathogenic variability is also present in eastern and southern Africa (Aggarwal et al., 2004).

The pathogen has been divided into Andean and Mesoamerican groups (Pastor-Corrales et al., 1998; Mahuku et al., 2002a). The Andean isolates of *P. griseola* exhibit a narrow host range by attacking the common beans belonging to the Andean gene pool only, while the Mesoamerican isolates are more virulent and aggressive on the Mesoamerican beans, and also attack the Andean beans (Guzman et al., 1995; Mahuku et al., 2002b). Another group of *P. griseola* was identified in Africa as the Afro-Andean (Wagara et al., 2004), which has similar characteristics to the Andean group. The existence of the Afro-Andean group was shown to be as a result of mutation, recombination and ecological adaptation of the Andean group under the different conditions found in Africa (Mahuku et al., 2002b). Molecular markers have been used to group the *P. griseola* isolates into their respective gene pools (Pastor-Corrales et al., 1998; Guzman et al., 1999).

The variation in virulence of *P. griseola* has also been attributed to the intensification of common bean production, thus leading to differences in the pathogenic and ecological adaptation of the pathogen (Mahuku et al., 2002b). In Kenya there is a high genetic diversity in *P. griseola* isolates whereby 44 physiological races were reported by Wagara et al. (2004). These races belong to the Andean and Mesoamerican groups. The presence of the two groups was attributed to the common bean genotypes grown in Kenya. The study also revealed that genetic differentiation between the two major groups Andean and Mesoamerican was low. This was attributed to lack of a strong influence of the common bean on population structure of the ALS pathogen in Kenya, due to the cultivation of bean mixtures (Wagara et al., 2004).

The high levels of pathogen variability in different production regions may affect the resistance in common bean genotypes. Variability of the pathogen makes it difficult to breed common bean genotypes for resistance to only one type of *P. griseola* race. Hence, it is important to breed for resistance against several *P. griseola* races, by either pyramiding resistance genes or targeting

non-race specific resistance (quantitative). However resistance developed by gene pyramiding may not last long with the occurrence of the different races, as it is still specific to a few and not all races of the pathogen. Combining Andean and Mesoamerican resistance genes in the same background has been proposed as a way of prolonging ALS resistance (Mahuku and Iglesias, 2009). However, the most effective resistant genotypes could be achieved through breeding for durable resistance, where minor genes (quantitative resistance) are involved.

1.12 Sources of resistance to angular leaf spot

Several common bean lines have been evaluated for resistance to many races of the ALS pathogen and used as sources of resistance in breeding programmes. The Centro Internacional de Agricultura Tropical (CIAT) established an international nursery with sources of resistance that were evaluated in several countries. Ultimately, several accessions with good levels of resistance to different isolates of *P. griseola* were identified (Pastor-Corrales et al., 1998; Mahuku et al., 2003b).

Common bean genotypes have been identified as sources of resistance to ALS and genetic studies have revealed different types of gene action depending on the parents and the pathogen races. Genotypes AND 277, MAR-2, Mexico 54, BAT 332, and Cornell 49242 were shown to have single dominant genes that governed plant resistance to certain races of *P. griseola* (Carvalho et al., 1998; Nietsche et al., 2000; Sartorato et al., 2000; Aggarwal et al., 2004; Caixeta et al., 2005). Genotype Ouro Negro was reported to have a dominant gene that controlled resistance to *P. griseola* races 63-39 and 31-23 where the dominant gene was shown to be different from that found in AND 277, BAT 332, Cornell 49242, MAR-2 and Mexico 54 (Sanglard et al., 2013). Control of resistance in US genotype Pinto 111 against pathogen race 31-23 was found to be a single recessive gene (Correa et al., 2001). The G10474 common bean was shown to have a single dominant gene conditioning resistance to two *P. griseola* pathotypes (Mahuku et al., 2004). Two dominant and complementary genes were shown to condition resistance to ALS in G10909 when crossed with susceptible common bean cultivar Sprite, against pathogen race 63-63 (Mahuku et al., 2003a; Mahuku et al., 2011).

Previous studies have shown that the choice of the parental susceptible lines, and pathogen race used, influences the genetic reaction observed (Pastor-Corrales et al., 1994). For example, a single gene with a dominant allele was observed for the resistance to pathogen race 63-19, when the genotype Mexico 54 was crossed with the Ruda cultivar (Mesoamerican) (Sartorato et

al., 1999). On the other hand, Mahuku et al. (2002a) described the resistance of genotype Mexico 54 to pathogen race 31-55 as a single gene with the resistance due to the recessive allele, when crossed with a snap bean cultivar. Caixeta et al. (2002) showed that the genotype Mexico 54 had three dominant genes and BAT 332 had one dominant gene, while using pathogen race 61-41. Resistant line BAT 332 was crossed with susceptible cultivar Ruda and the resulting segregating populations when tested against race 61-41 showed that a single dominant gene confers resistance (Caixeta et al., 2003) to ALS disease. Studies by Mahuku and Iglesias (2009) revealed that three dominant and complementary genes conditioned resistance of common bean genotype G5686 to *P. griseola* race 31-0. Resistance to ALS disease has also been shown to be inherited quantitatively. Oblessuc et al. (2012) showed the existence of seven QTLs that had variable magnitudes of phenotypic effects under different environments. This showed the complex and quantitative pattern of inheritance of ALS in common bean genotype CAL 143. Allelism tests have shown that the genotype AND 227 has four angular leaf spot resistance genes designated as *Phg-1*^{a}, *Phg-2*^{2}, *Phg-3*^{2}, and *Phg-4*^{2}, while Mexico 54 has three *(Phg*-2, *Phg*-5 and *Phg*-6) and MAR-2 has two (*Phg*-4, *Phg*-5) (Mahuku et al., 2004; Caixeta et al., 2002).

These sources of resistance are conditioned by major genes and they have been shown to be susceptible in certain regions. For example, CAL 143 was shown to be resistant in Malawi, South Africa, Zambia and Tanzania, but it was susceptible in Uganda (Aggarwal et al., 2004). Hence there is need to breed common beans for resistance that is non-race specific.

1.13 Breeding common beans for resistance to angular leaf spot

Breeding common beans for resistance contributes greatly to management of the disease, since resistant varieties are the most practical and easily adopted strategy by the small holder farmers. The advantage of host resistance is that once the technology has been developed, it is packaged in seed which is easy to disseminate and does not require any additional handling by the farmers, other than the normal crop production practices.

1.13.1 Types of disease resistance

Plant species have a defence mechanism to avoid and resist pathogens and pests (Parlevliet, 2002). Plant resistance has been defined as the ability of the host plant to hinder the growth and or development of the pathogen (Parlevliet, 1979). Van der Plank (1963) classified host resistance as vertical and horizontal. He defined vertical resistance (VR) as race specific

resistance which is characterised by the presence of genetic interaction between the host and pathogen races. On the other hand, horizontal resistance is non-race specific and is characterised by the absence of genetic interaction between the host and the pathogen races.

Tolerance to disease, according to Politowski and Browning (1978), is "…e*ndurance and implies that 'A' undergoes the same stress as 'B' but withstands it better. In relation to plant diseases, a cultivar has 'true tolerance' if it has a susceptible infection type and supports the same amount of the pathogen inoculum as another cultivar, but has significantly better yield and quality (each relative to its disease free check), or if it has the same yield and quality as another cultivar, but supports significantly more of the pathogen*".

Durable resistance has been defined as the resistance that remains effective over long periods of widespread agricultural use and under conditions favourable for disease development (Johnson, 2000). Monogenic or major gene resistance (vertical resistance) has been widely used by breeders, but the high selection pressure has led to rapid emergence of new virulent strains (McDonald and Linde, 2002). The use of extensive monoculture and other practices that favour pathogen proliferation increase the evolution of virulent strains that cause significant yield losses and devastating epidemics (Boyd et al., 2012). Hence, there is need for durable resistance in crops. Some researchers have classified resistance conditioned by major genes that has lasted for many years as durable resistance. For example, research on durable resistance to downy mildew in sunflower showed the existence of 50 inbred lines that expressed partial resistance to two major races of the causative pathogen *Plasmopara halstedii* over four years (Tourvieille de Labrouhe et al., 2008). Durable resistance has been shown to exist as was proved with the woolly aphid resistance in apple cultivars (Niks et al., 1993) and also the phylloxera aphid resistance of the grape rootstocks (Pouget, 1990). Oligogenic durable resistance to coffee berry disease exists in arabica coffee (*Coffea arabica* L.) in Kenya, since it has lasted for over twenty years (Van der Vossen and Walyaro, 2009).

Polygenic resistance (horizontal resistance), on the other hand, is attributed to minor genes, and is termed durable or quantitative since each gene contributes a small additive effect to the overall resistance, so any virulence gene that might overcome that effect will have only a small selective advantage in the pathogen (Parlevliet, 1995). Polygenic resistance has been shown to include phenotypes that range from partial to full resistance and is effective against multiple strains of a pathogen and determined by several to many genes with small additive effects (Stahl et al., 1999; Ballini et al., 2008). This type of resistance is believed to remain effective

over long periods of time because of the diffuse selection pressures on the pathogen (Stuthman et al., 2007), but little research has been done to prove this (St. Clair, 2010).

There is not much work that has been done to breed for durable resistance that is conditioned by minor genes in common bean. Examples of research on durable resistance using minor genes on other crops include; Singh et al. (2011), who bred spring wheat cultivars for non-race specific resistance to rust diseases by deploying cultivars that had adult plant resistance which was based on minor, slow rusting genes. Inheritance of durable resistance to stripe and leaf rusts in Australian wheat cultivar 'cook' was shown as quantitative due to additive interaction of linked slow-rusting genes (Navabi et al., 2005). In the French wheat cultivar Apache, its durable resistance was attributed to three resistance genes (Paillard et al., 2012).

1.13.2 Breeding for minor gene resistance

There are no exact breeding strategies that have been proposed to develop resistance conditioned by minor genes. In addition, it is difficult to identify the major or minor gene resistance in the field since both types can occur simultaneously in the breeding population (Parlevliet and van Ommeren, 1988). Parlevliet (1985), and Parlevliet and van Ommeren (1988) recommended selection against complete resistance to eliminate resistance conditioned by major genes. This is because the presence of the major genes confounds the selection for the minor genes during breeding for non-race specific resistance (Parlevliet, 1983). Parlevliet (1981) suggested that if the starting population is conditioned by minor gene resistance, then any increase in resistance is non-race specific. A mixture of races that varies over the years (pathogen population is not defined) can be used as long as the starting population is of intermediate resistance (Parlevliet, 1983; Parlevliet and van Ommeren, 1988). Parlevliet (1985) described intermediate resistance, as the resistance that reduces levels of the pathogen sporulation despite being infected and termed it durable. Durable resistance is the resistance that will last for a long time (Johnson, 1981). However the length of time the resistance will last cannot be measured during the breeding process.

To breed for minor gene resistance, Robinson (1980) proposed accumulating minor genes of resistance by crosses between adapted local susceptible genotypes that may show transgressive segregation for higher levels of resistance. Parlevliet and van Ommeren (1988) accumulated partial resistance in barley to barley leaf rust and powdery mildew through three cycles of mild selection against susceptibility, and showed gain in resistance.

1.13.3 Gamete selection

Gamete selection is a breeding method that could be used to combine minor genes and favourable alleles into a single genotype. It was proposed by Singh (1994) as a method to simultaneously improve multiple traits in common bean through crossing of multiple parents followed by early generation testing and selection (Table 1.2). The multiple parents each contribute some favourable gene/allele which, when combined in a single genotype leads to genetic improvement.

Table 1.2 The method for gamete selection for the simultaneous improvement of multiple traits in common bean

Generation	Activities
"*Parents*	*Select contrasting parents, determine cross combinations, and produce single crosses*.
Parents and crosses	*Produce three-way, double and other types of crosses by using plant-to-plant paired hybridization.*
Parents and crosses	*Screen for desirable dominant and codominant alleles in heterogametic parents for production of final multiple-parent crosses, using plant-to-plant paired hybridization.*
F_1	*Screen for desirable dominant and codominant alleles and grow the remaining F_1 seed from each paired hybridization in separate hill plots. Record necessary data. Harvest seed from each surviving F_1 plant from a hill plot in a separate envelope.*
F_2	*Conduct a multilocational replicated yield trial in contrasting environments by organizing the F_1 derived F_2 families in random groups of three or more. Identify high yielding populations and discard undesirable populations. Alternatively, grow plant-to-progeny rows or hill plots in separate complementary nurseries under adequate and uniform pressure from important production constraints (e.g., anthracnose, angular leaf spot, common bacterial blight, and leaf hoppers). Bulk-harvest all resistant plants within selected families.*
F_3	*Evaluate surviving F_1 derived F_3 families from selected populations in separate, replicated complementary nurseries for each desirable trait. Discard low-yielding and susceptible families. Bulk-harvest resistant plants within selected families.*
F_4	*Repeat evaluation of F_1 derived F_4 families in replicated yield trials under each production constraint. Harvest in bulk resistant plants from high-yielding families possessing other desirable traits.*
F_5	*Space-plant and make maximum number of single plant harvests from selected families. Discard plants with undesirable morphological, seed and adaptation traits.*
F_6	*Grow plant-to-progeny rows. Check for uniformity of flower colour, growth habit, maturity, and seed adaptation traits. Harvest in bulk plants from selected uniform families.*
F_7	*Grow separate complementary nurseries for each desirable trait. Discard susceptible, undesirable, and inferior lines.*
F_{8-10}	*Evaluate in replicated yield trials under pressure from abiotic and biotic constraints in contrasting environments to identify new cultivars*".

Source: Singh (1994)

The effectiveness of gamete selection in the introgression of ALS resistance, using a multiple parent population, is not known. Multiple parent crosses and gamete selection have been used before to improve seed yield, seed quality and resistance to bean common mosaic virus (BCMV) and rust (Singh et al., 2008). It was also effective in improving plant architecture and resistance to five diseases (ALS, anthracnose, BCMV, bean golden mosaic virus, and common bacterial blight) and one insect-pest (leafhopper) (Singh et al., 1998). Teran and Singh, (2009) also used gamete selection to improve resistance to white mould disease in common bean. Gamete selection was also successful in improving resistance to common and halo bacterial blights in common bean inter-gene pool populations (Asensio et al., 2006).

1.14 Importance of landraces in breeding

Several definitions have been used to describe landraces. Mansholt (1909) described landraces as having high "*stability of their characteristics*" and great "*resistance capacity to tolerate adverse influences*". Teshome et al. (1997) also described a landrace as "*variable plant populations adapted to local agro-climatic conditions which are named, selected and maintained by the traditional farmers to meet their social, economic, cultural and ecological needs"*. A landrace has also been defined as a variety with a high potential to tolerate biotic and abiotic stress, resulting in high yield stability and an intermediate yield under low input agricultural systems (Zeven, 1998). A landrace differs from a cultivar since yield stability is the major characteristic of a landrace and a cultivar is characterised by a high yielding capacity under optimal conditions (Falcinelli et al., 1994).

Since the introduction of common bean to the eastern African coast by the Portuguese, farmers have used the crop to develop farming practices that are adapted to local conditions. Hence they have exploited useful alleles in the crop, which have resulted in a wide range of morphologically diverse landraces (Singh et al., 1991a; Wortmann et al., 1998). The genetic diversity helps to broaden the genetic base of new cultivars and hence maximises the available germplasm resources (Escribano et al., 1998). Genetic diversity has been shown to be present in common bean landraces in Italy (Piergiovanni and Lioi, 2010), Bulgaria and Portugal (Stoilova et al., 2005), in Galicia, Spain (Escribano et al., 1998), Mexico and Central America, using random amplified polymorphic DNA (RAPD) (Beebe et al., 2000), in Nilgiris, India using RAPD analysis (Jose et al., 2009) and in Ethiopia and Kenya using micro-satellite marker analysis (Asfaw et al., 2009). Blair et al. (2012) evaluated wild accessions and landraces of common bean using simple sequence repeat markers (SSR) that showed their genetic diversity. In

Bulgaria and Portugal landraces are still important genetic resources that are in use by the small-scale farmers, and have been used in common bean improvement programmes (Stoilova et al., 2005). In Tanzania, common bean landraces were improved for resistance to angular leaf spot and anthracnose (Mongi et al., 2009). Different regions have specific temperatures, humidity and other production requirements, and hence each landrace may not be grown successfully in regions where they are not traditionally cultivated (Piergiovanni and Lioi, 2010). Hence this should be considered during breeding.

1.15 Participatory plant breeding

Plant breeding should be carried out with the participation of farmers to ensure that released varieties meet their demands and are easily adopted. Participatory plant breeding techniques are being used to develop, multiply and distribute seed of improved common bean varieties (Almekinders et al., 2007). This approach to plant breeding allows the participation of farmers in the development, evaluation and selection of bean breeding lines (Morris and Bellon, 2004). Morris and Bellon (2004) noted that participatory plant breeding is well suited for the development of a variety that possesses a unique combination of traits, such as a specific bean type for a niche market.

Conventional and centralized plant breeding programmes have been shown to have significant impact in high input areas, but low impact in the marginal and small scale farming sector (Morris and Bellon, 2004). Ceccarelli and Grando (2007) showed that decentralized and demand driven research was essential, especially for the poor farmers in low input farming systems. They reported that this would help farmers choose the varieties that do well in their environmental conditions and hence adopt newly released varieties. Participatory plant breeding has been shown to have the potential to develop crop varieties that are better adapted to farmers' local environmental conditions and with farmer preferred traits (Sthapit et al., 1996). In the Andean region, farmers were involved as participants in selection of advanced materials rather than selection of finished varieties. This encouraged the use of locally adapted cultivars, incorporation of farmer preferred cultivars and the Andean cropping systems (Danial et al., 2007).

Positive results have been reported with important contributions by farmers, when the farmers are involved during selection in the breeding process. For example, Sperling et al. (1993) reported that lines selected by farmers yielded higher than those selected by breeders. Farmers

were shown to visually select higher yielding barley lines than the breeders (Ceccarelli et al., 2001). Hence involving farmers during selection leads to improvement of the breeding process. In another study, Fufa et al. (2010) tested the efficiency of selection by farmers in a barley breeding program. They compared farmers' and breeders' selection of varieties for different regions and realised that farmers chose varieties that were better adapted to their specific regions, while breeders selected for broad environments. They emphasised the importance of decentralized participatory plant breeding in increasing and stabilizing productivity and maintaining genetic diversity. Courtois et al. (2001) carried out a farmer participation study on rain fed rice in eastern India and showed that varietal evaluation (by ranking) on farmers' fields was better than when they were evaluated by breeders on the breeding stations. They concluded that combining efforts by farmers and breeders leads to varieties more suitable to the farmers.

Consultation with farmers before or during the breeding process has led to better adoption of newly released varieties. Surveys, interviews, and participatory rural appraisal have been used to determine farmers' preferred traits in crops. The information has successfully been used in the breeding of common bean for resistance to bean fly (Ojwang' et al., 2009) and resistance to fusarium root rot (Mukankusi, 2008). Asfaw et al. (2012) compared the use of focus group discussions, interviews and participatory variety selection (PVS) to assess information from farmers, on their preferences for drought tolerant common bean varieties in southern Ethiopia. They found that active selection of drought tolerant genotypes on farmers' fields was fast, efficient and accurate. Women play a key role in most farming systems in Africa, since they are involved in production and also in the utilisation (cooking). Women smallholder farmers in eastern Ethiopia were able to make significant contributions in identification of superior common bean cultivars when they evaluated them on-farm (Assefa et al., 2005). Hence farmers can be involved during the breeding process at the beginning, where farmer preferences are evaluated, and also at the end when varieties are tested on the farmers' fields.

1.16 Summary

From the review of literature it can be concluded that common bean is a major crop of importance in Kenya produced mainly by small scale farmers for home consumption. It provides a cheap source of proteins for consumers. However, its production is affected by several constraints of which ALS is most important leading to yield losses of up to 80%. The most affordable method for management of the disease is the use of resistant varieties. Breeding for

resistant varieties has been made difficult by the high pathogenic variability of *P. griseola* that occurs in Kenya. Major genes have been used to breed for resistance to the disease, but the released new varieties have become susceptible over time.

An effective way of breeding for durable resistance to the highly variable pathogen is by use of minor genes. There is no specific breeding method that has been suggested for durable resistance for ALS in common beans. The use of landraces is essential because of their wide genetic diversity. Small scale farmers in Kenya still use landraces because they value traits such as seed colour, seed shape, and cooking time which are no longer considered during the breeding process. Farmers have acquired knowledge through experience from years of growing common beans. It is essential that farmer knowledge, perceptions and needs are incorporated into a breeding programme. This ensures that newly released varieties are widely adopted.

References

Abawi, G.S. 1989. Root rots, p. 105-158, *In* H.F. Shwartz and M.A. Pastor-Corrales, eds. Bean production problems in the tropics. CIAT, Cali, Colombia.

Abawi, G.S., and F.V. Agudelo. 1989. Nematodes, p. 433-454, *In* H.F. Shwartz and M.A. Pastor-Corrales, eds. Bean production problems in the tropics. CIAT, Cali, Colombia.

Aggarwal, 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.

Allen, D.J., J.K.O. Ampofo, and C.S. Wortmann. 1996. Pests, diseases, and nutritional disorders of the common bean in Africa: A field guide. CIAT, Cali, Colombia.

Almekinders, C.J.M., E. Graham, E. Thiele, and D.L. Danial. 2007. Can cultivars from participatory plant breeding improve seed provision to small-scale farmers? Euphytica 153:363-372.

Asensio, S.M.M.C., C. Asensio, and S.P. Singh. 2006. Gamete selection for resistance to common and halo bacterial blights in dry bean intergene pool populations. Crop Science 46:131-135.

Asfaw, A., M.W. Blair, and C. Almekinders. 2009. Genetic diversity and population structure of common bean (*Phaseolus vulgaris* L.) landraces from the east African highlands. Theoretical and Applied Genetics 120:1-12.

Asfaw, A., C.J.M. Almekinders, M.W. Blair, and P.C. Struik. 2012. Participatory approach in common bean (*Phaseolus vulgaris* L.) breeding for drought tolerance for southern Ethiopia. Plant Breeding 131:125-134.

Assefa, T., G. Abebe, C. Fininsa, B. Tesso, and A.M. Al-Tawaha. 2005. Participatory bean breeding with women and small holder farmers in eastern Ethiopia. World Journal of Agricultural Sciences 1:28-35.

Ballini, E., J.B. Morel, G. Droc, A. Price, and B. Courtois. 2008. A genome-wide meta-analysis of rice blast resistance genes and quantitative trait loci provides new insights into partial and complete resistance. Molecular Plant-Microbe Interaction 21:859-868.

Becerra Vela´squez, V.L., and P. Gepts. 1994. RFLP diversity in common bean (*Phaseolus vulgaris* L.). Genome 37:256-263.

Beebe, S., P.W. Skroch, J. Tohme, M.C. Duque, F. Pedraza, and J. Nienhuis. 2000. Structure of genetic diversity among common bean landraces of Middle American origin based on correspondence analysis of RAPD. Crop Science 40:264-273.

Blair, M.W., A. Soler, and A.J. Corte's. 2012. Diversification and population structure in common beans (*Phaseolus vulgaris* L.). PLoS ONE 7: e49488.doi:10.1371/journal.pone.0049488.

Boyd, L.A., C. Ridout, D.M. O'Sullivan, J.E. Leach, and H. Leung. 2012. Plant-pathogen interactions: Disease resistance in modern agriculture. Trends Genetics 29:233-240.

Caixeta, E.F., A. Bor´em, N.G. de Morais Silvia, R.C. Rocha, E.G. de Barros, and M.A. Moreira. 2002. Teste de alelismo para genes do feijoeiro que conferem resistencia ao fungo *Phaeoisariopsis griseola*. VII Congresso Nacional de Pesquisa de Jeijiao. Universidade Federal de Vicosa, Vicosa-MG, Brazil.

Caixeta, E.T., A. Bor´em, S. de Azevedo 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 134:297-303.

Caixeta, E.T., A. Bore´m, A. L. Alzate-Marin, S. de Azevedo Fagundes, M. G. de Morais Silva, E. G. de Barros, and M.A. Moreira. 2005. Allelic relationships for genes that confer resistance to angular leaf spot in common bean. Euphytica 145:237-245.

Carvalho, G.A., T.J. Paula, A.L. Alzate-Marin, S. Nietsche, E.G. de Barros, and M.A. Moreira. 1998. Inheritance of resistance to angular leaf spot of common bean in AND 277 to race 63–23 of *Phaeoisariopsis griseola* and identification of a RAPD marker linked to the resistance gene. Fitopatologia Brasil 23:482-485.

Ceccarelli, S., and S. Grando. 2007. Decentralized-participatory plant breeding: An example of demand driven research. Euphytica 155:349-360.

Ceccarelli, S., S. Grando, E. Bailey, A. Amri, M. El-Felah, F. Nassif, S. Rezgui, and A. Yahyaoui. 2001. Farmer participation in barley breeding in Syria, Morocco and Tunisia. Euphytica122:521-536.

Correa, R.X., P.I.V. Good-God, M.L.P. Oliveira, S. Nietsche, M.A. Moreira, and E.G. Barros. 2001. Inheritance of resistance on the common bean angular leaf spot and identification of molecular markers flanking the resistance locus. Fitopatologia Brasil 26:27-32.

Courtois, B., B. Bartholome, D. Chaudhary, G. McLaren, C.H. Misra, N.P. Mandal, S. Pandey, T. Paris, C. Piggin, K. Prasad, A.T. Roy, R.K. Sahu, V.N. Sahu, S. Sarkarung, S.K. Sharma, A. Singh, H.N. Singh, O.N. Singh, N. K. Singh, R. K. Singh, R.K. Singh, S. Singh, P.K. Sinha, B.V.S. Sisodia, and R. Takhur. 2001. Comparing farmers and breeders rankings in varietal selection for low-input environments: A case study of rainfed rice in eastern India. Euphytica 122:537-550.

Damasceno e Silva, K.J., E.A. Souza, A. Sartorato, and C.S. Freir. 2008. Pathogenic variability of isolates of *Pseudocercospora griseola*, the cause of common bean angular leaf spot, and its implications for resistance breeding. Journal of Phytopathology 156:602-606.

Danial, D., J. Parlevliet, C. Almekinders, and G. Thiele. 2007. Farmers' participation and breeding for durable disease resistance in the Andean region. Euphytica 153:385-396.

Debouck, D. 1991. Systematics and morphology, p. 55-118, *In* A. Van Schoonhoven and O. Voysest, eds. Common beans: Research for crop improvement. CIAT, Cali, Colombia.

Duke, J.A. 1981. Handbook of Legumes of World Economic Importance Plenum Press, New York, USA.

Escribano, M.R., M. Santalla, P.A. Casquero, and A.M. Ron. 1998. Patterns of genetic diversity in landraces of common bean (*Phaseolus vulgaris* L.) from Galicia. Plant Breeding 117:49-56.

Evans, A.M. 1980. Structure, variation, evolution, and classification in *Phaseolus.*, p. 337–347, *In* R. J. Summerfield and A. H. Bunting, eds. Advances in legume science. Royal Botanic Gardens, Kew, UK.

Falcinelli, M., L. Russi, V. Negri, and F. Verones. 1994. Variation within improved cultivars and landraces of lucerne in Central Italy. Euphytica 77:199-203.

Freyre, R., R. Rı´os, L. Guzma´n, D. Debouck, and P. Gepts. 1996. Ecogeographic distribution of *Phaseolus* spp. (Fabaceae) in Bolivia. Economic Botany 50:195-215.

Fufa, F., S. Grando, O. Kafawin, Y. Shakhatreh, and S. Ceccarelli. 2010. Efficiency of farmers' selection in a participatory barley breeding programme in Jordan. Plant Breeding 129:156-161.

Galvez, G.E., and F.J. Morales. 1989. Aphid transmitted viruses, p. 333-362, *In* H.F. Shwartz and M.A. Pastor-Corrales, eds. Bean production problems in the tropics. CIAT, Cali, Colombia.

Gepts, P., and D. Debouck. 1991. Origin, domestication and evolution of common bean (*Phaseolus vulgaris* L.), p. 7-54, *In* A. Van Shoonhoven and O. Voysest, eds. Common beans: Research for crop improvement. CIAT, Cali, Colombia.

Graham, P.H., and P. Ranalli. 1997. Common bean (*Phaseolus vulgaris* L.). Field Crops Research 53:131-146.

Guzman, P., P. Gepts, S. Temple, A.B. Mkandawire, and R.L. Gilbertson. 1999. Detection and differentiation of *Phaeoisariopsis griseola* isolates with the polymerase chain reaction and group specific primers. Plant Disease 83:37-42.

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

Johnson, R. 1981. Durable resistance: Definition of, genetic control and attainment in plant breeding. Phytopathology 71:567-568.

Johnson, R. 2000. Classical plant breeding for durable resistance to diseases. Plant Pathology 82:3-7.

Jose, F.C., M.M.S. Mohammed, G. Thomas, G. Varghese, N. Selvaraj, and M. Dorai. 2009. Genetic diversity and conservation of common bean (*Phaseolus vulgaris* L., Fabaceae) landraces in Nilgiris. Current Science 97:227-235.

Karel, A.K., and A. Antrique. 1989. Insects and other pests in Africa, p. 455-504, *In* H.F. Shwartz and M. A. Pastor-Corrales, eds. Bean production problems in the tropics. CIAT, Cali, Colombia.

Koenig, R., and P. Gepts. 1989. Allozyme diversity in wild *Phaseolus vulgaris*: further evidence for two major centers of diversity. Theoretical and Applied Genetics 78:809-817.

Koinange, E.M.K., and P. Gepts. 1992. Hybrid weakness in wild *Phaseolus vulgaris* L. Journal of Heredity 83:135-139.

Kwak, M., and P. Gepts. 2009. Structure of genetic diversity in the two major gene pools of common bean (*Phaseolus vulgaris* L., Fabaceae). Theoretical and Applied Genetics 118:979-992.

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.

Mahuku, G., C. Jara, H. Teran, and S. Beebe. 2003a. Inheritance of angular leaf spot resistance in selected common bean genotypes. Annual Report Bean Improvement Cooperative 46:151-152.

Mahuku, G.S., and A.M. Iglesias. 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. 2002a. Genetic variability within *Phaeoisariopsis griseola* from Central America and its implications for resistance breeding. Plant Pathology 51:594-604.

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

Mahuku, G.S., C. Jara, C. Cajiao, and S. Beebe. 2003b. 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. Montoya, M.A. Henrı´quez, C. Jara, H. Teran, and S. Beebe. 2004. Inheritance and characterization of the angular leaf spot resistance gene in the common bean accession, G 10474 and identification of an AFLP marker linked to the resistance gene. Crop Science 44:1817-1824.

Mahuku, G.S., M.A. Henrı´quez, C. Montoya, C. Jara, H. Teran, and S. Beebe. 2011. Inheritance and development of molecular markers linked to angular leaf spot resistance genes in the common bean accession G10909. Molecular Breeding 28:57-71.

Mansholt, U.J. 1909. Van Pesch Plantenteelt, beknopte handleiding tot de kennis van den nederlandschen landbouw plantenteelt. Zwolle, Netherlands.

McDonald, B.A., and C. Linde. 2002. Pathogen population genetics, evolutionary potential, and durable resistance. Annual Review Phytopathology 40:349-379.

Mongi, R.J., F. Mwalyego, A. Elanga, and B. Kiwovele. 2009. Introgressing genes conferring resistance to angular leaf spot and anthracnose in common bean landraces in southern highlands of Tanzania. African Crop Science Conference Proceedings 9:517-520.

Morris, M.L., and M.R. Bellon. 2004. Participatory plant breeding research: Opportunities and challenges for the international crop improvement system. Euphytica 136:21-34.

Mukankusi, C.M. 2008. Improving resistance to fusarium root rot [*Fusarium solani* (Mart.) Sacc. f. sp. *phaseoli* (Burkholder) W.C. Snyder & H.N. Hans.] in common bean (*Phaseolus vulgaris* L.). PhD Thesis, University of KwaZulu-Natal, Pietermaritzburg, South Africa.

Mwang'ombe, A.W., I.N. Wagara, J.W. Kimenju, and R.A. Buruchara. 2007. Occurence and severity of angular leaf spot of common bean in Kenya as influenced by geographical location, altitude and agroecological zones. Plant Pathology 6:235-241.

Myers, J.R. 2000. Tomorrow's snap bean cultivars, p. 39-51, *In* S. P. Singh, ed. Bean research production and utilization. University of Idaho, USA.

Navabi, A., J.P. Tewari, R.P. Singh, B. McCallum, A. Laroche, and K.G. Briggs. 2005. Inheritance and QTL analysis of durable resistance to stripe and leaf rusts in an Australian cultivar, *Triticum aestivum* 'Cook'. Genome 48:97-107.

Nietsche, S., A. Borém, G.A. Carvalho, R.C. Rocha, T.J. Paula-Jr, E.G. Barros, and M.A. Moreira. 2000. RAPD and SCAR markers linked to a gene conferring resistance to angular leaf spot in common bean. Journal of Phytopathology 148:117-121.

Nietsche, S., A. Bor´em, G.A. de Carvalhos, T.J. de Paula J´unior, C. Fortes Ferreira, E. Goncalves de Barros, and M.A. Moreira. 2001. Genetic diversity of *Phaeoisariopsis griseola* in the state of Minas Gerais, Brazil. Euphytica 117:77-84.

Niks, R.E., P.R. Ellis, and J.E. Parlevliet. 1993. Resistance to parasites, p. 422-447, *In* M.D. Hayward and N.O. Bosemark, eds. Plant breeding, principles and prospects. Chapman & Hall, London, UK.

Oblessuc, P.R., R.M. Baroni, A.A.F. Garcia, A.F. Chioratto, S.A.M. Carbonell, L.E.A. Camargo, and L.L. Benchimol. 2012. Mapping of angular leaf spot resistance QTL in common bean (*Phaseolus vulgaris* L.) under different environments. BMC Genetics 13:50:1-9.

Ojwang', P.P.O., R. Melis, J.M. Songa, M. Githiri, and C. Bett. 2009. Participatory plant breeding approach for host plant resistance to bean fly in common bean under semi-arid Kenya conditions. Euphytica 170:383-393.

Paillard, S., G. Trotoux-Verplancke, M.R. Perretant, F. Mohamadi, M. Leconte, S. Coedel, C. de Vallavieille-Pope, and F. Dedryver. 2012. Durable resistance to stripe rust is due to three specific resistance genes in French bread wheat cultivar Apache. Theoretical and Applied Genetics 125:955-965.

Parlevliet, J.E. 1979. Components of resistance that reduce the rate of epidemic development. Annual Review Phytopathology 17:203-222.

Parlevliet, J.E. 1981. Disease resistance in plants and its consequences for plant breeding, p. 309-364, *In* K. J. Frey, ed. Plant Breeding II. Iowa State University Press, Iowa, USA.

Parlevliet, J.E. 1983. Can horizontal resistance be recognized in the presence of vertical resistance in plants exposed to a mixture of pathogen races? Phytopathology 73:379.

Parlevliet, J.E. 1985. Resistance of the non-race-specific type, p. 501-525, *In* A.P. Roelfs and W. R. Bushnell, eds. The cereal rusts vol. II. Academic Press, New York, USA.

Parlevliet, J.E. 1995. Genetic and breeding aspects of durable resistance of crops to pathogens. African Crop Science Journal 3:1-13.

Parlevliet, J.E. 2002. Durability of resistance against fungal, bacterial, and viral pathogens; present situation. Euphytica 124:147-156.

Parlevliet, J.E., and A. van Ommeren. 1988. Accumulation of partial resistance in barley to barley leaf rust and powdery mildew through recurrent selection against susceptibility. Euphytica 37:261-274.

Pastor-Corrales, M.A., and J.C. Tu. 1989. Anthracnose, p. 77-104, *In* H.F. Shwartz and M.A. Pastor-Corrales, eds. Bean production problems in the tropics. CIAT, Cali, Colombia.

Pastor-Corrales, M.A., and A.W. Saettler. 1989. Angular leaf spot, p. 59-76, *In* H.F. Shwartz and M.A. Pastor-Corrales, eds. Bean production problems in the tropics. CIAT, Cali, Colombia.

Pastor-Corrales, M.A., C. Jara, and S.P. 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.

Pastor-Corrales, M.A., O.A. Erazo, E.L. Estrada, and S.P. Singh. 1994. Inheritance of anthracnose resistance in common bean accession G 2333. Plant Disease 78:959-962.

Piergiovanni, A.R., and L. Lioi. 2010. Italian common bean landraces: History, genetic diversity and seed quality. Diversity 2:837-862.

Politowski, K., and J.A. Browning. 1978. Tolerance and rsistance to plant disease:an epidemiology study. Phytopathology 68:1177-1185.

Pouget, R. 1990. Histoire de la lutte contre le Phylloxera de la vigne en France (1868-1895). INRA, Paris, France.

Robinson, R.A. 1980. New concepts in breeding for disease resistance. Annual Review of Phytopathology 18:189-210.

Rutger, J.N., and L.S. Beckham. 1970. Natural hybridisation of *Phaseolus vulgaris* x *Phaseolus coccineus L.* Journal of the American Society for HortScience 95:659-661.

Saettler, A.W. 1989. Common bacterial blight, p. 261-284, *In* H.F. Shwartz and M.A. Pastor-Corrales, eds. Bean production problems in the tropics. CIAT, Cali, Colombia.

Sanglard, D.A., C.A.G. Ribeiro, B.P. Balbi, K.M.A. Arruda, E.G. Barros, and M.A. Moreira. 2013. Characterisation of the angular leaf spot gene present in common bean cultivar Ouro Negro. Journal of Agricultural Science 5:19-23.

Sartorato, A., S. Nietsche, E.G. Barros, and M.A. Moreira. 1999. SCAR marker linked to angular leaf spot resistance gene in common bean. Annual Report of the Bean Improvement Cooperative 42:23-24.

Sartorato, A., S. Nietsche, E.G. Barros, and M.A. Moreira. 2000. RAPD and SCAR markers linked to resistance gene to angular leaf spot in common bean. Fitopatologia Brasil 25:637-642.

Shwartz, H.F. 1989. Halo blight, p. 285-302, *In* H.F. Shwartz and M.A. Pastor-Corrales, eds. Bean production problems in the tropics. CIAT, Cali, Colombia.

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

Shwartz, H.F., and J.R. Steadman. 1989. White mould, p. 211-230, *In* H.F. Shwartz and M.A. Pastor-Corrales, eds. Bean production problems in the tropics. CIAT, Cali, Colombia.

Shwartz, H.F., J.R. Steadman, R. Hall, and R. Forester. 2005. Compendium of bean diseases. American phytopathology society press, USA.

Shwartz, H.F., V.F. Correa, D.D.A. Pineda, M.M. Otoya, and M.J. Katherman. 1981. Ascochyta, angular and whitefly leaf spots in Colombia. Plant Disease 65:494-496.

Singh, R.P., J. Huerta-Espino, S. Bhavani, S.A. Herrera-Foessel, D. Singh, P.K. Singh, G. Velu, R.E. Mason, Y. Jin, P. Njau, and J. Crossa. 2011. Race non-specific resistance to rust diseases in CIMMYT spring wheats. Euphytica 179:175-186.

Singh, S.P. 1994. Gamete selection for simultaneous improvement of multiple traits in common bean. Crop Science 34:352-355.

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

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

Singh, S.P., R. Nodari, and P. Gepts. 1991b. Genetic diversity in cultivated common bean. I. Allozymes. Crop Science 31:19-23.

Singh, S.P., C. Cardona, F.J. Morales, M.A. Pastor-Corrales, and O. Voysest. 1998. Gamete selection for upright Carioca bean with resistance to five diseases and a leafhopper. Crop Science 38:666-672.

Singh, S.P., H. Teran, M. Lema, M.F. Davis, R. Hayes, and C. Robinson. 2008. Breeding for slow darkening, high yielding, broadly adapted dry bean pinto 'Kimberly' and 'Shoshone'. Journal of Plant Registration 2:181-186.

Sperling, L., M.E. Loevinsohn, and B. Ntabomvura. 1993. Rethinking the farmers' role in plant breeding: Local bean experts and on-station selection in Rwanda. Experimental Agriculture 29:509-519.

St. Clair, D.A. 2010. Quantitative disease resistance and quantitative resistance loci in breeding. Annual Review Phytopathology 48:247-268.

Stahl, E.A., G. Dwyer, R. Mauricio, M. Kreitman, and J. Bergelson. 1999. Dynamics of disease resistance polymorphism at the *Rpm1* locus of *Arabidopsis*. Nature 400:667-671.

Stavely, J.R. 1989. Rust, p. 159-194, *In* H.F. Shwartz and M.A. Pastor-Corrales, eds. Bean production problems in the tropics. CIAT, Cali, Colombia.

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. Advanced Applied Microbiology 52:209-243.

Sthapit, B.R., K.D. Joshi, and J.R. Witcombe. 1996. Farmers participatory crop improvement. III. Participatory plant breeding, a case study for rice in Nepal. Experimental Agriculture 32:479-496.

Stoetzer, H.A. 1984. Natural cross-pollination in bean in Ethiopia. Annual Report of the Bean Improvement Cooperative 27:99-100.

Stoilova, T., G. Pereira, M.M.T. de Sousa, and V. Carnide. 2005. Diversity in common bean landraces (*Phaseolus vulgaris L.)* from Bulgaria and Portugal. Journal of Central European Agriculture 6:443-448.

Stuthman, D.D., K.J. Leonard, and J. Miller-Garvin. 2007. Breeding crops for durable resistance to disease, p. 319-367, *In* D. L. Sparks, ed. Advances in Agronomy, Vol. 95. Academic Press, New york, USA.

Teran, H., and S.P. Singh. 2009. Gamete selection for improving physiological resistance to white mold in common bean. Euphytica 167:271-289.

Teshome, A., B.R. Baum, L. Fahrig, J.K. Torrance, T.J. Arnason, and J.D. Lambert. 1997. Sorghum (*Sorghum bicolor* (L.) Moench) landrace variation and classification in North Shewa and South Welo, Ethiopia. Euphytica 97:255-263.

Tourvieille de Labrouhe, D., F. Serre, P. Walser, S. Roche, and F. Vear. 2008. Quantitative resistance to downy mildew (*Plasmopara halstedii*) in sunflower (*Helianthus annuus*). Euphytica 164:433-444.

Van der Plank, J.E. 1963. Plant diseases: Epidemics and control. Academic Press, New York, USA.

Van der Vossen, H.A.M., and D.J. Walyaro. 2009. Additional evidence for oligogenic inheritance of durable host resistance to coffee berry disease (*Colletotrichum kahawae*) in arabica coffee (*Coffea arabica* L.). Euphytica 165:105-111.

Voysest, O., and M. Dessert. 1991. Bean cultivars: Classes and commercial seed types, p. 119-162, *In* H.F. Shwartz and M.A. Pastor-Corrales, eds. Bean production problems in the tropics. CIAT, Cali, Colombia.

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.

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

Zeven, A.C. 1998. Landraces: A review of definitions and classifications. Euphytica 104:127-139.

CHAPTER 2

Evaluation of common bean production systems, constraints, and farmer varietal preferences in Kenya

Abstract

Common bean (*Phaseolus vulgaris* L.) production in Kenya is practiced in most agro-ecological zones, mainly by small scale farmers. However, there is limited information on common bean production systems and constraints facing the small scale farmers in Kenya and hence this study. The aim of the study was to determine the farmers' knowledge on common bean production systems, constraints, and their preferred traits in new common bean varieties. The study was carried out in two districts (Kiambu and Thika) in Kiambu county. A sample of 181 farmers participated in the semi-structured interviews and focus group discussions. The results showed that the farmers practice common bean farming on small land holdings (< 0.5 ha). They grow several market class varieties (GLP & KAT series) and landraces ('Kiboland' and 'Mukura na oke'). Common bean production is practiced during both the long and short rains either as a monocrop (29%) or as an intercrop (71%) with several other crops, the three main ones being maize (91%), coffee (85%), and bananas and coffee (40%). The farmers' main source of seed is retained seed (70%) and, where they are not self-sufficient, they source seed from the local market (19%), neighbouring farmers (8%) and seed traders (2%). The farmers experience several constraints to production which include abiotic constraints such as low rainfall (29%), excessive rainfall (12%), while the biotic constraints include diseases (94%) and insect-pests (92%). The main insect-pests identified were black aphids (80%), whiteflies (60%) and bollworms (29%). Diseases of major importance were angular leaf spot (71%), ascochyta blight (46%) and powdery mildew (23%). The farmers did not use any pesticides to control the insect-pests and diseases due to the high cost involved and only relied on cultural practices such as weeding and roguing. Farmer preferred common bean traits are high yield (80%), resistance to insect-pests and diseases (72%), type I growth habit (determinate) (52%), early maturity (68%), seed size (medium/large) and colour (red/cream) (21%) and short cooking time (20%). Knowledge of farmers' production constraints is essential for success of a breeding programme. Farmers' varietal preferences should be taken into consideration, to ensure better adoption of the released varieties.

2.1 Introduction

Common bean is ranked as the most important legume crop in Kenya in terms of production and utilisation (MOA, 2013). Kiambu county is one of the largest producers of common bean with 28 thousand ha under production. The crop is grown by small scale farmers at a subsistence level, and these farmers experience a wide range of biotic, abiotic and socio-economic constraints. Hence the farmers have accumulated knowledge and experience over time on production systems and how to cope with the constraints. It is therefore essential to utilise this knowledge from the farmers. Plant breeders should thus involve the farmers in the breeding process to ensure that the released varieties meet the farmers' needs and hence can readily be adopted (Sthapit et al., 1996; Ceccarelli and Grando, 2007). Sperling et al. (2001) reported that participatory plant breeding involves all the stakeholders including scientists, farmers, agriculture organisations, the industries and consumers, in the process. Biggs (1989) suggested that participation by farmers in the breeding process could be in several ways; by consultation, on contract where farmers are paid, by collaboration whereby they are active partners in research, or where groups of farmers cooperate to lead research.

Several studies have been carried out to assess the impact of farmer involvement in breeding programmes. Conventional and centralized plant breeding programmes have been shown to have significant impact in high input areas, but low impact in the marginal and small scale farming sector (Morris and Bellon, 2004). Ceccarelli and Grando (2007) showed that decentralized and demand driven research is essential, especially for the poor farmers in low input farming systems, where farmers choose the varieties that do well under local environmental conditions. Fufa et al. (2010) emphasised the importance of decentralized participatory plant breeding in increasing and stabilizing productivity and maintaining genetic diversity. Research in the Andean region of South America has shown that certain varieties (potato, maize, wheat and barley) were not accepted by farmers due to poor grain quality traits or susceptibility to disease (Danial et al., 2007). Hence it is important to involve farmers in the early stages of the breeding process rather than at the end during selection of advanced lines (Danial et al., 2007).

Positive results have been reported with important contributions by farmer involvement during selection in the breeding process. For example, Sperling et al. (1993) reported that lines selected by farmers yielded higher than those selected by breeders. Farmers were shown to visually select higher yielding barley lines than the breeders (Ceccarelli et al., 2001). Hence involving farmers during selection leads to improvement of the breeding process. In another

study, Fufa et al. (2010) compared farmers' and breeders' selection of barley varieties for different regions and realised that farmers chose varieties that were better adapted to their specific regions, while breeders selected for broad environmental adaptation. Courtois et al. (2001), in a study on rain fed rice in eastern India, showed that farmer varietal evaluation (by ranking) on farmers' fields was better than when they were evaluated by breeders on station. Were (2011) showed that farmers were able to select their preferred traits in cassava from concealed landraces and improved varieties in the field. Thus combining efforts by farmers and breeders leads to varieties that are more suitable to the farmers' preferences.

Consultation with farmers before or during the breeding process has led to better adopted varieties. Surveys, interviews and participatory rural appraisal have been used to determine farmers' preferred traits in crops. The information has successfully been used in the breeding process to develop common beans for resistance to bean fly (Ojwang' et al., 2009) and resistance to fusarium root rot (Mukankusi, 2008). Asfaw et al. (2012) compared the use of focus group discussions, interviews and participatory variety selection (PVS) to assess information from farmers on their preferences for drought tolerant common bean varieties in southern Ethiopia. They found that active selection of drought tolerant genotypes, on farmers' fields was fast, efficient and accurate. Williams et al. (2012) used farmers' fields for peanut yield trials in East Timor, where farmers did the actual production with guidance from the breeders. Their results showed high adoption levels of up to 75% a year after the trials. They emphasized the importance of combining the breeding process with reliable seed systems to ensure continuity. Therefore, farmers' participation is able to give insight into trait preferences, constraints related to production and marketing, so that they can be addressed during the breeding process and hence enhance the adoption rate of newly released varieties (Ceccarelli et al., 2003).

This study was therefore undertaken to identify the common bean cropping systems, the sources of seed, as well as the production constraints experienced by the farmers. In addition, the study aimed to identify the farmer preferred traits that could be considered in a common bean breeding programme.

2.2 Materials and Methods

2.2.1 Study area

The study was conducted in the common bean growing areas of Thika and Kiambu districts of Kiambu county, in Kenya. Kiambu district covers an area of 1324 km². The district lies between latitudes 0°75′ and 1°20′ south of equator and longitudes 36°54′ and 36°85′ east. Altitude ranges from 1400 m above sea level (masl) in the southeast to 2400 masl in the north. The rainfall is bimodal with peaks in April/May and October/November. The average rainfall is 1100 mm per year. The most predominant soils are the nitosols (red Gikuyu loams). The combination of good soils, suitable climate, well-developed infrastructure and the proximity to the country's main market (Nairobi) makes the district one of the best farming regions in the country. Thika district covers an area of 1960 km². The district lies between latitudes 3°53′ and 1°45′ south of equator and longitudes 36°35′ and 37°25′ east at an altitude between 1555-2400 masl. The rainfall is bimodal with the peaks in April/May and October/November. The average rainfall is 700 mm. The predominant soil types are nitosols and vertisols (black cotton soils). The two districts produce common bean as a source of both food and income.

2.2.2 Farmer surveys and focus group discussions

A semi-structured questionnaire (Appendix 2.1) was administered to individual farmers and farmer group representatives in the form of interviews. The interviews covered the main common bean varieties grown, cropping systems, source of seed, seed selection criteria, constraints to production, major insect-pests and diseases, and farmer-preferred traits for improvement during breeding. The focus group discussions were conducted with farmer groups which were comprised mainly of women. In each location there was one focus group that comprised of 10-15 farmers. The farmer groups discussed the cropping calendar, preferred common bean varieties and reasons for preference, ranking of production constraints and major diseases in order of importance. Field observations were also done by the researcher and farmers were asked to identify the diseases in the field. A total of 181 farmers from seven locations of Kiambu and Thika districts participated in the study (Table 2.1). The participants included 3-5 representatives of farmer groups, as well as individual farmers. The study was carried out in collaboration with the Ministry of Agriculture officials.

Table 2.1: Number of farmers who participated during the farmer survey and discussions

District	Location	Farmers
Kiambu	Kabete	32
	Kiangotho	34
	Kikuyu	20
	Ndeiya	20
	Riabai	20
	Ndumberi	15
Thika	Kiganjo	40

2.2.3 Data analysis

Data was analysed using the Statistical Package for the Social Sciences (SPSS) version 15.0, statistical software (SPSS, 2006).

2.3 Results

2.3.1 Common bean production and cropping systems

Common bean farming in Kiambu county is mainly practiced by women while the men concentrate on cash crops such as coffee and tea. The farmers produce common bean during both the long and short rains seasons. The long rains are from March to July while the short rains are from September to December. The farmers reported that they obtain better yields during the short rains than during the long rains. The main activity during the common bean season is weeding, which is carried out twice, just before flowering and after pod set. During the discussions, farmers reported that they do not use any agro-chemicals to control insect-pests and diseases. They depend on good agronomic practices such as weeding and clearing of plant debris during the production season. Harvesting is carried out manually where whole crops are uprooted when dry (Figure 2.1), threshed and stored without further cleaning. The seed is used for consumption as well as for the next planting.

Figure 2.1: Common bean farmer in an interview during harvest

The farmers practice common bean farming on small land holdings (< 0.5 ha). The common beans are planted as an intercrop by 71% of the farmers and as a pure stand by 29% of the farmers. The farmers intercrop common bean with several other crops including maize (91%), coffee (85%), bananas and coffee (40%), potatoes (25%), macadamia (12%) and leafy vegetables (8%) (Figures 2.2, 2.3 and 2.4).

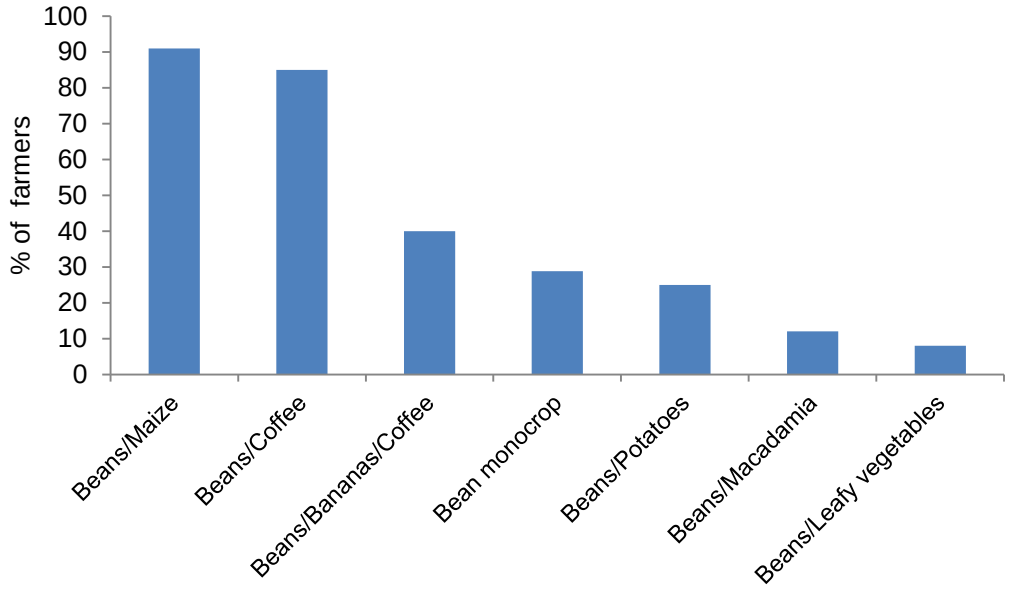

Figure 2.2: Common bean cropping systems

Figure 2.3: Common bean intercrop with maize

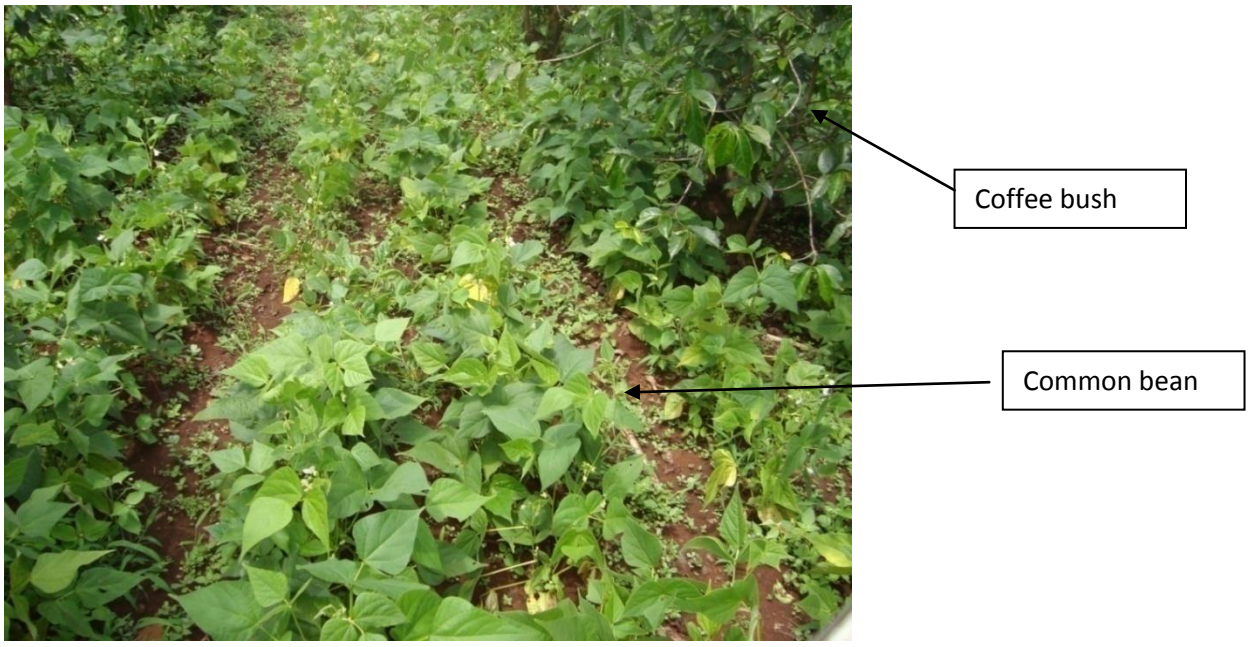

Figure 2.4: Common bean intercropped with coffee

2.3.2 Sources of seed and varietal preferences by farmers

During the focus group discussions, the farmers reported that availability of good quality seed is important and directly contributes to yield. The farmers have several ways in which they source their common bean seed for planting. About 70% of the farmers mentioned that their main source of seed is retained seed. Those who are not self-sufficient in seed source it from their neighbouring farmers (8%), the local market (19%), the seed traders (seed merchants) (2%) and the Ministry of Agriculture (MoA) (1%) (Figure 2.5).

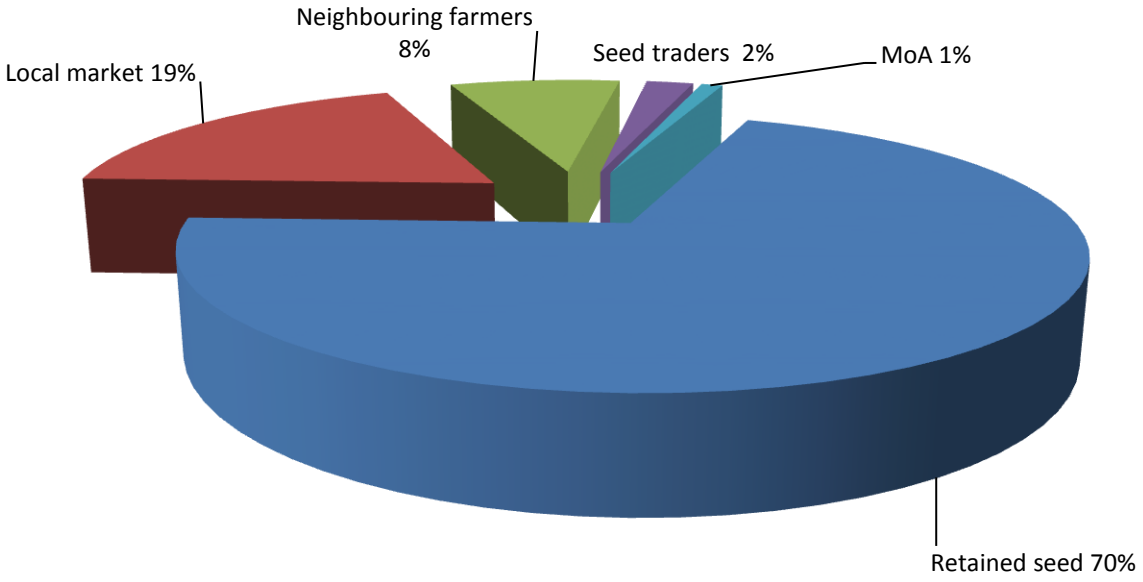

Figure 2.5: Sources of common bean seed

The varieties of common bean grown by farmers in Thika and Kiambu are different. The farmers in Thika grow a range of common bean varieties which include GLP X92 (Mwitemania), GLP 2 (Rosecoco), GLP 585 (Wairimu), GLP 1127 (Mwezi moja), KAT X56 and KAT X69. In Kiambu, farmers grow GLP 2, GLP 585, GLP 24 (Canadian wonder/Gituru), GLP X92, 'Mukura na oke', 'Kiboland' and 'Gikaara'. The farmers gave various reasons for their varietal preferences which include, seed size, shape, colour, marketability, cooking time and taste, early maturity and tolerance to disease and insect-pests (Table 2.2).

Table 2.2: Common bean varieties under production and reasons for preference

Common bean variety	Seed type	Reasons for preference
GLP 585 Wairimu	Small red haricot	High yield, early maturity, drought tolerant, short cooking time, good colour to food, used in githeri (traditional meal of maize and beans)
GLP 2 Rosecoco/Nyayo/Saitoti	Large red/purple mottle calima	Highly marketable, good taste in food, tolerant to disease
GLP 1127 Mwezimoja	Medium purple speckled	Early maturity, high yield
GLP X92 Mwitemania,	Attractive large pinto	Early maturing, preferred by consumers, drought tolerant
Kat X56	Red kidney	High yield, tolerant to disease, good taste
Kat X69	Very large red calima	High yield, tolerant to disease, good taste and food colour
GLP 24 Canadian wonder (Gituru)	Large dark red kidney.	High yield, large seed, gives food good colour
Mukura na oke	Large yellow and black stripes	Early maturing, short cooking time, tolerant to disease and insect pests
Gikaara	Large white and black stripes	Large seed, mixed in stews, tolerant to disease and insect pests
Kiboland	White with black stripes	Short cooking time and it is floury (not mashy), sweet when eaten green

2.3.3 Common bean production constraints experienced by the farmers

The farmers reported several biotic and abiotic constraints that hinder high yield during production (Figure 2.6). The abiotic stresses include low rainfall (29%), excessive rainfall (12%), low soil fertility (8%) and high cost of inputs (10%). The most important constraints mentioned are biotic stresses that lead to crop failure once the common beans are infected. The biotic constraints included diseases (94%) and insect-pests (92%). The biotic constraints are more important than the abiotic constraints.

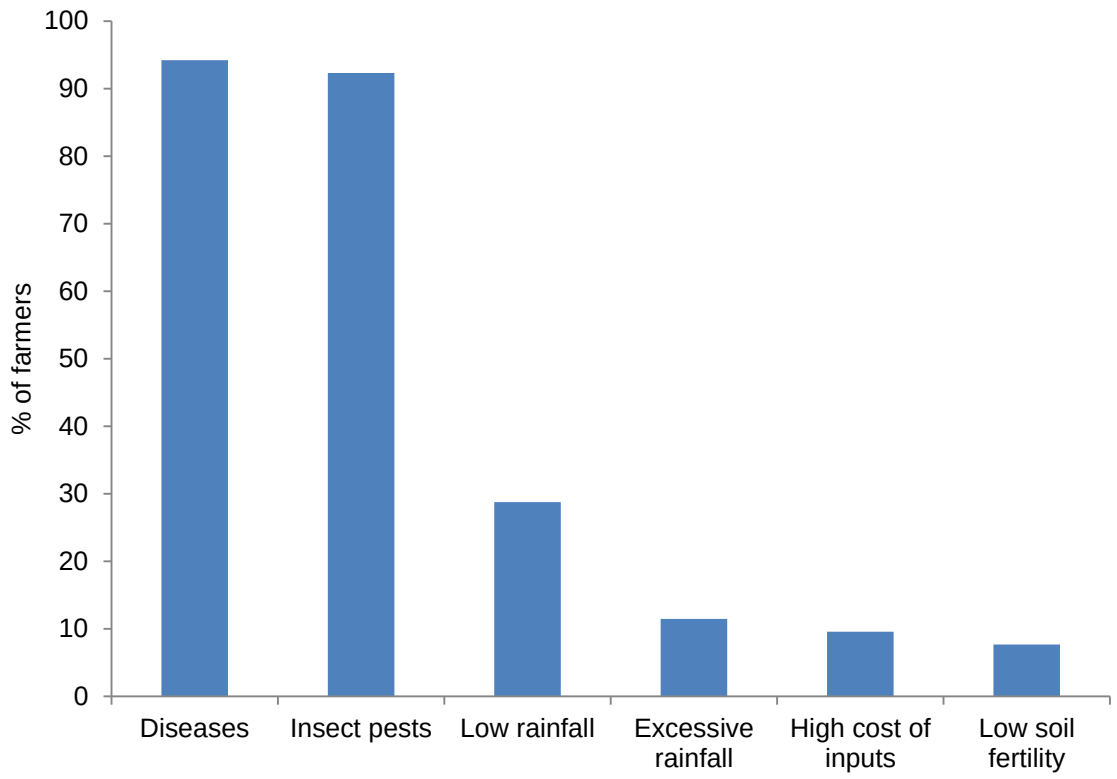

Figure 2.6: Constraints to common bean production

The farmers were asked to identify insect-pests and diseases during field visits in the short rains season and also from identification charts provided. The insect-pests identified include black aphids (80%), whiteflies (60%), bollworms (29%), and leafminers (6%) (Figures 2.7 and 2.8).

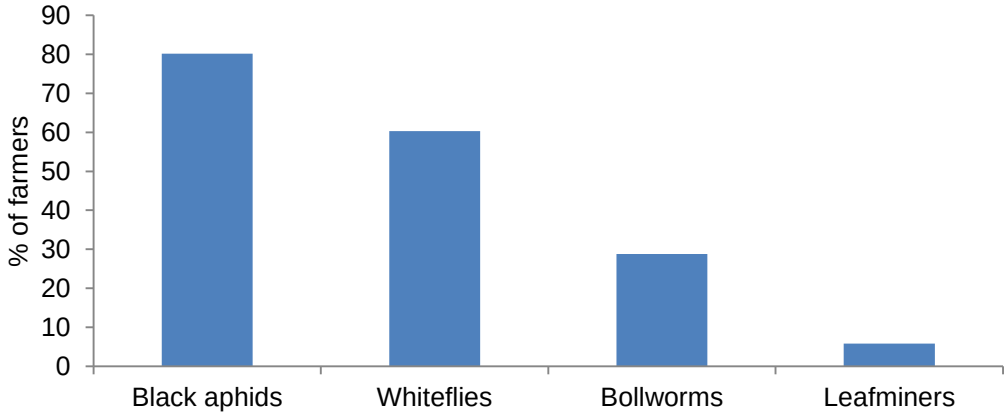

Figure 2.7: Insect-pests affecting common bean production

Figure 2.8: Common bean leaf showing black aphid infestation

The main diseases identified are angular leaf spot (71%), ascochyta blight (46%), powdery mildew (23%), bean common mosaic virus (BCMV) (8%), and rust (6%) (Figures 2.9, 2.10 and 2.11). Farmers do not know the names of these diseases and could only give a description of the symptoms.

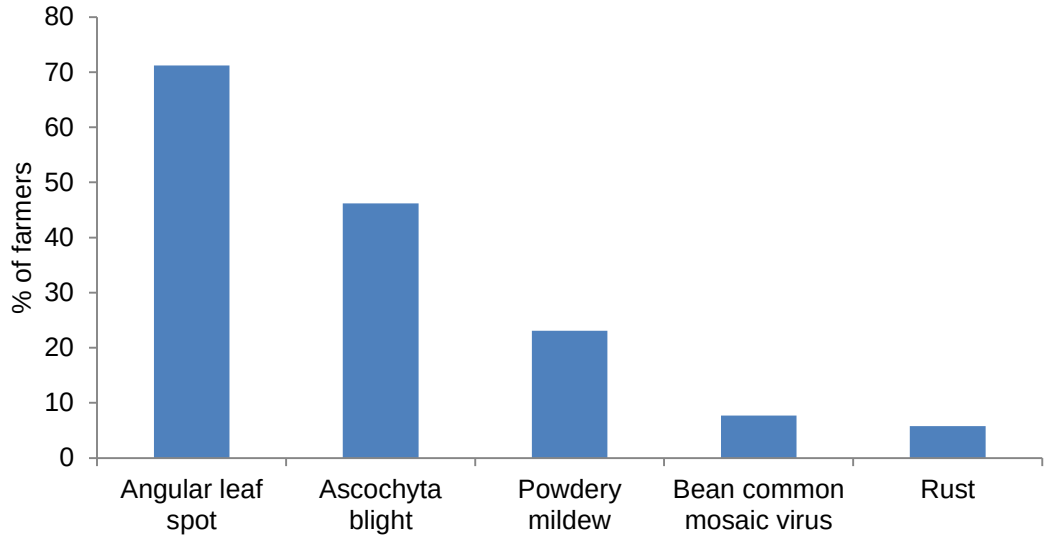

Figure 2.9: Major common bean diseases affecting common bean production

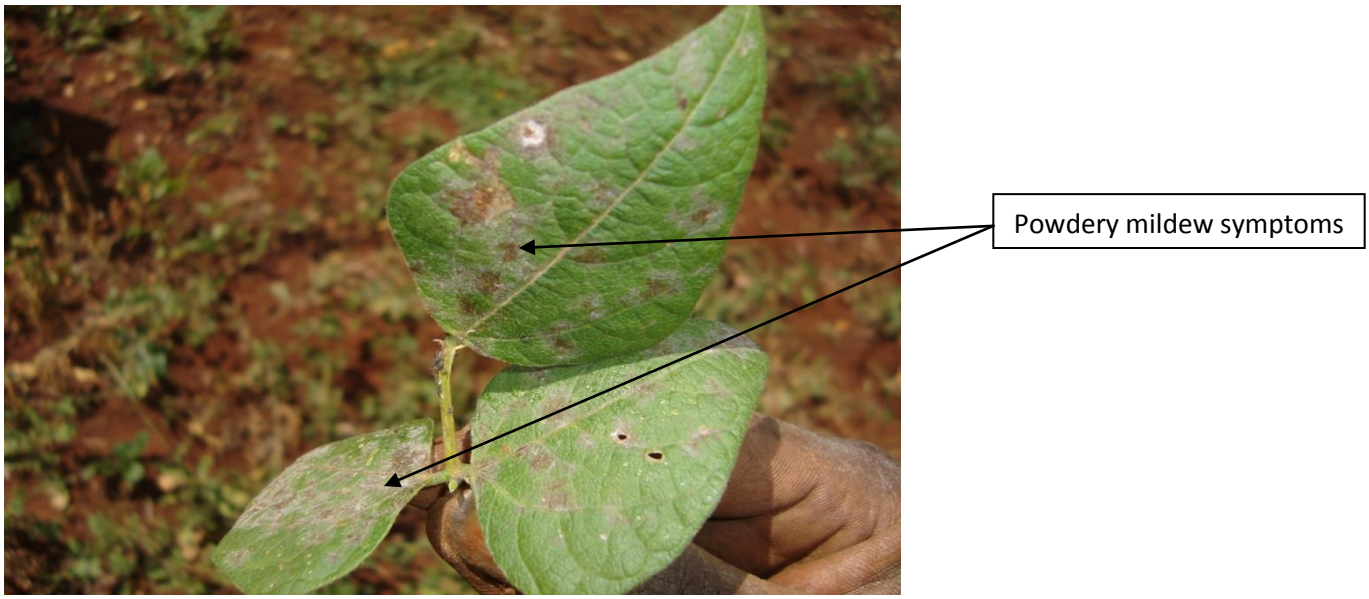

Figure 2.10: Powdery mildew symptoms on common bean leaf on farmer's field

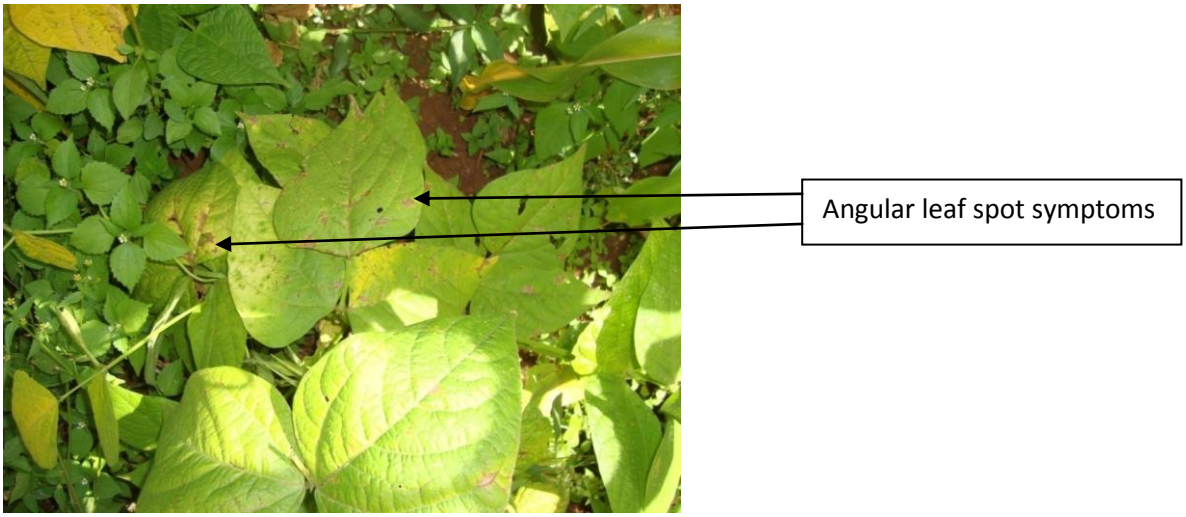

Figure 2.11: Common bean showing angular leaf spot symptoms on a farmer's field

2.3.4 Farmer preferred common bean traits for improvement during breeding

The farmers have preferences on the kind of trait improvement they would like incorporated in new common bean varieties. The preferred traits include high yielding ability (80%), resistance to insect-pests and diseases (72%), early maturity (68%), type I growth habit (52%), seed colour (red, red mottled, cream speckled) and size (medium/large) (21%), and short cooking time (20%) (Figure 2.12).

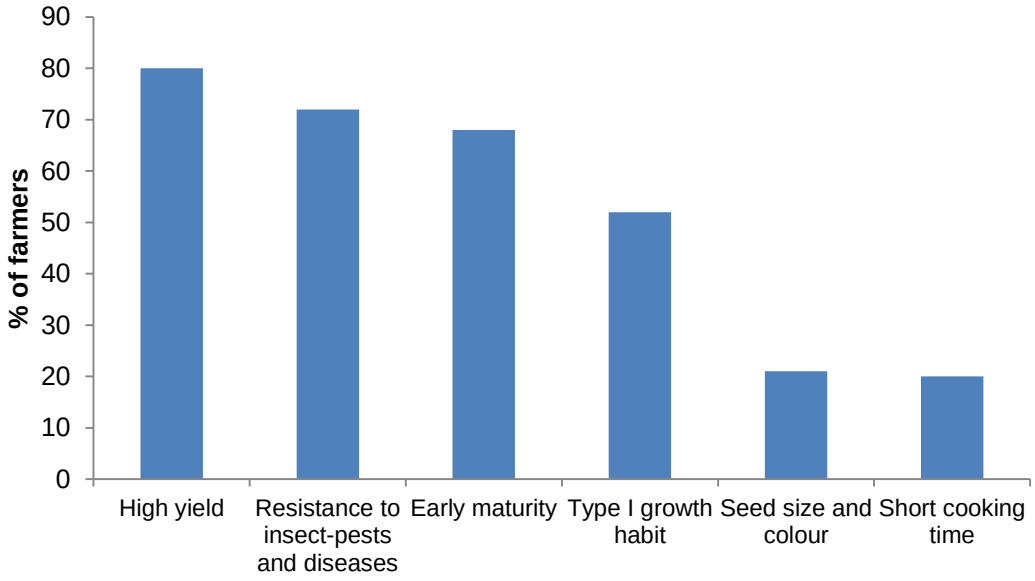

Figure 2.12: Farmer preferred common bean traits for improvement during breeding

2.4 Discussion and conclusion

Farmers are knowledgeable about the constraints of production and have their own ways of managing the diseases either by early planting, planting in different cropping systems, planting seed mixtures and good agronomic practices such as weeding. The aim of the study was to identify the common bean cropping systems, constraints and farmer preferred varietal traits.

The common bean farmers in Kiambu county obtain better common bean yields during the short rains. This could be attributed to a more even distribution of rainfall during the short rains as compared to the long rains, where heavy downpours occur in the first two months followed by a dry spell and cold weather. Due to the need for food, the farmers plant the common bean in both rainy seasons. Katungi et al. (2009), in a survey of common bean production in Kenya reported that farmers in central province grow common bean in both rainy seasons, while in western and rift valley production is only in the long rain season. The farmers plant before the onset of the rains and practice weeding before flowering and after pod set to ensure that the flowering stage is not disrupted. After harvest and threshing, the farmers do not entirely clean the common bean seed, which helps to protect the seed from damage by storage insects. The monocropping of common bean is done on a small scale for purposes of crop rotation. Crop rotation is not practiced widely due to the small size of the farms which predisposes the crop to diseases. The main reasons farmers gave for intercropping with bananas, coffee and macadamia was to maximise on the space since the fruit trees are widely spaced. Intercropping with maize, potatoes and leafy vegetables helps the farmers spread the risk in case of one crop failure. Intercropping has been reported to contribute to weed control, reduction of the spread of pathogens, and is an effective use of land area (Graham and Ranalli, 1997).

The farmers' main source of seed is retained seed. They select the best seed after harvest, remove off-types and ensure the seed is free from insect-pest damage. Farmers also source seed from their neighbours who have a superior variety. After a poor harvest farmers utilise all their seed for home consumption, and source seed from the local market. Some farmers receive small quantities of seed of a new variety on trial from the MoA extension staff. The farmer groups multiply the seed and later share amongst themselves. The only time the farmers buy seed from the seed companies is when there is a new variety release. They purchase the new variety as a group, select one farm and plant the seed as a pure stand for multiplication purposes, after which they share the seed for production on individual farms. It has been shown that farmers' seed and the informal seed sources are the major sources for planting seed (Almekinders and Louwaars, 2002). The formal seed sector in developing countries has not

contributed fully to the development of small scale farming (Lipton and Longhurst, 1989; Thiele, 1999; Tripp, 2001).

Farmers produced a range of common bean varieties, mostly improved varieties (GLP and KAT series). In Thika distict, the farmers grow the KAT series and GLP 1127 which are better adapted to hot and drier areas. The farmers in Kiambu still grow landraces because they are early maturing and resilient to biotic and abiotic stresses. They plant the landraces in small sections of the land because, even in adverse weather conditions, the landraces still give a stable yield. The seed colours of the landraces are different from those of improved varieties. Most of the improved varieties have a red colour, while the landraces have the characteristic zebra stripes. 'Kiboland is specifically preferred for its floury nature and hence not mashy when prepared in stews. It is also eaten at the mature soft seed stage for its good taste. Landraces are adapted to their local conditions and are therefore able to tolerate disease and adverse weather conditions (Zeven, 1998; Stoilova et al., 2005).

The farmers experience a range of production constraints caused by both abiotic and biotic stresses. During the long rains, the excessive rainfall leads to reduced yield due to flower abortion. On the other hand, low rainfall leads to reduced moisture available for the crops and hence low yield. Continuous cropping on the same piece of land without rotating the crops has resulted in low soil fertility. Due to the high cost of inputs, farmers do not use fertilizer or pesticides, which lead to less vigorous crops that become vulnerable to both abiotic and biotic stresses.

Several insect-pests affect yield especially during the short rains. The black aphids were noted as the major insect-pest that causes the leaves to roll and eventually fall under heavy infestation. Diseases are more severe during the long rains most importantly angular leaf spot. The farmers do not use any agro-chemicals for the control of the disease. This, coupled with lack of crop rotation and continued use of farmers' retained seed, predisposes the crop to several diseases. It has been reported that in the tropical areas, the warm, humid environment is conducive for pathogen development, and planting of two to three crop cycles per year allows for continuity of the inoculum (Graham and Ranalli, 1997). Mukankusi (2008) showed that small scale farmers in Uganda are faced with similar abiotic and biotic constraints during common bean production, while in Kenya the farmers in eastern province (Ojwang' et al., 2009) and western and rift valley (Katungi et al., 2009) have similar constraints.

The farmers have several preferred traits for improvement in common bean. High yielding varieties are preferred by farmers so that they can sell the surplus to generate income. Varieties resistant to insect-pests and diseases would benefit the farmers since they do not use pesticides. The resistant varieties would also result in high yields. Varieties with greater tolerance to abiotic and biotic stress can help farmers to produce more stable common bean yields under unfavourable conditions (Miklas et al., 2006). Early maturing varieties are an answer to food insecurity, since the farmers could have three or more crop cycles per year. In addition, early maturing varities escape drought, insect-pests and diseases and hence higher yields could be expected. The farmers preferred the type I growth habit (determinate) for intercropping. The type I growth habit is preferred in central and eastern parts of Kenya, while the type IV growth habit (indeterminate climbing vine) is more common in Nyanza (Wachenje, 2002; Gichangi et al., 2012). The farmers preferred common bean seed types such as red kidney, large red mottled, large red calima, and the small red haricot. They explained that these seed types are more marketable, have a short cooking time, and give a good colour to the food. A fast to cook common bean variety would save the farmers on fuel costs. Farmers in other parts of the country also consider traits such as seed size, seed colour and cooking ability, to be important (Katungi et al., 2009).

In conclusion, the involvement of farmers through surveys and focus group discussions has given valuable insights into local common bean production systems, production constraints, and varietal preferences. Farmers prefer common bean varieties with resistance to disease, high yield, early maturity, large and medium seed type, with red, red mottled, cream speckled seed colour and a short cooking time. The information obtained from farmers is of value to breeding programmes for smallholder farmers in Kenya.

References

Almekinders, C., and N.P. Louwaars. 2002. The importance of the farmers' seed system in a functional national seed sector, p. 15-33, *In* N. P. Louwaars, ed. Seed policy, legislation and law: widening a narrow focus. Food Products Press, New York, USA.

Asfaw, A., C.J.M. Almekinders, M.W. Blair, and P.C. Struik. 2012. Participatory approach in common bean (*Phaseolus vulgaris* L.) breeding for drought tolerance for southern Ethiopia. Plant Breeding 131:125-134.

Biggs, S.D. 1989. Resource-poor farmer participation in research: A synthesis of experiences from nine national agricultural research systems. OFCOR Comparative Study Paper No. 3, The Hague: International Service for National Agricultural Research, The Netherlands.

Ceccarelli, S., and S. Grando. 2007. Decentralized-participatory plant breeding: An example of demand driven research. Euphytica 155:349-360.

Ceccarelli, S., S. Grando, E. Bailey, A. Amri, M. El-Felah, F. Nassif, S. Rezgui, and A. Yahyaoui. 2001. Farmer participation in barley breeding in Syria, Morocco and Tunisia. Euphytica 122:521-536.

Ceccarelli, S., S. Grando, M. Singh, M. Michael, A. Shikho, M. Al Issa, A. Al Saleh, G. Kaleonjy, S.M. Al Ghanem, A.L. Al Hasan, H. Dalla, S. Basha, and T. Basha. 2003. A methodological study on participatory barley breeding II. Response to selection. Euphytica 133:185-200.

Courtois, B., B. Bartholome, D. Chaudhary, G. McLaren, C.H. Misra, N.P. Mandal, S. Pandey, T. Paris, C. Piggin, K. Prasad, A.T. Roy, R.K. Sahu, V.N. Sahu, S. Sarkarung, S.K. Sharma, A. Singh, H.N. Singh, O.N. Singh, N.K. Singh, R.K. Singh, S. Singh, P.K. Sinha, B.V.S. Sisodia, and R. Takhur. 2001. Comparing farmers and breeders rankings in varietal selection for low-input environments: A case study of rainfed rice in eastern India. Euphytica 122:537-550.

Danial, D., J. Parlevliet, C. Almekinders, and G. Thiele. 2007. Farmers' participation and breeding for durable disease resistance in the Andean region. Euphytica 153:385-396.

Fufa, F., S. Grando, O. Kafawin, Y. Shakhatreh, and S. Ceccarelli. 2010. Efficiency of farmers' selection in a participatory barley breeding programme in Jordan. Plant Breeding 129:156-161.

Gichangi, A., S.N. Maobe, D. Karanja, A. Getabu, C.N. Macharia, J.O. Ogecha, M.K. Nyang'au, E. Basweti, and L. Kitonga. 2012. Assessment of production and marketing of climbing beans by smallholder farmers in Nyanza region, Kenya. World Journal of Agricultural Sciences 8:293-302.

Graham, P.H., and P. Ranalli. 1997. Common bean (*Phaseolus vulgaris* L.). Field Crops Research 53:131-146.

Katungi, E., A. Farrow, J. Chianu, l. Sperling, and S. Beebe. 2009. Common bean in Eastern and Southern Africa: A situation and outlook analysis, CIAT, Cali, Colombia.

Lipton, M., and R. Longhurst. 1989. New seeds and poor people. Unwin and Hyman, London, UK.

Miklas, P.N., J.D. Kelly, S.E. Beebe, and M. Blair. 2006. Common bean breeding for the resistance against biotic and abiotic stresses: From classical to marker assisted selection breeding. Euphytica 147:105-131.

MOA. 2013. Economic Review of Agriculture. CPPMU, Nairobi, Kenya.

Morris, M.L., and M.R. Bellon. 2004. Participatory plant breeding research: Opportunities and challenges for the international crop improvement system. Euphytica 136:21-34.

Mukankusi, C.M. 2008. Improving resistance to fusarium root rot [*Fusarium solani* (Mart.) Sacc. f. sp. *phaseoli* (Burkholder) W.C. Snyder & H.N. Hans.] in common bean (*Phaseolus vulgaris* L.). PhD Thesis, University of KwaZulu-Natal, Pietermaritzburg, South Africa.

Ojwang', P.P.O., R. Melis, J.M. Songa, M. Githiri, and C. Bett. 2009. Participatory plant breeding approach for host plant resistance to bean fly in common bean under semi-arid Kenya conditions. Euphytica 170:383-393.

Sperling, L., M.E. Loevinsohn, and B. Ntabomvura. 1993. Rethinking the farmers' role in plant breeding: Local bean experts and on-station selection in Rwanda. Experimental Agriculture 29:509-519.

Sperling, L., J.A. Ashby, M.E. Smith, E. Weltzien, and S.M. Guire. 2001. A framework for analysing participatory plant breeding approaches and results. Euphytica 122:106-119.

SPSS Inc. 2006. Statistical package for the social sciences for Windows. Release 15.0. SPSS Inc., Chicago, IL, USA.

Sthapit, B.R., K.D. Joshi, and J.R. Witcombe. 1996. Farmers participatory crop improvement. III. Participatory plant breeding, a case study for rice in Nepal. Experimental Agriculture 32:479-496.

Stoilova, T., G. Pereira, M.M.T. de Sousa, and V. Carnide. 2005. Diversity in common bean landraces (*Phaseolus vulgaris L.)* from Bulgaria and Portugal. Journal of Central European Agriculture 6:443-448.

Thiele, G. 1999. Informal potato seed systems in the Andes: Why are they important and what should we do with them. World Development 27:83-99.

Tripp, R. 2001. Seed provision and agricultural development. ODI, London, UK.

Wachenje, C.W. 2002. Bean production constraints, bean seed quality and effect of intercropping on floury leaf spot disease and yields in Taita Taveta district, Kenya. M.Sc. Thesis, University of Nairobi.

Were, V.W. 2011. Cassava breeding through complementary, conventional and participatory approaches in Western Kenya. PhD Thesis, University of KwaZulu-Natal, Pietermaritzburg, South Africa.

Williams, R., R. Andersen, A. Marcial, L. Pereira, L. Almeida, and W. Erskine. 2012. Exploratory agronomy within participatory variety selection: The case of peanut in East Timor. Experimental Agriculture 48:272-282.

Zeven, A.C. 1998. Landraces: A review of definitions and classifications. Euphytica 104:127-139.

Appendix 2.1: Semi-structure questionnaire used during the survey

COMMON BEAN RESEARCH

Farm Production

1. Basic data

Date form filled (dd/mm/yy) PINT ☐☐ ☐☐ ☐☐☐☐

Name of enumerator ……..

Form filled at: VSITE 1. Kiambu ☐ 2. Thika ☐

Name of district _____

Name of location _____

Name of village _____

GPS coordinates _____

2. Education and occupation of the respondent

Respondent name _____ Age PAGE ☐☐

Sex of respondent PSEX ☐ 1male ☐ 2 female

Respondent's main occupation POCC ☐ 1 Farmer ☐ 2 Farm labour service ☐ 3 Non farm service ☐ 4 Business

Number of years experience in independent farming PEXP ☐☐

Is the respondent organized in any farmer's groups? PGR 0 No 1 Yes

3. Land details (indicate units)

Land owned and cultivated LOWN ☐☐☐

Land Leased-in LREN ☐☐

Total cultivation area LCUL ☐☐

4. **Production and Cropping system**

Which is your major crop of production? Rank in order of importance

1 - Most important
2 - Important
3 - Moderately important
4 - Least important

Crop of importance	**Rank**	**Area under production** (indicate units)	**Use** 1. Sale 2. Home consumption

If common bean, why? _____

_____

Do you practice crop rotation on the areas under common bean production?

☐1Yes ☐ 0 No

How do you cultivate the common bean, 1 Pure stand ☐ 2 Intercrop ☐
Reasons for how crop is cultivated? _____

_____

_____

Production			
	Varieties of common bean cultivated	Source of seed	Yield (units)
i)			
ii)			
iii)			
iv)			
v)			
vi)			

5. Problems you encounter in common bean production PCON

What are the major constraints to production of common bean in your farm?

i)

ii)

iii)

iv)

6. Common bean insect-pests and diseases on your farm

Insect pest / disease Farmer description	Variety affected	Symptoms	Part of common bean attacked	Stage of growth	Season when most damage occurs	Severity 1 High 2moderate 3 low	Control measures
i)							
ii)							
iii)							
iv)							

7. Varietal preferences, adoption, and development

What varieties of common bean did you grow when you started common bean production?

Which varieties of common bean are you currently growing?

Have you ever grown improved varieties of common bean? 1Yes ☐ 0 No ☐

If No, give reasons _____

_____

Where do you obtain information on improved bean varieties? VINFO

☐ 1 Friends/neighbours
☐ 2 extension staff
☐ 3 farmers association
☐ 4 Other (specify)

a) Local varieties grown

Local variety in order of preference	Year first grown	Reasons for Preference	If stopped, year and why
1 -			
2 -			
3 -			
4 -			
5 -			

b) Improved varieties grown

Improved variety in order of preference	Year first grown	Reasons for Preference	If stopped, year and why
1 -			
2 -			
3 -			
4 -			
5 -			

1 – most preferred and 5 – least preferred

Where do you access the improved common bean varieties

☐ 1 KARI
☐ 2 Friends/neighbours
☐ 3 farmers association
☐ 4 extension staff
☐ 5 Seed company
☐ 6 NGO
☐ 7 Other (specify)

In future, would you like to grow improved common bean varieties?

☐ 1 Yes ☐ 0 No

What traits would you like to see improved in the common bean varieties that you are currently growing?

	Variety grown	Improvement
i)		
ii)		
iii)		
iv)		
v)		
vi)		
vii		

Have you ever been involved in development of new common bean varieties?

1 Yes☐ 0 No ☐

If yes, how were you involved and through which organization?

_____

8. Seed purchase

Are you self sufficient in dry seed?

1. Deficit
2. Self sufficient
3. Have market surplus

Do you purchase your improved common bean seed every year?

☐ 1 Yes ☐ 0 No

Reasons _____

_____

If you select your own seed for each season, fill table below

Table 8a.

Variety	When last purchased or given/ source	Time of Seed selection (**use code below**)	Characteristics Considered in selection

☐ 1 Pre-harvest ☐ 3 Both

☐ 2 Post-harvest ☐ 4 No selection

If you purchase seed for every growing season, fill table below

Table 8b.

Variety grown	Source of seed	Availability	Price (specify..per kilo..)

9. Common bean sales and income

Variety	Season harvested 1-Long Rain 2-Short Rain	Yield + unit	Quantity For Home consumption	Quantity sold	Month of sale	Sales price	Place of sale	Sold to whom

What problems do you face during common bean marketing?

_____

CHAPTER 3

Evaluation of local landraces and selected introductions of common bean for yield performance and resistance to angular leaf spot

Abstract

Breeding programmes for resistance to angular leaf spot (ALS) in common bean have relied on a range of sources of resistance. However, due to the occurrence of many different races of the pathogen in Kenya and other regions, the sources of resistance may not always be effective in all the regions. This study was conducted to identify new sources of resistance to ALS. A total of 200 common bean genotypes were evaluated for yield and ALS disease resistance in two locations; Kabete Field Station and KARI-Tigoni using an alpha-lattice (25 x 8) design. Disease evaluation was carried out through inoculation in the field in KARI-Tigoni and in the nethouse at Kabete. The genotypes were also evaluated for seed yield at two locations, Kabete Field Station and KARI-Thika during two seasons, short rains (2011) and long rains (2012) using an alpha lattice (25 x 8) design. The results showed that the response of the genotypes to ALS in the field in KARI-Tigoni and in the Kabete nethouse was similar. Most of the genotypes were susceptible to ALS. One Kenyan landrace, GBK 028123 and two Rwandan landraces, Minoire and Murangazi showed resistance in the field and in the nethouse. Such genotypes could be used either directly as varieties or in breeding programmes. On average, 22-32% of the Kenyan landraces showed intermediate resistance when evaluated in the Kabete nethouse and KARI-Tigoni field. These intermediate resistant landraces can be used in breeding programmes to develop durable resistant varieties. The medium seeded common bean genotypes had a lower percentage ALS susceptible plants (20%) compared to the large seeded genotypes (64%), though the correlation was non-significant. In the yield trials across two locations, Kabete Field Station and KARI-Thika, the top five high yielding genotypes were GLP 2 (766 kg ha^{-1}), Nyirakanyobure (660 kg ha^{-1}), GBK 028110 (654 kg ha^{-1}), GLP 585 (630 kg ha^{-1}) and Mukwararaye (630 kg ha^{-1}). These genotypes can be recommended for production across the two locations.

3.1 Introduction

The common bean (*Phaseolus vulgaris* L.) is an important food legume in Kenya. However, low yields are realised, which can be attributed to several biotic and abiotic constraints. Among the biotic stresses, angular leaf spot (ALS), caused by *Pseudocercospora griseola* (Sacc.) Crous & Braun, has been reported to cause major yield losses in Africa (Wortmann et al., 1998) and is prevalent in Kenya (Wagara et al., 2004; Mwang'ombe et al., 2007).

The control of ALS can be achieved through various technologies including integrated pest management (IPM), use of resistant varieties and application of fungicides (Liebenberg and Pretorius, 1997). However, integrated pest management is complex, while fungicides are expensive for small scale farmers in Kenya. The use of resistant varieties is the most economical and efficient strategy for reducing the losses caused by the disease. Several sources of resistance have been identified in various parts of the world through germplasm screening and they include: G10909 (Mahuku et al., 2011), G5686 (Mahuku et al., 2009), G10474 (Mahuku et al., 2004), Mexico 54, BAT 332 (Caixeta et al., 2003; Namayanja et al., 2006), MAR-2 (Ferreira et al., 2000), AND 277 (Carvalho et al., 1998; Aggarwal et al., 2004; Caixeta et al., 2005) and CAL 143 (Aggarwal et al., 2004). However, some of the resistant sources are only resistant in some locations or against specific races. For example, CAL 143 was only resistant in Tanzania, South Africa and Zambia, but susceptible in Uganda. AND 277 was resistant only in Malawi and South Africa (Aggarwal et al., 2004) as well as Brazil against eight races only (Caixeta et al., 2005). It has been shown that resistance can easily break down when a new race of the pathogen appears in a different geographical region or through a mutation of an existing race. This was evident when the maize rust (*Puccinia polysora*) could not infect maize beyond the tropics of Cancer and Capricorn, but caused major epidemics in Africa (Robinson, 1987)

In Kenya, the common bean landraces are an important genetic resource maintained by farmers. Some of the important characteristics of landraces include adaptation to local climatic conditions and cultural practices, yield stability and tolerance to diseases and insect-pests (Zeven, 1997; Zeven, 1998; Stoilova et al., 2005). It is essential therefore to exploit the genetic diversity that characterise the landraces. Landraces have been selected by local farmers and are often well adapted to local conditions. The objective of this study was to evaluate common bean landraces and introductions in order to identify genotypes with high yield and/or resistance to ALS.

3.2 Materials and Methods

3.2.1 Study sites

Disease evaluation at Kabete nethouse

The study was conducted at Kabete Field Station of the University of Nairobi. Kabete is located at coordinates, 01°14'59.7"S; 036°44'28.8"E at an altitude of 1820 m above sea level (masl). The area receives an average rainfall of 1046 mm annually, and has mean maximum temperature of 23°C and mean minimum temperature of 12°C. The soils are dark red or brown friable clay.

Disease and yield evaluation at KARI-Tigoni

The experiment was conducted in the field at KARI-Tigoni located at coordinates 01° 09' 7.22"S; 036°41'8.72"E, at an altitude of 2051 masl. Tigoni receives an average rainfall of 1100 mm annually and has a mean maximum temperature of 24°C and a mean minimum temperature of 12°C. The soils are humic nitosols.

Yield evaluation at KARI-Thika and Kabete Field Station

These experiments were initially conducted to evaluate the genotypes for both yield and ALS and the experiments were inoculated with *Pseudocercospora griseola* (*P. griseola*). However, due to the dry weather conditions the infestation levels were too low and hence the experiments were mainly for yield performance of the genotypes.

The field experiments were carried out at two sites during two production seasons. The first site was at KARI-Thika located at coordinates 00° 59'18.4"S; 037°05'06.9"E, at an altitude of 1548 masl. Thika receives a mean annual rainfall of 900 mm, and has a mean maximum temperature of 25°C and mean minimum temperature of 14°C. The soils are verto-luvic phaeozems. The second site was at Kabete Field Station of the University of Nairobi. The studies at the two sites were carried out concurrently during the 2011 short rains and 2012 long rains.

3.2.2 Plant materials

A total of 200 genotypes were used in the experiments. These included 157 Kenyan landraces sourced from the National Gene Bank of Kenya (the landraces had originally been collected from Kiambu and Thika counties where ALS is prevalent), 23 Rwandan landraces sourced from the East and Central Africa Bean Research Network (ECABREN) (Rwandan landraces were

used for their tolerance to diseases in Kenya (Kimani, P.M., *personal communication*[2]), 12 market class varieties from the Kenya Agricultural Research Institute (KARI), which served as checks and four ALS resistant and four ALS susceptible cultivars from ECABREN (Table 3.1).

Table 3.1: Source and seed size of 200 common bean genotypes used in this study

Nursery	Source	Seed size			
		Large	Medium	Small	Total
Kenya landraces	Genebank Kenya	112	24	21	157
Rwandan landraces	ECABREN	7	6	10	23
ALS resistant cultivars	ECABREN	1	2	1	4
ALS susceptible cultivars	ECABREN	1	3	0	4
Market class varieties	KARI	4	7	1	12

Market class varieties: Improved varieties
Seed size = 100-seed weight $^{-1}$; Large = > 40 g, medium = 25-40 g and small = < 25 g

3.2.3. Pathogen isolation and inoculum preparation

Pseudocercospora griseola (*P. griseola*), was isolated from infected common bean plants showing characteristic angular shaped spots on leaves that were collected from Kabete Field Station. Two types of media were used, namely agar-agar (20 g l^{-1} of sterile water) and the V8 medium (200 ml V8 juice, 20 g agar-agar, and 800 ml sterile water). A small piece of agar mounted on a sharp sterile needle (attached to a long handle) was used to lightly touch the lesions (abaxial side of leaf) in order for the spores to attach to the medium. The block of agar was then placed on a petri dish containing agar-agar medium. Four small blocks of agar were placed onto the medium and using a sterile wire loop, the conidia were spread evenly onto the media. The petri dishes were then incubated in a non-illuminated incubator at 22^{o}C for 14 days. They were then sub-cultured and placed under the same conditions for 10 days. To prepare the inoculum, sterile water was then poured onto the growing colonies, they were gently scraped and the suspension filtered through a double muslin cloth. The concentration of the inoculum was adjusted to 2.0x10^{4} conidia per ml.

[2] Kimani, P.M. Department of Crop Science, University of Nairobi, P.O Box 30197-00100, Nairobi, Kenya

3.2.4 Experimental procedures

At all sites (KARI-Tigoni, Kabete nethouse, Kabete Field Station and KARI-Thika), 200 genotypes were planted in experimental plots of 3 m long single rows of 20 plants each, 50 cm between rows and 15 cm between plants. The experiments were arranged in a 25 x 8 alpha-lattice design with three replications. The experiments were planted on the following dates: KARI-Tigoni, 3rd October 2012; Kabete nethouse, 4th October 2012; Kabete Field Station, 17th October 2011 (short rain) and 3rd April 2012 (long rain); KARI-Thika, 19th October 2011 (short rain) and 5th April 2012 (long rain).

At planting, fertiliser (diammonium phosphate, 80 kg ha $^{-1}$) was applied (at Kabete nethouse and KARI-Tigoni). In addition, chicken manure was applied to the soil at Kabete nethouse. For the yield evaluations at Kabete Field Station and KARI-Thika, fertiliser was not applied. Weeding at all sites was carried out three times: two weeks after seedling emergence, before flowering and after podding. The pesticide Confidor (200 g l^{-1} Imidacloprid) was used to control whiteflies and leafminer in the Kabete nethouse.

The genotypes were inoculated with the *P. griseola* at a concentration of $2.0x10^4$ conidia per ml (prepared as described in section 3.2.3) at the V3 stage of development (where first trifoliate leaf is open and the second trifoliate leaf appears). The first trifoliate leaf was inoculated on both sides of the leaf until runoff, using a hand sprayer. On symptom appearance (10-14 days after inoculation), data was collected four times at three day intervals. A random sample of four plants per replication per genotype was scored for ALS disease. The score on the last day was used for the analysis. The reaction to the disease was rated using a CIAT scale, with severity scores ranging between 1 and 9; 1-3 being resistant, 4-6 intermediate resistant and 7-9 susceptible (Table 3.2; Figure 3.1) (Van Schoonhoven and Pastor-Corrales, 1987). These disease severity scores were used to classify the genotypes as resistant, intermediate resistant or susceptible.

For the field experiment at KARI-Tigoni, Kabete Field Station and KARI-Thika, data were collected on seed yield (kg ha^{-1}) and seed size (100-seed $weight^{-1}$) per plot per replication.

Table 3.2: CIAT scale (1-9) for angular leaf spot disease severity

Score	Symptoms
1	No visible disease symptoms (0-<1%)
3	Presence of a few small non-sporulating lesions that cover approximately 2% of the leaf or pod surface area
5	Presence of several, generally small lesions with limited sporulation that cover approximately 5% of the leaf or pod surface area
7	Abundant and generally large sporulating lesions that cover approximately 10% of the leaf or pod surface area. On the foliage the lesions may coalesce to produce larger infected areas associated with chlorotic tissue. Lesions may also be found on the stem and branches
9	Twenty-five percent or more of the leaf or pod surface area is covered by large sporulating and often coalescing lesions. Leaf tissues are generally chlorotic resulting in severe premature defoliation. Infected pods are often deformed and shrivelled and contain a low number of seeds. Abundant sporulating lesions are present on stem and branches

Source: Van Schoonhoven and Pastor-Corrales (1987)

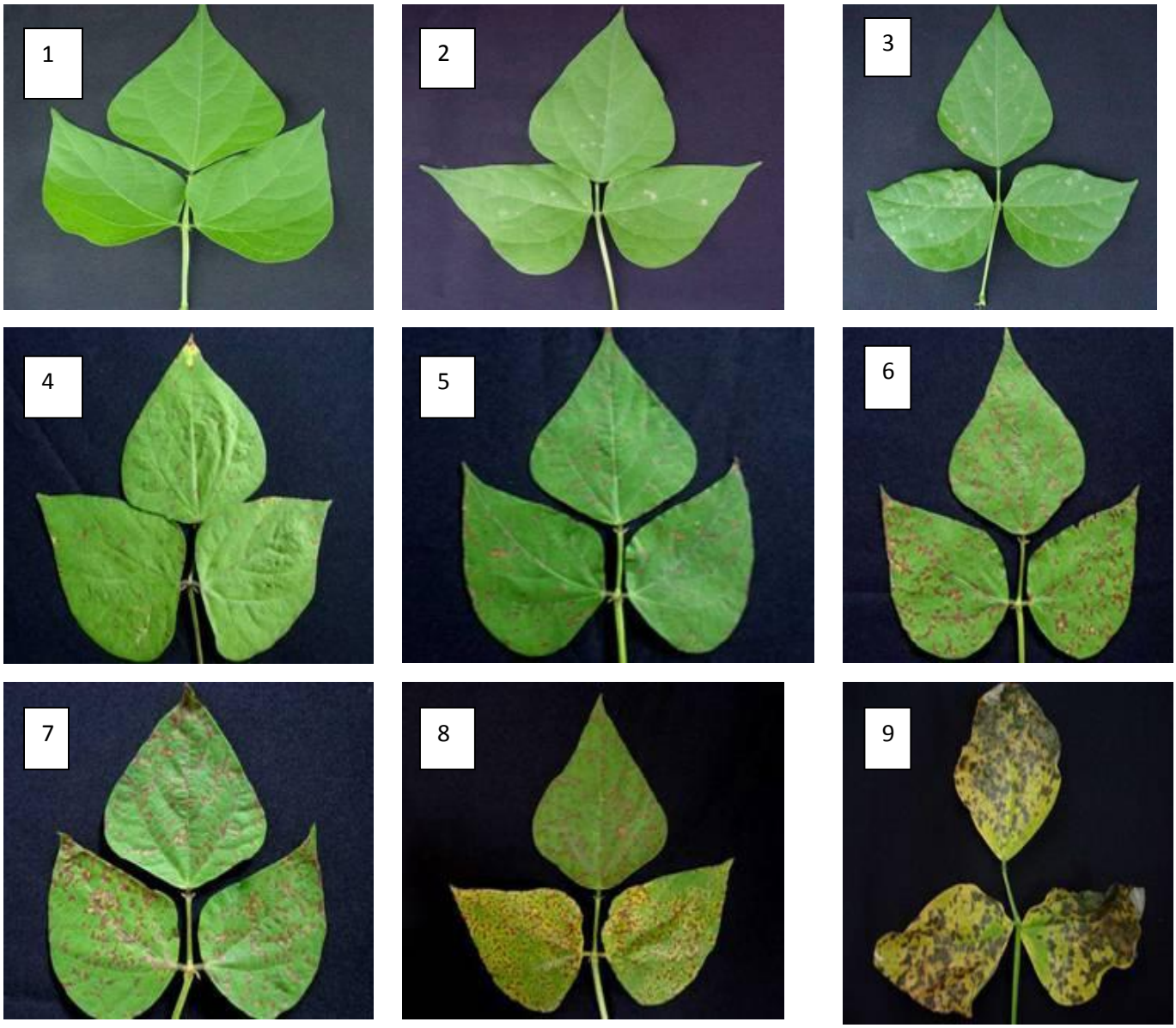

Figure 3.1: Pictorial presentations of angular leaf spot symptoms rating scale

3.2.5 Data analysis

Data for disease severity scores from Kabete nethouse and KARI-Tigoni as well as yield (KARI-Tigoni) were analysed using REML (restricted or residual maximum likelihood) in the Genstat 12th edition statistical package (Payne et al., 2009). The blocks within replications and replications were considered as random terms, while the genotypes were considered as the fixed terms. For the yield evaluation at KARI-Thika and Kabete Field Station, the replications and blocks were considered as random terms while the genotypes, season/year, location and their interactions were the fixed terms.

The model is as shown below;

$Y_{ijk} = \mu + \alpha_i + \rho_j + \beta_{jk} + \varepsilon_{ijk}$

where,

Y_{ijk} is the observation of line i in the k-th incomplete block within the j-th replicate

μ = the overall mean

α_i = the fixed effect of the i-th line

ρ_j = the effect of level j-th replicate

β_{jk} = the effect of the k-th incomplete block within the j-th replicate

$\varepsilon_{ijk\,i}$ = error associated with line i in the k-th incomplete block within the j-th replicate

3.3 Results

3.3.1 Rainfall and mean temperatures

Data on the mean temperature and total rainfall received during 2011 short rain and 2012 long rain seasons are presented in Table 3.3. Tigoni was cool with high rainfall; Thika was warm with low rainfall; while Kabete was intermediate for mean temperature and rainfall.

Table 3.3: Mean temperature and rainfall at Kabete, Thika and Tigoni

Month Year	Location					
	Kabete Field Station		KARI-Thika		KARI-Tigoni	
	Mean temp	Rainfall	Mean temp	Rainfall	Mean temp	Rainfall
2011						
August	17	27	19	10	-	-
September	19	33	20	39	-	-
October	19	154	21	135	-	-
November	19	176	21	182	-	-
December	19	246	21	63	-	-
2012						
January	19	0	20	0	-	-
February	20	16	21	21	-	-
March	20	5	22	0	19	49
April	20	352	22	249	17	690
May	19	262	20	185	17	375
June	18	40	22	38	14	521
July	17	23	18	8	14	26
August	17	42	23	41	16	100
September	18	9	21	20	23	113
October	19	242	21	51	24	413
November	19	262	22	177	22	248
December	19	245	21	168	22	292

Temperature ^{o}C and rainfall in mm

3.3.2 Angular leaf spot evaluation at Kabete nethouse

At Kabete nethouse the disease symptoms appeared between 11-14 days after inoculation. The analysis of variance of Wald tests statistics showed that the genotypes were significantly different (P<0.001) in their reaction to ALS (Table 3.4).

Table 3.4: Analysis of variance (Wald tests for fixed effects) for angular leaf spot severity scores for 200 common bean genotypes in Kabete nethouse

Sequentially adding terms to fixed model

Fixed term	Wald statistic	df	Wald/df	chi pr
Genotype	8166.79	199	41.04	<0.001

Dropping individual terms from full fixed model

Fixed term	Wald statistic	df	Wald/df	chi pr
Genotype	8166.79	199	41.04	<0.001

df degree of freedom, chi pr = chi probability

The 200 genotypes evaluated at the Kabete nethouse had a mean disease severity score of 6.8 (Appendix 3.1). Of the 200 genotypes evaluated, 4% had disease severity scores ranging between 1.0 and 3.0, 23% between 4.0 and 6.0, while 73% had disease severity scores ranging between 7.0 and 9.0 (Figure 3.2).

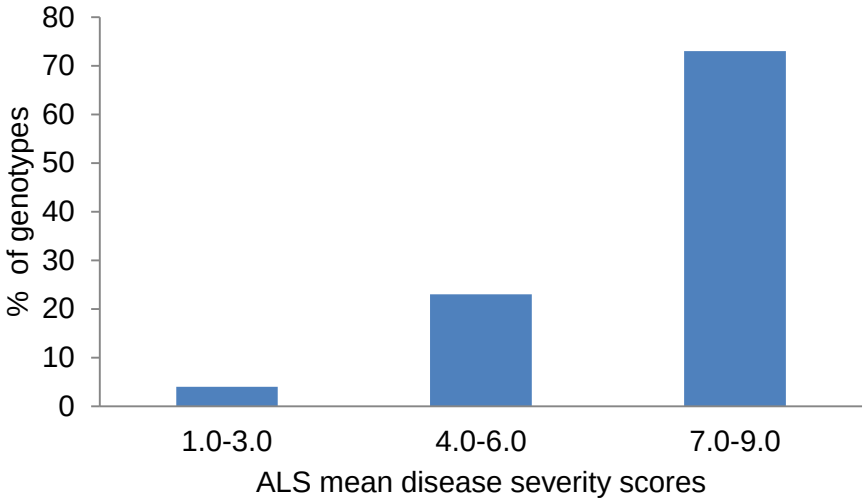

Figure 3.2: Reaction of 200 genotypes to angular leaf spot at Kabete nethouse

The ALS resistant checks Mexico 54, G10909 and MAR-2 had disease severity scores of 1.8, 2.2 and 2.8 respectively, while resistant check AND 277 had a disease score of 7.8. The susceptible check CAL 96, BRB 191 and MCM 5001 showed susceptibility with disease severity scores of 8.2, 7.8 and 6.8 respectively, while Kanyebwa showed intermediate resistance with a score of 4.8. Reaction of the landraces and market class varieties to ALS was varied (Figure 3.3). The mean disease severity score of the Kenyan landraces was 7.0. The Kenyan landraces GBK 028123 and GBK 052129 had low disease severity scores of 2.9, and 3.3 respectively. The Rwandan landraces had a mean disease severity score of 6.0. Rwandan landraces Minoire and Murangazi had low scores of 2.9, and 3.2 respectively. The market class varieties had a mean disease severity score of 7.1 whereby, GLP series, KAT series, Super-rosecoco and New-rosecoco had disease severity scores of between 7.0 and 9.0, apart from KAT 69 which had a score of 5.0.

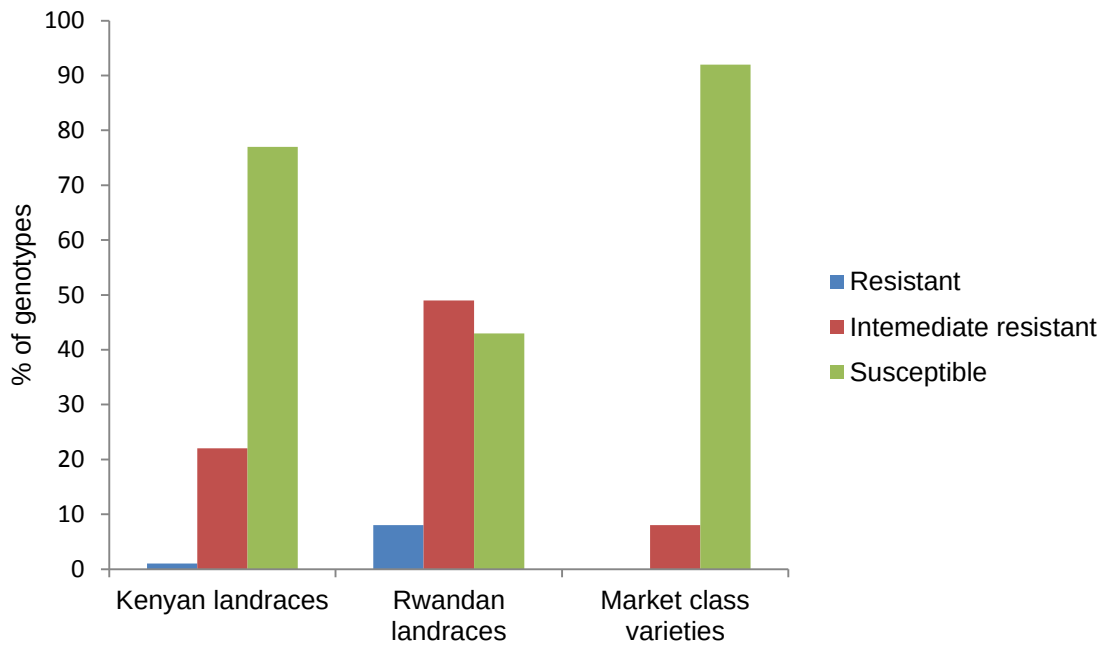

Figure 3.3: Reaction of landraces and market class varieties to angular leaf spot at Kabete nethouse

3.3.3 Angular leaf spot and yield evaluation at KARI-Tigoni

At the field in KARI-Tigoni, ALS disease symptoms appeared 10-14 days after inoculation. The analysis of variance of Wald tests statistics showed that the genotypes were highly significantly different (P<0.001) in their reaction to ALS (Table 3.5).

Table 3.5: Analysis of variance (Wald tests for fixed effects) for angular leaf spot severity scores for 200 common bean genotypes at KARI-Tigoni

Sequentially adding terms to fixed model				
Fixed term	Wald statistic	df	Wald/df	chi pr
Genotype	7049.41	199	35.42	<0.001
Dropping individual terms from full fixed model				
Fixed term	Wald statistic	df	Wald/df	chi pr
Genotype	7049.41	199	35.42	<0.001

df degree of freedom, chi pr = chi probability

The 200 genotypes evaluated at KARI-Tigoni had a mean disease severity score of 6.6 (Appendix 3.2). The genotypes had varied reaction to ALS. Of the 200 genotypes evaluated, 3% had disease severity scores ranging between 1.0 and 3.0, 31% between 4.0 and 6.0, while 66% had disease severity scores ranging between 7.0 and 9.0 (Figure 3.4).

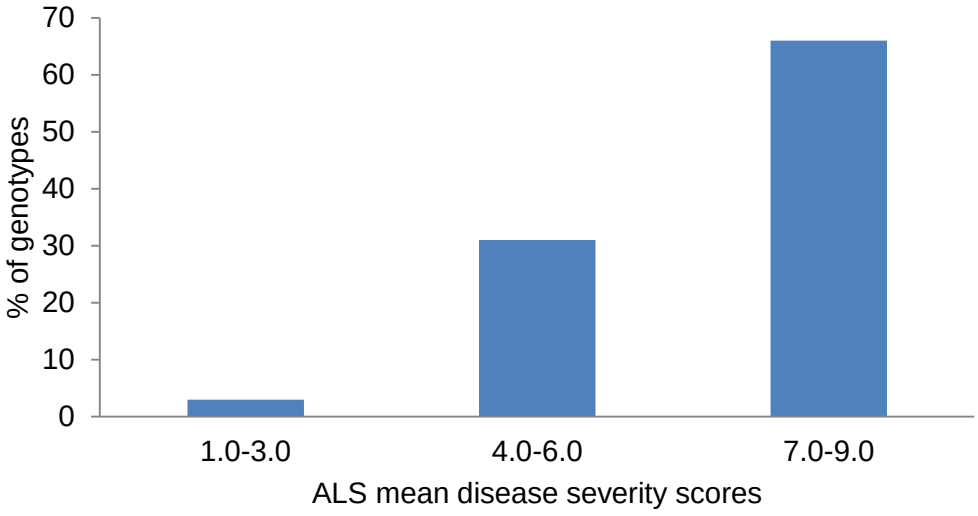

Figure 3.4: Reaction of 200 genotypes to angular leaf spot at KARI-Tigoni

The ALS resistant checks Mexico 54, G10909 and MAR-2 had disease severity scores of 2.0, 2.2 and 3.0 respectively, while resistant check AND 277 had a high disease severity score of 7.8. The susceptible checks CAL 96, MCM 5001 and BRB 191 had disease severity score of 7.9, 7.3 and 7.3 respectively, while Kanyebwa had a score of 4.9. The landraces and market class varieties had varied reaction to ALS (Figure 3.5). The Kenyan landraces had a mean disease severity score of 6.8. Kenya landrace GBK 028123 had a low disease severity score of 2.8. The Rwandan landraces had a mean disease severity score of 6.0. Minoire and Murangazi had scores of 2.6 and 2.9 respectively. The market class varieties had mean disease severity score of 7.1 whereby, GLP series, KAT series, Super-rosecoco and New-rosecoco had disease severity scores of between 7.0 and 9.0, apart from KAT 69 which had a score of 4.9.

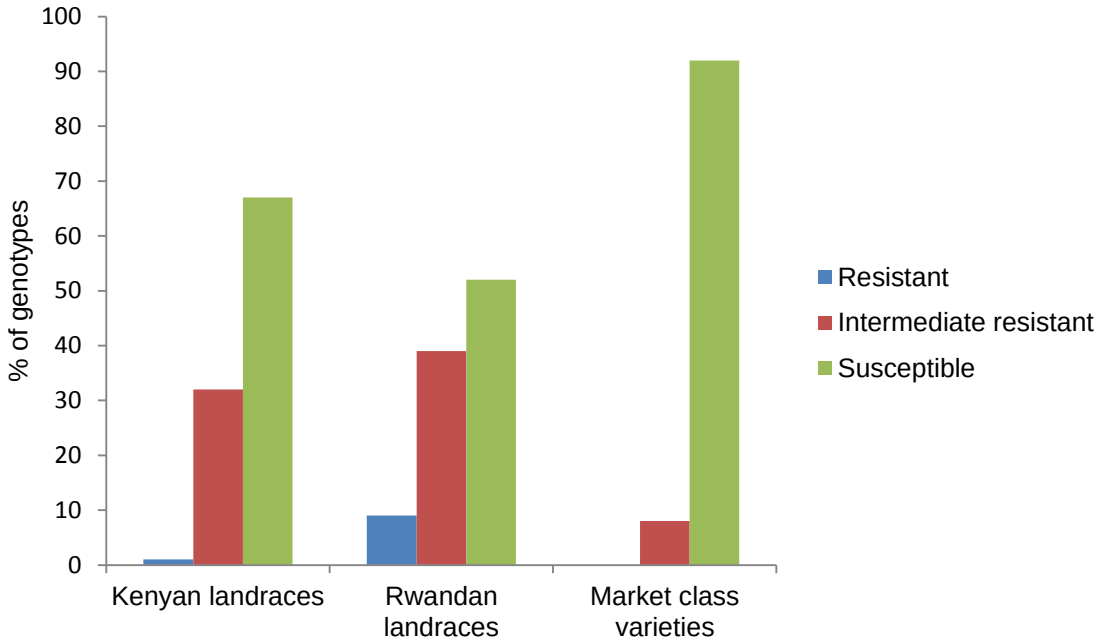

Figure 3.5: Reaction of landraces and market class varieties to angular leaf spot at KARI-Tigoni

There was a significant (P<0.001) difference among the genotypes for seed yield (Table 3.6).

Table 3.6: Analysis of variance (Wald tests for fixed effects) for yield for 200 common bean genotypes at KARI-Tigoni

Sequentially adding terms to fixed model				
Fixed term	Wald statistic	df	Wald/df	chi pr
Genotype	1777.16	199	8.93	<0.001
Dropping individual terms from full fixed model				
Fixed term	Wald statistic	df	Wald/df	chi pr
Genotype	1777.16	199	8.93	<0.001

df degree of freedom, chi pr = chi probability

The 200 genotypes evaluated at KARI-Tigoni had a mean seed yield of 559 kg ha^{-1} (Appendix 3.2). Of the 200 genotypes; 5% had a mean yield of > 700 kg ha^{-1}, 92% had a mean yield that ranged between 401 and 700 kg ha^{-1}, while 3% had a mean yield that ranged between 100 and 400 kg ha^{-1} (Figure 3.6). The five genotypes with the highest mean yield were GLP 585 (844 kg ha^{-1}), Mukwararaye (797 kg ha^{-1}), GBK 028011 (773 kg ha^{-1}), Nyirabukara (742 kg ha^{-1}) and Mufiki (733 kg ha^{-1}). The performance in mean yield of the landraces and market class varieties was varied (Figure 3.7). The Kenyan landraces had a mean yield of 557 kg ha^{-1}, the Rwandan landraces had a mean yield of 575 kg ha^{-1} and the market class varieties a mean yield of 592 kg ha^{-1}.

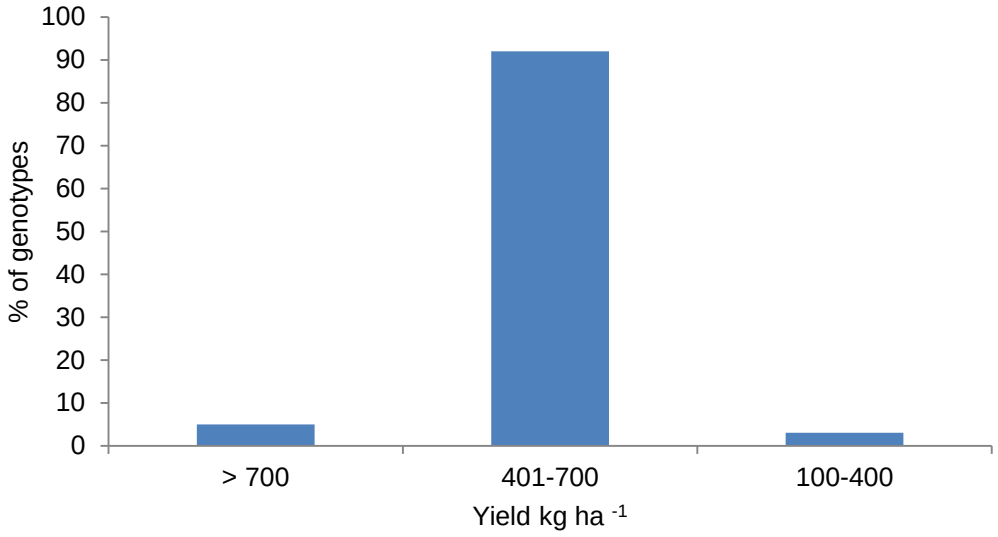

Figure 3.6: Mean yield performance of 200 genotypes at KARI-Tigoni

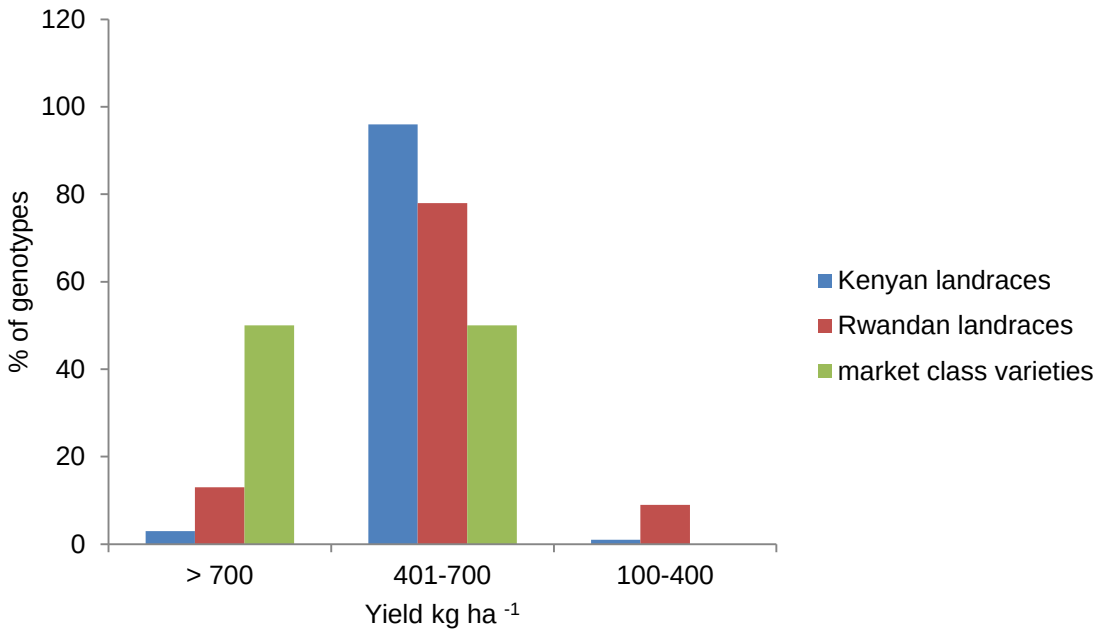

Figure 3.7: Mean yield performance of landraces and market class varieties at KARI-Tigoni

Comparison of mean disease severity and mean seed yield among genotypes

There was a negative non-significant and weak correlation (r = -0.002, P=0.970) between disease severity and seed yield. On average the resistant genotypes had a mean yield of 525 kg ha^{-1}, the intermediate resistant genotypes had a mean yield of 566 kg ha^{-1}, and the susceptible cultivars had a mean yield of 557 kg ha^{-1}. The five highest yielding genotypes reacted differently to ALS. GLP 585 had a disease severity score of 7.7, while Mukwararaye, GBK 028011, Nyirabukara, and Mufiki had disease severity scores of 7.3, 4.5, 5.3 and 6.7 respectively.

Comparison of mean disease severity and seed size among genotypes

There was a positive non-significant and weak correlation (r = 0.064, P = 0.383) between the disease severity and seed size of genotypes. Of the resistant genotypes; 33% were small seeded, 50% medium seeded and 17% large seeded. Of the moderate resistant genotypes; 16% were small seeded, 21% were medium seeded and 63% were large seeded. Of the susceptible genotypes; 16% were small seeded, 20% were medium seeded, and 64% were large seeded.

3.3.4 Yield evaluation at Kabete Field Station and KARI-Thika

Results from the analysis of variance of Wald tests statistics showed highly significant differences (P<0.001) in yield among the genotypes (Table 3.7). The genotype mean yield of the locations were also significantly different (P<0.001). The year (seasons; long rain and short rain) were not significantly different for mean yield (P=0.707). The interactions of genotype x location, genotype x year, location x year, genotype x location x year were significant (P<0.001).

Table 3.7: Analysis of variance (Wald tests for fixed effects) for yield of common bean genotypes at Kabete Field Station and KARI-Thika

Sequentially adding terms to fixed model				
Fixed term	Wald statistic	df	Wald/df	chi pr
Genotype	4764.11	199	23.94	<0.001
Location	1882.52	1	1882.52	<0.001
Year	0.14	1	0.14	0.707
Genotype x Location	1794.95	199	9.02	<0.001
Genotype x Year	710.03	199	3.57	<0.001
Location x Year	90.43	1	90.43	<0.001
Genotype x Location x Year	550.33	199	2.77	<0.001

df = degrees of freedom, chi pr = chi probability

The mean yield of the genotypes at Kabete Field Station during the short rains (2011) and long rains (2012) was 528 kg ha^{-1} and 501 kg ha^{-1} respectively. At KARI-Thika, the mean yield of the genotypes during the short and long rains was 402 kg $ha^{-1}$and 373 kg ha^{-1}, respectively. The mean yield of the 200 genotypes in each location during the two seasons and the mean yield during each season are presented in Appendix 3.3. The mean yield of the 200 genotypes at Kabete Field Station over the two seasons was 514 kg ha^{-1} and 388 kg ha^{-1} at KARI-Thika. At the two sites, the performance in yield of the 200 genotypes varied (Figure 3.8). The five genotypes with the highest mean yield at Kabete Field Station were GLP 2 (993 kg ha^{-1}), Mukwararaye (818 kg ha^{-1}), GBK 028012 (817 kg ha^{-1}), Nyirakanyobure (802 kg ha^{-1}) and Mufiki (733 kg ha^{-1}). The five genotypes with the highest mean yield at KARI-Thika were GBK 028110 (607 kg ha^{-1}), GBK 035065 (567 kg ha^{-1}), GBK 027984 (556 kg ha^{-1}), Nyirabukara (550 kg ha^{-1}) and Gitsindayogi (540 kg ha^{-1}).

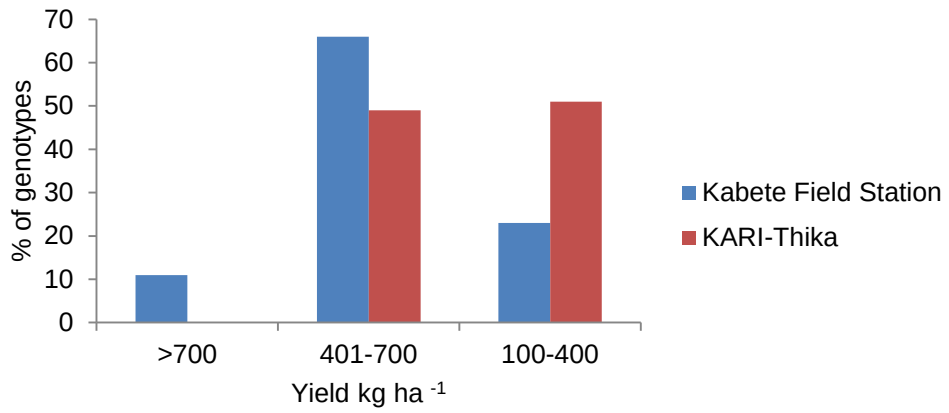

Figure 3.8: Mean yield performance of 200 genotypes at Kabete Field Station and KARI-Thika

The mean yield during the short rain (2011) was 450 kg ha^{-1} and 452 kg ha^{-1} during the long rains (2012). The high yielding genotypes during the short rain (2011) across the two locations were GLP 2 (939 kg ha^{-1}), GBK 027934 (719 kg ha^{-1}), GBK 028010 (710 kg ha^{-1}), Nyirakanyobure (692 kg ha^{-1}) and Mufiki (675 kg ha^{-1}). During the long rains, GBK 28110 (646 kg ha^{-1}), Nyirakanyobure (628 kg ha^{-1}), GBK 035065 (614 kg ha^{-1}), GLP 24 (612 kg ha^{-1}) and GLP 585 (610 kg ha^{-1}) had the highest yield.

Overall, across the two locations and two seasons, only one genotype, GLP 2, had a mean yield of >700 kg ha^{-1}, while 68% of the genotypes ranged between 401-700 kg ha^{-1} and 31% between 100-400 kg ha^{-1} (Figure 3.9). The high yielding genotypes in the two locations during the two seasons were GLP 2 (766 kg ha^{-1}), Nyirakanyobure (660 kg ha^{-1}), GBK 028110 (654 kg ha^{-1}), GLP 585 (630 kg ha^{-1}) and Mukwararaye (630 kg ha^{-1}).

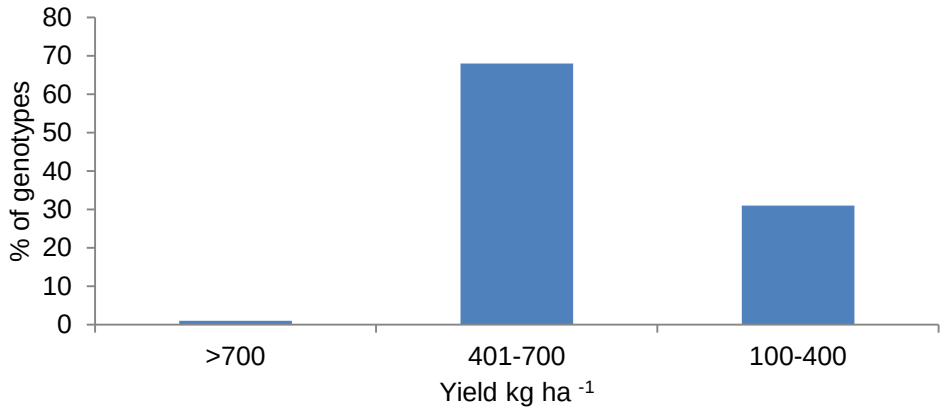

Figure 3.9: Mean yield performance of genotypes across Kabete Field Station and KARI-Thika

3.4 Discussion and conclusion

Common bean landraces are genetically diverse and possess important characteristics that include adaptation to local climatic conditions and cultural practices, and tolerance to diseases and insect-pests. The aim of the study was to identify landraces with high yield and/or resistance to ALS, and compare them to the market class varieties.

The trials at Kabete nethouse and KARI-Thika showed that only 3-4% of the genotypes had a resistant reaction to ALS. Most of the genotypes were susceptible (66-71%), while a substantial number had intermediate resistance (23-31%). None of the entries was immune to ALS, including the resistant checks Mexico 54, G10909 and MAR-2 which developed disease symptoms at low levels. These resistant checks have been used in several studies as sources of major gene resistance (Mahuku et al., 2011). The use of minor genes for durable resistance has been recommended by several authors (Van der Plank, 1968; Robinson, 1980). Disease tolerant genotypes have been shown to have stable yields, even when infected by diseases (Politowski and Browning, 1978). Genotype AND 277, which had been reported to be resistant to ALS (Goncalves-Vidigal et al., 2011), was susceptible in this study, which shows that a resistant genotype in one region may be susceptible in another. It is therefore essential to look for sources of resistance in locally adapted cultivars, such as landraces, as was done in the study.

Most of the landraces were susceptible, which shows that ALS is present in Kenya. However, three landraces, GBK 028123, Minoire and Murangazi, were resistant to ALS. Genotype GBK 052129, which was resistant in the Kabete nethouse, had intermediate resistance in KARI-Tigoni, which could be attributed to differences between the two sites or the presence of different races. Resistance in the landraces can be exploited and used locally as a source of resistance in a breeding programme or promoted as resistant genotypes. The high number of intermediate resistant genotypes can be utilised as sources for minor gene resistance in a durable resistance breeding programme. Sources of quantitative resistance in landraces have also been identified in several studies and the resistance accumulated through crossing and subsequent selection (Danial et al., 2007). Mmbaga et al. (1992) identified new sources of non-race specific resistance to rust and common bacterial blight from common bean landraces. The Rwandan genotypes performed better than the Kenyan landraces in their response to ALS. This can be attributed to either the lack of races that could break down the resistance or they are actually resistant in Kenya. A higher percentage of the Rwandan genotypes had intermediate resistance to ALS as compared to the Kenyan landraces. The Rwandan landraces

Minoire and Muragazi were resistant in Kenya. The market class varieties, which included GLP series, KAT series, Super-rosecoco and New-rosecoco were all susceptible to ALS disease. This could be attributed to the fact that the GLP and KAT series were released between 1980 and 1984 and the physiological races of the pathogen have changed over time, rendering them susceptible. Breeding of improved varieties in Kenya has targeted yield improvement rather than disease resistance. Hence there is need to improve market class varieties for ALS resistance.

At KARI-Tigoni the yield and seed size varied significantly among the genotypes. Market class variety GLP 585 and Kenyan landrace GBK 028011 and Rwandan landraces Mukwararaye, Nyirabukara and Mufiki were among the highest yielding entries. A high percentage (96%) of the Kenyan landraces had a moderate yield, which shows the adaptability and tolerance of these landraces. The market class varieties showed a higher mean yield as compared to the Kenyan and Rwandan landraces. This showed their ability as varieties that have been improved for yield. In this study, there was no significant correlation between disease severity and yield of the genotypes. The resistant genotypes had a lower mean yield as compared to the intermediate resistant and the susceptible genotypes. This could have been attributed to the fact that most of the resistant genotypes were the exotic resistant checks which are poorly adapted to the Kenyan conditions hence low yield. Similar results were reported by Filho et al. (1997) who found that yield of carioca common bean was not correlated with the disease severity and area under disease progress curve. Large seeded genotypes were more susceptible to ALS than the medium seeded genotypes, even though the correlation was not significant. Previous studies have shown that the large seeded common beans are susceptible to both the Mesoamerican and Andean races of the pathogen (Guzman et al., 1995). In addition, there are few Andean sources (CAL 143 and AND 277) of resistance and they are not universally resistant since they are susceptible in some parts of eastern Africa (Aggarwal et al., 2004). Andean source of resistance G5686 was found to be susceptible to some *P. griseola* races from Kenya (Mahuku et al., 2009). In Kenya there are predominantly large seeded common beans under production by the farmers (Katungi et al., 2009) and therefore breeding for durable resistance in large seeded beans, conditioned by minor genes, will be appropriate.

Yield evaluation at Kabete Field Station and KARI-Thika over the two seasons, showed the genotypes had a higher mean yield at Kabete Field Station than at KARI-Thika. Thika is drier and hotter when compared to Kabete and this could have affected genotype performance. Rainfall during the seasons was higher at Kabete than at Thika. The best performing genotypes

with the highest mean yield in Kabete were GLP 2, Mukwararaye, GBK 028012, Nyirakanyobure and Mufiki, and these can be recommended for production at Kabete. At Thika the best performing genotypes, GBK 028110, GBK 035065, GBK 027894, Nyirabukara and Gitsindayogi, could be recommended for production, in Thika. Overall, across the two locations the best performing and high yielding genotypes were GLP 2, Nyirakanyobure, GBK 028110, GLP 585 and Mukwararaye. However the genotype x environment interaction (genotype x location, genotype x year, location x year, and genotype x location x year) was significant meaning that there was variation in seed yield performance of the genotypes in the different environments. The stable genotypes across the four environments in the presence of the interactions were GBK 027869, GBK 028017, GBK 028147, GBK 028140 and GBK 028136. Hence it is important to carry out a stability analysis if a breeder wants to recommend a variety across a wide environment. Previous studies have shown that genotype by environment interaction affects common bean yield in Brazil and Ethiopia (Abreu et al., 1990; Mekbib, 2003; Carbonell et al., 2004).

In conclusion, landraces are an important source of genetic diversity as they are adapted to local conditions and have farmers' preferred traits. Three landraces Nyirakanyobure, GBK 028110, and Mukwararaye had a high mean yield, comparable to the market class varieties and they can be recommended in both Thika and Kabete. Several resistant landraces were identified such as GBK 028123, Minoire and Murangazi. These resistant landraces, as well as the large number of landraces with intermediate resistance, can be a valuable resource in local breeding programmes for durable resistance to ALS.

References

Abreu, A.F.B., M.A.P. Ramalho, J.B. dos Santos, and I.A.P. Pereira-Filho. 1990. Effect of Genotype x Environment interaction on estimations of genetic and phenotypic parameters of common beans. Brazil Journal of Genetics 13:75-82.

Aggarwal, 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.

Caixeta, E.T., A. Bor´em, S. de Azevedo 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 134:297-303.

Caixeta, E.T., A. Bore´m, A.L. Alzate-Marin, S. de Azevedo Fagundes, M.G. de Morais Silva, E. G. de Barros, and M.A. Moreira. 2005. Allelic relationships for genes that confer resistance to angular leaf spot in common bean. Euphytica 145:237-245.

Carbonell, S.A.M., J.A.A. Filho, L.A.S. Dias, A.A.F. Garcia, and L.K. Morais. 2004. Common bean cultivars and lines interactions with environments. Science Agriculture (Piracicaba, Brazil.) 61:169-177.

Carvalho, G.A., T.J. Paula, A.L. Alzate-Marin, S. Nietsche, E.G. de Barros, and M.A. Moreira. 1998. Inheritance of resistance to angular leaf spot of common bean in AND 277 to race 63–23 of *Phaeoisariopsis griseola* and identification of a RAPD marker linked to the resistance gene. Fitopatologia Brasil 23:482-485.

Danial, D., J. Parlevliet, C. Almekinders, and G. Thiele. 2007. Farmers' participation and breeding for durable disease resistance in the Andean region. Euphytica 153:385-396.

Ferreira, C.F., A. Borém, G.A. Carvalho, S. Nietsche, T.J. Paula-Jr, 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.

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, healthy leaf area and yield. Phytopathology 87:506-515.

Goncalves-Vidigal, M.C., A.S. Cruz, A. Garcia, J. Kami, P.S. Vidigal Filho, L.L. Sousa, P. McClean, 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 122:893-903.

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

Katungi, E., A. Farrow, J. Chianu, l. Sperling, and S. Beebe. 2009. Common bean in Eastern and Southern Africa: A situation and outlook analysis, CIAT, Cali, Colombia.

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.

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. Montoya, M.A. Henrı´quez, C. Jara, H. Teran, and S. Beebe. 2004. Inheritance and characterization of the angular leaf spot resistance gene in the common bean accession, G 10474 and identification of an AFLP marker linked to the resistance gene. Crop Science 44:1817-1824.

Mahuku, G.S., M.A. Henrı´quez, C. Montoya, C. Jara, H. Teran, and S. Beebe. 2011. Inheritance and development of molecular markers linked to angular leaf spot resistance genes in the common bean accession G10909. Molecular Breeding 28:57-71.

Mekbib, F. 2003. Yield stability in common bean (*Phaseolus vulgaris* L.) genotypes. Euphytica 130:147-153.

Mmbaga, M.T., E.A. Santana, J.R. Steadman, and D.P. Coyne. 1992. New sources of nonspecific resistance to rust and common bacterial blight in dry bean landrace Pompadour. Euphytica 61:135-144.

Mwang'ombe, A.W., I.N. Wagara, J.W. Kimenju, and R.A. Buruchara. 2007. Occurence and severity of angular leaf spot of common bean in Kenya as influenced by geographical location, altitude and agroecological zones. Plant Pathology 6:235-241.

Namayanja, A., R. Buruchara, G.S. 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.

Payne, R.W., D.A. Murray, S.A. Harding, D.B. Baird, and D.M. Soutar. 2009. GenStat for Windows (12th Edition) Introduction. VSN International, Hemel Hempstead, UK.

Politowski, K., and J.A. Browning. 1978. Tolerance and resistance to plant disease: An epidemiology study. Phytopathology 68:1177-1185.

Robinson, R.A. 1980. New concepts in breeding for disease resistance. Annual Review of Phytopathology 18:189-210.

Robinson, R.A. 1987. Plant pathosystems. Springer-Verlag, Berlin, Heidelberg, New York, USA.

Stoilova, T., G. Pereira, M.M.T. de Sousa, and V. Carnide. 2005. Diversity in common bean landraces (*Phaseolus vulgaris L.)* from Bulgaria and Portugal. Journal of Central European Agriculture 6:443-448.

Van der Plank, J.E. 1968. Disease resistance in plants. Academic Press, New York, USA.

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

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.

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

Zeven, A.C. 1997. The introduction of the common bean (*Phaseolus vulgaris* L.) into Western Europe and the phenotypic variation of dry beans collected in the Netherlands in 1946. Euphytica 94:319-328.

Zeven, A.C. 1998. Landraces: A review of definitions and classifications. Euphytica 104:127-139.

Appendices

Appendix 3.1: Mean values of angular leaf spot disease severity score for 200 genotypes at Kabete nethouse

Genotype	ALS	Genotype	ALS	Genotype	ALS	Genotype	ALS	Genotype	ALS
Mexico 54	1.8	GBK028028	5.0	GBK028015	7.1	GBK027981	7.6	GBK028142	7.9
G10909	2.2	KAT69	5.0	GBK028150	7.1	GBK028029	7.6	GBK035065	7.9
MAR 2	2.8	Nyirabukara	5.1	Mamesa	7.1	GBK035059	7.6	GLP585	7.9
GBK028123	2.9	GBK028133	5.5	Umubano	7.1	Nyirakanyobure	7.6	GLPX92	7.9
Minoire	2.9	GBK028006	5.6	GBK027869	7.2	GBK027992	7.7	GBK027896	8.0
Murangazi	3.2	GBK027867	5.8	GBK027889	7.2	GBK028032	7.7	GBK027953	8.0
GBK052129	3.3	Mufiki	5.9	GBK027920	7.2	GBK028039	7.7	GBK027963	8.0
Inconnue8	3.8	GBK027864	6.1	GBK027975	7.2	GBK028101	7.7	GBK028038	8.0
Gosorera	3.8	GBK028122	6.2	GBK028057	7.2	GBK028163	7.7	GBK028044	8.0
GBK028019	4.3	GBK028135	6.2	GBK027916	7.3	GBK035446	7.7	GBK028109	8.0
GBK027917	4.4	Kinyaruka	6.3	GBK028130	7.3	BRB191	7.8	GBK028118	8.0
GBK028102	4.4	GBK027965	6.3	GBK028154	7.3	GBK027918	7.8	GBK028136	8.0
GBK028162	4.4	GBK027893	6.4	Mushaka	7.3	GBK028003	7.8	GBK035355	8.0
GBK027894	4.5	GBK028023	6.6	Newrosecoco	7.3	GBK028010	7.8	Nyiramabuye	8.0
GBK028042	4.5	GBK028083	6.7	GBK027987	7.3	GBK028041	7.8	GBK027937	8.1
GBK028098	4.5	GBK035081	6.7	GBK027996	7.3	GBK028089	7.8	GBK027962	8.1
GBK028141	4.5	GLP1004	6.7	GBK028016	7.3	GBK028145	7.8	GBK027966	8.1
GBK036478	4.5	GBK027912	6.8	GBK028106	7.3	GBK035464	7.8	GBK027970	8.1
Gitsindayogi	4.5	GBK027976	6.8	GBK027863	7.4	Mukwararaye	7.8	GBK028020	8.1
GBK027926	4.6	GBK027958	6.8	GBK027927	7.4	Super rosecoco	7.8	GBK028026	8.1
GBK027955	4.6	GBK027961	6.8	GBK027933	7.4	AND277	7.8	GBK035161	8.1
GBK027974	4.6	GBK028021	6.8	GBK028007	7.4	GBK027900	7.8	Inconnue2	8.1
GBK028108	4.6	MCM5001	6.8	GBK028017	7.4	GBK028099	7.8	RWR2154	8.1
GBK028140	4.6	RWR1802	6.8	GBK028097	7.4	GBK028128	7.8	CAL96	8.2
GBK035024	4.6	GBK027901	6.9	GBK028107	7.4	GBK028134	7.8	GBK027891	8.2
GBK027919	4.7	GBK028132	6.9	GBK028112	7.4	GBK028152	7.8	GBK027895	8.2
GBK028115	4.7	GLP1127	6.9	GBK028126	7.4	GBK028178	7.8	GBK027924	8.2
Inconnue3	4.7	KAT56	6.9	GLP2	7.4	GBK035022	7.8	GBK027956	8.2
Kagondo	4.7	KATB1	6.9	GLP24	7.4	ABA136	7.9	GBK028035	8.2
GBK027948	4.8	GBK027890	7.0	GBK027870	7.5	GBK027872	7.9	GBK028139	8.2
GBK028005	4.8	GBK027903	7.0	GBK027914	7.5	GBK027931	7.9	GBK028013	8.3
GBK028137	4.8	GBK027930	7.0	GBK027983	7.5	GBK027936	7.9	GBK035341	8.3
GBK035119	4.8	GBK027988	7.0	GBK027984	7.5	GBK027959	7.9	GBK028045	8.3
GBK028011	4.8	GBK028147	7.0	GBK027994	7.5	GBK027968	7.9	GBK035090	8.3
GBK028036	4.8	GBK035357	7.0	GBK028110	7.5	GBK027977	7.9	GBK028004	8.4
GBK028144	4.8	Inconnue7	7.0	GBK028119	7.5	GBK027993	7.9	GBK027898	8.5
Kanyebwa	4.8	KatB9	7.0	GBK028179	7.5	GBK028018	7.9	GBK027921	8.5
Mukara	4.8	GBK027928	7.1	GBK035001	7.5	GBK028053	7.9	GBK028104	8.7
GBK027934	4.9	GBK027932	7.1	GBK027866	7.6	GBK028079	7.9	GBK028012	8.8
Inconnue6	4.9	GBK027973	7.1	GBK027979	7.6	GBK028086	7.9	GBK027952	8.8
								Grand mean	6.8
								sed	0.3

ALS = disease severity score; 1.0-3.0 = resistant, 4.0-6.0 = intermediate resistant, 7.0-8.0 = susceptible

Appendix 3.2: Mean values of angular leaf spot severity scores, yield and seed size for 200 genotypes at KARI-Tigoni field

Genotype	ALS	Yield	Seed size	Genotype	ALS	Yield	Seed size	Genotype	ALS	Yield	Seed size
Mexico 54	2.0	556	M	GBK027963	6.4	603	L	GBK027912	7.7	530	M
G10909	2.2	421	M	GBK027981	6.5	517	L	GBK027952	7.7	481	L
Minoire	2.6	604	S	GBK028007	6.5	565	L	GBK028013	7.7	709	M
GBK028123	2.8	476	L	GBK028097	6.6	665	L	GBK028128	7.7	714	L
MAR-2	2.9	482	M	KAT56	6.7	454	M	GBK035341	7.7	547	S
Murangazi	2.9	613	S	KATB1	6.7	527	M	GLP585	7.7	844	S
GBK052129	3.6	511	S	KATB9	6.7	460	M	RWR2154	7.7	466	M
Inconnue8	4.0	567	L	Mufiki	6.7	733	L	Super-rosecoco	7.7	690	L
Gosorera	4.2	535	M	GBK027864	6.8	466	L	ABA136	7.8	564	S
GBK028011	4.5	773	L	GBK035081	6.8	503	S	AND277	7.8	585	L
GBK027934	4.6	666	M	Mushaka	6.8	256	S	GBK027870	7.8	500	L
GBK028108	4.6	475	L	Umubano	6.8	570	S	GBK027889	7.8	555	M
GBK036478	4.6	563	S	GBK027920	6.8	609	L	GBK027896	7.8	458	L
GBK027894	4.7	585	L	GBK027933	6.8	667	L	GBK027900	7.8	534	S
GBK027917	4.7	543	L	GBK027976	6.8	526	L	GBK027937	7.8	561	S
GBK027919	4.7	554	L	GBK027987	6.8	571	L	GBK027959	7.8	630	M
GBK028019	4.7	582	L	GBK028130	6.8	601	L	GBK028017	7.8	514	L
GBK028115	4.7	538	L	GBK028154	6.8	574	L	GBK028018	7.8	502	S
GBK035024	4.7	575	L	GBK035357	6.8	671	S	Nyirakanyobure	7.8	692	S
Gitsindayogi	4.7	679	M	Inconnue7	6.8	460	L	GBK027866	7.8	551	L
Kagondo	4.7	656	M	Kinyaruka	6.8	388	L	GBK027927	7.8	649	L
GBK027955	4.8	536	L	GBK027903	6.9	424	L	GBK027956	7.8	470	S
GBK027974	4.8	564	L	GBK027930	6.9	552	L	GBK027965	7.8	482	L
GBK028006	4.8	601	L	GBK028106	6.9	569	L	GBK027984	7.8	496	L
GBK028036	4.8	506	S	GBK028147	6.9	576	L	GBK027993	7.8	628	L
GBK028089	4.8	533	S	Newrosecoco	6.9	614	L	GBK028003	7.8	652	L
GBK028162	4.8	535	L	RWR1802	6.9	630	L	GBK028010	7.8	597	L
GBK035119	4.8	426	L	GBK027968	7.0	605	M	GBK028109	7.8	550	L
GBK027926	4.8	643	S	GBK028021	7.0	439	L	GBK028139	7.8	491	L
GBK028005	4.8	586	L	GBK028132	7.0	680	L	GBK028152	7.8	566	M
GBK028042	4.8	630	L	GBK028145	7.0	517	M	GBK035355	7.8	655	S
GBK028098	4.8	688	L	GBK035090	7.0	470	L	Mamesa	7.8	424	S
GBK028102	4.8	526	L	GBK035446	7.0	498	L	CAL96	7.9	587	L
GBK028144	4.8	569	L	GBK027890	7.1	508	M	GBK027924	7.9	555	L
GBK027948	4.9	475	L	GBK027988	7.1	569	S	GBK027931	7.9	545	L
GBK028140	4.9	439	M	GBK028112	7.1	609	L	GBK027936	7.9	546	S
GBK028141	4.9	396	M	GBK028163	7.1	540	L	GBK027962	7.9	520	L
Kanyebwa	4.9	361	M	GBK028044	7.2	545	S	GBK028053	7.9	560	L
KAT69	4.9	480	L	GBK028045	7.2	644	M	GBK028057	7.9	577	L
Mukara	4.9	655	S	GBK028086	7.2	520	L	GBK028107	7.9	518	L
GBK028028	5.0	635	M	GBK028178	7.2	639	L	GBK028118	7.9	514	M
GBK028122	5.0	471	L	GBK028179	7.2	457	M	GLPX92	7.9	550	M
GBK028137	5.0	664	S	GBK027979	7.3	545	L	Nyiramabuye	7.9	475	M
Inconnue3	5.0	580	L	GLP1004	7.3	707	M	GBK027891	8.0	539	L
Inconnue6	5.1	518	M	MCM5001	7.3	396	M	GBK028126	8.0	548	M
GBK027961	5.2	601	L	Mukwararaye	7.3	797	L	GBK028134	8.0	537	L
Nyirabukara	5.3	742	S	BRB191	7.3	601	M	GBK028136	8.0	542	L
GBK028099	5.4	639	L	GBK027869	7.3	523	L	GBK035059	8.0	675	S

Genotype	ALS	Yield	Seed size	Genotype	ALS	Yield	Seed size	Genotype	ALS	Yield	Seed size
GBK028135	5.4	493	L	GBK027872	7.3	532	L	Inconnue2	8.0	625	S
GBK027932	5.7	564	L	GBK027901	7.3	365	L	GBK027916	8.1	560	L
GBK027958	5.8	547	M	GBK028101	7.3	547	L	GBK027918	8.1	543	L
GBK027867	5.8	453	L	GBK028119	7.3	530	L	GBK027921	8.1	549	S
GBK027928	5.8	516	M	GBK035001	7.3	570	L	GBK027970	8.1	628	L
GBK028035	5.8	600	L	GBK027863	7.4	559	L	GBK027994	8.1	659	L
GBK027973	5.9	679	L	GBK028004	7.4	543	L	GBK028012	8.1	676	L
GBK028032	5.9	556	L	GLP2	7.4	653	L	GBK028020	8.1	509	L
GBK028041	5.9	647	L	GBK027895	7.5	542	M	GBK027983	8.2	579	L
GBK028079	5.9	550	M	GBK027914	7.5	575	L	GBK027992	8.2	566	L
GBK028133	5.9	647	L	GBK027953	7.5	416	L	GBK028142	8.2	491	L
GBK028029	6.0	528	L	GBK027966	7.5	530	L	GBK028150	8.2	643	M
GBK028039	6.0	582	L	GLP1127	7.5	511	M	GBK035065	8.2	503	L
GBK027996	6.1	676	S	GLP24	7.5	608	M	GBK035464	8.2	574	L
GBK028015	6.2	623	L	GBK027898	7.6	483	L	GBK027975	8.3	508	L
GBK028038	6.2	497	M	GBK028016	7.6	471	L	GBK028104	8.3	624	M
GBK027893	6.3	487	L	GBK028026	7.6	632	L	GBK035022	8.4	563	L
GBK028023	6.3	563	L	GBK028110	7.6	702	L	GBK027977	8.5	495	L
GBK028083	6.3	487	S	GBK035161	7.6	471	S	Mean	6.6	559	
								sed	0.3	39.3	

sed = standard error difference, seed size = 100-seed weight^{-1}; small <25 g, medium 25-40g, large >40 g, yield = kg ha^{-}1, ALS = disease severity score; 1.0-3.0 = resistant, 4.0-6.0 = intermediate resistant, 7.0-9.0 = susceptible

Appendix 3.3: Mean values of yield for 200 common bean genotypes at Kabete Field Station and KARI-Thika

Genotype	Kabete	Thika	SR2011	LR2012	Gen. mean	Genotype	Kabete	Thika	SR 2011	LR 2012	Gen. mean
GLP 2	993	539	939	594	766	GBK 028006	564	441	520	486	503
Nyirakanyobure	802	518	692	628	660	GBK 028107	653	347	456	544	500
GBK 028110	701	607	663	646	654	GBK 027917	608	389	511	487	499
GLP 585	758	502	650	610	630	MCM 5001	503	493	504	492	498
Mukwararaye	818	442	657	602	630	GBK 027963	520	475	588	406	497
GBK 028133	723	535	666	592	629	GBK 027894	492	500	484	508	496
GBK 028010	756	498	710	544	627	GBK 028020	526	457	473	510	491
GBK 027934	753	494	719	528	623	GBK 027900	455	528	529	453	491
Mufiki	773	470	675	568	621	Nyiramabuye	489	492	524	457	491
GBK 035065	673	567	626	614	620	RWR 1802	574	395	492	476	484
GBK 028011	768	472	634	606	620	GBK 028035	609	352	458	503	481
GBK 035355	741	494	636	599	618	GBK 028130	475	480	468	487	477
GBK 028012	817	402	627	592	610	GBK 028145	544	409	481	472	476
Nyirabukara	666	550	629	587	608	GBK 028115	526	424	442	508	475
Gitsindayogi	674	540	657	557	607	GBK 027983	509	436	497	448	473
GLP 24	719	488	595	612	604	GBK 028045	635	309	485	458	472
GBK 027925	667	533	605	594	600	GBK 027992	555	386	452	490	471
GBK 028128	737	452	580	609	595	GBK 027898	425	516	445	496	470
GBK 027994	753	433	646	540	593	GBK 027993	478	462	500	440	470
BRB 191	847	334	738	444	591	GBK 028016	601	339	495	445	470
GBK 028162	665	511	626	550	588	GBK 027937	564	369	492	442	467
GBK 028150	711	464	656	519	588	GBK 028041	539	395	471	462	467
GBK 028098	706	454	630	531	580	GBK 028097	554	379	489	443	466
GBK 027863	719	441	606	554	580	GBK 027895	500	433	424	508	466
GBK 027988	676	482	644	514	579	KAT 56	493	439	517	414	466
GBK 035341	700	456	620	536	578	GBK 027948	503	426	449	480	464
GBK 027959	716	430	611	535	573	GBK 028102	519	407	473	453	463
GBK 027926	622	521	565	579	572	GBK 028154	484	441	475	449	462
GBK 028137	658	481	598	541	570	GBK 028079	559	363	443	479	461
GBK 027973	708	424	554	578	566	GBK 028018	510	411	444	477	461
GLP 1004	637	486	589	535	562	GBK 028122	474	447	488	433	460
Inconnue 2	613	510	582	542	562	GBK 035464	548	370	474	444	459
GBK 028013	582	525	555	553	554	GLP X92	507	410	461	456	458
GBK 027996	664	442	574	532	553	GBK 027927	529	385	374	540	457
GBK 027984	544	556	580	521	550	GBK 027976	474	438	487	425	456
GBK 028015	635	460	530	565	548	Kinyaruka	493	418	473	438	455
Minoire	610	479	595	495	545	GBK 028021	449	458	455	451	453
GBK 027931	739	350	538	552	545	Umubano	507	389	419	477	448
Super rosecoco	577	507	563	521	542	GBK 027965	581	306	472	414	443
GBK 027970	596	488	558	526	542	GBK 028026	491	394	447	437	442
GBK 027962	611	462	575	498	537	GBK 027975	486	396	404	479	441
GBK 028042	668	401	550	519	535	GBK 027893	499	382	409	472	440
GBK 028132	576	493	569	500	534	GBK 035081	432	448	413	467	440
GBK 027974	666	402	564	504	534	GBK 027968	500	377	446	431	438
Mukara	573	495	525	543	534	GBK 035059	503	373	423	453	438
Gosorera	571	495	552	514	533	GBK 035022	483	392	411	463	437
GBK 028005	594	470	521	544	532	Inconnue 8	598	259	390	467	428
GBK 035024	545	508	571	482	526	GBK 035446	518	337	393	462	427
GBK 028003	612	439	519	533	526	GBK 028044	551	303	412	441	427
New Rosecoco	604	443	548	499	524	GBK 028086	431	418	450	399	424
Muragazi	635	411	555	490	523	GBK 028109	489	357	438	408	423
GBK 028139	627	417	515	530	522	GBK 027979	506	339	403	442	422
Inconnue 7	555	488	576	467	522	GBK 028179	536	308	402	442	422
GBK 027914	587	453	514	526	520	GBK 027870	549	294	439	404	422
GBK 028099	631	409	494	545	520	GBK 027872	530	312	458	385	421
GBK 027961	621	410	532	499	515	CAL 96	541	299	423	417	420
GBK 028089	597	430	499	529	514	GBK 028135	591	248	424	415	419
GBK 028029	521	504	514	511	512	GBK 027890	521	317	408	431	419
GBK 027921	647	375	574	448	511	GBK 027896	481	356	376	461	419
GBK 027918	590	431	581	440	511	AND 277	603	234	425	412	419
Kagondo	548	469	492	524	508	GBK 027981	366	470	416	421	418
GBK 027977	486	527	527	486	507	GBK 028178	683	153	413	423	418
GBK 035161	541	470	530	480	505	GBK 027952	391	445	384	452	418

Genotype	Kabete	Thika	SR2011	LR2012	Gen. mean	Genotype	Kabete	Thika	SR 2011	LR 2012	Gen. mean
GBK 028038	443	393	409	427	418	GBK 027953	353	358	269	443	356
GBK 028144	481	354	430	405	418	GBK 027901	428	282	332	378	355
Mamesa	384	442	425	401	413	KAT 69	436	270	328	378	353
Inconnue 3	422	404	374	452	413	GBK 027936	323	383	307	399	353
GBK 028126	416	406	435	387	411	GBK 028104	349	351	331	370	350
ABA 136	468	350	443	376	409	MA2R	380	321	358	342	350
GBK 027966	420	397	402	416	409	GBK 028106	407	281	303	385	344
GBK 027987	468	344	406	407	406	GBK 027903	398	287	344	340	342
GBK 027920	562	251	354	458	406	GBK 028019	376	290	296	371	333
GBK 028147	356	454	409	401	405	GBK 027889	434	231	256	408	332
GBK 028140	371	438	376	434	405	GBK 028007	436	228	286	378	332
GBK 027956	467	335	395	407	401	GBK 028053	335	329	316	347	332
GBK 028083	414	386	349	451	400	GBK 028039	378	281	268	391	330
GBK 028134	396	395	377	414	395	GBK 027912	335	321	278	378	328
GBK 028141	388	401	389	401	395	GBK 027955	347	307	293	361	327
GBK 028017	332	455	380	406	393	GBK 027866	513	139	298	354	326
GBK 027869	326	458	385	398	392	GBK 028108	324	325	302	347	324
GBK 035088	393	389	371	412	391	KAT B9	346	303	307	341	324
GBK 036478	506	266	294	479	386	KAT B1	364	282	294	351	323
GBK 027924	442	327	371	398	385	GBK 028023	338	300	296	343	319
GBK 027933	435	334	343	426	384	GBK 028036	342	295	248	389	318
GBK 028032	393	372	408	357	383	GLP 1127	343	292	280	355	317
GBK 035357	378	387	387	377	382	GBK 028123	303	331	281	354	317
GBK 028004	347	415	385	377	381	GBK 035001	341	281	283	339	311
Inconnue 6	448	310	505	253	379	GBK 035090	307	310	301	316	309
GBK 027928	529	221	310	440	375	RWR 2154	281	330	251	361	306
GBK 028057	422	317	331	409	370	GBK 027930	302	306	273	335	304
GBK 028152	530	209	317	421	369	GBK 027932	437	166	265	339	302
GBK 027919	489	242	335	396	366	GBK 028112	289	309	261	338	299
GBK 027916	428	302	327	404	365	GBK 028118	384	212	233	362	298
GBK 028101	357	373	461	269	365	GBK 028142	326	233	259	300	280
GBK 028136	325	402	338	389	364	GBK 027958	288	257	238	308	273
GBK 028028	444	283	291	436	364	GBK 027864	249	246	190	305	248
GBK 028163	336	389	338	387	363	Kanyebwa	173	263	196	239	218
GBK 028119	476	249	330	395	362	GBK 052129	198	197	172	224	198
GBK 027867	455	266	311	410	360	GBK 035119	200	183	186	197	191
GBK 027891	421	291	327	385	356	Mushaka	219	148	180	187	184
						Grand mean	514	388	450	452	
						Sed	2.9		2.9		

Yield in kg ha^{-1}, Mean yield for each specific location as indicated, Gen. mean = mean value for yield of genotype across the sites and seasons

CHAPTER 4

Genetic analysis of resistance to angular leaf spot in common bean

Abstract

Angular leaf spot (ALS) caused by *Pseudocercospora griseola* (Sacc.) Crous & Braun is a major cause of common bean yield losses in Kenya. The pathogen is highly variable and 44 different races have been reported in the country. Understanding the genetics of resistance to the disease is important for a breeding programme. The objective of the study was to determine the mode of inheritance of resistance to ALS in common bean. Leaves infected with ALS were collected from a common bean field at Kabete Field Station. Twelve ALS differentials were used to identify the isolates. The most virulent of the isolates was characterised as Mesoamerican race 63-39 and was used to inoculate the F_1, F_2, BC_1P_1, and BC_1P_2 generations developed. Three crosses: Super-rosecoco x Mexico 54, Wairimu x G10909, and Wairimu x Mexico 54 were made. Genotypes Mexico 54 and G10909 were ALS resistant, whilst the susceptible genotypes were Super-rosecoco and Wairimu. For each cross, the two parents (P_1 and P_2), F_1, F_2, BC_1P_1, and BC_1P_2 generations were evaluated for resistance to ALS in a randomised complete block design in the field at Kabete. The results showed that both the additive and dominance gene effects were important. The additive gene effects were higher than the dominance gene effects. This indicated that resistance to angular leaf spot in genotypes Mexico 54 and G10909 was quantitative in nature. The narrow sense heritability estimate for resistance was moderately high (52.9 - 71.7%). The predominance of additive gene effects and the moderately high narrow sense heritability estimates observed imply that progress would be made through selection.

4.1 Introduction

Common bean is the third most important food legume crop in the world, after soybean and peanut. It is an important source of protein for the small scale farmers who practice subsistence farming. Angular leaf spot, caused by the fungus *Pseudocercospora griseola* (*P. griseola*), is a major disease of common bean worldwide (Pastor-Corrales and Saettler, 1989). The disease occurs in most common bean growing areas and lack of adequate control methods has led to yield losses of up to 80% in Africa (Wortmann et al., 1998; Stenglein et al., 2003). It is widespread in Africa, especially in Malawi, Ethiopia, Kenya, Uganda, Tanzania and the Great Lakes region (Pastor-Corrales et al., 1998). In Kenya, the disease is prevalent in all the agro-ecological zones where common bean is grown (Mwang'ombe et al., 2007). The most practical, economical and environmentally friendly way for the management of ALS is the use of resistant varieties.

Resistant varieties have been shown to have the ability to hinder the growth and/or development of various pathogens (Parlevliet, 1979). Knowledge of the availability of different sources of resistance and the mode of inheritance of resistance is essential to plant breeders. This will assist in identifying the type of resistance to breed for in a breeding programme. Several sources of resistance to ALS have been identified through germplasm screening in various parts of the world (Caixeta et al., 2003; Mahuku et al., 2004; Namayanja et al., 2006; Mahuku and Iglesias, 2009; Mahuku et al., 2011). In addition, high levels of pathogenic and genetic variation have been shown to occur in *P. griseola* in different regions (Pastor-Corrales et al., 1998; Mahuku et al., 2002a), including Kenya (Wagara et al., 2004). This high pathogenic variability renders varieties that are resistant in one location/year to be susceptible in another (Pastor-Corrales et al., 1998).

The high pathogenic variability of *P. griseola,* has led to the search and characterisation of new sources of resistance and a better understanding of the genetics behind reaction to the pathogen (Borel et al., 2011). Genetic studies have revealed different types of gene action depending on the parents and the pathogen races used. Genotypes AND 277, MAR-2, Mexico 54, BAT 332 and Cornell 49-242 were shown to have single dominant genes that governed resistance to certain races of *P. griseola* (Carvalho et al., 1998; Nietsche et al., 2000; Sartorato et al., 2000; Caixeta et al., 2005). Genotype Ouro Negro was reported to have a dominant gene that controlled resistance to *P. griseola* races 63-39 and 31-23. The dominant gene was shown to be different from that found in AND 277, BAT 332, Cornell 49242, MAR-2 and Mexico 54

(Sanglard et al., 2013). Control of resistance in US genotype Pinto 111 against race 31-23 was found to due to single recessive gene (Correa et al., 2001). The G10474 common bean was shown to have a single dominant gene conditioning resistance to two *P. griseola* races (Mahuku et al., 2004). Two dominant and complementary genes condition resistance to ALS in G10909 against *P. griseola* race 63-63 (Mahuku et al., 2003; Mahuku et al., 2011). Allelism tests have shown that genotype AND 227 has four angular leaf spot resistance genes designated as *Phg-1*^{a}, *Phg-2*^{2}, *Phg-3*^{2} and *Phg-4*^{2}, while Mexico 54 has three *(Phg*-2, *Phg*-5 and *Phg*-6) resistance genes and MAR-2 has two genes (*Phg*-4, *Phg*-5) (Mahuku et al., 2004; Caixeta et al., 2002).

This type of resistance conditioned by dominant genes is race-specific. It breaks down quickly and therefore new sources of resistance must always be sought. It is also important to determine the inheritance of resistance in these new sources. These sources of resistance must therefore be exposed to the existing pathogen races variation in the different common bean production areas (Milgroom and Fry, 1997)**.** This will facilitate the development of new varieties with resistance to any new pathogen races that are identified. Each source of resistance has been shown to react differently to the various pathogen races found in a region. The sources of resistance also react differently depending on the susceptible variety used (Pastor-Corrales et al., 1994; Mahuku et al., 2011). The type of gene action identified will enable breeders to decide on whether to breed for race specific resistance, if a resistance source is conditioned by dominance gene effects or non-race specific resistance if the source is conditioned by additive gene effects. Such studies have not been conducted in Kenya and hence this study. The objective of this study was to identify the mode of inheritance of the genes that confer resistance to angular leaf spot in common bean genotypes Mexico 54 and G10909.

4.2 Materials and Methods

4.2.1 Study site

The study was conducted at Kabete Field Station of the University of Nairobi. Kabete is located at coordinates 01^{o}14'59.7"S; 036^{o}44'28.8"E, with an altitude of 1820 m above sea level. The area receives an average rainfall of 1046 mm annually, with a mean maximum temperature of 23^{o}C and mean minimum temperature of 12^{o}C. The soils are dark red or brown friable clay.

4.2.2 Isolation and identification of *Pseudocercospora griseola* races

The causative pathogen, *P. griseola* was isolated from the leaves of infected common bean plants showing characteristic angular shaped spots that were collected from Kabete Field Station. Two types of media were used, agar-agar (20 g l^{-1} of sterile water) and the V8 medium (200 ml V8 juice, 20 g agar-agar, and 800 ml sterile water). A small piece of agar mounted on a sharp sterile needle (attached to a long handle) was used to gently and lightly touch the lesions (abaxial side of leaf) in order for the spores to attach to the medium. The block of agar was then placed on a petri dish containing agar-agar medium. Four small blocks of agar were placed onto the medium and using a sterile wire loop, the conidia were spread evenly onto the media. The petri dishes were then incubated in a non-illuminated incubator at 22^{o}C and observed daily under a dissecting microscope. After day one the conidia were visible. Single conidia were gently cut out of the medium and singly transferred onto V8 medium plates. These were incubated at 22^{o}C for 14 days and then sub-cultured separately and placed under the same conditions for another 10 days. To prepare the inoculum, sterile water was then poured onto the growing colonies, they were gently scraped and the suspension filtered through a double muslin cloth. The concentration of the inoculum was adjusted to 2.0x10^{4} conidia per ml.

Pathogen races (pathotypes) were identified using a set of twelve approved ALS common bean differential genotypes (CIAT, 1995) (Table 4.1).

Table 4.1: Common bean differential genotypes used to characterise *Pseudocercospora griseola* isolates and the binary numbers used to assign isolates to pathogen races.

Differential cultivar	Notation	Seed size	Bean gene pool	Binary value
Don Timoteo	A	L	Andean	1
G11796	B	L	Andean	2
Bolon Bayo	C	L	Andean	4
Montcalm	D	L	Andean	8
Amendoim	E	L	Andean	16
G5686	F	L	Andean	32
PAN 72	G	S	Mesoamerican	1
G2858	H	M	Mesoamerican	2
Flor de Mayo	I	S	Mesoamerican	4
Mexico 54	J	M	Mesoamerican	8
BAT 332	K	S	Mesoamerican	16
Cornell 49242	L	S	Mesoamerican	32

Seed size in 100-seed weight $^{-1}$; L = large (>40 g), M = medium 25-40 g, S = small <25 g

This race identification experiment was carried out in the greenhouse. Each differential was planted in four pots, two seeds per pot, with three replications. Each of the thirteen isolates was tested on the differentials in separate experiments though the general procedures applied were the same for all. The differentials were inoculated at V3 stage of development (first trifoliate leaf open and second trifoliate leaf appears) using a hand sprayer on both the adaxial and abaxial sides of the trifoliate leaf, until runoff. The plants were then covered with a plastic sheet and misted for 4 days to ensure a high relative humidity for disease infection. The plastic cover was removed after 4 days. Scoring for disease was done four times at 3 day intervals from the onset of disease symptoms. Disease severity was based on scores of between 1 (resistant) and 9 (susceptible). The score on the last day was used to classify the differentials as either resistant or susceptible. Differential cultivars with disease scores of 1-3 were classified as resistant, and scores of 4-9 were susceptible (Van Schoonhoven and Pastor-Corrales, 1987). The most virulent race among the isolates tested was used to inoculate the six generations (P_1, P_2, F_1, F_2, BC_1P_1 and BC_1P_2) developed for the generation means analysis trial.

4.2.3 Generation means analysis

Plant materials

The parental genotypes used in this study were obtained from the East and Central Africa Bean Research Network (ECABREN). They included two genotypes resistant (R) to ALS (Mexico 54 and G10909) and two genotypes susceptible (S) to ALS (Super-rosecoco and Wairimu). Mexico 54 and G10909 are of Mesoamerican origin with a type III growth habit (indeterminate, semi-vine). Super-rosecoco has a type I growth habit (determinate), red mottled seeds and is from the Andean gene pool while Wairimu (GLP 585) has a type I growth habit, red kidney seed of Mesoamerican gene pool.

Developing the generations

The four parental genotypes were planted in experimental plots of 3 m rows, 50 cm between rows and 15 cm between plants. They were planted three times at 1 week interval in order to synchronise the flowering of the genotypes. Three crosses, namely Super-rosecoco (S) x Mexico 54 (R), Wairimu (S) x Mexico 54 (R) and Wairimu (S) x G10909 (R), were made using the hooking method (Buishand, 1956). The F_1 seed harvested from each cross was planted in similar experimental plots, with three replications. At flowering the plants were allowed to self pollinate to generate F_2. The F_1 plants were also cross pollinated to their parents P_1 (susceptible) and P_2 (resistant) to get the backcross generations BC_1P_1 and BC_1P_2 respectively (Figures 4.1 and 4.2).

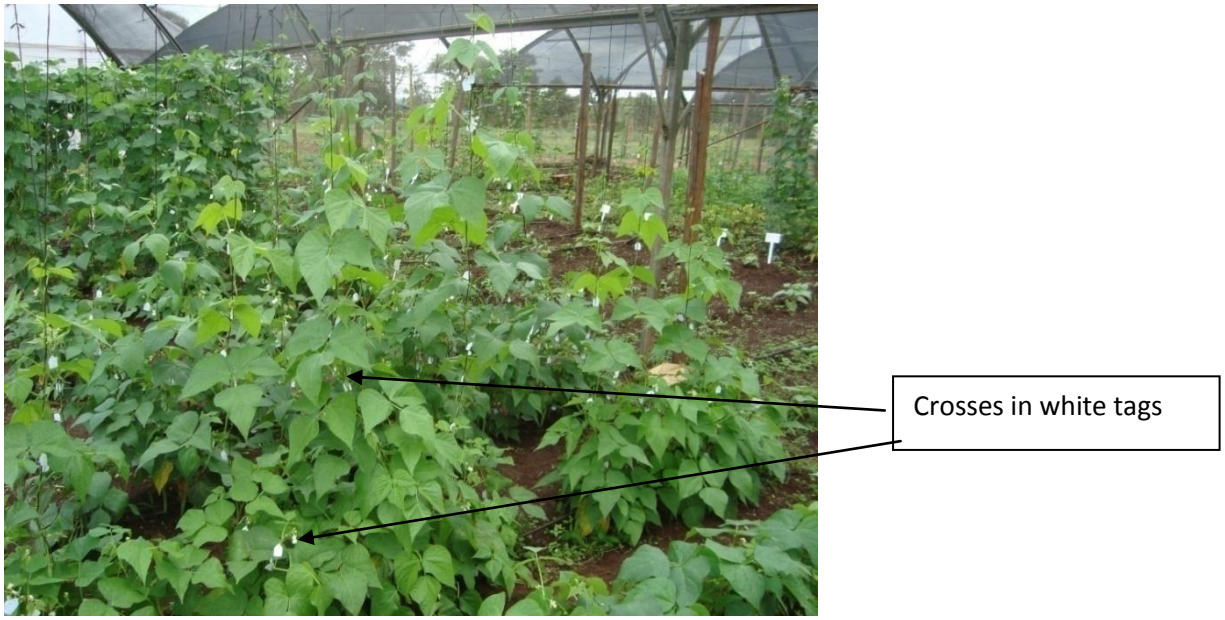

Figure 4.1: Crosses made to develop the generations

Figure 4.2: Successful crosses ready for harvest (in white tags)

Field evaluation of generations and their parental genotypes

The six generations, P_1, P_2, F_1, F_2, BC_1P_1, and BC_1P_2 from each of the three crosses were planted in June 2012 at the Kabete Field Station (Figure 4.3). Each of the three crosses was planted and evaluated in a separate experiment, though the general procedures applied were the same for all. The experiments were laid out in a randomized complete block design with three replications. The experimental plots consisted of 3 m long rows, 50 cm between rows and 15 cm between plants. For each cross per replication there was one row of 25 plants for P_1, P_2 and F_1 generations, four rows of 25 plants each for the F_2 generation, and two rows of 25 plants each for the BC_1P_1 and BC_1P_2 generations. The segregating generations (F_2 and backcrosses) had more plants than the parents and F_1.

All plants were inoculated with the most virulent race of *P. griseola* that was isolated and identified using ALS common bean differentials (section 4.2.2.). All plants were scored for disease severity on symptom appearance, four times at 3 day intervals. A scale of 1 (resistant) to 9 (susceptible) was used. Plants with disease severity scores of 1-3 were classified as resistant, scores of 4-6 were intermediate resistant, and scores of 7-9 were susceptible (Van Schoonhoven and Pastor-Corrales, 1987). The score on the last day was used for the analysis.

Figure 4.3: Evaluation of the Super-rosecoco x Mexico 54 generations

4.2.4 Data analysis

Analysis of variance for each of the three crosses was carried out separately. Where the ANOVA showed significant ($P<0.05$) differences among the generations, separation of means was done using Tukey's procedure for multiple comparisons.

The disease severity scores for the crosses with contrasting parents were subjected to generation means analysis using the methodology proposed by Mather and Jinks (1971) based on the following model:

$g_k = m + (\alpha_k)a + (\delta_k)d + (\alpha_k)^2aa + (\alpha_k\delta_k)ad + (\delta_k)^2dd,$

where,

g_k = mean of generation k

m = mean of the parental homozygotes

α_k and δ_k = coefficients determined by the degree of relationship of generation k

a = additive gene effects

d = dominant gene effects

aa = epistatic effects of additive x additive type

ad = epistatic effects of additive x dominant type

dd = epistatic effects of dominant x dominant type

Estimates of the generation means used in the analysis were obtained after averaging over the replicates.

A stepwise linear regression analysis was carried out using the statistical package Genstat 12th edition (Payne et al., 2009). The regression analysis was weighted based on the inverse of the variance of means and the matrix of coefficient of genetic effects (Mather and Jinks, 1971). The parameters that were acceptable within the model were tested using the R^2 and the "goodness of fit" (F-test) (Ceballos et al., 1998)

The formula used for the F-test was as below:

$$Fc = \frac{[(\text{SSq general model}) - (\text{SSq reduced model})]/\text{difference in df}}{\text{SSq residual from the general model / df residual from the general model}}$$

Where SSq = sums of squares, df = degrees of freedom, Fc = F calculated

The importance of the additive, dominance, and the epistatic effects was determined by analysing the sequential sum of squares derived from addition of each genetic effect in the model. This was determined based on the ratio between the sequential sum of squares and the total sum of squares, after sequentially entering the different effects into the model (Cukadar-Olmedo and Miller, 1997). Significance of the genetic estimates was also determined by comparing the estimated values with their standard errors. The estimate was considered significantly different from zero if its absolute value exceeded twice its standard error. The following genetic parameters were also estimated using formulae from Mather and Jinks (1971):

Environmental variance or error: $\sigma^2e = \frac{1}{4}(\sigma^2P_1 + \sigma^2P_2 + (2\sigma^2F_1))$

Genotypic (G) variance in F_2: $\sigma^2G\ (F_2) = \sigma^2F_2 - \sigma^2e$

Additive (A) variance in F_2: $\sigma^2A\ (F_2) = (2\sigma^2F_2) – [\sigma^2BC_1\ P_1 + \sigma^2BC_1P_2]$

Variance of dominance (D) in F_2: $\sigma^2D\ (F_2) = \sigma^2G\ (F_2) - \sigma^2A\ (F_2)$

Heritability of the traits were calculated as follows,

Broad sense heritability: $H = 100(\sigma^2G\ (F_2) / \sigma^2\ (F_2))$

Narrow sense heritability: $h^2 = 100\ (\sigma^2A\ (F_2) / \sigma^2\ (F_2))$

Where: σ^2P_1 = variance of parent 1; σ^2P_2 = variance of parent 2; σ^2F_1 = variance of F_1; σ^2F_2 = variance of F_2 generation; $\sigma^2BC_1P_1$ = variance of backcross to parent 1; $\sigma^2BC_1P_2$ = variance of backcross to parent 2.

The minimum number of genes involved in controlling resistance to ALS were estimated using the formula by Wright (1968):

$N=(X_1-X_2)^2/8*(\sigma^2F_2 - \sigma^2e)$, where $\sigma^2F_2 - \sigma^2e = \sigma^2G$

N = number of genes, X1 = mean resistance of parent 1, X2 = mean resistance of parent 2, σ^2F_2 = variance of F_2 generation, and σ^2e = environmental variance within the family.

The assumption was that all genes controlling the resistance are unlinked, they affect resistance equally in size and direction, and that there are no dominance or epistasis effects involved.

4.3 Results

4.3.1 Identification of *Pseudocercospora griseola* races

The isolates that were used in the study were pathogenic and varied in their virulence on the common bean differentials. Depending on their virulence reactions, the thirteen isolates were characterised into five physiological races. A race was identified with two numbers separated by a dash (CIAT, 1995). The first and second numbers were obtained by adding the binary values of the susceptible (denoted with 'S') Andean and Mesoamerican differential genotypes respectively. Hence the five physiological races, identified were of the Mesoamerican virulence group and included, 61-37, 62-3, 62-23, 62-39 and 63-39 (Table 4.2).

Table 4.2: Reaction of common bean differentials to selected *Pseudocercospora griseola* isolates

	Differential genotype												Race	Virulence group
	Andean						Mesoamerican							
	A	B	C	D	E	F	G	H	I	J	K	L		
Isolate	1	2	4	8	16	32	1	2	4	8	16	32		
1	S	R	S	S	S	S	S	R	S	R	R	S	61-37	Mesoamerican
2	R	S	S	S	S	S	S	S	R	R	R	R	62-3	Mesoamerican
3	R	S	S	S	S	S	S	S	S	R	S	R	62-23	Mesoamerican
4	R	S	S	S	S	S	S	S	S	R	R	S	62-39	Mesoamerican
5	S	S	S	S	S	S	S	S	S	R	R	S	63-39	Mesoamerican

S = susceptible; R = resistant, Andean differential genotypes: A = Don Timoteo, B = G11796, C = Bolon Bayo, D = Montcalm, E = Amendoim, F = G5686. Mesoamerican differential genotypes: G = PAN 72, H = G2858, I = Flor de Mayo, J = Mexico 54, K = BAT 332, and L = Cornell 49242

For example, race 63-39 was coded as follows; the first value 63 was derived by summing the binary values of the susceptible Andean differential genotypes; Don Timoteo, G11796, Bolon Bayo, Montcalm, Amendoim and G5686 (1+2+4+8+16+32=63). The second value, 39, was a sum of the susceptible Mesoamerican differential genotypes; PAN 72, G2858, Flor de Mayo and Cornell 49242 (1+2+4+32=39). The most virulent isolate identified and used in this study was Mesoamerican race 63-39.

4.3.2 Comparison of means among generations

Analysis of variance of ALS severity scores from the six generations showed highly significant differences among the generations for all the crosses (Table 4.3)

Table 4.3: ANOVA for angular leaf spot severity scores among generations in the crosses, Wairimu x Mexico 54, Super-rosecoco x Mexico 54 and Wairimu x G10909

Treatment	df	SS	Mean square	Fpr
Wairimu x Mexico 54	5	2724.670	544.934	<0.001
Super-rosecoco x Mexico 54	5	3709.319	741.864	<0.001
Wairimu x G10909	5	2955.735	591.147	<0.001

df = degrees of freedom, SS = sum of squares, Fpr = F probability

Results of the mean separation using the Tukey's studentized range test are presented in Table 4.4. For all the three crosses, the disease severity scores of P_2, BC_1P_2 and F_1 were not significantly different from each other, while the severity scores of P_1, BC_1P_1 and F_2, were significantly different from each other and from the severity scores of P_2, BC_1P_2 and F_1 generations.

For the cross Wairimu (S) x Mexico 54 (R) the susceptible parent, Wairimu (P_1) had a disease severity score of 8.1. The resistant parent, Mexico 54 (P_2), had a disease severity score of 2.0. The F_1 disease severity score was 2.2 and it was not significantly different from BC_1P_2 and P_2. The F_2 disease severity score was 4.0 and it was significantly different from P_1, P_2, F_1, BC_1P_1 and BC_1P_2. The backcross to the susceptible parent, BC_1P_1, had a disease severity score of 6.8 and was significantly different and lower than that of its recurrent parent P_1. The backcross to the resistant parent BC_1P_2 had a disease severity score of 2.2 and it was higher but not significantly different from the resistant parent P_2.

For the cross Wairimu (S) x G10909 (R) the susceptible parent, Wairimu (P_1), had a disease severity score of 7.9. The resistant parent, G10909 (P_2), had a disease severity score of 1.9. The F_1 disease severity score was 2.1 and it was not significantly different from the BC_1P_2 and P_2. The F_2 disease severity score was 5.1 and it was significantly different from P_1, P_2, F_1, BC_1P_1 and BC_1P_2. The backcross to the susceptible parent, BC_1P_1, had disease severity score of 6.9 and was significantly different and less than that of its recurrent parent P_1. The backcross to the resistant parent BC_1P_2 had a disease severity score of 2.1 which was not significantly different from the resistant parent P_2.

For the cross Super-rosecoco (S) x Mexico 54 (R), the susceptible parent, Super-rosecoco (P_1), had a disease severity score of 8.0. The resistant parent, Mexico 54 (P_2), had a disease severity score of 1.7. The F_1 disease severity score was 2.0 and it was not significantly different from the BC_1P_2 and P_2. The F_2 disease severity score was 5.6 and it was significantly different from P_1, P_2, F_1, BC_1P_1 and BC_1P_2. The backcross to the susceptible parent, BC_1P_1, had a disease severity score of 7.1 and was significantly different and less than that of its recurrent parent P_1. The backcross to the resistant parent BC_1P_2 had a disease severity score of 1.8 which was not significantly different from the resistant parent P_2.

Table 4.4: Tukey's studentized range test for comparison of angular leaf spot disease severity score means ± standard errors in three S x R crosses

Generation	Wairimu(S) x Mexico 54 (R)	Wairimu (S) x G10909 (R)	Super-rosecoco (S) x Mexico 54 (R)
P_1	8.1 ±0.11 A	7.9 ±0.12 A	8.1 ±0.12 A
BC_1P_1	6.8 ±0.17 B	6.9 ±0.15 B	7.1 ±0.17 B
F_2	4.0 ±0.11 C	5.1 ±0.09 C	5.6 ±0.11 C
F_1	2.2 ±0.13 D	2.1 ±0.12 D	2.0 ±0.13 D
BC_1P_2	2.2 ±0.07 D	2.7 ±0.07 D	1.8 ±0.08 D
P_2	2.0 ±0.10 D	1.9 ±0.10 D	1.7 ±0.09 D

Means followed by the same letter for each cross are not significantly different at $P<0.05$. R and S = Resistant and susceptible, respectively.

The frequency distributions for the three crosses are presented in Figures 4.4, 4.5 and 4.6. For the cross Wairimu (S) x Mexico 54 (R), the frequency distribution for P_1 (Wairimu) was skewed to the right (higher disease severity score), while that of P_2 (Mexico 54) was skewed to the left (lower disease severity score). The distribution of the BC_1P_1 was skewed to the right, while that of BC_1P_2 was skewed to the left, similar to their recurrent parents. The F_1 plants had disease severity scores that were similar to the resistant parent, hence skewed towards the P_2. The F_2 generation showed a more continuous distribution.

For the cross Wairimu (S) x G10909 (R) the frequency distribution for P_1 (Wairimu) was skewed to the right (higher disease severity score), while that of P_2 (G10909) was skewed to the left (lower disease severity score). The distribution of the BC_1P_1 was skewed to the right, while that of BC_1P_2 was skewed to the left, similar to their recurrent parents. The F_1 plants had disease

severity scores that were similar to the resistant parent hence skewed towards the P_2. The F_2 generation showed a more continuous distribution.

The cross Super-rosecoco (S) x Mexico 54 (R) had a frequency distribution for P_1 (Super-rosecoco) that was skewed to the right (higher disease severity score), while that of P_2 (Mexico 54) was skewed to the left (lower disease severity score). The distribution of the BC_1P_1 was skewed to the right, while that of BC_1P_2 was skewed to the left, similar to their recurrent parents. The F_1 plants had disease severity scores that were similar to the resistant parent hence skewed towards the P_2. The F_2 generation showed a more continuous distribution.

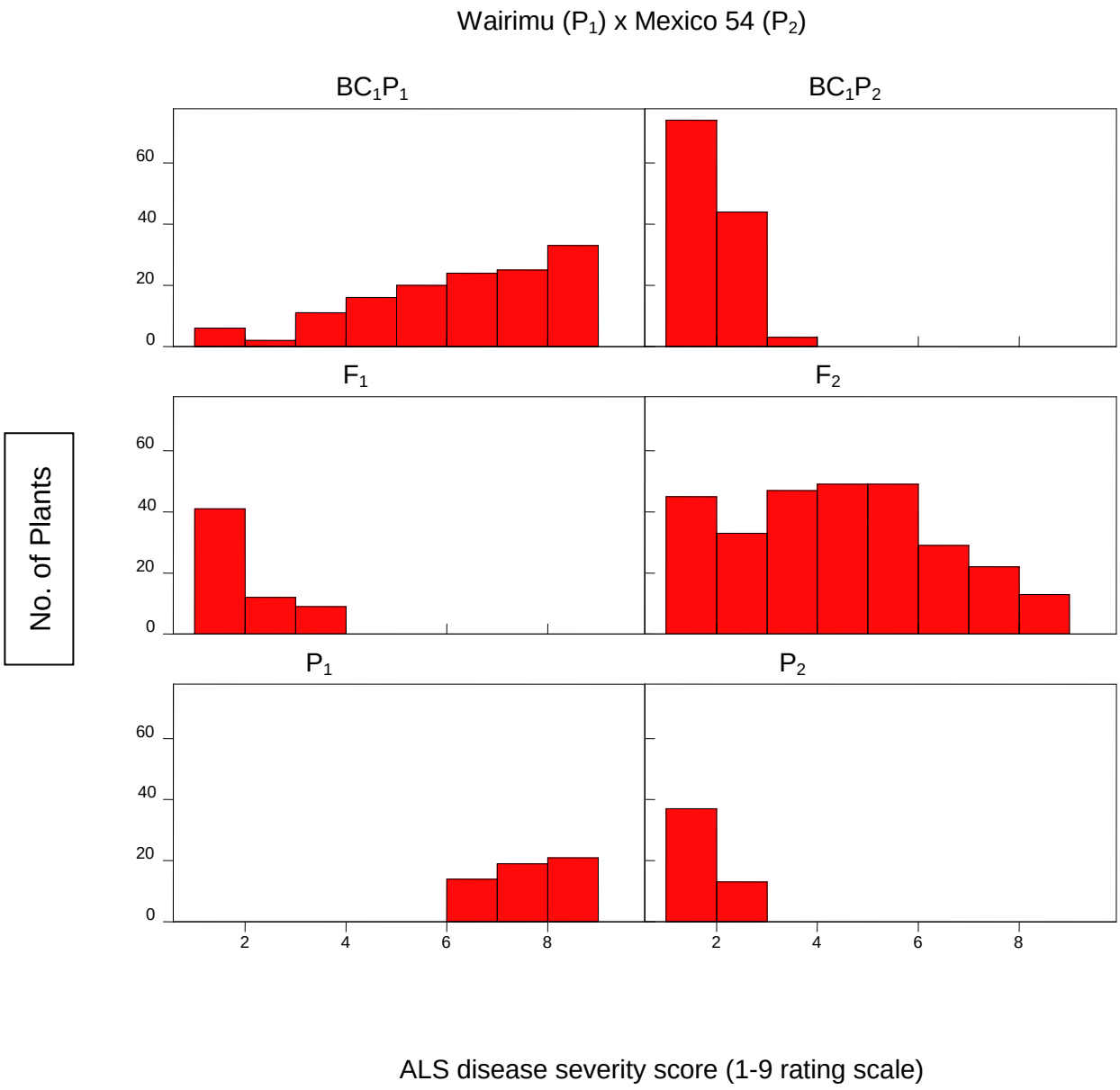

Figure 4.4: Frequency distribution of angular leaf spot disease severity scores for six generations of the cross Wairimu (S) x Mexico 54 (R)

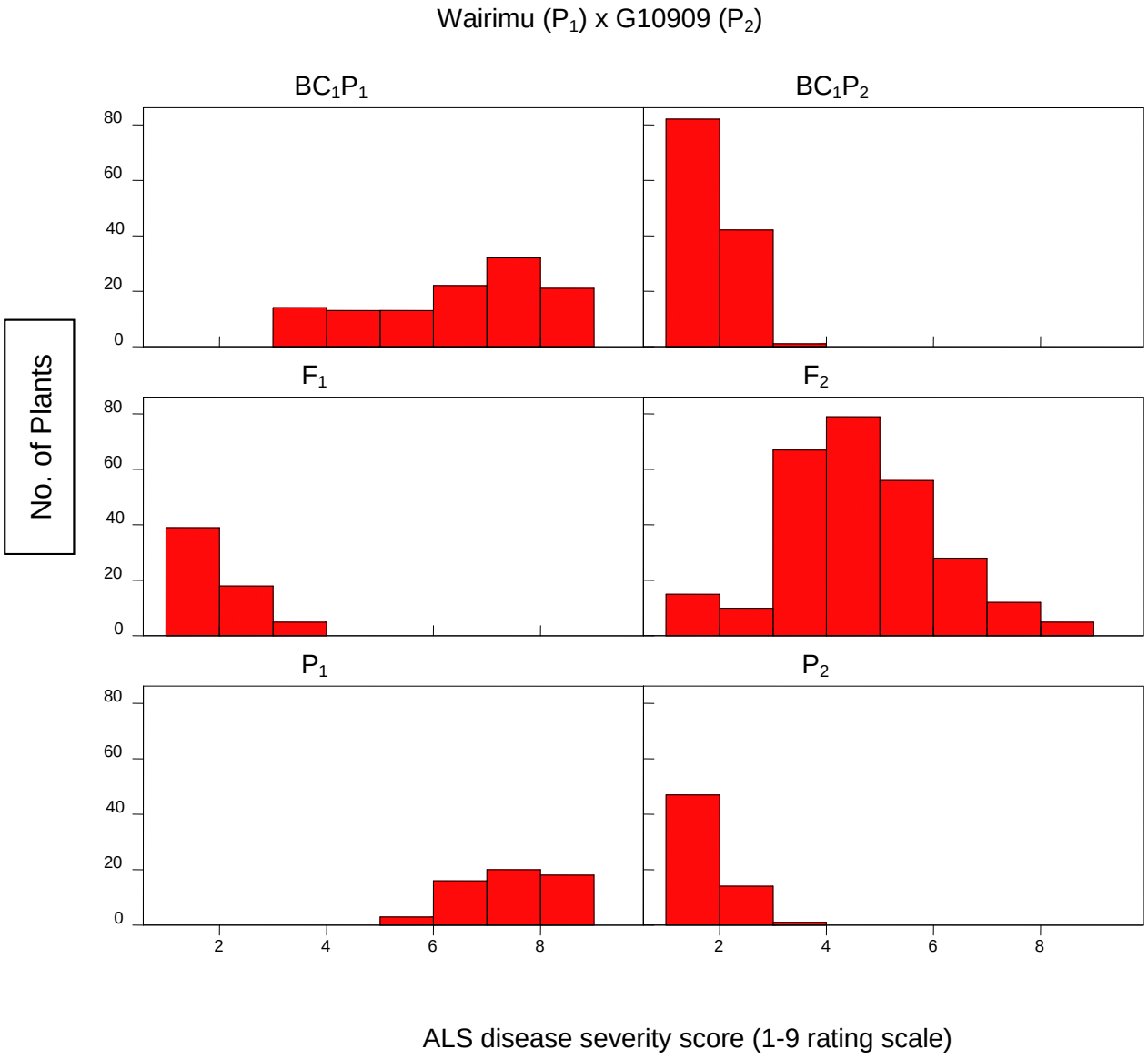

Figure 4.5: Frequency distribution of angular leaf spot disease severity scores for six generations for the cross Wairimu (S) x G10909 (R)

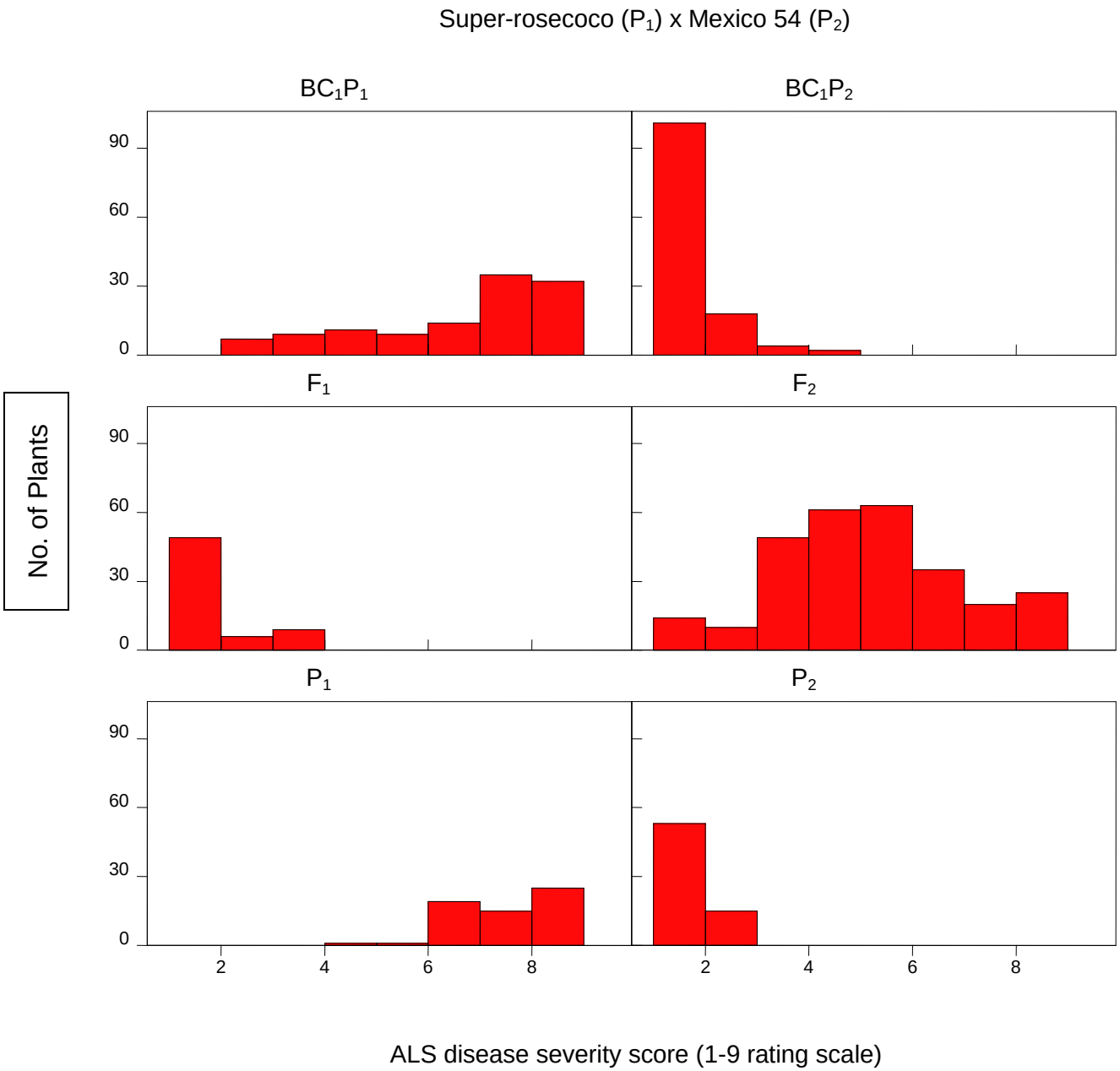

Figure 4.6: Frequency distribution of angular leaf spot disease severity scores for six generations of the cross Super-rosecoco (S) x Mexico 54 (R)

4.3.3 Gene effects for disease resistance

For each of the three crosses, a total of nine regression models were fitted. On the basis of R^2 and 'goodness of fit' (F test), a three parameter model (m + a + d) estimating additive (a) and dominance (d) genetic effects was chosen as the best.

The sum of squares calculated for the full model indicated that the additive and dominance gene effects contributed more to the sum of squares as compared to the interaction effects (Table 4.5). For the cross Wairimu x Mexico 54, the additive and dominance gene effects contributed 95.0% of the total sum of squares, while for Super-rosecoco x Mexico 54, and Wairimu x G10909, the gene effects contributed 86.4% and 90.1% respectively. The combined interaction effects contributed 5.0% to the sum of squares in the cross between Wairimu x Mexico 54, 13.6% in Super-rosecoco x Mexico 54, and 9.9% in Wairimu x G10909.

Table 4.5: Percentage contribution of gene effects to total sum of squares in the full model for the three crosses

	Wairimu x Mexico 54		Super-rosecoco x Mexico 54		Wairimu x G10909	
Gene effects	Model SSQ	% SSQ	Model SSQ	% SSQ	Model SSQ	% SSQ
a	85.6	81.1	95.5	76.5	85.8	79.2
d	14.7	13.9	12.3	9.9	11.8	10.9
aa	1.0	0.9	11.4	9.1	6.6	6.1
ad	2.8	2.7	5.6	4.5	4.0	3.7
dd	1.5	1.4	0.02	0.02	0.1	0.1
Total SSQ	105.6		124.8		108.3	

Gene effects; a = additive gene effects, d = dominance gene effects, aa = epistatic effects of additive x additive type, ad = epistatic effects of additive x dominance type, dd = epistatic effects of dominance x dominance type. SSQ = sum of squares; %SSQ = % relative contribution to the model SSQ

The estimates of gene effects, R^2 and the dominance ratio are presented in Table 4.6. The additive-dominant model had a coefficient of variation R^2 of 98.1% in the Wairimu x Mexico 54 cross. The R^2 for the Super-rosecoco x Mexico 54, and the Wairimu x G10909 crosses was 93.9% and 94.9% respectively.

Table 4.6: Estimates of gene effects for resistance to angular leaf spot for three common bean crosses fitted to a three parameter model

Crosses	m	a	d	R^2	d/a
Wairimu x Mexico 54	5.1	3.2±0.12**	-2.7±0.26**	98.1	0.9
Super-rosecoco x Mexico 54	4.9	3.3±0.24***	-2.7±0.47***	93.9	0.8
Wairimu x G10909	5.0	3.2±0.22***	-2.5±0.41***	94.9	0.8

Significance based on t-test; **, *** indicates term is significant at p<0.001 and p<0.0001 respectively. m = midparent value, a = additive gene effects, d = dominance gene effects, R^2 = coefficient of variation, d/a = dominance ratio

The additive gene effects were 3.2, 3.3, and 3.2 for the crosses, Wairimu x Mexico 54, Super-rosecoco x Mexico 54 and Wairimu x G10909 respectively. The dominance effects were all negative and the crosses Wairimu x Mexico 54, Super-rosecoco x Mexico 54, and Wairimu x G10909 had values of -2.7, -2.7, and -2.5 respectively. The dominance to additive gene effects ratio was 0.9 for Wairimu x Mexico 54, 0.8 for Super-rosecoco x Mexico 54, and 0.8 for Wairimu x G10909.

4.3.4 Heritability estimates and minimum number of genes controlling disease resistance

For the cross Wairimu x Mexico 54, the genetic variance estimate σ^2_A was 2.1, while σ^2_D was 0.4 (Table 4.7). The other estimates of genetic variance σ^2_G and σ^2_E were 2.5 and 0.8 respectively. The broad-sense heritability estimate (H) was 75.4% while the narrow sense heritability estimate (h^2) was 64.2%. For the cross Super-rosecoco x Mexico 54 the genetic variance estimate σ^2_A was 2.4, while σ^2_D was 0.02. The other estimates of genetic variance σ^2_G and σ^2_E were 2.5 and 0.9 respectively. The broad-sense heritability estimate (H) was 72.4% while the narrow sense heritability estimate (h^2) was 71.7%.

For the cross Wairimu x G10909 the genetic variance estimate σ^2_A was 1.2, while σ^2_D was 0.2. The other estimates of genetic variance σ^2_G and σ^2_E were 1.4 and 0.8 respectively. The broad-sense heritability estimate (H) was 73.6% while the narrow sense heritability estimate (h^2) was 52.9%. The minimum number of genes controlling resistance to ALS for the cross Wairimu x Mexico 54 was 2, while for Super-rosecoco x Mexico 54 and Wairimu x G10909, resistance was controlled by 2 and 3 genes respectively.

Table 4.7: Estimates of genetic variance, broad and narrow sense heritability, and minimum number of genes estimates for resistance to angular leaf spot in three crosses of common bean

Population	σ^2_A	σ^2_D	σ^2_G	σ^2_E	H	h^2	MNG
Wairimu x Mexico 54	2.1	0.40	2.5	0.8	75.4	64.2	2
Superosecoco x Mexico 54	2.4	0.02	2.5	0.9	72.4	71.7	2
Wairimu x G10909	1.2	0.20	1.4	0.8	63.6	52.9	3

σ^2_A = additive genetic variance estimate; σ^2_D = dominance genetic variance estimate; σ^2_E = environmental variance estimate; H = broad sense heritability estimate; h = narrow sense heritability estimate; MNG = minimum number of genes

4.4 Discussion and conclusion

Genotypes that have been identified as sources of resistance react differently to the pathogen races found in a region. The aim of the study was to identify the different pathogen races in Kabete and use the most virulent race to determine the mode of inheritance of genes that confer resistance to angular leaf spot in common bean genotypes Mexico 54 and G10909. A single pathogen race has been used in previous studies to study mode of inheritance of resistance to ALS in common bean genotypes (Mahuku et al., 2011; Sanglard et al., 2013). The use of only one race is essential to ensure that the inheritance studies are accurate. It gives insight as to whether the resistance is conditioned by major genes or minor genes. With this information breeders can utilise the source of resistance to breed for either race specific or non-race specific resistance.

Results from this study indicated high virulence variability among the *P. griseola* isolates. Of the thirteen isolates used, five physiological races 61-37, 62-3, 62-23, 62-39 and 63-39, of the Mesoamerican virulence group were characterised. The Mesoamerican races have been shown to infect both the Andean and the Mesoamerican gene pools of the common bean hence their occurrence in large numbers than the Andean races. The occurrence of the Mesoamerican races could also be attributed to the different production practices whereby farmers produce common bean varieties from both the Andean and Mesoamerican gene pools. Co-evolution of the pathogen and the common beans has led to the existence of races corresponding to the common bean gene pools (Guzman et al., 1995; Pastor-Corrales, 1996; Mahuku et al., 2002b; Wagara et al., 2005)**.** Of the five races identified, only one, race 63-39 was used in the study because it was the most virulent of the five.

The analysis of variance for response to ALS in each of the three crosses showed that the six generations (P_1, P_2, F_1, F_2, BC_1P_1 and BC_1P_2), were significantly different. In the three crosses, Wairimu (S) x Mexico 54 (R), Super-rosecoco (S) x Mexico 54 (R), and Wairimu (S) x G10909 (R), the disease severity scores of both parents (P_1 and P_2) were contrasting, with both extremes of resistance and susceptible. This implies the pattern of response was due to genetic differences among the two parents P_1 and P_2. The susceptible parents Wairimu and Super-rosecoco are common bean varieties currently grown by the farmers in Kenya and hence need their resistance improved. The P_2 disease severity scores were low for all the crosses indicating a resistant reaction. This showed that, even though Mexico 54 and G10909 have been identified as good sources of resistance, they are not immune to the pathogen. The results

confirmed that the parents chosen for the study were contrasting in respect to disease reaction, which is essential for a generation means analysis, as proposed by Mather and Jinks (1971).

The disease severity for the F_1 generation of the three crosses was low but similar to that of the resistant parent (P_2). This shows that resistance in P_2 was dominant over susceptibility in P_1. The F_2 generation had disease severity scores that were intermediate and similar to the mid-parent value. In addition, the variation in the F_2 generation for all the three crosses was approximately normal with a continuous distribution; a pattern consistent with quantitative resistance. This segregation makes it possible to select for the resistant plants which can be advanced to F_3 in a breeding programme. The backcross, BC_1P_1 generation for the three crosses had disease severity scores that showed a susceptible reaction to the disease and were similar to P_1, though the disease severity mean score was lower than the parents. The backcross BC_1 P_2 generation for the three crosses had a disease severity score that was not significantly different from the P_2 which showed resistance.

Based on the best model chosen (additive-dominance) in this study and the contribution to the total sum of squares; the dominance and additive effects (predominant) were more important in controlling ALS compared to the digenic interactions. This implies epistasis was not important in the inheritance of disease resistance in the genotypes used. The importance of the additive and dominance effects was also shown by the high R^2 values obtained for the three crosses. The estimates of the additive effects were higher than the estimates of the dominance effects meaning that the additive effects contributed more to the control of ALS resistance than the dominance effects. The dominance effects for the three crosses were all negative. This indicated that the dominance effects decreased the disease severity score and hence increased resistance. The dominance to additive effects ratio was on average 0.8 for the three crosses indicating incomplete dominance for resistance.

The results are similar to those of Borel et al. (2011), who showed that genetic control of angular leaf spot reaction in the common bean leaves and pods of the cross Carioca MG x ESAL 686, was dominated by additive gene effects. Similar results were reported from Tanzania in crosses between four resistant genotypes (Mexico 54, BAT 332, Amendoim and G5686) and two susceptible local genotypes (Kablanketi and Spenjeli) (Fivawo et al., 2013). In this study epistatic effect had a small and non-significant contribution to ALS disease resistance in the three crosses. Other studies have also shown that the additive, or additive-dominance effects are more prevalent than epistatic effects in generation means analysis for common beans traits

such as disease and insect-pest resistance, heat tolerance, and climbing ability (Hanson et al., 1993; Park et al., 1994; Rainey and Griffiths, 2005; Checa et al., 2006; Borel et al., 2011; Ojwang' et al., 2011; Fivawo et al., 2013).

The additive genetic variance estimates (σ^2_A) were higher than the dominance genetic variance estimates (σ^2_D) for the three crosses. In addition, heritability estimates both in the narrow sense and the broad sense were moderately high (ranging between 53% and 75%). This high narrow sense heritability estimate suggests a large contribution of the additive genetic effects on the phenotypic expression of the ALS resistance and that selection of the traits would be highly efficient. Heritability estimates are population-specific and are influenced by environmental conditions and hence, variations among studies. Borel et al. (2011) reported high heritability estimates for ALS using genotypes ESAL 686 and Cornell 69242, (H 61%, h 60%) as well as ESAL 686 and Carioca MG, (H 92 %, h 81%) A cross between common bean genotypes 'Kablanketi' and Mexico 54 had an estimated heritability of 72% (Tryphone et al., 2012). Oblessuc et al. (2012), showed that heritability varied with the conditions in the dry season (H=51%), wet season (H=81%) and in the greenhouse (H=69%) for the cross IAC-UNA x CAL 143. The minimum number of genes controlling resistance to ALS ranged between 2 and 3 for all the three crosses. Resistance to ALS disease has been shown to be inherited quantitatively (Caixeta et al., 2002; Mahuku et al., 2004; Oblessuc et al., 2012).

In conclusion, knowledge on the type of gene action conditioning resistance to ALS will enable breeders to develop race specific or non-race specific resistant varieties. In the two genotypes Mexico 54 and G10909, both dominance and additive gene action were significant in the expression of resistance to ALS. However, additive gene action was more important than dominance gene action. Hence it could be quantitatively inherited. The moderately high narrow sense heritability estimate shows that gain in selection for ALS resistance is possible in early generations**.**

References

Borel, J.C., M.A.P. Ramalho, A.F.B. Abreu, and L.G.S. Maia. 2011. Genetic control of angular leaf spot reaction in common bean leaves and pods. Scientia Agricola 68:661-664.

Buishand, T.J. 1956. The crossing of beans. Euphytica 5:41-50.

Caixeta, E.F., A. Bor´em, N.G. de Morais Silvia, R.C. Rocha, E.G. de Barros, and M.A. Moreira. 2002. Teste de alelismo para genes do feijoeiro que conferem resistencia ao fungo *Phaeoisariopsis griseola*. VII Congresso Nacional de Pesquisa de Jeijiao. Universidade Federal de Vicosa, Vicosa-MG, Brazil.

Caixeta, E.T., A. Bor´em, S. de Azevedo 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 134:297-303.

Caixeta, E.T., A. Bore´m, A.L. Alzate-Marin, S. de Azevedo Fagundes, M.G. de Morais Silva, E.G. de Barros, and M.A. Moreira. 2005. Allelic relationships for genes that confer resistance to angular leaf spot in common bean. Euphytica 145:237-245.

Carvalho, G.A., T.J. Paula, A.L. Alzate-Marin, S. Nietsche, E.G. de Barros, and M.A. Moreira. 1998. Inheritance of resistance to angular leaf spot of common bean in AND 277 to race 63–23 of *Phaeoisariopsis griseola* and identification of a RAPD marker linked to the resistance gene. Fitopatologia Brasil 23:482-485.

Ceballos, H., S. Pandey, L. Narro, and J.C. Perez-Velazquez. 1998. Additive, dominant and epistatic effects for maize grain yield in acid and non-acid soils. Theoretical and Applied Genetics 96:662-668.

Checa, O., H. Ceballos, and M.W. Blair. 2006. Generation means analysis of climbing ability in common bean (*Phaseolus vulgaris* L.). Journal of Heredity 97:456-465.

CIAT. 1995. Annual Bean report, Bean Progam. CIAT, Cali, Colombia.

Correa, R.X., P.I.V. Good-God, M.L.P. Oliveira, S. Nietsche, M.A. Moreira, and E.G. Barros. 2001. Inheritance of resistance on the common bean angular leaf spot and identification of molecular markers flanking the resistance locus. Fitopatologia Brasil 26:27-32.

Cukadar-Olmedo, B., and J.F. Miller. 1997. Inheritance of the stay green trait in sunflower. Crop Science 37:150-153.

Fivawo, N.C., S.N. Msolla, and R. Vats. 2013. Heritability of angular leaf spot resistance in populations of common bean developed using G5686, Mexico 54, Amendoim and BAT 332, as donor parents. International Journal of Science Innovations and Discoveries 3:38-42.

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

Hanson, P.M., M.A. Pastor-Corrales, and J.L. Kornegay. 1993. Heritability and sources of ascochyta blight resistance in common bean. Plant Disease 77:711-714.

Mahuku, G., C. Jara, H. Teran, and S. Beebe. 2003. Inheritance of angular leaf spot resistance in selected common bean genotypes. Annual Report Bean Improvement Cooperative 46:151-152.

Mahuku, G.S., and A.M. Iglesias. 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. 2002a. Genetic variability within *Phaeoisariopsis griseola* from central America and its implications for resistance breeding. Plant Pathology 51:594-604.

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

Mahuku, G.S., C. Montoya, M.A. Henrı´quez, C. Jara, H. Teran, and S. Beebe. 2004. Inheritance and characterization of the angular leaf spot resistance gene in the common bean accession, G 10474 and identification of an AFLP marker linked to the resistance gene. Crop Science 44:1817-1824.

Mahuku, G.S., M.A. Henrı´quez, C. Montoya, C. Jara, H. Teran, and S. Beebe. 2011. Inheritance and development of molecular markers linked to angular leaf spot resistance genes in the common bean accession G10909. Molecular Breeding 28:57-71.

Mather, K., and L. Jinks. 1971. Biometrical genetics. Cornell University Press, Ithaca, New York, USA.

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

Mwang'ombe, A.W., I.N. Wagara, J.W. Kimenju, and R.A. Buruchara. 2007. Occurence and severity of angular leaf spot of common bean in Kenya as influenced by geographical location, altitude and agroecological zones. Plant Pathology 6:235-241.

Namayanja, A., R. Buruchara, G.S. 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. Borém, G.A. Carvalho, R.C. Rocha, T.J. Paula-Jr, E.G. Barros, and M.A. Moreira. 2000. RAPD and SCAR markers linked to a gene conferring resistance to angular leaf spot in common bean. Journal of Phytopathology 148:117-121.

Oblessuc, P.R., R.M. Baroni, A.A.F. Garcia, A.F. Chioratto, S.A.M. Carbonell, L.E.A. Camargo, and L.L. Benchimol. 2012. Mapping of angular leaf spot resistance QTL in common bean (*Phaseolus vulgaris* L.) under different environments. BMC Genetics 13:50:1-9.

Ojwang', P.P.O., R. Melis, S.M. Githiri, and J.M. Songa. 2011. Genetic analysis for resistance to bean fly (*Ophiomyia phaseoli*) and seed yield among common bean genotypes in a semi-arid environment. Field Crops Research 120:223-229.

Park, S.J., P.R. Timmins, D.T. Quiring, and P.Y. Jui. 1994. Inheritance of leaf area and hooked trichome density of the first trifoliate leaf in common bean. Canadian Journal of Plant Science 74:235-240.

Parlevliet, J.E. 1979. Components of resistance that reduce the rate of epidemic development. Annual Review Phytopathology 17:203-222.

Pastor-Corrales, M.A. 1996. Traditional and molecular confirmation of the coevolution of beans and pathogens in Latin America. Annual Report Bean Improvement Cooperative 39:46-47.

Pastor-Corrales, M.A., and A.W. Saettler. 1989. Angular leaf spot, p. 59-76, *In* H.F. Shwartz and M. A. Pastor-Corrales, eds. Bean production problems in the tropics. CIAT, Cali, Colombia.

Pastor-Corrales, M.A., C. Jara, and S.P. 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.

Pastor-Corrales, M.A., O.A. Erazo, E.L. Estrada, and S.P. Singh. 1994. Inheritance of anthracnose resistance in common bean accession G 2333. Plant Disease 78:959-962.

Payne, R.W., D.A. Murray, S.A. Harding, D.B. Baird, and D.M. Soutar. 2009. GenStat for Windows (12th Edition) Introduction. VSN International, Hemel Hempstead, UK.

Rainey, K.M., and P.D. Griffiths. 2005. Inheritance of heat tolerance during reproductive development in snap bean (*Phaseolus vulgaris* L.). American Society of Horticultural Science 130:700-706.

Sanglard, D.A., C.A.G. Ribeiro, B.P. Balbi, K.M.A. Arruda, E.G. Barros, and M.A. Moreira. 2013. Characterisation of the angular leaf spot gene present in common bean cultivar Ouro Negro. Journal of Agricultural Science 5:19-23.

Sartorato, A., S. Nietsche, E.G. Barros, and M.A. Moreira. 2000. RAPD and SCAR markers linked to resistance gene to angular leaf spot in common bean. Fitopatologia Brasil 25:637-642.

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. Advanced Applied Microbiology 52:209-243.

Tryphone, G.M., L.A. Chilagane, P.M. Kusolwa, and S. Nchimbi-Musola. 2012. Inheritance of angular leaf spot [*Phaeoisariopsis griseola* (Sacc.) Ferr] resistance in common bean (*Phaseolus vulgaris* L.) population developed from Kablanketi x Mexico 54. Journal of Agricultural Science and Technology 2:856-862.

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

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

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.

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

Wright, S. 1968. The genetics of qualitative variability. *In* S. Wright, ed. Evaluation and genetics of populations. Volume 1. Genetics and Biometrics Foundation. University of Chicago Press, Chicago, USA.

CHAPTER 5

Development of a breeding method for durable resistance to angular leaf spot in common bean

Abstract

Durable resistance, conditioned by minor resistance genes, has been described as more reliable in managing ALS in the long term. The aim of this study was to develop a breeding method for durable ALS resistance in common bean and use it to accumulate minor genes into single genotypes. Four genotypes with intermediate resistance to ALS were selected from an initial 182 genotypes and used to generate a double cross segregating population. The F_1 was planted in a nethouse and inoculated with a mixture of races of the ALS pathogen. The genotypes that showed intermediate resistance to ALS were subsequently selected from the F_1 to the F_3 segregating populations. In the F_3 generation resistant plants were selected and the F_4 plants were evaluated, together with the parents and several market class varieties. Data were collected on ALS disease severity, seed yield, days to physiological maturity, seed size, seed colour and growth habit. Ten F_4 advanced lines with enhanced levels of resistance (disease score 1.9-3.2) were selected. These lines had improved resistance when compared to their parents (disease score 4.6-4.8) and market class varieties (disease score 5.4-8.2), which confirmed breeding progress for resistance to ALS. Simultaneous selection was done for seed yield, seed size, farmer preferred seed type and growth habit. The results of the study have shown that it is possible to develop ALS resistant common bean lines through the double cross method, using a mixture of ALS races.

5.1 Introduction

The angular leaf spot (ALS) pathogen *Pseudocercospora griseola* (*P. griseola*) has been shown to be highly variable in Kenya (Wagara et al., 2005) and other parts of the world (Pastor-Corrales et al., 1998; Mahuku et al., 2002; Damasceno e Silva et al., 2008). Hence breeding for resistance is complex due to the occurrence of many races of the pathogen (Mahuku et al., 2002). The host resistance to *P. griseola* could be inherited as monogenic, oligogenic (Mahuku et al., 2009; Mahuku et al., 2011) or polygenic.

The use of minor genes of resistance (polygenic) has been recommended for durable resistance breeding programmes (Van der Plank, 1968; Robinson, 1980). However, it is difficult to differentiate the expression of resistance conditioned by major and minor genes (Parlevliet and van Ommeren, 1988). The presence of the major genes confounds selection for the minor genes during breeding (Parlevliet, 1983). There is, therefore, need to separate the two types of resistance so as to be able to accumulate the minor genes in the absence of the major genes. In a breeding programme for minor gene resistance, selection against major genes could be done by removing the resistant or immune plants and selecting genotypes with intermediate resistance (Parlevliet and van Ommeren, 1988). A mixture of races can be used on a starting population with intermediate resistance (Parlevliet, 1983; Parlevliet and van Ommeren, 1988). Parlevliet (1985) described intermediate resistance, as the resistance that reduces levels of the pathogen sporulation, despite being infected and termed it durable. Durable resistance is the resistance that will last for a long time (Johnson, 1981). However, the length of time the resistance will last cannot be determined during the breeding process.

Gamete selection was proposed by Singh (1994) as a method to improve traits with alleles originating from multiple parents. He indicated that the basis for gamete selection is the multiple parent crosses that produce heterogametes in the male and female parents of the double cross. This method has been successfully used to improve several traits in common bean, including seed yield, seed quality and resistance to bean common mosaic virus (BCMV) and rust (Singh et al., 2008); plant architecture and multiple resistance to five diseases (ALS, anthracnose, bean common mosaic, bean golden mosaic and common bacterial blight) and the leafhopper (Singh et al., 1998); resistance to white mould (Teran and Singh, 2009); multiple resistance to common and halo bacterial blights (Asensio et al., 2006); and resistance to different bacterial, fungal and viral diseases in one variety (Teran et al., 2013).

The aim of this study was to develop a breeding method for durable ALS resistance in common bean using a double cross population. The method was then used to accumulate minor genes of resistance to ALS into single genotypes.

5.2 Materials and Methods

5.2.1 Study site and parental selection

Parental selection was conducted in the greenhouse at Kabete Field Station. Kabete is located at coordinates 01^{o}14'59.7"S; 036^{o}44'28.8"E with an altitude of 1820 m above sea level. The area receives an average rainfall of 1046 mm annually, with a mean maximum temperature of 23^{o}C and mean minimum temperature of 12^{o}C. The soils are dark red or brown friable clay.

5.2.2 Parental selection

A total of 182 genotypes were screened for resistance to angular leaf spot. They included 159 Kenyan landraces sourced from the Kenyan Genebank, and 23 Rwandan landraces sourced from ECABREN.

The 182 genotypes were planted on 8th November 2010 in five pots (size, 18x18x18 cm) each, two seeds per pot and replicated three times. The soil in the pots was collected from Kabete Field Station and mixed with chicken manure at a ratio of 3:2. The genotypes were inoculated with a mixture of the *P. griseola* races (where pathogen population is not defined) (isolated as indicated in Chapter 3, section 3.2.3 of this Thesis) at the V3 stage of development (where the first trifoliate leaf is open and the second trifoliate leaf appears). The first trifoliate leaf was inoculated on both sides of the leaf until runoff, using a hand sprayer. The plants were then covered for 4 days using clear polythene to increase the relative humidity and allow for the pathogen to infect the plants. On symptom appearance, data were collected on disease severity four times at 3 day intervals. The score on the last day was used in the analysis. Disease severity was based on scores of between 1 and 9, where 1 was resistant and 9 was susceptible. The scores were further classified as follows: 1-3 was resistant, 4-6 intermediate resistant and 7-9 susceptible (Van Schoonhoven and Pastor-Corrales, 1987).

Thirty intermediate resistant genotypes (disease score 4-6) were selected and planted on 30th March 2011 in five pots each, two seeds per pot and replicated three times. These were then screened again for ALS resistance using a mixture of *P. griseola* races. Four intermediate resistant genotypes, two from each common bean gene pools (Andean and Mesoamerican),

were subsequently selected and used as parents to develop an inter-gene pool double cross segregating population.

5.2.3 Development of the inter-gene pool double cross population and advancement to F_3

The four selected parents were planted on 28th June 2011 at the Kabete nethouse, in single row plots of 10 plants, spaced at 15 cm between plants and 50 cm between rows and replicated three times. The parents were crossed as follows: Parent A x Parent B and Parent C x Parent D to produce single crosses, $F_{1\ (AB)}$ and $F_{1\ (CD)}$. The single crosses were planted on 11th October 2011 at the Kabete nethouse in pots (18x18x18 cm). The F_1 single cross progeny were then crossed as $F_{1\ (AB)}$ X $F_{1\ (CD)}$ (Figure 5.1) to generate the double cross population, $F_{1\ (ABCD)}$ and subsequent generations were developed as shown in Table 5.1.

Figure 5.1: Successful cross $F_{1(AB)}$ x $F_{1(CD)}$

Table 5.1: Methodology used to accumulate minor genes of resistance to angular leaf spot

Season	Cross	Cross	Description
Season 1	AxB ↓ $F_{1(AB)}$	CxD ↓ $F_{1\ (CD)}$	Four parents (with intermediate resistance to ALS and from different gene pools) were selected and single crosses produced (heterogametic parents)
Season 2	$F_{1(AB)}$ x $F_{1\ (CD)}$ ↓ $F_{1(ABCD)}$		Hybridization was done to produce a double cross population (assumed to have accumulated minor genes of resistance)
Season 3	$F_{1(ABCD)}$ ↓ F_2		$F_{1\ (ABCD)}$ was planted and evaluated for resistance to angular leaf spot. The intermediate resistant plants were advanced to F_2.
Season 4	F_2 ↓ F_3		The F_2 seeds were planted in a plant to progeny row and evaluated for ALS resistance. Intermediate resistant plants within each selected family were harvested, bulked and advanced to F_3
Season 5	F_3 ↓ F_4		Selected families were planted in rows and screened for resistance to ALS. Selection of resistant plants within and between families was carried out, and they were advanced to F_4
Season 6	Evaluation of F_4		The selected F_4 plants from the population, their parents (A, B, C, D), and selected market class varieties were evaluated for resistance, yield and other agronomic traits under three replications.

NB: Only single plant selection was done from F_1 to F_4

The double cross $F_{1(ABCD)}$ seed was planted on 21st June 2012 in 3 m single row plots of 20 plants, spaced at 15 cm between plants and 50 cm between rows (Figure 5.2). They were inoculated with a mixture of *P. griseola races* (isolated as indicated in Chapter 3, section 3.2.3 of this Thesis) at the V3 stage of development. The intermediate resistant lines were selected and advanced to F_2. The F_2 seed was planted on 10th October 2012 as plant to progeny rows in 3 m single row plots of 20 plants per row spaced at 15 cm between plants and 50 cm between rows. They were inoculated with a mixture of *P. griseola* races and intermediate resistant plants within each selected family were harvested, bulked and advanced to F_3. The selected F_3 families were planted on 16th January 2013 in 3 m single row plots of 20 plants spaced at 15 cm between plants and 50 cm between rows. They were inoculated with a mixture of *P*. *griseola* isolates and ALS resistant plants selected and advanced to F_4.

Figure 5.2: $F_{1(ABCD)}$ planted at Kabete nethouse

5.2.4 Experimental design and evaluation of F_4 lines

Eleven F_4 lines, the four parental genotypes used in the double cross and seven market class varieties were evaluated in the Kabete nethouse. The market class varieties included Super-rosecoco, GLP 24, GLP 2, GLP 585, New-rosecoco, GLP X92 and KAT 69. The experiment was set up in a randomized complete block design with three replications. The common beans were planted on 26th April 2013 in five pots (18x18x18 cm in size) per genotype, two seeds per pot. The plants were inoculated with a mixture of *P. griseola* races, at the V3 stage of development, on both sides of the first trifoliate leaf until runoff using a hand sprayer. On symptom appearance, disease severity data was recorded four times at three day intervals. The score on the last day was used for the analysis. Severity scores were on a scale of 1-9, where 1-3 was resistant, 4-6 intermediate resistant and 7-9 susceptible (Van Schoonhoven and Pastor-Corrales, 1987). Data were recorded on: seed yield (g plant^{-1}), days to physiological maturity (days after planting where 50% of the plants have pods, and 50% of the pods have lost their pigmentation and begin to dry. At this stage the seeds begin to develop their typical varietal colour), 100-seed weight^{-1}, seed colour and growth habit.

5.2.5 Data analysis

Data were analysed using Genstat 12th edition statistical package (Payne et al., 2009). Separation of means was carried out using Tukey's studentized range test.

5.3 Results

5.3.1 Selected parents for the inter-gene pool double cross population

The four parents selected to develop the double cross population were, GBK 028162 (parent A), Gitsindayogi (parent B), GBK 028011 (parent C) and GBK 027934 (parent D), whose disease severity scores were between 4.6 and 4.8 (Table 5.2). Of the four parents, parent A (GBK 028162) and parent C (GBK 028011) belong to the Andean gene pool, while parent B (Gitsindayogi) and parent D (GBK 027934) belong to the Mesoamerican gene pool. The parents had different growth habit, seed type and colours (Table 5.2).

Table 5.2: Characteristics of the selected parental genotypes

	Parent	ALS score	100-seed weight^{-1} (g)	Growth habit	Gene pool	Seed colour
A	GBK 028162	4.8	72.8	Type I	Andean	Cream with red stripes
B	Gitsindayogi	4.8	33.7	Type II	Mesoamerican	Cream with black specks
C	GBK 028011	4.8	78.8	Type I	Andean	Purple
D	GBK 027934	4.6	29.5	Type I	Mesoamerican	Cream with black and brown specks

Growth habit type I = determinate, type II = indeterminate bush, erect stem and branches

5.3.2 Selection from F_1 to F_4 generation

There were 1073 F_1 plants, whereby 125 were resistant, 85 were intermediate resistant and 863 were susceptible to ALS. The intermediate resistant plants were selected and advanced to F_2. In the F_2, 41 intermediate resistant families were selected and advanced to F_3. In the F_3 generation 11 resistant common bean plants were selected and advanced to F_4. During the evaluation, line 6 was infected by bean common mosaic virus and thus discarded. Analysis was therefore carried out on the remaining ten common bean advanced lines.

5.3.3 Analysis of variance and mean values of advanced lines for angular leaf spot severity score, seed yield, days to maturity and 100-seed weight^{-1}

The analysis of variance (Table 5.3) showed that the F_4 advanced lines, their parents and the market class varieties were significantly different at ($p < 0.001$) for all the traits (ALS severity score, seed yield, days to maturity, and 100-seed weight^{-1}). Results for the mean values are presented in Table 5.4.

Table 5.3: Analysis of variance of F_4 lines for angular leaf spot severity score, seed yield, days to maturity and 100-seed weight^{-1}

Trait	Source of variation	df	ss	ms	vr	Fpr
ALS severity score	Common bean entries	20	3565.24	178.26	258.00	<0.001
Seed yield	Common bean entries	20	136.87	6.84	8.05	<0.001
Days to maturity	Common bean entries	20	1462.98	73.15	19.12	<0.001
100-seed weight^{-1}	Common bean entries	20	12357.99	617.90	91.41	<0.001

df = degree of freedom, ss = sum of squares, ms = mean square, vr = variance ratio, Fpr = F probability

Table 5.4: Mean values of angular leaf spot severity score and selected agronomic traits

	ALS score	Seed yield	Physiological maturity	100seed weight^{-1}	Seed size	Growth habit
Market class varieties						
GLP 585	8.0	12.9	85	23.4	small	I
New-rosecoco	7.5	11.6	81	48.7	large	I
GLP 24	8.2	10.2	89	37.4	medium	I
KAT 69	5.4	11.4	77	54.5	large	I
Super-rosecoco	7.9	8.4	85	47.9	large	I
GLP X92	8.2	11.6	78	32.3	medium	I
GLP 2	8.1	9.3	81	49.3	large	I
Mean of varieties	7.6	10.7	82	41.9		
Parents						
A-GBK 028162	4.8	7.6	76	72.8	large	I
B-Gitsindayogi	4.8	9.8	73	33.7	medium	II
C-GBK 028011	4.8	7.7	77	78.8	large	I
D-GBK 027934	4.6	11.4	84	29.3	medium	I
Mean of parents	4.8	9.1	77	53.7		
Advanced lines						
Line 1	3.2	10.7	74	44.8	large	I
Line 2	2.1	9.1	76	38.1	medium	I
Line 3	2.4	10.3	72	38.5	medium	II
Line 4	2.0	9.4	71	28.7	medium	II
Line 5	2.1	8.7	83	37.8	medium	II
Line 7	3.3	11	77	47.1	large	II
Line 8	2.0	7.5	74	31.6	medium	II
Line 9	2.2	8.8	75	47.6	large	II
Line 10	1.9	8.9	73	55.0	large	II
Line 11	3.2	8.5	75	63.0	large	II
Mean of lines	2.4	9.3	75	43.2		
Grand mean	4.6	9.8	78	44.8		
LSD (0.05)	0.4	1.5	3.2	4.3		

ALS severity score = 1.0-9.0 rating scale, where 1.0-3.0 = resistant, 4.0-6.0 = intermediate resistant and 7.0-9.0 = susceptible. Seed yield in g plant^{-1}, Seed size = 100-seed weight^{-1}; Small = < 25 g, Medium = 25-40 g, Large = > 40 g**.** Growth habit type I = determinate, type II = indeterminate bush, erect stem and branches. df = degrees of freedom

The parents had intermediate resistance to angular leaf spot. The disease severity scores of parents A (GBK 028162), B (Gitsindayogi) and C (GBK 028011) were 4.8 and not significantly different (P≤0.05) from each other, but different from parent D (GBK 027934) which had a score of 4.6. The ten F_4 common bean advanced lines had a mean disease severity score of 2.4. The

advanced line 10 had the lowest disease severity score (disease score 1.9). Advanced lines 2, 3, 4, 5, 8, 9 and 10 were not different from each other in the disease severity score. Advanced lines 1, 7 and 11 which had a disease severity score of 3.2, 3.2, and 3.3 respectively, were significantly different (P≤0.05) from all the other common bean entries but not from each other. The market class varieties had a disease severity score of between 5.4 and 8.2. The variety KAT 69 had a disease severity score of 5.4 and hence showed intermediate resistance. KAT 69 was significantly different (P≤0.05) from the other market class varieties, New-rosecoco, Super-rosecoco, GLP 585, GLP 2, GLP 24, and GLP X92, which had disease severity scores between 7.5 and 8.2.

The market class variety GLP 585 had the highest mean yield of 12.9 g plant^{-1}, which was significantly different (P≤0.05) from the low yielding common bean entries, advanced line 8 (7.5 g plant^{-1}), parent A (GBK 028162) (7.6 g plant^{-1}), and parent C (GBK 028011) (7.7 g plant^{-1}). The mean yield of GLP 585 was not significantly different from New-rosecoco (11.6 g plant^{-1}), GLP X92 (11.6 g plant^{-1}), parent D (GBK 027934) (11.4 g plant^{-1}), KAT 69 (11.4 g plant^{-1}), advanced line 7 (11.0 g plant^{-1}), advanced line 1 (10.7 g plant^{-1}), advanced line 3 (10.3) and GLP 24 (10.2 g plant^{-1}). On the other hand, advanced line 8, parent A (GBK 028162) and parent C (GBK 028011) which had comparatively low mean yield, were not significantly different from Super-rosecoco (8.4 g plant^{-1}), advanced line 11 (8.5 g plant^{-1}), advanced line 5 (8.7 g plant^{-1}), advanced line 9 (8.8 g plant^{-1}), advanced line 10 (8.9 g plant^{-1}), advanced line 2 (9.1 g plant^{-1}), GLP 2 (9.3 g plant^{-1}), advanced line 4 (9.4 g plant^{-1}) and parent B (Gitsindayogi) (9.8 g plant^{-1}).

The earliest maturing common bean was advanced line 4, which attained maturity in 71 days. It was significantly different (P≤0.05) from the later maturing common bean entries GLP 2 (81 days) and New-rosecoco (81 days), advanced line 5 (82 days), parent D (GBK 027934) (84 days), Super-rosecoco (85 days), GLP 585 (85 days) and GLP 24 (89 days). Advanced line 4 was not significantly different in mean days to maturity from advanced line 3 (72 days), advanced line 10 (73 days), parent B (Gitsindayogi) (73 days), advanced line 1 (74 days), advanced line 8 (74 days), advanced line 9 (75 days), advanced line 11 (75 days), parent A (GBK 028162)(76 days), advanced line 2 (76 days), parent C (GBK 028011) (77 days), KAT 69 (77 days) and advanced line 7 (77 days).

The small seeded market class variety GLP 585 had a mean 100-seed weight^{-1} of 23.4 g which was significantly different (P≤0.05) from the large seeded parent A (GBK 028162) and parent C (GBK 028011), which had a mean 100-seed weight^{-1} of 72.8 g and 78.8 g respectively. The

common bean entries, classified as medium seeded with a mean 100-seed weight^{-1} of between 25-40 g, were GLP 24 (37.4 g), GLP X92 (32.3 g), parent B (Gitsindayogi) (33.7 g), parent D (GBK 027934) (29.5 g), advanced line 2 (38.1 g), advanced line 3 (38.5 g), advanced line 4 (28.7 g), advanced line 5 (37.8 g), and advanced line 8 (31.6 g). The large seeded common bean entries were New-rosecoco (48.7 g), KAT 69 (54.5 g), Super-rosecoco (47.9 g), GLP 2 (49.3 g), parent A (GBK 028162) (72.8 g), parent C (GBK 028011) (78.8 g), advanced line 1 (44.8 g), advanced line 7 (47.1 g), advanced line 9 (47.6 g), advanced line 10 (55.0 g), and advanced line 11 (63.0 g). Five of the advanced lines were medium seeded and the other five large seeded. The growth habit of the three parents used in the double cross was determinate type I for parent A (GBK 028162), parent C (GBK 028011), and parent D (GBK 027934), while parent B (Gitsindayogi) had an indeterminate type II growth habit. In F_4, advanced lines 3, 4, 5, 7, 8, 9, 10, and 11 were indeterminate type II while advanced lines 1 and 2 had the determinate growth habit. The market class varieties had a type I growth habit (Table 5.4).

5.3.4 Seed types of the parents, advanced lines and market class varieties

Parent A (GBK 028162) had a large cream seed with red stripes, parent B (Gitsindayogi) had a kidney shaped, cream seed with black speckles, parent C (GBK 028011) had a large purple seed and parent D (GBK 027934) a small kidney shaped cream seed with light brown and black specks (Figure 5.3). The seed of advanced lines 1 and 7 were cream and black striped, similar to parent A (GBK 028162) which had red stripes. Seed of advanced line 2 was cream coloured with black specks, similar to parent B (Gitsindayogi), but had a larger shape similar to parent A (GBK 028162) and parent C (GBK 028011). Seed of advanced line 3 was cream with black specks similar to parent B (Gitsindayogi) and parent D (GBK 027934). The seed of advanced lines 4 and 9 were 'sugars', kidney shape, pink in colour, with black and red specks. This was similar to the red on parent C (GBK 028011), and the black specks of parent D (GBK 027934). Seed of advanced line 5 was cream with black specks similar to parent B (Gitsindayogi) and parent D (GBK 027934). Seed of advanced line 8 was purple in colour with black stripes, similar to parent C (GBK 028011) and parent A (GBK 028162), which also had stripes though they were red. Seed of advanced line 10 was cream coloured with black specks, similar to parent B (Gitsindayogi) but advanced line 10 had larger seeds. The market class varieties have different seed colours. Super-rosecoco, New-rosecoco, KAT 69, and GLP 2 had red mottled seeds. Market class variety GLP 24 had a large and purple seed. The GLP 585 seed was a small red kidney, while GLP X92 seed had a kidney shape, cream in colour with brown specks**.** Advanced lines 2, 3, 5, 10 and 11 had similar seed colour to GLP X92 seed.

Parent A – GBK 028162

Parent B - Gitsindayogi

Parent C – GBK 028011

Parent D – GBK 027934

Line 1

Line 2

Line 3

Line 4

Line 5

Line 7

Line 8

Line 9

Line 10

Line 11

Super-rosecoco

New-rosecoco

GLP 2

GLP 24

GLP 585

GLP X92

KAT 69

Figure 5.3: Seed types of parents, advanced lines and market class varieties

5.4 Discussion and conclusion

The aim of this study was to develop a breeding method for durable resistance to ALS for common beans. The method was used to accumulate minor genes of resistance into single genotypes. Four intermediate resistant common bean genotypes were used to develop a double cross inter-gene pool population. The F_1 population was evaluated for resistance to ALS using a mixture of *P. griseola* races and the intermediate resistant plants were advanced to F_2. Selection against resistant and susceptible plants was also carried out to advance the intermediate resistant plants from F_2 to F_3. At F_3 resistant lines were advanced to F_4 where they were evaluated and compared to their four parents and selected market class varieties. The assumption was made that the F_3 plants had accumulated enough minor genes for resistance hence selection of only resistant plants at the F_3 generation.

A mixture of races rather than a single virulent race was used to inoculate the segregating populations. Parlevliet (1983) suggested the use of a single race with the broadest virulence on a host population that varies in both major and minor gene resistance so as to distinguish between the two. However, in this study the starting host population was of intermediate resistance and by using a mixture of races and selecting against resistant and susceptible plants, it was possible to eliminate major genes for resistance in the early generations. Parlevliet and van Ommeren (1988), in their study on accumulation of partial resistance in barley using recurrent selection against susceptibility, tested a single race and a mixture of races on a host population that had partial resistance and another with both major and minor genes resistance. They suggested that the effectiveness of selection could be enhanced if the highly resistant plants are removed when using a mixture of races.

During advancement of the double cross generations, minor genes for resistance were accumulated into single genotypes. The continuous selection against resistance during the advancement stage of the double cross $F_{1\ (ABCD)}$ to F_2 and F_2 to F_3 was designed to eliminate the major genes of resistance from the population. This implies that at F_3, the resistance present in the plants was conditioned mainly by the minor genes. This approach was taken in line with Parlevliet (1995), who suggested that for durable resistance to be achieved, selection should be done against both susceptible and resistant genotypes. The choice of an inter-gene pool double cross population aimed at creating a wide genetic variability, by utilising parents from both the Andean and Mesoamerican gene pools, so as to maximise gains from selection and increase durability of resistance (Singh, 2001). Inter-gene pool crosses have been successfully used before to breed for varieties resistant to common and halo bacterial blights in common bean

(Asensio et al., 2006). The genetic diversity and inherent resistance available in the landraces was also taken into account in the choice of parents for the double cross. Danial et al. (2007) have shown that sources of quantitative resistance have been identified in local cultivars and used in breeding programmes to accumulate resistance genes through crossing and subsequent selection.

A high selection pressure (8%) was applied whereby both resistant and susceptible plants were removed from F_1 to F_2 generation. Selection was made possible by the segregation that occurred in the F_1 generation of the double cross and subsequent generations, whereby susceptibility, intermediate resistance and resistance were expressed. By selecting only intermediate resistant plants from F_1 and F_2 populations, quantitative resistance conditioned by minor genes was probably accumulated. Resistance to ALS can gradually be built up/accumulated from a segregating intermediate resistant population with transgressive segregation. Robinson (1987) emphasised that there is 'no good source' of resistance when breeding for horizontal resistance thus justifying the need to use intermediate resistant parents.

The advanced lines developed in this study are presumed to have durable resistance that can remain effective for a long time in regions where ALS is prevalent. Robinson (1980) suggested that horizontal resistance could be durable, and can be achieved by increasing the frequency of (+) alleles in a genetically flexible gene pool by population breeding. The assumption is that the intermediate resistance is inherited polygenically and hence raising the levels of the resistance is possible through accumulation.

Simultaneous selection for ALS resistance and certain agronomic and farmer preferred traits was possible in this breeding method. Developing an inter-gene pool population from landraces increased the diversity from which to choose preferred traits. In this study the advanced lines had a higher mean yield than their parents. Several market class varieties had higher yields than the advanced lines, but they were susceptible to ALS. This is attributed to common bean breeding that emphasised on yield rather than resistance to disease (Kimani, P.M., personal communication[3]). The advanced lines matured earlier than the parents and the market class varieties, in particular advanced line 4. Early maturity is an important trait for common bean farmers in most parts of Kenya (Katungi et al., 2009; Ojwang' et al., 2009). Five of the advanced lines were medium seeded and the other five were large seeded. Preference of large and medium seeded common bean in Kenya has been reported (Katungi et al., 2009; Ojwang' et al.,

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2009; Gichangi et al., 2012). Inter-gene pool crosses have been shown to generate large genetic variation in the segregating population, but performance of the best line does not always exceed that of the best parent (Gonza´ lez et al., 2009) in seed size. Common bean advanced lines 1 and 2 had the type I growth habit while the other eight advanced lines had the type II growth habit. During the interviews (Chapter 2, this Thesis), farmers indicated that they preferred the type I growth habit of common bean. Growth habit has been reported as an important trait in different common bean growing regions of Kenya (Katungi et al., 2009; Gichangi et al., 2012). Farmers also preferred common bean varieties similar to the already existing market class varieties hence seeds of advanced lines 4 and 9 could easily be accepted by the farmers. Seeds of advanced lines 3, 2, 5, 10 and 11 were similar to seeds of market class variety GLP X92. The seed colour of advanced lines 1 and 7 that were cream with black zebra stripes were similar to the seed of landrace 'Mukura na oke' which is cultivated by the farmers.

In conclusion, a new durable resistance breeding method was developed, whereby four landraces with intermediate resistance to ALS, representing two diverse common bean gene pools were used to develop a double cross population. Advanced lines were developed with minor gene resistance to ALS and with farmer preferred traits, showing significant breeding progress. These lines need to be advanced to homozygous lines, which need to be further tested in multi-locational trials over several years, to confirm the durability of the resistance to a range of ALS races.

References

Asensio, S.M.M.C., C. Asensio, and S.P. Singh. 2006. Gamete selection for resistance to common and halo bacterial blights in dry bean intergene pool populations. Crop Science 46:131-135.

Damasceno e Silva, K.J., E.A. Souza, A. Sartorato, and C.S. Freir. 2008. Pathogenic variability of isolates of *Pseudocercospora griseola*, the cause of common bean angular leaf spot, and its implications for resistance breeding. Journal of Phytopathology 156:602-606.

Danial, D., J. Parlevliet, C. Almekinders, and G. Thiele. 2007. Farmers' participation and breeding for durable disease resistance in the Andean region. Euphytica 153:385-396.

Gichangi, A., S.N. Maobe, D. Karanja, A. Getabu, C.N. Macharia, J.O. Ogecha, M.K. Nyang'au, E. Basweti, and L. Kitonga. 2012. Assessment of production and marketing of climbing beans by smallholder farmers in Nyanza region, Kenya. World Journal of Agricultural Sciences 8:293-302.

Gonza´ lez, A.M., A.P. Rodin'o, M. Santalla, and A.M.D. Ron. 2009. Genetics of intra-gene pool and inter-gene pool hybridization for seed traits in common bean (*Phaseolus vulgaris* L.) germplasm from Europe. Field Crops Research 112:66-76.

Johnson, R. 1981. Durable resistance: Definition of, genetic control and attainment in plant breeding. Phytopathology 71:567-568.

Katungi, E., A. Farrow, J. Chianu, l. Sperling, and S. Beebe. 2009. Common bean in Eastern and Southern Africa: A situation and outlook analysis, CIAT, Cali, Colombia.

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. Plant Pathology 51:594-604.

Mahuku, G.S., M.A. Henrı´quez, C. Montoya, C. Jara, H. Teran, and S. Beebe. 2011. Inheritance and development of molecular markers linked to angular leaf spot resistance genes in the common bean accession G10909. Molecular Breeding 28:57-71.

Ojwang', P.P.O., R. Melis, J.M. Songa, M. Githiri, and C. Bett. 2009. Participatory plant breeding approach for host plant resistance to bean fly in common bean under semi-arid Kenya conditions. Euphytica 170:383-393.

Parlevliet, J.E. 1983. Can horizontal resistance be recognized in the presence of vertical resistance in plants exposed to a mixture of pathogen races? Phytopathology 73:379.

Parlevliet, J.E. 1985. Resistance of the non-race-specific type, p. 501-525, *In* A .P. Roelfs and W. R. Bushnell, eds. The cereal rusts vol. II. Academic Press, New York, USA.

Parlevliet, J.E. 1995. Genetic and breeding aspects of durable resistance of crops to pathogens. African Crop Science Journal 3:1-13.

Parlevliet, J.E., and A. van Ommeren. 1988. Accumulation of partial resistance in barley to barley leaf rust and powdery mildew through recurrent selection against susceptibility. Euphytica 37:261-274.

Pastor-Corrales, M.A., C. Jara, and S.P. 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.

Payne, R.W., D.A. Murray, S.A. Harding, D.B. Baird, and D.M. Soutar. 2009. GenStat for Windows (12th Edition) Introduction. VSN International, Hemel Hempstead, UK.

Robinson, R.A. 1980. New concepts in breeding for disease resistance. Annual Review of Phytopathology 18:189-210.

Robinson, R.A. 1987. Plant pathosystems. Springer-Verlag, Berlin, Heidelberg, New York, USA.

Singh, S.P. 1994. Gamete selection for simultaneous improvement of multiple traits in common bean. Crop Science 34:352-355.

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

Singh, S.P., C. Cardona, F.J. Morales, M.A. Pastor-Corrales, and O. Voysest. 1998. Gamete selection for upright Carioca bean with resistance to five diseases and a leafhopper. Crop Science 38:666-672.

Singh, S.P., H. Teran, M. Lema, M.F. Davis, R. Hayes, and C. Robinson. 2008. Breeding for slow darkening, high yielding, broadly adapted dry bean pinto 'Kimberly' and 'Shoshone'. Journal of Plant Registration 2:181-186.

Teran, H., and S.P. Singh. 2009. Gamete selection for improving physiological resistance to white mold in common bean. Euphytica 167:271-289.

Teran, H., C. Jara, G. Mahuku, S. Beebe, and S.P. Singh. 2013. Similtaneous selection for resistance to five bacterial, fungal and viral diseases in three Andean x Middle American inter-gene pool common bean populations. Euphytica 189:283-292.

Van der Plank, J.E. 1968. Disease resistance in plants. Academic Press, New York, USA.

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

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

CHAPTER 6

General overview of the study and implications to plant breeding

6.1 Introduction

Resistant varieties are a major component in the management of insect-pests and diseases. The type of resistance is essential to ensure that the varieties are resistant for a long period of time. This study was therefore focused on breeding for durable resistance to angular leaf spot (ALS) of common bean in Kenya. The study was conducted in four parts, which included one survey and three experiments. The first one involved carrying out a survey in Kiambu county, a common bean growing region in Kenya, where farmers' perceptions on common bean production systems, constraints, and their preferred traits were evaluated. The second one was carried out to screen common bean landraces and selected introductions so as to identify local common bean genotypes that could be used as sources of resistance to ALS or used as resistant varieties. Yield was also evaluated at two sites (Kabete and KARI-Thika) during two seasons (short rains 2011 and long rains 2012) to evaluate the yield performance of the landraces. The third experiment was conducted to identify the mode of inheritance and gene action that conditions resistance to ALS. In the fourth experiment, a breeding method for durable ALS resistance was developed through accumulation of minor resistance genes. The method was used to develop common bean lines with durable resistance to ALS.

This chapter thus highlights the major findings and the future breeding implications of the study.

6.2 Summary of the major findings

A participatory rural appraisal using a semi-structured questionnaire and focus group discussion was carried out in Thika and Kabete districts of Kiambu county, which are major common bean production areas in Kenya. The study aimed to identify the farmers' knowledge on common bean production, their cropping systems, constraints to production and their preferred traits. The main findings were;

- The farmers grow both improved common bean varieties (GLP and KAT series) and landraces ('Gikaara', 'Kiboland', and 'Mukura na oke').
- Common bean production by the small scale farmers is carried out during the long and short rains, though the highest yields are realized during the short rains. This is because there is reduced disease pressure during the short rains.
- The farmers do not apply any agrochemicals (fertilizers and pesticides) during production because of the expense involved. They depend on good agricultural practices, mainly weeding to ensure insect-pest and disease management.
- Due to the small size of land, the farmers prefer to intercrop common bean with other crops such as maize, coffee and fruit trees so as to maximize on the space usage.
- The farmers commonly retain seed for the next planting season. This reduces their production expenses. In cases where the crop yield was not high, they purchase seed from the local market and from their neighbours. The Ministry of Agriculture also supplies them with free seed when introducing new varieties.
- The major production constraints cited by the farmers were insect-pests and diseases. The aphids were a major constraint during the short rains, while angular leaf spot disease caused losses during the long rain season.
- Resistance to diseases was a major trait of preference in improved varieties. The farmers also preferred early maturing varieties that will be able to escape disease and also perform well during the short rains. High yielding varieties were also preferred, with short cooking time.

Common bean landraces were evaluated at Kabete nethouse and KARI-Tigoni field for resistance to ALS. The landraces were also evaluated for seed yield at KARI-Thika and Kabete Field Station in two seasons (short rains 2011 and long rains 2012). The study aimed to identify local landraces with resistance to ALS, and also the highest yielding genotypes at the two locations. The main results were as follows;

- The reaction of common bean landraces was varied with some having resistance, intermediate resistance and others susceptible reaction to ALS. Three common bean landraces (GBK 028123, Minoire and Murangazi) had low ALS severity scores at Kabete (nethouse) and KARI-Tigoni (field) and they can be used as sources of resistance or resistant varieties.
- The resistant check genotype AND 277 was susceptible in Kenya.
- About 32% of the Kenyan landraces had intermediate resistance to the disease as compared to the market class varieties that were all susceptible, except KAT 69 which had intermediate resistance. This intermediate resistance can be exploited positively to accumulate the minor genes for durable resistance.
- The genotypes had variable yield. The three high yielding genotypes across locations were GLP 2, Nyirakanyobure and GBK 028110. In specific locations, the three high yielding genotypes at Kabete were GLP 2, Mukwararaye, and GBK 028012, while at Thika they were GBK 028110, GBK 035065, and GBK 027984.

Inheritance and gene action conditioning resistance to ALS was evaluated at Kabete Field Station. Resistant genotypes Mexico 54 and G10909 were crossed to susceptible genotypes Super-rosecoco and Wairimu. Generations F_1, F_2, BC_1P_1, BC_1P_2 were developed for each cross. The generations and the parents for each cross were evaluated separately in the field for resistance to ALS. The main findings were that;

- Additive gene effects were more important than the dominance gene effects implying that inheritance of resistance to ALS is quantitatively inherited.
- Narrow sense heritability ranged between 59-71% and this implies that improvement for ALS resistance would be possible through selection in the early segregating generations.

A breeding method for durable resistance to ALS was developed through a double cross of landraces of intermediate resistance, followed by selection against resistant genotypes in early segregating generations. It is assumed that this method accumulated minor genes of resistance to ALS into single genotypes. These were advanced to F_4 and evaluated for resistance to ALS and other agronomic traits. The major findings were;

- The new durable resistance breeding method was successful in accumulating minor genes of resistance in ten advanced common bean lines.
- The advanced lines had improved resistance (disease score 1.9-3.2) when compared to their parents (disease score 4.6-4.8) which had intermediate resistance. They also performed better in resistance to ALS than the market class varieties which were susceptible (disease score 7.6-8.2) to ALS. These newly selected advanced lines can be tested further in multi-locational trials to confirm the durability of the resistance to a range of races.
- Simultaneous selection of agronomic traits was possible, whereby the yield of the common bean advanced lines was an average 9.3 g plant^{-1} and compared well with the parents (9.1 g plant^{-1}) and the market class varieties (10.7 g plant^{-1}).
- The common bean advanced lines were early maturing (75 days) compared to the parents (77 days) and the market class varieties (82 days).
- Five of the advanced lines (lines 1, 7, 9, 10 and 11) were large seeded while five (lines 2, 3, 4, 5 and 8) were medium seeded.
- The growth habit differed and two advanced lines (lines 1 and 2) had type I, while the other eight advanced lines had type II growth habit.

6.3 Breeding implications and future research needs

Participatory plant breeding is essential for all breeding programmes to ensure that the varieties released are adaptable to the farmers' conditions and hence more readily adopted. The common bean farmers have acquired knowledge on common bean production through the continuous cultivation of the crop. Hence this knowledge can be utilized to improve common bean in the breeding programmes. The farmer preferences should also be incorporated.

Landraces are genetically diverse and this diversity can be exploited positively in plant breeding. In addition, landraces are better adapted to local conditions and thus survive adverse weather conditions. With the adverse effects that climate change is having on the agriculture sector and thus threatening food security, landraces should be incorporated in the breeding programmes. Intermediate resistant landraces were successfully used to accumulate the minor genes for resistance to ALS into ten single genotypes. Hence the use of landraces with intermediate resistance can be beneficial in future breeding work to select for durable resistance. Other suitable traits in landraces such as seed colour, early maturity, cooking ability and taste can be exploited based on farmers' preferences.

The good performance of the Rwandan landraces in respect of resistance to ALS in Kenya means that breeders can utilise resistant genotypes from other regions that differ in their climatic conditions. They can be used in the breeding programmes and their resistance should last longer before their matching pathogen races appear in the country. The Rwandan landraces Minoire and Muragazi and Kenyan landrace GBK 028123 were identified as resistant. Studies can be done to identify markers associated with QTLs that have effects on resistance to ALS and if different from other known sources of resistance, this resistance could be pyramided into one genotype thus ensuring durable resistance. The three landraces, Minoire, Muragazi and GBK 028123 can also be released as resistant varieties.

The susceptible nature of AND 277 showed that resistance governed by major genes is not durable. Again all resistance sources should be tested against all races, and if it is not possible, they should be recommended for specific areas only. In this study, a high percentage of the large seeded common beans were susceptible, yet large seeded common beans are most popular and widely grown in Kenya and Africa in general. Their widespread use is what could have made them susceptible, hence the need for additional Andean resistant sources. There are no excellent sources of resistance from the Andean gene pool in Kenya and therefore research should focus towards this.

High stable yield across environments should be considered when breeding. Recommendation of suitable common bean varieties can be based on their performance across several locations or performance in a specific environment. In the study reported here, the genotypes GLP 2, Nyirakanyobure and GBK 028110 can be recommended in both Kabete and Thika locations. In the specific locations genotype GLP 2, Mukwararaye and GBK 028012, can be recommended in Kabete and genotypes GBK 028110, GBK 035065 and GBK 027984 in Thika.

Two very different resistance breeding methods have been the focus of this study. In the first method, known ALS resistant genotypes were used in a backcrossing programme to introgress ALS resistance into susceptible Kenyan market class varieties. This breeding approach is commonly used by breeders and in some cases will be used to pyramid genes from several sources into susceptible varieties. The gene pyramiding is criticized by some authors for breeding resistance to fungal diseases such as ALS, as the resistance is considered non-durable (Parlevliet, 2002). However, this study has shown that resistance in genotypes Mexico 54 and G10909 is quantitative and could be used in the development of durable resistant varieties.

The second resistance breeding approach, using a double cross method, does not come natural to breeders. In particular the removal of ALS resistant progeny from segregating populations goes against the breeder's natural instinct to select for resistance. However, the double crossing of landraces with intermediate resistance, followed by selection of F_1 and F_2 progeny with intermediate resistance under infestation of a mixture of ALS races, has proved to be a promising new method to develop common bean lines with potential for durable minor gene resistance to ALS. It will be a challenge to breeders to experiment with the different breeding approach in order to design the best strategy for durable ALS resistance breeding.

Reference

Parlevliet, J.E. 2002. Durability of resistance against fungal, bacterial, and viral pathogens; present situation. Euphytica 124:147-156