Journal of Agricultural Science and Technology A 2 (2012) 856-862
Earlier title: Journal of Agricultural Science and Technology, ISSN 1939-1250

Inheritance of Angular Leaf Spot [*Phaeoisariopsis griseola* (Sacc.) Ferr] Resistance in Common Bean (*Phaseolus vulgaris* L.) Population Developed from Kablanketi × Mexico 54

George Muhamba Tryphone, Luseko Amos Chilagane, Paul Mbogo Kusolwa and Susan Nchimbi-Msolla

Department of Crop Science and Production, Faculty of Agriculture, Sokoine University of Agriculture, P.O. Box 3005, Chuo Kikuu, Morogoro, Tanzania

Received: February 3, 2012 / Published: July 20, 2012.

Abstract: The genetic resistance to angular leaf spot (ALS) caused by *Phaeoisariopsis griseola* in the common bean cultivar Mexico 54 was investigated on disease reactions in parental, F_1, F_2 and backcross generations derived from crosses between a resistant cultivar Mexico 54 and a susceptible cultivar Kablanketi under screen house conditions. The heritability (h^2) estimate was as high as 0.719 indicating a successful transfer of ALS resistance among progenies and thus selection can be performed in early generation. High heritability coupled with high expected genetic advance of 39.5% is considered to be more useful in predicting the outcome of selecting the best individuals. Chi-square values were computed to determine whether the observed ratios for disease reactions deviated from expected Mendelian ratios for a single, dominant gene controlling resistance to angular leaf spot in common bean. Based on the resistance of the F_2, and the backcross generation to the resistant parent, a 3 resistant: 1 susceptible segregation ratio in the F_2 and a 1 resistant: 1 susceptible segregation ratio in the backcross generation to the susceptible parent was obtained implying that resistance to the isolate of *Phaeoisariopsis griseola* is governed by a single, dominant gene.

Key words: *Phaeoisariopsis griseola,* heritability, genetic advance, inheritance, common bean.

1. Introduction

Common bean (*Phaseolus vulgaris* L.) is an important grain legume for direct human consumption [1]. It is a staple food for more than 100 million people in Africa with per capita consumption of 60 kg/person/year in the Great Lakes Regions (GLR) [2]. The crop represents one of the principal crops in East Africa in terms of total area planted and number of farmers involved in production. It is an important source of dietary protein and starch in Africa and a primary staple in parts of the GLR. It is estimated that over 75% of rural households in Tanzania depend on it for daily dietary requirements [3]. Bean production also provides farm households with both a source of income and food for nutrition. It has the nutritional benefits such as high source of proteins and high mineral contents especially Fe and Zn which combat high prevalence related micronutrient deficiencies [4]. Consuming beans also have medicinal benefits that contribute to treating human aliments like cancer, diabetes, and heart diseases [5].

The average yield of common bean is around 500 kg/ha in Africa while the potential yields being at 1,500 kg/ha [6]. Across farming systems, biotic and abiotic stresses continue to present the major constraints for increasing bean production and high yields, and bean diseases represent the major

Corresponding author: George Muhamba Tryphone, M.Sc., research field: agronomy/breeding for resistance. E-mail: muhamba@suanet.ac.tz.

constraints to production by reducing yields and seed quality [6]. Angular leaf spot (ALS) disease caused by the fungus *Phaeoisariopsis griseola* (Sacc.) Ferr. is one of the most devastating diseases of common bean in the tropical and subtropical countries [7]. In Tanzania the disease is endemic in low to high altitude bean producing zones. Angular leaf spot disease causes an estimated yield loss of 389,900 t/year in sub-Saharan Africa, 263,600 t/year in East Africa, Tanzania inclusive, and 125,300 t/year in Southern Africa [1].

Angular leaf spot disease development occurs over a wide range of temperature with the optimum development at 24 °C [8]. High relative humidity (90%-100%) favours the growth of the fungi. Infection may not occur if the humidity is below 85% [1]. The disease affects all aerial plant parts especially pods, seeds, leaf petioles and lower surface of the leaflets, causing premature leaf drop, foliar and stem necrosis which often result to poorly filled seeds and reduce seed quality [9]. The lesions on leaves can cause severe defoliation and decreased leaf area index [10] resulting to yield losses of up to 80% under severe infection [11, 12].

Control strategies for ALS include chemical applications, cultural practices, and genetic resistance. However, among the existing strategies, genetic resistance is considered most appropriate, safe and cost effective for small scale bean growers [13]. Selection of bean genotypes resistant to ALS is important for improving common bean production in terms of quality and quantity [1, 14]. Thus the need to breed for high resistance levels to ALS is paramount. Such techniques as introgression of resistant genes in locally adapted cultivars such as Kablanketi (in case of Tanzania) will optimize resistance levels of the variety since the mode of gene action and inheritance is subject to the background used. This study aimed at incorporating ALS resistance in locally adapted and preferred common bean cultivar and to determine inheritance of ALS resistance.

2. Materials and Methods

2.1 Sources of Resistance

The resistant parents were obtained from the Kawanda National Agricultural Research Institute, Uganda. The cultivar Mexico 54 was used donor parent for resistance to ALS while the landrace Kablanketi/Soya was used as recurrent farmers preferred cultivar and was collected from farmers' fields in Mbeya Region.

The cultivar Mexico 54 is an indeterminate strong climber resistant to most African isolates of ALS disease [15]. This cultivar is therefore, a potential source of resistance in breeding work. Kablanketi is the landrace which has a good market class and displays other good agronomic traits but is generally susceptible to various diseases including ALS. It is medium sized with the growth habit type II and gives low yields.

2.2 Hybridization

The crosses were made in the screen house at SUA. The crosses were made between susceptible cultivar and resistant parent where ALS disease resistance was incorporated into the adapted but susceptible cultivar Kablanketi (♀) × Mexico 54 (♂). Then, the backcrosses were developed and F_2 population produced for the inheritance studies. The backcrosses produced were both for susceptible (Kablanketi × F_1) and resistant (Mexico 54 × F_1) parents. The left parent is female (♀) and the right parent is male (♂). The seeds of F_1, F_2 and backcrosses were harvested and used for ALS evaluation.

2.3 Plant Establishment and Evaluation for ALS

The parents, F_1, F_2 and backcross populations were grown in plastic pots with one plant per pot filled with sterilized sandy loam soil. Inocula used were collected from SUA-crop museum bean plots. Spore was isolated and grown into V8 agar and purified using single spore culture in another V8 agar and multiplied. The number of spores in suspension was determined using haemocytometer and the concentration adjusted to 10^4/mL using distilled water containing 0.05% (v/v) Tween 20 (polyoxyethylene sorbitan monooleate). Plants were inoculated at the age of 17 days from sowing when they had 2-3 sets of fully expanded trifoliolate leaves. The inoculated plants were covered with plastic sheets for 72 h to create humidity. After 72 h, the plastic sheets were removed and plants put on tables in screenhouse and plants monitored daily for disease development and appearance of first symptoms, seven days after inoculation disease severity was scored at weekly intervals for a total of four ratings, using a CIAT 1-9 visual scale [16]. Plants with a score of 1-3 were considered resistant, 4-6 were regarded as intermediate while scores greater than 6 were considered susceptible.

2.4 Pathogenicity Test

Susceptible bean genotype locally called *Maini* was used for pathogenicity test. The cultivar *Maini* is a Mesoamerican, growth habit type II with creamy seed, it cooks fast and has very good palatability. Plants were grown in the screenhouse and inoculated at second trifoliolate leaf. Observations of symptoms on susceptible genotype were used for confirmation of the reaction of ALS isolate. The aggressive isolate was identified and used in an experiment.

2.5 Data Collection and Analysis

Data were collected on the reaction of bean line (severity) for ALS disease and analyzed using the GENSTAT statistical package to generate means, variance, standard deviation, standard error and coefficient of variation. The variances of parents, F_1, F_2 and backcrosses were generated and used to estimate the heritability in narrow sense based on scaling test as described by Falcon and Mackay [17]. Narrow sense heritability (h^2) was determined from the variances of the parents, F_1's, F_2's and the backcross generations where $h^2 = 1/2D/VF_2$, V_{F2} is the total variance of F_2 and 1/2D is the additive genetic component of variance of F_2 calculated from the variances of the backcrosses and the total variance of F_2 and $1/2D = 2\,(V_{F2}) - (V_{B1} + V_{B2})$. Inheritance was calculated based on the crosses generated. The genetic gain was also calculated as the product of heritability, phenotypic standard deviation and selection differential (Genetic gain/advance (GA) = $h^2\ \sigma_p\ K$) where h^2 = heritability; K = selection differential at 5% (2.06) intensity of selection; σ_p = the phenotypic standard deviation. MS Excel was used to generate the frequency graphs and estimate Chi-square. Chi-square values were computed on numbers of resistant and susceptible plants in each generation to determine whether the observed ratios deviated from the expected Mendelian ratios for segregation expectations according to gene action type and number of genes controlling resistance to angular leaf spot in the common bean.

3. Results and Discussion

3.1 Heritability of Angular Leaf Spot Resistance

The heritability (h^2) estimated for progenies from the cross Kablanketi × Mexico 54 for angular leaf spot was 0.719. The present study reflects that sufficient variation for disease resistant exists in F_2 population. However, other authors reported the heritability for this trait (angular leaf spot resistance) to range from medium to high. Amaro et al. [18] reported the heritability for the reaction of common bean to angular leaf spot varying from 0.4444 to 0.5886. The result of this study shows that the heritability for disease in this study is higher. High h^2 implies that there is higher contribution of the genes to this trait (additive gene effects) than the environmental effects and it is a reflection of the good general combining ability suggesting that resistance could be improved by selection. Estimate of heritability from segregating population is useful in understanding the genetic consequences of hybridization and inbreeding. They can help the breeder in selecting and utilizing the superior individuals from a population. Heritability is the most important parameter in the prediction of selection response since the effectiveness of selection depends on the additive portion of genetic variance in relation to total variance [17]. Traits with relatively high heritability have been reported to respond highly to selections for effectiveness in crop improvement at early generation [17, 19]. It is suggested that phenotypic selection of the promising plants in large F_2 population followed by progeny testing would increase productivity. However, low heritability can be obtained depending on the susceptible parents used. In such situation the application of molecular marker linked to the genes to be transferred to the susceptible background is appropriate [18].

The genetic gain in selection for angular leaf spot resistance was estimated to be 39.5% categorized as high. This high genetic advance is an efficient indicator of the nature of variability for the trait under study and the possible breeding progress expected under selection. However, the effectiveness of selection depends upon genetic advance of the character selected along with heritability [20]. Thus, the ultimate goal of the plant breeder is to have higher genetic advance for the material selected.

3.2 Inheritance Pattern

Average disease severity data for angular leaf spot of all generations from the parents and three different crosses (F_1, F_2 and backcrosses) were recorded (Fig. 1). The frequency distributions of observed leaf lesion reactions of the Mexico 54, Kablanketi, F_1, F_2, Kablanketi × F_1 and Mexico 54 × F_1 populations illustrated several aspects of the genetic system conditioning ALS resistance in Mexico 54. Majority of the plants in the Mexico 54 population exhibited a resistant reaction (leaf lesion score ≤ 3). All plants in the Kablanketi population exhibited a susceptible reaction (leaf lesion score ≥ 4). Majority of plants in the F_1 population exhibited a resistant reaction with the scores of 1-3, suggesting that the ALS resistance is inherited as a dominant trait. The distribution frequencies in the F_2 population and Kablanketi × F_1 backcross population indicated that ALS resistance is mostly conditioned by qualitative rather than a quantitative genetic system (Fig. 1). The monogenic inheritance of resistance indicates that pedigree breeding would be adequate for transferring the resistance to the susceptible genotypes. The disease grade distributions (Fig. 1) clearly indicated a monogenic type of inheritance of resistance to *P. griseola*.

The segregation data indicated that ALS resistance in Mexico 54 is conditioned by a major gene (Table 1). While segregation for resistance in the F_2 population was consistent with a ratio of 3:1 as resistant: susceptible plants in a population ($\chi^2 = 0.015$, $P > 0.05$) and that the backcross to susceptible parent (Kablanketi) fits a 1:1 ratio ($\chi^2 = 0.177$, $P > 0.05$) (Table 2). These results support the hypothesis that a single dominant gene in Mexico 54 controls resistance to *P. griseola* as reported by Muthomi et al. [21]. Similar results were reported using the same source of resistance on modes of inheritance to *P. griseola* in Mexico 54 indicating a single dominant gene effect for controls resistance [12]. Caixeta et al. [22] identified monogenic inheritance using BAT 332 as a source of resistance to *P. griseola*.

However, several studies in common bean cultivars have demonstrated or reported that *P. griseola* is resistance may be controlled by 1, 2 or 3 dominant or recessive genes [7, 22, 23]. It has been established however, that genetic resistance to *P. griseola* in different cultivars as source of resistance is monogenic and dominant [21]. This implies that the transfer of this trait to elite cultivars by backcross. The cultivars that are resistant sources to *P. griseola* include AND 277 [23], MAR 2 [24], Cornell 49-242 [25], Mexico 54 [26, 27], BAT 332 [22, 27] and CAL 143 [28]. The total of nine genes has been identified in some of the resistant cultivars after allelism tests. These genes include *Phg-1*^{a}, *Phg-2*^{2}, *Phg-3*^{2}, and *Phg-4*^{2} for cultivar AND 227, *Phg-2, Phg-5* and *Phg-6*

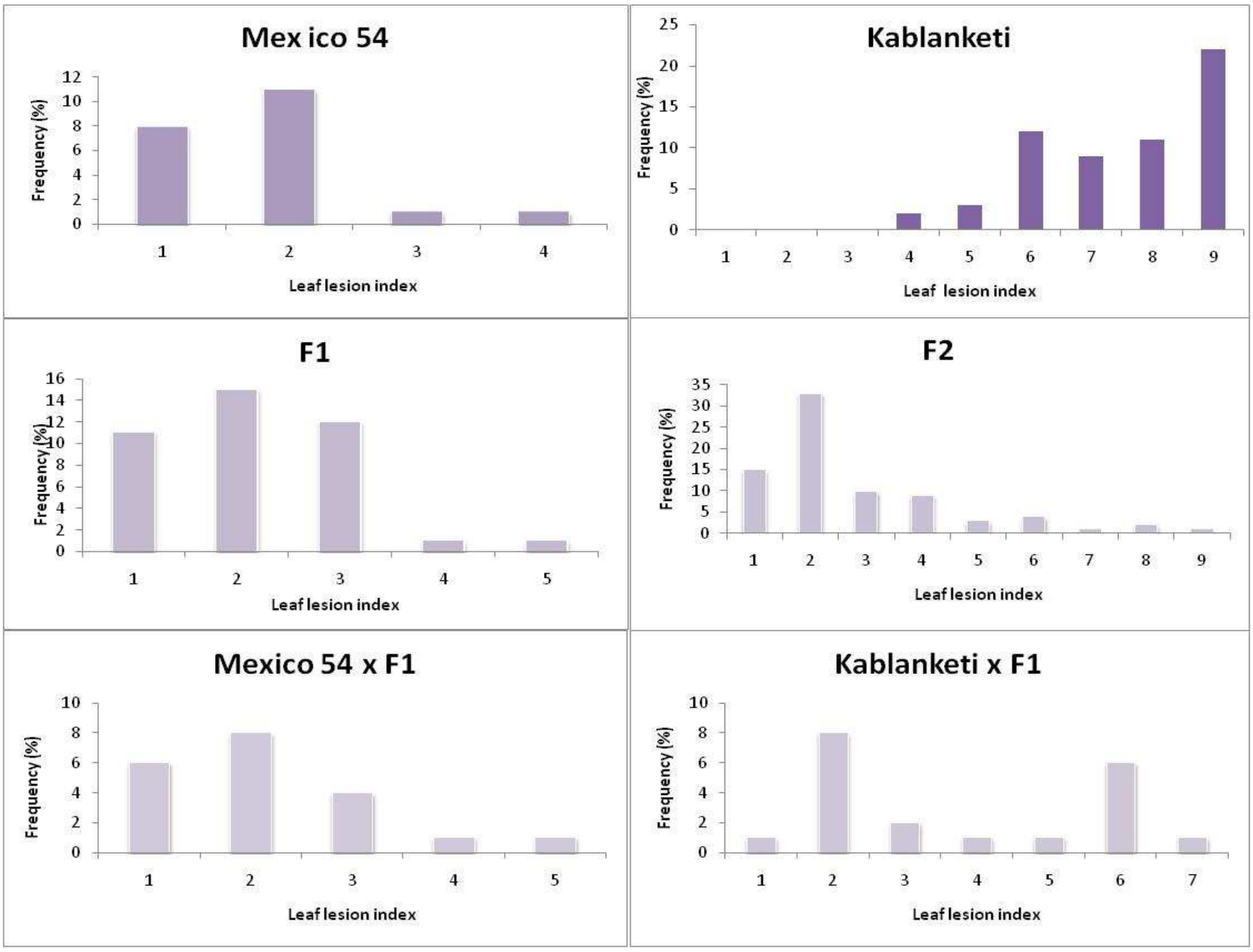

Fig. 1 Comparative frequency distributions of reaction to *P. griseola* for parental, F_1, F_2 and backcross populations of Kablanketi (♀) × Mexico 54 (♂) grown in screenhouse (1 = no visible symptoms/lesions (resistant), 9 = chlorotic leaf tisue (susceptible)).

Table 1 Segregation for resistance to angular leaf spot (*Phaeoisariopsis griseola*) in parental, F_1, F_2 and backcross populations of the cross "Kablanketi" × "Mexico 54".

Parents/Population	Observed number of Plants		Expected radio (R:S)	Chi-square	Probability
	Resistant (R)	Susceptible (S)			
Kablanketi (P1)	0	59			
Mexico 54 (P2)	61	2			
F_1 (Kablanketi × Mexico 54)	65	6			
F_2 (Kablanketi × Mexico 54)	67	23	3:1	0.015	0.903
$BC_{1-1}F_1$ ($P_1 \times F_1$)	27	24	1:1	0.177	0.659
$BC_{1-2}F_1$ ($P_2 \times F_1$)	34	6	1:0	0.000	1.000

Resistant (R): number of resistant plants (leaf lesion index ≤ 3); Susceptible (S): number of susceptible plants (leaf lesion index ≥ 4); on CIAT scale of 1-9.

for Mexico 54 and MAR 2 has *Phg-4* and *Phg-5* resistance genes [29].

4. Conclusions

Selection for traits having high heritability would be very effective as there would be a close correspondence between genotype and phenotype. This work has demonstrated that the heritability for ALS disease is higher indicating that the trait was transferred to the offspring and selection can be performed in early generation. High heritability coupled with high expected genetic advance is considered to be more useful in predicting the outcome of selection. The study also revealed that resistance of *P. griseola* is due to monogenic dominant gene using Mexico 54 as source of resistance. The screening technique coupled with the simple inheritance of angular leaf spot resistance would lead to easy selection of breeding lines when the source of resistance is independent of inocula density. The breeding lines identified to have ALS resistance with scores of 1-3 and those lines can be picked as breeding lines for multiplexing other resistance genes into Kablanketi.

Acknowledgments

This paper is part of a Ph.D. research thesis to be submitted at Sokoine University of Agriculture, Morogoro. Financial support for this work was provided by Kirkhouse Trust. Authors are grateful to CIAT-Uganda for providing the resistant bean cultivar (Mexico 54).

References

[1] C.S. Wortmann, R.A. Kiluby, C.A. Eledu, D.J. Arron, Atlas of Common Bean (*Phaseolus vulgaris* L.), Production in Africa, CIAT, Cali. Columbia, 1998, p. 133.

[2] CTA, Techinical Center for Agricultural and Rural Cooperation (Dry Beans: Grains of Hope), Spore 146, Apr., 2010.

[3] CIAT, The impact of improved bean production technologies in Northern Tanzania [Online], 2008, http//www.ciat.cgiar.org/work/Africa/Documents/highlig hts42.pdf.

[4] G.M. Tryphone, S. Nchimbi-Msolla, Diversity of common bean (*Phaseolus vulgaris* L.) g enotypes in iron and zinc contents under screenhouse conditions, African J. Agric. Res. 5 (8) (2010) 738-747.

[5] L.A. Hangen, M.R. Bennink, Consuption of black beans and navy beans (*Phaseolus vulgaris*) reduced ozoxymethane-induced colon cancer in rats, Nutr. Cancer 44 (2003) 60-65.

[6] R.J. Hillocks, C.S. Madata, R. Chirwa, E.M. Minja, S. Msolla, Phaseolus bean improvement in Tanzania, 1959-2005, Euphytica 150 (2006) 215-231.

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

[8] R.B. Bassanezi, L. Amorim, A. Bergamin Filho, B. Hau, Effects of bean line pattern mosaic virus on the monocyclic components of rust and angular leaf spot of *Phaseolus* bean at different temperatures, Plant Pathololgy 47 (1998) 289-298.

[9] M.M. Liebenberg, Z.A. Pretorius, A review of angular leaf spot of common bean (*Phaseolus vulgaris* L.), Africa Plant Protection 3 (1997) 81-106.

[10] W.C. de Jesus Junior, F.X.R. do Vale, R.R. Coelho, B. Hau, L. Zambolim, L.C. Costa, et al., Effects of angular leaf spot and rust on yield loss of *Phaseolus vulgaris*, Phytopathology 91 (2001) 1045-1053.

[11] R. Hall, Compedium of Bean Diseases, APS Press, The American Phytopathological Society, 2000, p. 73.

[12] A. Namayanja, R. Buruchara, G. Mahuku, P. Rubaihayo, P. Kimani, S. Mayanja, et al., Inheritance of resistance to angular leaf spot in common bean and validation of the utility of resistance linked markers formarker assisted selection outside the mapping population, Euphytica 151 (2006) 361-369.

[13] S. Liu, K. Yu, S.J. Park, R.L. Conner, P.M. Balasubramanian, H.H. Mündel, et al., Marker-assisted selection of common beans for multiple disease resistance, Annual Report of the Bean Improvement Cooperative (BIC) 48 (2005) 82-83.

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

[15] G.S. Mahuku, M.A. Henriquez, J. Munoz, R.A. Buruchara, Molecular markers dispute the existence of the Afro-Andean Group of the bean angular leaf spot pathogen, *Phaeoisariopsis griseola*, Phytopathology 92 (2002) 580-589.

[16] Van Schoonhoven, Pastor-Corrales, Standard System for the Evaluation of Bean Germplasm, Cali, Colombia, 1987, p. 44.

[17] D.S. Falconer, T.F.C. Mackay, Introduction to Quantitative Genetics, 4th ed., Benjamin Cummings, England, 1996.

[18] G.B. Amaro, A.F.B. Abreu, M.A.P. Ramalho, F.B. Silva, Phenotypic recurrent selection in the common bean (*Phaseolus vulgaris* L.) with carioca-type grains for resistance to the fungi *Phaeoisariopsis griseola*, Genet, Mol. Biol. 30 (3) (2007) 584-588.

[19] N. Ali, J.C. Wynne, Heritability estimates and correlation studies of early maturity and other agronomic traits in two crosses of peanut (*Arachis hypogaea* L.), Pakistan J. Bot. 26 (1) (1994) 75-82.

[20] R.W. Allard, Principles of Plant Breeding, 1st ed., John Wiley and Sons, Inc. New York, 1960, p. 485.

[21] J.W. Muthomi, K.K. Muimui1, P.M. Kimani, Inheritance of resistance to angular leaf spot in yellow beans, African Crop Sci. J. 19 (4) (2011) 267-275.

[22] E.T. Caixeta, A. Bor´em, S.A. Fagundes, S. Niestche, E.G. de Barros, M.A. Moreira, Inheritance of angular leaf spot resistance in common bean line BAT 332 and identification of RAPD markers linked to the resistance gene, Euphytica 134 (2003) 297-303.

[23] G.A. Carvalho, T.J. Paular, A.L. Alzate-Marin, S. Nietsche, E.G. Barros, M.A. Moreira, Inheritance of line resistance AND277 of common bean plants of the race 63-23 of *Phaeoisariopsis griseola* and identification of RAPD markers linked to the resistant gene, Fitopatologia Bras. 23 (1998) 482-485.

[24] C.F. Ferreira, A. Borém, G.A. Caravalho, S. Neitsche, T.J. Paula, E.G. de Barros, et al., Inheritance of angular leaf spot resistance in common bean and identification of a RAPD marker linked to a resistance gene, Crop Sci. 40 (2000) 1130-1133.

[25] S. Nietsche, A. Borém, G.A. Carvalho, R.C. Rocha, T.J. Paula, E.G. de Barros, et al., RAPD and SCAR markers linked to a gene conferring resistance to angular leaf spot in common bean, Phytopathology 148 (2000) 117-121.

[26] A. Sartorato, S. Nietsche, E.G. Barros, M.A. Moreira, RAPD and SCAR markers linked to resistance gene to angular leaf spot in common beans, Fitopatologia Bras. 25 (2000) 637-642.

[27] G. Mahuku, C. Montoya, M.A. Henríquez, C. Jara, H. Teran, S. Beebe, Inheritance and characterization of angular leaf spot resistance gene presence in common bean accession G 10474 and identification of an AFLP marker linked to the resistance gene, Crop Sci. 44 (2004) 1817-1824.

[28] B.Y.E. Chataika, J.M. Bokosi, M.B. Kwapata, R.M. Chirwa, V.M. Mwale, P. Mnyenyembe, et al., Performance of parental genotypes and inheritance of angular leaf spot (*Phaeosariopsis griseola*) resistance in the common bean (*Phaseolus vulgaris*), African J. Biotechn. 9 (28) (2010) 4398-4406.

[29] E.F. Caixeta, A. Bore´m, N.G. de Morais Silvia, R.C. Rocha, E.G. de Barros, M.A. Moreira, Teste de alelismo para ggenes do feijoeiro que conferem resisteˆncia ao fungo *Phaeoisariopsis Griseola*: VII. Congresso nacional de pesquisa de jeija˜ o., Universi-dade Federal de Vicosa, Vicosa-MG, Brazil, Sept., 2002, pp. 8-12.