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#### INHERITANCE OF RESISTANCE TO ANGULAR LEAF SPOT IN YELLOW BEANS

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### ABSTRACT

Angular leaf spot (*Phaeoisariopsis griseola* (Sacc) is an important disease of common bean (*Phaeolus vulgaris* L.) in most parts of Africa, causing yield losses of 40-80%. This study was carried out to determine the inheritance of resistance to angular leaf spot in yellow beans. Biparental crosses were done between susceptible yellow bean genotypes and angular leaf spot resistant parents to generate  $F_1$ ,  $F_2$  and backcrosses. Resistance was evaluated in a screen house and field after inoculation with *Phaeoisariopsis griseola*. The  $F_1$  were resistant, indicating that resistance was dominant. There was no significant deviation from the expected 3:1 ratio for resistant to susceptible in the  $F_2$  population, confirming that resistance to angular leaf spot was both monogenic and dominant. The backcross to Lusaka Yellow showed a 1:1 segregation ratio, while the backcrosses to Mexico 54 were all resistant : susceptible  $F_2$  populations. All backcrosses to Mexico 54 were resistant, confirming that resistance to angular leaf spot in Mexico 54 is controlled by a single dominant gene. The results of the study showed that resistance to angular leaf spot in yellow beans is governed by a single dominant gene. This can be used to improve the local landraces by incorporating angular leaf spot resistance and high yield traits.

Key Words: Monogenic, Phaeoisariopsis griseola, Phaseolus vulgaris

# RÉSUMÉ

La tâche agulaire des feuilles (Phaeoisariopsis griseola (Sacc) est une maladie importante du haricot commun (Phaseolus vulgaris L.) dans la plus part de regions africaines, causant des pertes de rendement d'environ 40-80%. Cette étude était conduite pour déterminer l'acquisition de la résistance des haricots jaunes à la maladie de tâche angulaire. Les croisements biparentaux étaient effectués entre les génotypes du haricot jaune susceptible et les parents résistants à la génération F, F, ainsi que de croisements récurrents. La résistance était évaluée en serre et au champ après inoculation avec Phaeoisariopsis griseola. Les F, étaient résistantes, indicant que la résistance était dominante. Il n'avait aucune déviation significative du rapport attendu 3:1 pour la résistance, au susceptible dans les populations F., confirmant que la résistance à la tâche angulaire de feuilles était monogénique et dominant. Les produits du croisement en retour de Lusaka Yellow a montré un rapport de ségrégation 1:1, pendant que ceux de Mexico 54 étaient tous résistants. Pempela était susceptible à la tâche angulaire, alors que les F, étaient résistantes. Les progénies F, ségrégées en un rapport 3:1 pour résistant au susceptible et le backcross (croisement en retour) au Pempela avait donné un rapport 1:1 pour résistant au susceptible. Tous les produits du croisement en retour du Mexico 54 étaient résistants, confirmant que la résistance à la tâche angulaire des feuilles dans Mexico 54 est controllée par un seul gène dominant. Les résultats de cette étude ont montré que la résistance à la tâche angulaire dans les haricots jaunes est gouvernée par un seul gène dominant. Ceci peut être utilisé pour améliorer les variétés locales indigènes par incorporation de la résistance à la tâche angulaire des feuilles et les traits à rendement élevé.

Mots Clés: Monogénique, Phaeoisariopsis griseola, Phaseolus vulgaris

### INTRODUCTION

Common bean (Phaseolus vulgaris L.) is the most widely grown legume and it is the second most important source of dietary protein in Eastern and Southern Africa (Pachico, 1993). Major seed classes grown include red mottled, large red kidney, small red, yellow, navy, purples, black and sugars (Wortmann et al., 1998). However, yellow bean cultivars are susceptible to diseases such as angular leaf spot (Phaeoisariopsis griseola) resulting in low yields. In Africa, angular leaf spot is considered to be the most widely spread and economically important disease of beans (Wortmann and Allen, 1994). The disease significantly reduces the number of seeds per pod as well as seed weight. It also leads to premature defoliation, shrivelled pods and shrunken seeds (Santos - Filho et al., 1976). Yield losses can reach 80% under severe conditions of infection (Schwartz et al., 1981).

Management of angular leaf spot and other foliar diseases of beans is constrained by the high cost of chemicals, the ability of the pathogen to survive in plant debris for a long time and land un availability for crop rotation (Deeksha et al., 2009). The current focus is to develop varieties that have multiple-constraint resistance (Miklas et al., 2002; Sharma et al., 2007). Therefore, breeding programmes aim at pyramiding or accumulating several resistance sources in a variety as a way of developing broad and durable resistance in common bean in general, and in preferred bean classes in particular. To realise this goal, it is important to identify sources of resistance to the diseases, determine their mode of inheritance and deploy the resistance genes in order to improve the preferred but low yielding types in the market classes.

Diverse sources of resistance to angular leaf spot in bean genotypes have been reported (Correa *et al.*, 1989; Beebe and Pastor-Corrales, 1991). Examples of resistant cultivars include A 75, A 140, A 152, A 175, A 229, BAT 76, BAT 431, BAT 1432, BAT 1458 and G5686 (CIAT,1984). Regagnin *et al.* (2005) found angular leaf spot resistance in AND 277, while Nietsche *et al.*, (2000) found it in Cornell 49-242. Mahuku *et al.* (2004) found resistance in G 10474. CIAT (2003) reported resistance in Mexico 54. Sources of resistance reported from Africa include GLP 24, GLP X-92, GLP-806 and GLP 77 (CIAT, 1984).

Resistance to various diseases is monogenically determined, but cases of duplicate, complementary and other interactions have been reported (Singh and Muñoz, 1999). The objective of this study was to develop segregating populations of yellow beans with resistance to angular leaf spot and determine the inheritance of the resistance.

### MATERIALS AND METHODS

Generation of experimental populations. The experimental materials consisted of angular leaf spot susceptible Zambian varieties (Lusaka Yellow and Pembela), resistant line (Mexico 54) and their crosses (F<sub>1</sub>, F<sub>2</sub>, backcrosses BC<sub>1</sub>P<sub>1</sub> and  $BC_1P_2$ ). The progeny derived from backcrossing  $F_1$  to the female parent was designated  $BC_1P_1$  and those from the backcrossing to the male parent as BC<sub>1</sub>P<sub>2</sub>. Lusaka Yellow and Pembela, which are widely grown and preferred in Zambia, but are susceptible to angular leaf spot and common bacterial blight, were used as females (Table 1); while the source of resistance was Mexico 54 whose seed was obtained from the Regional Bean Programme at the University of Nairobi. The two crosses were Lusaka Yellow X Mexico 54 and Pembela X Mexico 54.

Emasculation and pollination was done either early in the morning or evening as described by Tumwesigye (1988). Buds, which were plump, showing colour and would open the following day were chosen as the female flowers. After emasculation of the female flower, pollination was done immediately using the rubbing or hooking method (Buishand, 1956; CIAT, 1987). Freshly opened flowers were chosen from donor parents to provide pollen.

After crossing, a cotton thread and a tag labelled with the pedigree of the cross was tied loosely on the flower stalk. At maturity, the pods were harvested together with their identification tags. These were sun-dried and threshed to give  $F_1$  seed. Part of the  $F_1$  seed from each cross was sown in the screen house to produce  $F_2$  seeds and also backcrossed to both parents.

Genotype	Origin	Seed colour	Growth habit <sup>a</sup>	Seed size	Days to flowering	Reaction to ALS
Mexico 54 Lusaka Yellow	CIAT Zambia	Pink Yellow	 	Medium Medium	43 41	Resistant Susceptible
Pembela	Zambia	Yellow	II	Medium	33	Susceptible

TABLE 1. Characteristics of parental lines used in the study

II -indeterminate erect; III -indeterminate semi-prostate; ALS = Angular leaf spot

Isolation of Phaeoisariopsis griseola and plant inoculation. Isolation of angular leaf spot was made from lesions of naturally infected bean leaves showing fungal sporulation. In the case of non-sporulating lesions, the fungus was induced to sporulate by incubating the infected tissues in moist chambers. Small pieces of infected tissues were surface sterilised with 3% (v/v) sodium hypochlorite for 5 minutes and plated on bean leaf decoction agar (BLDA, Karanja et al. 1994). The emerging fungal growth was subcultured on fresh BLDA medium. The inoculum was harvested in sterile distilled water and the suspension was filtered through a triple layer of muslin and adjusted to 2x106 conidia ml-1 using a haemocytometer.

Three weeks old bean seedlings which were planted in inoculation chambers maintained at high humidity of over 90% 24 hours before inoculation. High humidity in the inoculation chambers was ensured by lining with polythene sheets and wet absorbent clothing. Using a hand atomiser, the plants were inoculated mainly on the abaxial side of the first primary and trifoliate simple leaves at a distance of 10-15 cm until run off. Controls consisted of plants treated with sterile distilled water without the pathogen inoculum. A repeat inoculation was done at the start of flowering. After inoculation, the plants were maintained under high relative humidity to enhance the development of angular leaf spot symptoms.

Green house and field experiments lay out. Treatments consisted of angular leaf spot susceptible parents (Lusaka yellow and Pembela), resistant parent (Mexico 54) and their  $F_1$ ,  $F_2$  and backcrosses. The  $F_1$  and  $F_2$  crosses were Lusaka yellow x Mexico 54 and Pembela x Mexico 54 while the backcrosses were (Lusaka yellow x Mexico 54) x Lusaka yellow, (Lusaka yellow x Mexico 54) x Mexico 54, (Pembela x Mexico 54) x Pembela and (Pembela x Mexico 54) x Mexico 54.

In the green house experiments, three to four seeds of each of the lines were planted in each of three pots per line and laid out in a completely randomised design with three replications. Sisal ropes were used to support the plants that showed some climbing tendency. Field experiments were conducted at the Faculty of Agriculture field station, University of Nairobi, located at latitude 11°4' 20" S and longitude 36°45' E with an altitude of 1820 metres above sea level. The site has an average annual rainfall of 1046 mm, mean maximum temperatures is 23°C and a minimum of 12º C. The soils are deep, well drained, darkreddish brown friable clays. Three metre rows of each of the parents,  $F_1$ ,  $F_2$  and the backcrosses were planted per plot at a spacing of 50 cm apart and 15 cm within rows. The experiment was laid out in a randomised complete block design and replicated three times. Fertilisation was done at planting by application of di-ammonium Phosphate (DAP - 18% N and 46%  $P_2O_5$ ) at the rate of 150 kg ha<sup>-1</sup>. Bean lines showing climbing growth habits were supported with sticks.

Angular leaf spot severity, plant height, days to flowering and maturity and yield data were recorded for each genotype. Disease severity assessment was initiated at onset of symptoms while plant height was assessed at pod set. The yield components assessed included the number of pods per plant, pod length and seed yield. At physiological maturity, each genotype was harvested, air-dried and threshed separately to determine the number of seeds per pod, number of seeds per plant, 100 seed weight and total seed weight.

Assessment of angular leaf spot severity. Angular leaf spot assessment was initiated at the onset of disease symptoms and each genotype was rated as resistant or susceptible based on a 1-9 assessment scale (van Schoonhoven and Pastor-Corrales, 1987) (Table 2). Scoring for the disease was done on three trifoliate leaves selected at the bottom, middle and top of each plant. The mean disease score for all the plants assessed was calculated for each genotype. Genotypes showing a disease severity rating of 1-3 were regarded as resistant, 4-6 as intermediate and 7-9 as susceptible.

**Statistical analysis.** Data collected were subjected to analysis of variance (ANOVA) using Genstat statistical package (GenStat 6.1). Genetic variances for plant height, days to flowering, days to maturity, yield and yield components were determined by analysis of variance. From the angular leaf spot data, Chi-square was calculated to obtain Mendelian segregation ratios while heritability was calculated as the proportion of total genetic variance in a progeny.

### RESULTS

**Inheritance of angular leaf spot.** All the plants of Lusaka Yellow were susceptible, while those of Mexico 54 were resistant. All the  $F_1$  plants showed resistant reactions to *P. griseola* (Table 3). The backcross to Lusaka Yellow showed 1:1

segregation ratio, while the backcross to Mexican 54 had all the progenies resistant. All Pembela plants were susceptible to the angular leaf spot while all Mexico 54 plants were resistant (Table 4). The  $F_1$  were all resistant, while the  $F_2$  segregated in the 3:1 ratio for resistance to susceptible. Backcrosses of the  $F_1$  to the susceptible parent produced progeny that showed a 1:1 resistant:susceptible ratio. However backcross progeny to the resistant parent were all resistant.

Inheritance of quantitative. All the Lusaka yellow x Mexico 54 crosses and the parents showed significant differences in all the quantitative traits (days to flowering, days to maturity, plant height pod length and yield (Table 5). The degrees of dominance for plant height and number of pods per plant were 0.74 and 0.70 with additive genetic differences of 61%. Pod length showed a degree of dominance of 31%. The degree of dominance for number of seeds per pod was 0.28. Seed size of the F<sub>1</sub> was better than the mid parent value by 6%. This trait showed an additive genetic difference of 65%, with a degree of dominance of .073. Grain Yield showed about 57% due additive genetic differences, while the degree of dominance was 0.45.

TABLE 2. General scale used to evaluate the reaction of bean germplasm to fungal pathogens

Rating	Category	Description	Comments
$\begin{bmatrix} 1\\2\\3 \end{bmatrix}$	Resistant	No visible symptoms or very light symptoms (5-10%)	Germplasm useful as parent or commercial variety
$\left\{\begin{array}{c}4\\5\\6\end{array}\right\}$	Intermediate	Visible and conspicuous symptoms resulting only in limited economic damage (10-60%)	Germplasm can be used as commercial variety or source of resistance to diseases
<sup>7</sup> 8 9	Susceptible	Severe to very severe symptoms causing considerable yield losses or plant death(60-100%)	Germplasm in most cases not useful as parents or commercial varieties

ABLE 3. Reaction of Lusaka ) abete, Kenya	Yellow and Mexico 54, their F <sub>1</sub> , F <sub>2</sub> , BC, P <sub>1</sub> a	ind BC <sub>1</sub> P <sub>2</sub> progenies to inoculation with isolate 63-55 of Phaeoisariopsis. griseola in greenhouse	experiments at
arent/cross	Generation	Number of plants X <sup>2</sup>	Pr

Parent/cross	Generation		Number of plants		X <sup>2</sup>	Pr
		Resistance	Susceptible	Expected ratio		
Lusaka Yellow (LY)	م	0	25			
Mexico 54	_ م_	27	0			
LY x Mexico 54	ц <sup>—</sup> і	37	0			
LY x Mexico 54	F 2	145	44	3:1	0.25	0.5-0.7
(LY x Mexico 54) x LY	BC <sub>1</sub> P <sub>1</sub>	63	56	1:1	0.54	0.3-0.5
(LY x Mexico 54) x Mexico 54	$BC_2P_2$	115	2	1:0	0.0	1.00
Parent/cross	Generation		Number of plants		<b>X</b> <sup>2</sup>	Pr
		Resistance	Susceptible	Expected ratio		
Pembela	۵_	0	29			
Mexico 54	P2	30	0			
Pembela x Mexico 54	LL <sup>-</sup> I	90	0			
Pembela x Mexico 54	т 2 2	120	40		0.13	0.5-0.7
(Pembela X Mexico 54) X Pembela		5 8 0 F	04 0		1.21	0.2-0.3 1 00
(Lellineia a ivieaiuu 24) a ivieaiuu u	4 υν <sub>2</sub>	0.4	۲	0.1	0.00	00.1

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Generation	Days to	Days to	Plant	Pods	Pod	Seeds	100 Seed	Grain yield
	flowering	maturity	height (cm)	plant <sup>1</sup>	length (cm)	-pod	weight (g)	(kg ha <sup>-1</sup> )
م.	41	85	91	13	13	Ð	38	2534
P2	44	89	151	24	11	5	40	2216
Ľ.	41	85	138	28	10	4	41	2618
BĊ,P,	43	87	130	28	11	4	38	1932
BC,P,	42	88	154	29	11	5	41	2746
F	43	87	142	20	11	4	38	2319
LSD(0.05)	1.605	2.485	30.10	12.37	1.114	0.666	NS	675.6
CV (%)	2.2	1.7	13.5	33.8	5.9	9.0	15.9	16.6

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Pembela x Mexico 54 crosses and parents were significantly different in days to maturity, plant height, pod and seed yield (Table 6). The crosses generally showed improved performance compared to parent 1. Pembela had slightly larger seeds compared to Mexico 54, but the seed sizes in the two parents were within the medium category of the CIAT classification (van Schoonhoven and Pastor-Corrales, 1987). An increase of 16% in seed size above the better parent and 21% above the mid parent value was observed in the F<sub>1</sub>. A heterosis of 11% in grain yield was observed above the better parent value. The crosses showed a higher heritability for number of pods per plant than grain yield (Table 7).

## DISCUSSION

The F<sub>1</sub> plants resulting from crosses between Lusaka yellow and Mexico 54 were all resistant (Tables 3 and 4) indicating that the resistance was governed by single dominant gene. The F<sub>2</sub> progenies segregated in a 3:1 ratio and the calculated Chi-square values gave a good fit for the ratio of 3 resistant to 1 susceptible. This type of segregation suggested that the resistance in Mexico 54 to angular leaf spot is governed by a single dominant gene as reported by Ferira et al. (2000) and Kimani et al. (2002). The BC<sub>1</sub>P<sub>1</sub> showed 1:1 segregation ratio and the  $BC_1P_2$  had all the progenies resistant. This agrees with the results by Namayanja (2003) and Mahuku et al. (2002) reported that the resistance in Mexico 54 was due to a single recessive gene. Mahuku et al (2003 and 2009) also reported three dominant genes  $(Phg_{G5686A}, Phg_{G5686B} and Phg_{G5686C})$  in Mexico 54. Resistance to specific isolates of P.griseola has been reported to be simply inherited and molecular markers have been identified for some these resistance genes (Ferreira et al., 2000; Mahuku et al., 2004; Miklas et al., 2005).

The non-significance of the 100 seed weight among the progenies of this cross indicated the closeness of the parents in terms of seed size. The dominance of genes from Mexico 54 governing days to flowering, maturity and plant height was observed in the progenies. Moreover, backcrosses to the donor parent had mean values above the mid parent, indicating the influence of

Mexico 54) x Mexico 54

TABLE 6. Mear	is for the selected tr	raits in $P_1$ , $P_2$ , $F_1$ , $F_2$ a	ind backcrosses of t	the cross Pembela x	Mexico 54 in field tria	als at Kabete, Kenya		
Generation	Days to flowering	Days to maturity	Plant height (cm)	Pods plant <sup>-1</sup>	Pod length (cm)	Seeds pod <sup>.1</sup>	100 Seed weight (g)	Grain yield (kg ha <sup>.1</sup> )
۳ 8 8 1 - 2 - 2 - 2 - 2 - 2 - 2 - 2 - 2 - 2 -	4 4 4 4 7 4 4 7 4 4 7 7 4 4 7 7 7 4 4 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7	85 89 89 88 88	59 156 163 138 145	16 33 25 23 23	9.5 1 1 2 1 1 1 0 1 1 1 0	ლ 4 ო ი 4	37 33 36 36 35	2670.6 2686.1 2977.5 3580.3 2899.3 2571.5
LSD(0.05) CV (%)	1.251 1.7	3.024 2.0	26.26 11.6	17.9 40.6	1.30 7.6	0.841 12.4	7.711 12.3	670.6 14.1
TABLE 7. Herita	bility (h²) (%) for dif	ferent traits in yellow	bean crosses in fiel	ld trials at Kabete in	Kenya			
Cross	Days to flowering	Days to maturity	Plant height	Pods plant -1 (cm)	Pod length (cm)	Seeds pod <sup>-1</sup>	100 Seed weight (g)	Grain yield (kg ha <sup>.1</sup> )
Lsk x Mex 54 Pem x Mex 54	22 56	52 64	42 48	61 64	39 24	41 17	65 48	57 35

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Lsk - Lusaka Yellow; Pem - Pembela.

the donor gene. The recombination of these two genotypes showed that it is possible to transfer resistance from the Mesoamerican to the Andean pool. The crosses showed a higher heritability for number of pods per plant compared to grain vield. This could indicate that selection for yield based on number of pods per plant would be less effective than based on the grain yield. Fernandez and Miller (1985), working with grain type cowpeas (Vigna unguiculata), also observed that pod number is the yield component most affected by the environment. The higher heritability observed in days to flowering, days to maturity, plant height, 100 seed weight and grain yield may indicates that the traits are useful in determining which family structure is best for maximising genetic gain over time (Holland et al., 2003).

The lack of difference in segregation of the F generation for resistance to susceptible to Phaeoisariopsis griseola of the crosses Lusaka Yellow x Mexico 54 and Pembela x Mexico 54 from the expected 3:1 ratio (Table 3 and 4) indicates that the resistance to angular leaf spot in Mexico 54 is governed by a single dominant gene. The existence of significant positive heterosis in the yield and yield components in the crosses is encouraging as it indicates that gene combinations exist which can result in enhanced yield performance. The performance of the parents could be used to predict the performance of the progenies. The top yielders among the F<sub>1</sub> had at least one of the parents with high grain yield per plot. This shows that for parents to enter a breeding programme they should be high yielding. High heritability values are important as they indicate that the selection of parents bearing particular measurements will produce progenies of the same phenotype (Holland et al., 2003).

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