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## Eight cycles of recurrent selection for resistance to angular leaf spot in common bean

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**ABSTRACT** - The genetic progress (GP) was evaluated after eight cycles of recurrent selection for resistance to angular leaf spot in common bean and indirect gain for yield and grain type. The base population (Cycle-0) was obtained from the diallel cross of seven lines with carioca grain and ten resistance sources. To obtain Cycle I, the most resistant  $S_0$  plants of Cycle 0 were intercrossed. The same procedure was adopted in the subsequent cycles. GP for resistance to the pathogen was estimated by the overall average of the progenies of each cycle in comparison with control Carioca MG and for productivity in comparison to the controls Carioca MG and Pérola. Lines of the first six cycles were used for grain type. No GP was detected for resistance to angular leaf spot. However, the indirect yield gain was 2.3% and 2.5% in the selection for grain type, with values similar to those obtained by direct selection for these traits.

**Key words:** *Phaseolus vulgaris*, *Pseudocercospora griseola*, heritability, genetic progress, disease resistance.

### INTRODUCTION

With the intensification of common bean cultivation in Brazil in the past 20 years, problems with diseases have increased. Among the pathogens, the causal agent of angular leaf spot, *Pseudocercospora griseola* (Sacc.) Crous and Braun (2006), has caused significant damage to the crop (Paula Júnior and Zambolim 2006). In the state of Minas Gerais its importance is on the rise. The primary means of control is the use of resistant cultivars (Silva et al. 2006, Couto et al. 2008, Ragagnin et al. 2009). However, the difficulty of obtaining lines with durable resistance is unquestioned, mainly due to the proven existence of numerous races of the pathogen (Sartorato and Alzate Marín 2004, Sartorato 2004, Silva et al. 2008, Balbi et al.

2009). The information available about the genetic control of resistance indicates that several genes must be involved (Miklas et al. 2006, Amaro et al. 2007, Mahuku et al. 2004, Mahuku et al. 2009).

In this situation, one of the possibilities to accumulate the different resistance alleles is by recurrent selection (Ramalho et al. 2001). This strategy can unite the greatest number of favorable alleles distributed in different parents in a single plant. In this case, where the trait heritability is relatively high (Pereira et al. 2004, Amaro et al. 2007, Couto et al. 2008), the selection of plants for recombination may be phenotypic already in the  $F_2$  generation. In 1999, Amaro et al. (2007) initiated a phenotypic recurrent selection program to produce new common bean lines that accumulate resistance alleles and simultaneously have high yields and

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grain with good market acceptance. On this background, this study was conducted to evaluate the genetic gain after eight cycles of phenotypic recurrent selection for resistance to angular leaf spot as well as to assess the indirect responses in yield and grain type.

## MATERIAL AND METHODS

The establishment of the base population and the recurrent selection program until the fifth cycle (C-V) were performed by Amaro et al. (2007). Initially, a partial diallel was established involving seven lines with carioca type grains (Carioca MG, CI-140, CI-128, ANPAT 8.12, IAPAR 81, ESAL 693, and Pérola) and 10 sources of resistance to *P. griseola griseola* (AN 512561, AND 277, Ouro Negro, Compuesto Negro Chimaltenango, CAL 143, MAR 2, MAR 1, G 5686, MA 4.137, and Jalo) with different grain types. The crosses were made in the greenhouse of the biology department of the Federal University of Lavras (UFLA), in the winter growing season of 1998 (sowing in July).

Of the 70 possible combinations, only 29 hybrids were obtained, largely due to the incompatibility of some crosses (Silva and Gutierrez 1984). The hybrids were sown in November of the same year to obtain the  $S_0$  seeds. Each of these 29  $S_0$  populations was treated independently, forming the base population or cycle 0 population (C-0). In March of 1999, this population was sown and at the end of the cycle, the most resistant plants from each cross were phenotypically selected.

To obtain cycle I (C-I), the 29 best  $S_{0;1}$  plants of C-0 were intercrossed, one per population, phenotypically selected for resistance to the fungus. Among these, lines were selected with carioca grain as similar as possible to the required market standard, i.e., grain with base color beige and light brown stripes. For this purpose, one plant per population was intercrossed with two others, according to the scheme proposed by Ramalho et al. (2001). Plants sown in July 2000 were recombined and the hybrid seeds obtained were sown in November of 2000, to obtain  $S_0$  seeds of cycle I (C-I). As shown, it is possible to conduct one selection cycle per year. The same procedure was used to obtain cycle II (C-II) through cycle VIII (C-VIII).

In the region, conditions for the development of the pathogen are favorable in growing season with sowing in February/March. The  $S_0$  populations in which plants were selected to be recombined to obtain the following cycle were therefore always conducted in this season. In each cycle, in the  $S_0$  generation, aside from the plants selected

for recombination, others were identified to generate progenies ( $S_{0;1}$ ) and continue the inbreeding process, to obtain lines. This procedure was adopted until C-V. From C-VI onwards, populations were conducted in bulk up to the  $S_3$  generation for the inbreeding process. For this purpose, the  $S_0$  generation was sown in February/March,  $S_1$  in July,  $S_2$  in October/November and  $S_3$  again in February/March, when the  $S_{3;4}$  progenies were obtained. By this strategy, resistance to the pathogen was selected before obtaining the progenies in  $S_0$  and also in  $S_3$ , which are the two generations grown in the season when the incidence of angular leaf spot is most severe.

The progenies  $S_{0;1}$  or  $S_{3;4}$  were always evaluated in experiments sown in February/March of the subsequent year in Lavras, MG, in 2-m row plots, always using 'Carioca MG' (susceptible to the pathogen) and 'Pérola' (tolerant) as control cultivars. Details about the number of progenies in each cycle of recurrent selection, the experimental design and the assessment year are shown in Table 1. In all experiments, the yield was evaluated in  $\text{kg ha}^{-1}$  and severity of angular leaf spot on a nine-grade scale proposed by CIAT and adapted by Nietzsche et al. (2000), where 1 refers to plants without disease symptoms and 9 indicates plants with severe symptoms, leading to premature leaf drop and death.

**Table 1.** Number of progenies of each cycle of recurrent selection, experimental design and year of sowing

Cycle	Progeny type	Number of progenies	Experimental design	Year
C-I	$S_{0;1}$	223	Simple lattice 15 x 15	2001
C-II	$S_{0;1}$	322	Simple lattice 18 x 18	2002
C-III	$S_{0;1}$	194	Simple lattice 14 x 14	2003
C-IV	$S_{0;1}$	287	Simple lattice 17 x 17	2004
C-V	$S_{0;1}$	254	Simple lattice 16 x 16	2005
C-VI	$S_{3;4}$	79	Simple lattice 9 x 9	2007
C-VII	$S_{3;4}$	254	Simple lattice 16 x 16	2008
C-VIII	$S_{3;4}$	194	Simple lattice 14 x 14	2009

The type of grain (of the same growing season) was evaluated in five lines of each of the first six cycles of recurrent selection, on a 1 - 5 scale, similar to that proposed by Ramalho et al. (2005). Lines with typical carioca grain, or light beige color with light brown stripes, with no halo, average weight of 100 grains of 22-24 g, and not flattened grains were graded 1 and lines with dark beige grains with dark brown stripes, with a halo, average weight of 100 seeds < 22 g, and flattened grains were graded 5. The grain was rated by three researchers familiar with the crop.

The data regarding severity of angular leaf spot (grades 1-9) and the grain yield of progenies from each cycle were subjected to analysis of variance, considering all effects as random, except of the mean. The genetic variance ( $\hat{\sigma}_G^2$ ), phenotypic variance ( $\hat{\sigma}_F^2$ ) and broad-sense heritability ( $h^2$ ) were also estimated, according to Ramalho et al. (1993). The confidence interval  $h^2$  was calculated by the equations proposed by Knapp et al. (1985).

The genetic progress (GP) achieved by recurrent selection in the traits reaction to angular leaf spot and grain yield was estimated by a methodology similar to that used by Amaro et al. (2007). For this purpose, linear regressions were obtained, always considering the selection cycles as independent variable (x). Genetic deviations of progenies ( $d_G$ ) were used as dependent variable (Y) for each trait in relation to the ordinary controls in order to mitigate the environmental effect, since the progenies of each cycle were evaluated in different years. For severity of angular leaf spot, the  $d_{Gi}$  ( $d_G$  in cycle i) was estimated considering only the average of the susceptible control ‘Carioca MG’ in cycle i. From this follows that  $d_{Gi} = (\text{mean of Carioca MG in cycle } i - \text{mean of the progenies in cycle } i)$ . For grain yield, the mean of two controls was used to compute  $d_{Gi}$ , that is,  $d_{Gi} = (\text{mean of progenies in cycle } i - \text{mean of controls in cycle } i)$ . Thus, the percentage of genetic progress was estimated as follows:  $GP(\%) = \left(\frac{b_i}{\bar{X}_{C_i}}\right) * 100$ , where  $GP(\%)$  is the percentage of genetic progress per cycle of recurrent selection in relation to the mean of the progenies of C-I;  $b_i$  is the coefficient of linear regression; and  $\bar{X}_{C_i}$  is the mean of the progenies of C-I.

This estimator was also used for grain type. A similar procedure as described above was used to estimate the genetic progress. However, for regression, the average grade of grains of the five lines of cycle i was considered as dependent (Y) and the selection cycles as independent variable (X).

## RESULTS AND DISCUSSION

Significant differences ( $P < 0.07$ ) were detected among the families in all cycles for grades of severity of angular leaf spot, indicating the existence of variability among the progenies in the resistance level to the pathogen (Table 2). The estimates of genetic and phenotypic parameters corroborated this observation. In most cases the  $h^2$  estimates exceeded 50% (Table 3). These values are consistent with those obtained by several authors who assessed the severity of the pathogen using different types of progenies (Pereira et

**Table 2.** Summary of analyses of variance for severity of angular leaf spot (1-9 grade scale) and grain yield (kg ha<sup>-1</sup>) of the progenies assessed in Lavras, from the first (C-I) to the eighth (C-VIII) cycle of recurrent selection

Cycle	Severity of angular leaf spot						CV(%)
	Progenies <sup>1</sup>			Error		Mean Progenies	
	df <sup>2</sup>	MS	Prob	df <sup>2</sup>	MS		
I	221	3.287	0.000	196	1.479	3.9	30.72
II	321	3.239	0.000	289	1.142	4.5	24.14
III	193	1.116	0.000	169	0.407	3.3	19.13
IV	286	1.329	0.000	256	0.391	3.8	16.38
V	253	0.347	0.029	225	0.271	2.8	18.30
VI	78	1.046	0.061	64	0.719	1.3	62.42
VII	253	1.317	0.000	225	0.550	4.6	16.01
VIII	193	2.519	0.000	169	1.442	4.3	27.94

  

Cycle	Grain yield						CV(%)
	Progenies <sup>1</sup>			Error		Mean Progenies	
	df <sup>2</sup>	MS	Prob	df <sup>2</sup>	MS		
I	221	531056.80	0.000	196	306031.60	1776	31.12
II	321	939776.10	0.000	289	473943.00	2638	26.10
III	193	778932.30	0.000	169	326905.60	2432	23.50
IV	286	480807.70	0.000	256	295401.40	2602	20.89
V	253	781277.00	0.012	225	580734.90	2575	29.66
VI	78	447798.75	0.066	64	310796.30	2001	27.90
VII	253	825187.55	0.000	225	478848.10	3310	21.09
VIII	193	740226.90	0.000	169	383913.15	2025	30.68

<sup>1</sup> Without check cultivars; <sup>2</sup> degrees of freedom associated to source of variation.

**Table 3.** Estimates of genetic ( $\hat{\sigma}_G^2$ ) and phenotypic variances ( $\hat{\sigma}_F^2$ ) between progenies and heritability in the broad sense ( $h_a^2$ ) with their respective lower (LL) and upper limits (UL), from the first to the eighth cycle of recurrent selection for grade of severity of angular leaf spot (1-9 grade scale) and grain yield (kg ha<sup>-1</sup>)

Cycle	Progeny types	Severity of Angular leaf spot				
		$\hat{\sigma}_G^2$	$\hat{\sigma}_F^2$	$h_a^2$ (%)	LL	UL
I	S <sub>0:1</sub>	0.904	1.644	55.00	40.97	65.78
II	S <sub>0:1</sub>	1.048	1.619	64.74	55.87	71.87
III	S <sub>0:1</sub>	0.354	0.558	63.52	48.81	72.83
IV	S <sub>0:1</sub>	0.469	0.665	70.58	62.66	76.85
V	S <sub>0:1</sub>	0.038	0.173	21.81	-0.64	39.52
VI	S <sub>3:4</sub>	0.163	0.523	31.24	-9.54	57.32
VII	S <sub>3:4</sub>	0.383	0.658	58.23	46.17	67.65
VIII	S <sub>3:4</sub>	0.538	1.259	42.75	23.37	57.35

  

Cycle	Progeny types	Grain yield				
		$\hat{\sigma}_G^2$	$\hat{\sigma}_F^2$	$h_a^2$ (%)	LL	UL
I	S <sub>0:1</sub>	112512.60	265528.40	43.37	24.39	56.18
II	S <sub>0:1</sub>	232916.55	469888.05	49.57	36.87	59.76
III	S <sub>0:1</sub>	226013.35	389466.15	58.03	43.82	68.73
IV	S <sub>0:1</sub>	92703.15	240403.85	38.56	22.03	51.66
V	S <sub>0:1</sub>	100271.05	390638.50	25.67	4.21	42.43
VI	S <sub>3:4</sub>	68499.98	223898.12	30.59	-10.58	56.92
VII	S <sub>3:4</sub>	173169.72	412593.78	41.97	25.22	55.06
VIII	S <sub>3:4</sub>	178156.88	370113.45	48.14	30.58	61.36

al. 2004, Amaro et al. 2007, Couto et al. 2008). The existence of variability in the progenies is also reflected in the magnitude of the mean estimates in each cycle (Table 4).

**Table 4.** Means of progenies (Prog) and of the check cultivars ‘Carioca MG’ (CMG) and ‘Pérola’, from the first to the eighth cycle of recurrent selection, genetic deviation ( $d_G$ ), coefficients of linear regression ( $b_0$  and  $b_1$ ) and of determination ( $R^2$ ) and genetic progress (GP) for severity of angular leaf spot (1-9 grade scale), yield (kg ha<sup>-1</sup>) and grain type (1-5 grade scale)

Cycle	Severity of Angular leaf spot				Yield			Grain type lines
	Prog	CMG	Pérola	$d_G^1$	Prog	Test	$d_G^2$	
I	3.9 (1.7-7.9) <sup>3</sup>	5.7	4.2	1.8	1776 (656-3934) <sup>3</sup>	1966	-190	3.400
II	4.5 (2.1-7.8)	7.6	5.2	3.1	2638 (1120-4910)	2057	581	2.900
III	3.3 (1.8-5.9)	7.2	3.1	3.9	2432 (950-4109)	1804	628	3.100
IV	3.8 (1.5-7.5)	6.5	4.9	2.7	2602 (904-3885)	2197	405	2.700
V	2.8 (1.8-4.3)	6.0	3.6	3.2	2575 (943-4365)	1864	711	2.900
VI	1.3 (1.0-4.4)	3.6	2.4	2.3	2001 (919-3084)	1892	109	2.900
VII	4.6 (2.9-6.7)	5.9	4.5	1.3	3310 (1568-5129)	2737	573	-
VIII	4.3 (2.0-7.4)	6.8	5.5	2.5	2025 (542-3674)	1528	497	-
$b_0$				3.033			224.55	3.260
$b_1$				0.098			41.55	-0.084
$R^2$ (%)				8.22			11.36	42.900
GP(%)				-2.49			2.34	-2.470

<sup>1</sup>  $d_G$  = (Mean of cultivar Carioca MG) - (Mean of the progenies).

<sup>2</sup>  $d_G$  = (mean of progenies) - (mean of the check cultivars).

<sup>3</sup> In brackets, variation in severity of angular leaf spot and grain yield.

The value of the overall mean of progenies throughout the selection cycles, tended to decrease up to C-VI (Table 4). The mean increased in C-VII and C-VIII, however the same was observed for controls, evidencing a fluctuation in pathogen severity among years. This fact may be due to some environmental factor and/or difference in the prevailing race in each year.

In the analysis of variance for grain yield significant differences ( $P < 0.07$ ) were also detected among the families in all cycles (Table 2). It appears that the  $h^2$  estimates in different cycles were, in most cases, over 40% (Table 3), similar to the values found for this trait under other conditions (Pereira et al. 2004, Amaro et al. 2007, Couto et al. 2008). The mean grain yield differed between cycles. The amplitude of variation was large within each cycle (Table 4).

Recurrent selection is an activity that needs to be periodically evaluated. Estimating genetic gain in these periods allows monitoring the gains achieved until then and to check whether the previously used procedures should be adapted. A major problem in comparative assessments of the severity of pathogen occurrence among years and/or different locations is the environmental effect. To mitigate this problem, a susceptible control such as cultivar ‘Carioca MG’ is mostly used. In assessments of the early progeny generations of each selection cycle, the average grade assigned to this

cultivar varied a little; the values reflected susceptibility of the cultivar, indicating, as mentioned above, that conditions in the dry growing season were favorable for the pathogen development (Table 4). Only in C-VI the pathogen incidence was low, as the average severity grade of ‘Carioca MG’ shows.

The estimation of genetic gain for severity of angular leaf spot showed that the linear regression coefficient ( $b_1$ ) was not different from zero (Table 4). Moreover, the coefficient of determination ( $R^2$ ) was only 8.2%, indicating that genetic differences in severity grades did not fit to the linear regression equation. It should be emphasized that in the case of this trait, there are some factors that may impede the smooth data fitting to the regression equation, one of which is the environmental effect. The use of a control mitigates the problem, but does obviously not eliminate it completely, especially when only one susceptible control is used. The trait measurement by grades is subjective. Even if the rater is experienced, the precision of the grades can vary from one cycle to another. Additionally, one should not forget that this pathogen has several races that differ in virulence (Nietsche et al. 2001, Sartorato and Alzate-Marin 2004, Silva et al. 2008). From one season to the next, the prevalent races may change and pathogen severity may therefore vary.

There are few reports on the use of recurrent selection for resistance to pathogens of common bean (Lyons et al. 1987, Garcia et al. 2003, Amaro et al. 2007). Amaro et al. (2007) had estimated a gain of 6.4% per cycle for resistance to *P. griseola* and an indirect response in grain yield of 8.9% up to the fifth recurrent selection cycle. Sufficient variability was also detected among the families allowing the prediction of the possibility of future selection gains. Since studies of the genetic control of resistance to certain *P. griseola* races suggest that resistance is monogenic and/or oligogenic (Caixeta et al. 2005, Mahuku et al. 2004), the use of recurrent selection could be questioned. However, this pathogen has several races (Nietsche et al. 2001, Sartorato and Alzate-Marin 2004, Silva et al. 2008) and, in the genetic control of resistance to all these races, surely a great number of genes will be involved. Furthermore, a gradient in symptoms was observed in the lines considered resistant. Part of this gradient can be attributed to environmental effects, however, the existence of polygenic control of the trait should not be ruled out.

As angular leaf spot affects the yield (Correa-Victoria et al. 1994, Jesus Júnior et al. 2001) the success of a recurrent selection program can also be assessed indirectly by estimating the gain for this trait. Thus, to obtain the indirect response in grain yield by the selection for pathogen resistance, the genetic gain for yield was estimated. The

estimated regression coefficient ( $b_1$ ) was 41.55 kg ha<sup>-1</sup>, but also associated with a low coefficient of determination (Table 4). The percentage gain in relation to the mean progeny yield in C-I was 2.3% per cycle. This value was similar or even superior to the values reported for direct selection for grain yield (Ranalli 1996, Singh et al. 1999, Ramalho et al. 2005). It was inferred that the focus of attention of common bean breeding programs should be the breeding of lines resistant to this pathogen, as a strategy to achieve gains in grain yield.

Aiming to quantify grain improvement over the recurrent selection cycles, genetic gain for this trait was estimated based on the average of five lines of each selection cycle, from the first to the sixth cycle. A decreasing trend of values in the selection cycles was noticed, i.e, the grain type was improved (Table 4). The estimated regression coefficient ( $b_1$ ) was -0.084, corresponding to an average percentage gain of -2.47% per cycle, compared to the grades of the lines in the first selection cycle. It is noteworthy that the likeliness of commercial acceptance of grains of the base population was very low. But since the grain type was considered in the identification of progenies for recombination or for

advancement of inbreeding, the trait was improved over the cycles. In other recurrent selection programs for grain yield and plant architecture, indirect gains for grain type were observed as well, since breeders always take this trait into consideration in the selection of progenies for recombination (Ramalho et al. 2005, Cunha et al. 2005).

## CONCLUSIONS

In seven of the eight wheat lines used as parents, the genotypes containing loci responsible for the grain color inheritance were fully or partially characterized. Among the genotypes evaluated, Frontana and Ônix have three genes for the determination of pre-harvest sprouting resistance, and this expression only occurs when all alleles are recessive homozygous.

The red seed color is not considered solely as a full guarantee of greater pre-harvest sprouting resistance.

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# Oito ciclos de seleção recorrente visando resistência à mancha-angular no feijoeiro

**RESUMO** - Foi avaliado o progresso genético (PG) após oito ciclos de seleção recorrente para resistência à mancha-angular no feijoeiro e o ganho indireto para produtividade e tipo de grãos. A população-base (Ciclo-0) foi obtida do dialeto entre sete linhagens de grãos tipo carioca e dez fontes de resistência. Para obtenção do Ciclo-I foram intercruzadas as plantas  $S_0$  do Ciclo-0 mais resistentes. O mesmo procedimento foi adotado nos ciclos seguintes. O PG para resistência ao patógeno foi estimado pela média geral das progênies de cada ciclo em relação à testemunha 'Carioca MG' e, para produtividade, em relação à média das testemunhas ('Carioca MG' e 'Pérola'). Para tipo de grão foram utilizadas linhagens dos seis primeiros ciclos. Não foi detectado PG para resistência à mancha-angular. Contudo, o ganho indireto na produtividade foi de 2,3% e de 2,5% para tipo de grãos, valores semelhantes aos obtidos com a seleção direta para esses caracteres.

**Palavras-chave:** *Phaseolus vulgaris*, *Pseudocercospora griseola*, herdabilidade, progresso genético, resistência à doenças.

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