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ARTICLE

Common bean breeding for resistance to anthracnose and angular leaf spot assisted by SCAR molecular markers

Gilmar Silvério da Rocha[1*], Luis Paulo Lelis Pereira[1], Pedro Crescêncio Souza Carneiro[2], Trazilbo José de Paula Júnior[3] and José Eustáquio de Souza Carneiro[1]

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Abstract - *The objective of the present study was to assess the genetic potential of inbred carioca common bean families from five populations derived from crossings involving elite lines and a disease-resistant line (Rudá-R), and to assess the efficiency of SCAR molecular markers in selecting plants resistant to anthracnose and angular leaf spot, at the time of bulk formation. Plant architecture, yield and grain type were assessed. Significant effect among families within population was observed, suggesting wide genetic variability for the characters assessed. Twenty-six superior families were selected. The families contained the greatest number of markers, identified by SCAR molecular markers in the* F_4 *generation. Eighteen of these families were resistant to the races 65 and 453 of* Colletotrichum lindemuthianum *and five were resistant to the race 63.23 of* Pseudocercospora griseola. *Thus selection assisted by SCAR markers, in the* F_4 *generation, was an important tool in common bean breeding.*

Key words: Phaseolus vulgaris, Colletotrichum lindemuthianum, Pseudocercospora griseola, *assisted selection, disease resistance.*

INTRODUCTION

Brazil is the main producer of common bean (*Phaseolus vulgaris*), followed by India, China, United States of America and Mexico (FAO 2008). However, Brazil presents one of the lowest average yields in the world, about 960 kg ha^{-1} (CONAB 2010). One of the factors that accounts for the low yield is the occurrence of several diseases that affect the common bean crop. They include anthracnose, caused by *Colletotrichum lindemuthianum*, rust, caused by *Uromyces appendiculatus* and angular leaf spot, caused by *Pseudocercospora griseola*, that deserve great attention because of the damage they cause and because of the high pathogenic variability of the pathogens (Paula Júnior and Zambolim 2006).

The use of resistant cultivars stands out among the various strategies used to control diseases because it is cheap and generally more efficient. The identification of molecular markers associated to the

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[1] Universidade Federal de Viçosa (UFV), Departamento de Fitotecnia, 36.570-000, Viçosa, MG, Brazil. *E-mail: gilmaragro2001@yahoo.com.br
[2] UFV, Departamento de Biologia Geral
[3] Empresa de Pesquisa Agropecuária de Minas Gerais (EPAMIG), Centro Tecnológico da Zona da Mata, C.P. 216, 36.570-000, Viçosa, MG, Brazil

resistance genes is an auxiliary tool in common bean breeding for resistance to diseases (Alzate-Marin et al. 2005). With the help of molecular markers, pyramided lines are obtained with resistance alleles to several pathogens, by means of assisted selection (Ragagnin et al. 2009, Costa et al. 2010). These lines are extremely useful in crossing programs to incorporate alleles with resistance to pathogens in elite lines and commercial cultivars.

Several strategies are used in breeding programs by using hybridization to conduct segregant populations. In the case of bulk strategy plant selection to obtain progenies is normally carried out phenotypically. In this sense, selection assisted by molecular markers for resistance to pathogens can also help to identify plants with desirable characters and prevents less promising plants from being submitted to later stages of selection.

Characters, such as plant architecture, yield and grain type, have also received great attention in common bean breeding programs (Collicchio et al. 1997, Cunha et al. 2005, Menezes Júnior et al. 2008). For the carioca-type common bean, the preference has been for grains with opaque tegument, cream color with light brown stripes and light underground. The cultivar Pérola, for example, the standard for grain type and yield, is the most cultivated in Brazil, even though it is susceptible to the main races of *C. lindemuthianum* and *P. griseola* that predominate in the producing regions in Brazil (Faleiro et al. 2001, Melo et al. 2008).

In this context, the present study was carried out with the objective of assessing the genetic potential of inbred families of the carioca-type common bean, from five populations derived from crossings involving the pyramided Rudá-R line, and to assess the efficiency of SCAR (sequence characterized amplified regions) molecular markers in the selection of plants resistant to anthracnose and angular leaf spot, at the time of bulk formation.

MATERIAL AND METHODS

The families assessed originated from crossings involving five elite lines (UTF-0013, CNFC 9437, OPS-82, Carioca 1070 and GEN 12-2) and the pyramided line Rudá-R (Ragagnin et al. 2009), that carries a pyramid of alleles that confer resistance to *C. lindemuthianum* (*Co-4*, *Co-6* and *Co-10*), *P. griseola* (*Phg-1*) and *U. appendiculatus* (*Ur*-ON) (Table 1). These families were derived from plants from the F_4 generation, previously genotyped by SCAR molecular markers (Table 2) associated to resistance genes to the pathogens of anthracnose and angular leaf spot.

The field experiments were carried out in the experimental station in the Crop Science Department (lat 20º 51' 24" S, long 42º 48' 10" W) at the Federal University of Viçosa (UFV), municipality of Coimbra, MG, in the 2006 winter growing season, 2007 dry growing season and 2007 winter growing season. Two hundred and fifteen families were assessed in the 2006 winter growing season, 35 of which belonged to the cross UTF-0013 x Rudá-R, 50 to the cross CNFC 9437 x Rudá-R and 70 to the cross GEN 12-2 x Rudá-R. The crosses OPS-82 x Rudá-R and Carioca 1070 x Rudá-R contributed with 30 families each. Eighteen families were selected from each population for the assessments in 2007, taking into consideration yield and grain type. Ten controls were used in the three experiments, involving the six parents and the cultivars Pérola, BRSMG Talismã, BRSMG Majestoso and BRSMG Madrepérola. A triple lattice design was used and the plots consisted of two 2m long rows.

Grain type and plant architecture were assessed in addition to yield. Grain type was assessed using a scale of scores ranging from 1 (typical carioca grain type, cream-colored with light brown stripes, light underground, cream halo, mass of 100 seeds of 22 to 24 g and non-flattened grains) to five, (cream-colored grain with dark brown stripes, dark underground, colored halo, mass of 100 seeds less than 22 g and flattened grain) (Ramalho et al. 1998). Plant architecture was evaluated using a scale of scores of 5 degrees similar to that proposed by Collicchio et al. (1997), where 1 refers to the type II plant, erect, with a stem and high insertion of the first pod and score 5 to the type III plant, with long internodes and very prostrated.

Grain yield (kg ha^{-1}) and plant architecture data were submitted to analysis of individual variance, considering the effects of treatments and the mean as fixed, following the lattice model: $Y_{ijk} = \mu + t_i + r_j + b_{k(j)} + e_{ijk}$, where Y_{ijk} is the observation for treatment i, in block k, within replication j; μ, the general mean; t_i, the effect of the treatment i (i = 1, 2, ..., 225 for the 2006 harvest; i = 1, 2, ..., 100 for the 2007 harvests); r_j, the effect of replication j (j = 1, 2, 3); $b_{k(j)}$, the effect of block k within replication j (k = 1, 2, ..., 15 for the 2006 harvest; k = 1, 2, ..., 10 for the 2007 harvests); e_{ijk}, the random error association to observation Y_{ijk}, assuming independent and normally distributed errors with zero mean and variance σ^2.

Table 1. Main characteristics of the common bean parents used to obtain the populations

Parents	Favorable characteristics	Unfavorable characteristics
UTF-0013	High yield and erect plants	Susceptibility to anthracnose
CNFC 9437	High yield and erect plants	Susceptibility to angular leaf spot and anthracnose (race 65 of *Colletotrichum lindemuthianum*)
OPS-82	High yield and resistance to rust	Susceptibility to anthracnose (race 65 of *C. lindemuthianum*)
Carioca 1070	Precocity	Susceptibility to anthracnose, rust and angular leaf spot
GEN 12-2	High yield and resistance to anthracnose	Susceptibility to rust
Rudá-R	Resistance to anthracnose, rust and angular leaf spot	Small grains, dark underground

Table 2. SCAR molecular markers used, alleles of resistance, distances and monitored common bean populations

Markers[1]	Alleles of resistance	Distances (cM)	Monitored populations
SCARY20$_{830c}$	*Co-4*	1.2	All, except GEN 12-2 x Rudá-R
SCARAZ20$_{940c}$	*Co-6*	7.1	Carioca 1070 x Rudá-R
SCARF10$_{1050c}$	*Co-10*	6.9	UTF-0013 x Rudá-R
SCARH13$_{520c}$	*Phg-1*	5.5	CNFC 9734 x Rudá-R

[1] c: coupling.

The analysis for grain type was carried out in randomized blocks, because the scores for this trait were attributed in the laboratory. Later, joint analysis of variance, using the means of the treatments (Ramalho et al. 2005) was carried out with the help of the softwares Genes (Cruz 2006) and MSTAT-C Michigan State University (1991).

The families with high performance in the field and with the molecular markers according to the information of the genotype of the F_4 plant that gave origin to them were inoculated with different pathogens. Fifteen plants of each family were first inoculated with the races 65 and 453 of *C. lindemuthianum*. The races used were chosen because the parents presented susceptibility reaction to at least one of them (except Rudá-R), according to the previous inoculation. The race 63.23 of *P. griseola* was inoculated only on plants of the families from the CNFC 9734 x Rudá-R population, which was monitored with the SCARH13$_{520a}$ marker. In this case, the referred marker was linked to the allele *Phg-1* (Queiroz et al. 2004), which confers resistance to several races of *P. griseola*.

The inoculum of *C. lindemuthianum* and *P. griseola* was prepared in the Molecular Plant Genetics Laboratory at the Institute of Biotechnology Applied to Agriculture (Bioagro/UFV). The steps related to disease assessment of inoculated plants were carried out in greenhouse and mist chambers. Inoculum production and inoculation of *C. lindemuthianum*, were carried out according to a methodology adapted from Pio-Ribeiro and Chaves (1975).

The inoculum of the race 63.23 of *P. griseola* was produced on Petri dishes with culture medium based on tomato extract, agar and calcium carbonate ($CaCO_3$). Inoculation was carried out after the appearance of the first trifoliate leaf, approximately 15 days after plant emergence, on both the leaf surfaces, with a suspension previously adjusted to the concentration of 2 x 10^4 conidia mL^{-1}. After quick drying in the air, the plants were transferred to a mist chamber (20 ± 1 ^{o}C and over 95 % relative humidity), where they remained for 48 hours with a 12 hour light period. After that, the plants were kept in a greenhouse (20 ± 5 ^{o}C) for 15 days.

The severity of anthracnose and angular leaf spot in the inoculated plants was assessed based on a scale of scores from 1 to 9 (Van Schoonhoven and Pastor-Corrales 1987), where 1 referred to absence of symptoms and 9 to severely attacked plants. The families that presented medium degrees of reaction from 1 to 3 were considered resistant and those with degree 3.1 or higher, susceptible.

RESULTS AND DISCUSSION

Significant differences were detected among populations, families and also among families within populations for the three characters, suggesting the existence of genetic variability in these populations (Table 3). In Brazil, it is common to make crosses

Table 3. Summary of the individual and joint analysis of variance of yield (YI, in kg ha^{-1}), plant architecture (PA) and grain type (GT) of the assessment of common bean families in the different growing seasons

Individual analysis										
Sources of variation	df	MS (winter 2006)		df	MS (dry season 2007)			MS (winter 2007)		
		YI	GT		YI	GT	PA	YI	GT	PA
Families	214	697114.84**	0.55**	89	507922.25**	0.58**	0.93**	732533.12**	0.64**	0.54**
Effective error	448 (406)[1]	141378.93	0.16	198 (171)[1]	275863.97	0.09	0.17	161469.79	0.12	0.20
CV (%)	-	16.60	18.10	-	13.19	12.82	11.73	12.76	12.25	13.23
General mean	-	2266	2.19	-	3983	2.36	3.52	3150	2.77	3.34

Joint analysis						
		Yield (kg ha^{-1})		Plant architecture		Grain type
Sources of variation	df	MS	df	MS	df	MS
Environments (E)	2	160169906.89**	1	4.91**	2	38.64**
Treatments (T)	99	883803.20**	99	1.34**	99	1.36**
Families (F)	89	804262.88**	89	1.21**	89	1.08**
UTF-0013 x Rudá-R	17	343211.18**	17	1.07**	17	0.28**
CNFC 9437 x Rudá-R	17	784622.40**	17	1.56**	17	0.70**
OPS-82 x Rudá-R	17	505273.76**	17	0.46**	17	0.41**
Carioca 1070 x Rudá-R	17	387214.75**	17	0.59**	17	0.23*
GEN 12-2 x Rudá-R	17	557698.60**	17	0.31*	17	0.46**
Among populations	4	6938261.22**	4	9.97**	4	15.15**
Controls (C)	9	1576493.23**	9	2.70**	9	4.18**
F *vs* C	1	1728681.51**	1	1.02*	1	0.73*
Treatments x E	198	562186.77**	99	0.28**	198	0.24**
F x E	178	532655.25**	89	0.26*	178	0.23**
(UTF-0013 x Rudá-R) x E	34	505922.65**	17	0.39**	34	0.22**
(CNFC 9437 x Rudá-R) x E	34	340875.26**	17	0.39**	34	0.33**
(OPS-82 x Rudá-R) x E	34	646995.50**	17	0.17	34	0.18
(Carioca 1070 x Rudá-R) x E	34	223495.19	17	0.17	34	0.19*
(GEN 12-2 x Rudá-R) x E	34	270839.12*	17	0.14	34	0.16
Among populations x E	8	3402036.44**	4	0.44**	8	0.44**
C x E	18	907417.41**	9	0.48**	18	0.36**
(F *vs* C) x E	2	83416.91	1	0.24	2	0.11
Mean error	748	176716.47	342	0.18	844	0.13
CV (%)	-	13.06	-	12.37	-	15.08
General mean	-	3220	-	3.43	-	2.39

[1] The value in parenthesis refers to the df number of the effective error of the YI and PA traits, analyzed in a lattice. * and ** Significant at 5 and 1 % probability, respectively, by the F test.

involving only parents of the carioca group to enhance the main characteristics that define this grain type. Singh and Urrea (1995) warned that genetic variability for crop yield was limited when the cross was carried out within the same genetic set. However, we found the presence of among and within population genetic variability, which could be useful to obtain superior lines.

The interaction between families and environments (F x E) and its partitions were significant for crop yield (Table 3). This fact indicated inconsistency in the performance of the families in the different growing seasons. The occurrence of the genotype x environment interaction for this character has been reported in studies with common bean (Carneiro et al. 2002, Bruzi et al. 2007, Mendes et al. 2009).

Lower occurrence of interaction of families x environment was observed for plant architecture and grain type, with three and two of the five populations with non-significant interaction, respectively (Table 3). Considering grain type, this fact was expected because it is less influenced by the environment (Pereira et al. 2004, Baldoni et al. 2006). However, some populations presented significant effect for the interaction families x environment. One of the possible causes of this interaction is the oscillation of the intermediate scores attributed to the families, as pointed out by Cunha et al. (2005). Thus, for greater reliability in the selective process, these characters should be assessed in more environments.

Families were observed with high yield potential and grains with good commercial type in all populations, superior than cultivars such as Pérola and BRSMG Majestoso, both recommended for the state of Minas Gerais (Table 4). The populations UTF-0013 x Rudá-R and CNFC 9437 x Rudá-R were outstanding, because they presented higher yield (3507 and 3337 kg ha^{-1}, respectively) than that of the cv. Pérola (3008 kg ha^{-1}), the reference and most cultivated variety of the carioca group in Brazil. Furthermore, the families of these populations were outstanding for the erect plant architecture. It is pointed out that among the parents used in the crosses, the lines UTF-0013 and CNFC 9437 have the most erect stand (Table 1). The populations CNFC 9437 x Rudá-R and GEN 12-2 x Rudá-R presented families with good commercial grain type, similar or even superior than 'Pérola' (Table 4).

Considering the mean performance of families and the information regarding the genotype of the F_4 plant that gave origin to them, 26 families were selected for inoculation with *C. lindemuthianum*. Eighteen of these 26 families presented resistance to the races 65 and 453. These results suggest that these families carried the alleles *Co-4* and *Co-10* and/or the allele *Co-6*, considering that the source of resistance used in the crosses (Rudá-R) carries these three alleles (Table 5). It is important to emphasize that race 65 of *C. lindemuthianum* has been reported as one of the most frequent in the common bean producing regions of Brazil (Carbonell et al. 1999, Talamini et al. 2004) and that the cultivars Pérola and Rudá are susceptible to this race (Melo et al. 2008).

The resistance of the families in the population CNFC 9437 x Rudá-R to the 63.23 race of *P. griseola* showed that the *Phg-1* allele was introgressed and that the selection assisted by the SCARH13$_{520a}$ marker in the F_4 generation was efficient (Table 5). It is important to mention that this race of *P. griseola* is widely distributed in Minas Gerais and Brazil (Nietsche et al. 2001).

Table 4. Yield means (kg ha^{-1}), plant architecture scores and grain type of five common bean populations and controls

Populations and controls	Yield[1]	Architecture	Grain type
UTF-0013 x Rudá-R	3507 (3098 - 3775)	3.1 (2.2 - 3.7)	2.4 (2.1- 2.7)
CNFC 9437 x Rudá-R	3337 (2493 - 3701)	3.2 (2.1 - 4.1)	2.2 (1.8 - 2.8)
OPS-82 x Rudá-R	3200 (2803 - 3620)	3.7 (3.1 - 4.3)	2.7 (2.4 - 3.1)
Carioca 1070 x Rudá-R	2948 (2680 - 3401)	3.3 (2.9 - 3.9)	2.7 (2.3 - 2.9)
GEN 12-2 x Rudá-R	3180 (2534 - 3483)	3.8 (3.4 - 4.2)	1.9 (1.7 - 2.5)
Controls	3088 (2330 - 3564)	3.3 (2.1 - 4.2)	2.5 (1.4 - 3.5)
Pérola	3008	3.0	2.0
BRSMG Majestoso	3133	3.2	1.8
BRSMG Talismã	2654	3.8	2.4

[1] Values in parenthesis refer to the lower and upper limits of each population and the controls.

Table 5. Yield means (YI, in kg ha^{-1}), plant architecture (PA) and grain type (GT); reaction[1] to *Colletotrichum lindemuthianum* (races 65 and 453) and *Pseudocercospora griseola* (race 63.23) and molecular characterization of the F_4 plants that gave rise to the common bean families assessed

Families and parents	Means			*C. lindemuthianum*		*P. griseola*	Markers linked to the respective resistance alleles			
	YI	PA	GT	Race 65	Race 453	Race 63.23	H13 (*Phg-1*)[2]	Y20 (*Co-4*)[3]	F10 (*Co-10*)[4]	AZ20 (*Co-6*)[5]
UTF-0013 x Rudá-R - 1	3545	2.9	2.5	R	R	-[6]	-	1	1	-
UTF-0013 x Rudá-R - 4	3721	3.5	2.2	R	R	-	-	1	1	-
UTF-0013 x Rudá-R - 5	3529	2.2	2.3	R	R	-	-	0	1	-
UTF-0013 x Rudá-R - 6	3731	2.8	2.6	R	R	-	-	1	1	-
UTF-0013 x Rudá-R - 11	3443	3.0	2.1	R/S	R	-	-	1	1	-
UTF-0013 x Rudá-R - 12	3386	2.7	2.6	R	R	-	-	1	1	-
UTF-0013 x Rudá-R - 13	3775	2.9	2.5	R	R	-	-	1	1	-
UTF-0013 x Rudá-R - 14	3469	2.8	2.3	R/S	R	-	-	1	1	-
UTF-0013 x Rudá-R - 18	3728	3.6	2.3	R	R	-	-	1	1	-
CNFC 9437 x Rudá-R - 20	3452	2.8	2.4	S	R	-	1[7]	0	-	-
CNFC 9437 x Rudá-R - 21	3418	2.6	2.4	R/S	R	R	1	0	-	-
CNFC 9437 x Rudá-R - 25	3701	2.1	2.6	R	R	R	1	0	-	-
CNFC 9437 x Rudá-R - 26	3577	2.8	2.5	R	R	R	1	0	-	-
CNFC 9437 x Rudá-R - 29	3096	3.8	2.5	R	R	R	1	1	-	-
CNFC 9437 x Rudá-R - 30	3381	2.6	2.1	R/S	R	R	1	0	-	-
CNFC 9437 x Rudá-R - 35	3639	3.5	2.3	R/S	R	R/S	1	1	-	-
OPS-82 x Rudá-R - 39	3620	3.3	2.6	R	R	-	-	1	-	-
OPS-82 x Rudá-R - 49	3405	3.8	2.6	R	R	-	-	1	-	-
OPS-82 x Rudá-R - 52	3371	3.7	2.4	R/S	R	-	-	1	-	-
OPS-82 x Rudá-R - 53	3484	3.9	2.4	R	R	-	-	1	-	-
Carioca 1070 x Rudá-R - 55	3121	3.0	2.4	R	R	-	-	0	-	0
Carioca 1070 x Rudá-R - 56	2784	3.3	2.6	R	R	-	-	1	-	1
Carioca 1070 x Rudá-R - 58	2987	3.1	2.9	R/S	R/S	-	-	1	-	1
Carioca 1070 x Rudá-R - 59	2908	2.9	2.3	R	R	-	-	1	-	1
Carioca 1070 x Rudá-R - 66	3192	3.3	2.6	R	R	-	-	1	-	1
Carioca 1070 x Rudá-R - 70	3031	2.9	2.6	R	R	-	-	0	-	0
UTF-0013	3398	2.2	2.7	R/S	S	-	1	0	0	0
CNFC 9437	2757	2.7	2.2	S	R	S	0	0	1	0
OPS-82	3556	3.9	2.2	S	R	-	0	0	0	0
Carioca 1070	2330	4.2	3.2	S	S	-	0	0	0	0
Rudá-R	3011	3.6	3.5	R	R	R	1	1	1	1

[1] R, resistant; S, susceptible; R/S, resistant and susceptible. [2] Allele that confers resistance to race 63.23 of *P. griseola*. [3] and [4] Alleles that confer resistance to races 65 and 453 of *C. lindemuthianum*, respectively. [5] Allele that simultaneously confers resistance to races 65 and 453. [6] Not assessed. [7] Value 1 and 0 for plants with presence and absence of the mark, respectively.

The results obtained with the inoculations in the selected families were consistent with those obtained in the molecular characterization of the F_4 plants that gave rise to them (Table 5). In the case of the population UTF-0013 x Rudá-R, all the families resistant to anthracnose under inoculation (races 65 and 453 of *C. lindemuthianum*) originated from F_4 plants, which presented the molecular marks associated to the alleles *Co-4* and *Co-10*, except for family 5, which presented only the mark linked to the allele *Co-10*. It could be explained because this family may have presented resistance to race 65 of *C. lindemuthianum* in function of the presence of the allele *Co-6*, also present in the Rudá-R. The same was observed for the population OPS-82 x Rudá-R, because all the families resistant to race 65 of *C. lindemuthianum* originated from plants carriers of the SCAR molecular markers associated to the allele *Co-4*.

Two families in the population Carioca 1070 x Rudá-R (55 and 70) presented resistance to *C. lindemuthianum* although they did not present molecular marks associated to the resistance in the F_4 generation (Table 5). This fact can be explained by the recombination between the resistance gene and the SCAR molecular markers, because the distance between them was 1.2 cM for the $SCARY20_{830a}$ marker and 7.1 cM for the $SCARAZ20_{940a}$ marker (Table 2).

Only seven of the total families assessed segregated, that is, presented plants resistant and susceptible to anthracnose and angular leaf spot (Table 5). This result was expected because SCAR is a dominant marker. Therefore plants with marks of the F_4 generation could be heterozygote.

CONCLUSIONS

The families of the population CNFC9437 x Rudá-R presented great potential for extraction of lines with carioca grain type, with erect plants, resistant to anthracnose and angular leaf spot and high yield potential.

The carioca line Rudá-R was an excellent source of resistance to the anthracnose and angular leaf spot pathogens for use in crossing programs.

Selection assisted by SCAR molecular markers was an important tool in common bean breeding for resistance to *C. lindemuthianum* and *P. griseola*.

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Melhoramento do feijoeiro visando resistência à antracnose e mancha angular assistido por marcadores moleculares SCAR

Resumo - *O objetivo deste trabalho foi avaliar o potencial genético de famílias endogâmicas de feijão carioca provenientes de cinco populações oriundas de cruzamentos envolvendo linhagens elites e uma fonte de resistência a doenças (Rudá-R), bem como avaliar a eficiência de marcadores moleculares SCAR na seleção de plantas resistentes a antracnose e mancha angular, por ocasião da abertura dos bulks. Foram avaliadas a arquitetura da planta, a produtividade e o aspecto de grãos. Observou-se efeito significativo entre famílias dentro de populações, indicando ampla variabilidade genética para os caracteres avaliados. Foram selecionadas 26 famílias superiores e que reuniam maior número de marcas, identificadas por marcadores SCAR na geração* F_4*. Dessas, 18 famílias foram resistentes às raças 65 e 453 de* Colletotrichum lindemuthianum *e cinco resistentes à raça 63.23 de* Pseudocercospora griseola*. Assim, a seleção assistida por marcadores SCAR, na geração* F_4*, mostrou ser uma ferramenta importante no melhoramento do feijoeiro.*

Palavras-chave: Phaseolus vulgaris, Colletotrichum lindemuthianum, Pseudocercospora griseola, *seleção assistida, resistência a doenças.*

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