

Selection of Carioca type common bean lines with anthracnose and angular leaf spot-resistance

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ABSTRACT - Objective of the present study was to select common bean lines with the traits anthracnose and angular leaf spot-resistance, high yield, Carioca type grains and an upright plant type. One hundred and fifty-three lines were selected from five segregating families derived from backcrosses using G2333 (donor parent) and the lines ESAL 696 and CI 140 (recurrent parents). The lines were evaluated and selected in three seasons under inoculation with the races 2047, 1545 and 81 of *C. lindemuthianum* and also tested with the SCAR marker of allele Co-5. Lines with high grain yield, Carioca type grains, upright plant type, and anthracnose-resistance were selected. The SCAR marker was not effective for the selection of resistant lines with Co-5 allele. No angular leaf spot-resistant lines were identified.

Key words: *Phaseolus vulgaris*, Carioca type, *Colletotrichum lindemuthianum*, *Phaeoisariopsis griseola*.

INTRODUCTION

In Brazil, common bean is affected by numerous biotic and abiotic factors. Among the biotic factors diseases such as anthracnose and angular leaf spot play a role by reducing the productivity and, consequently, the market supply of this leguminous crop. These diseases occur all over the aerial part of the plant and deprive the product quality. The use of genetic resistance in an integrated control system is of interest, since it does not incur costs or threat nature, the farmer, or the consumer. The capacity of pathogenic variation of the fungi has however hampered

work in this direction. It is imperative to have up-to-date information so that, by means of the exploitation of the existing variability in common bean, new resistant cultivars can be obtained (Rava et al. 1994).

It is of paramount importance to introduce novel sources of resistance alleles and obtain cultivars that have more than one resistance allele, in other words, a pyramid of alleles to be of protection against a large number of races and warrant a more durable resistance. One difficulty with the construction of the allele resistance pyramid is the lack of availability of a set of races that would allow the identification of all resistance alleles that will be

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pyramided in a single line. This difficulty can be overcome by the use of molecular markers such as the PCR-based (Polimerase Chain Reaction). Several markers of the resistance alleles have already been identified, mainly for anthracnose (Young et al. 1998, Alzate-Marin et al. 2001, Kelly and Vallejo 2004). One of the most important alleles is *Co-4²*, present in cultivar G2333 (Young et al. 1998), which confers resistance to all races identified in Brazil (Alzate-Marin and Sartorato 2004).

Another barrier for the use of most resistance sources is the fact that they are not adapted, making the use of backcrossings necessary. The common bean improvement program of the University Federal of Lavras has backcross populations available in which the allele pyramid against anthracnose, present in the non-adapted line G2333, was transferred to adapted lines with Carioca grain type (Hagiwara et al. 2001). Promising lines have been evaluated and selected which possess the pyramid of resistance alleles against *Colletotrichum lindemuthianum* and join other desirable agronomical phenotypes (Pereira et al. 2004).

In this context, the present study had the objectives: i) to identify, among the common bean lines derived from a backcrossing program, some with a pyramid of resistance alleles against *C. lindemuthianum* present in cultivar G2333; ii) to select common bean lines that unite, anthracnose resistance, high grain yield, Carioca grain type, resistance to angular leaf spot and an upright plant type.

MATERIAL AND METHODS

The experimental plant material consisted of 153 lines derived from backcrossings between the donor parent G2333 and recurrent parents ESAL 696 and CI 140. Among these, 19 lines were derived from a segregating $F_{4,7}RC_1$ [(G2333 X ESAL 696) X ESAL 696] family and 134 lines were derived from four segregating $F_{1,4}RC_2$ [(G2333 X ESAL 696) X ESAL 696] X CI 140} families.

G2333 is a Mexican line with various unfavorable phenotypes, but it carries a pyramid (*Co-4²*, *Co-5* and *Co-7*) which confers resistance to all *C. lindemuthianum* races that occur in Brazil, mainly through the presence of allele *Co-4²* in its constitution. Line ESAL 696 has some favorable phenotypes such as plant type II, grains similar to the Carioca cultivar, resistance to *Phaseoisariopsis griseola* and it is carrier of the *C. lindemuthianum*-resistance allele *Co-5*. Line CI140 is derived from an ongoing recurrent selection program at UFLA and stands out with an excellent grain type, similar to that of the

Carioca cultivar, but it has plant type III and is susceptible to both pathogens.

The 153 lines and 15 control lines selected by Pereira et al. (2004), plus the recently released high yield cultivar Talismã with Carioca grain type were evaluated in Lavras-MG in the winter season of 2003. The experiment was conducted in a 13 x 13 simple lattice square design, with two replications and plots of a 1m row. In the dry season of 2004, 120 lines previously selected in the winter season of 2003 as well as cultivar Talismã as control were evaluated in Ijaci-MG and Lambari-MG, considering mainly the grain type. A 11 x 11 lattice design was used with three replications and plots with two meter long rows. In the winter season of 2004 48 lines were evaluated in Lavras and Lambari, which were the most promising in the dry season of the same year, plus cultivar Talismã. The experiment had a 7 x 7 lattice design with three replications and plots of two rows of two meters, with 50 cm in-between rows and 15 seeds per meter.

All experiments were fertilized at sowing with 300 kg ha⁻¹ of the 8N-28P₂O₅-16K₂O formula plus 150 kg ha⁻¹ of ammonium sulfate in topdressing and were sprinkle-irrigated whenever necessary. Otherwise the normal culture treatments were used for the cultivation.

The severity of angular leaf spot was evaluated in a diagram of grades from one to nine (Sartorato and Thung 2002). This evaluation is generally realized in experiments during the dry period, since the pathogen finds favorable climatic conditions in this period for its development. For the evaluation of grain appearance we used a similar scale with grades from one (Carioca type grain) to five (non-standard grain) according to Ramalho et al. (1998). The weight of 100 seeds was also evaluated in the seasons of 2004, since consumers prefer grains of mean size with about 23 to 25 g (Ramalho and Abreu 1998). The plant type was evaluated on a descriptive scale of grades similar to the one proposed by Collichio et al. (1997), with scores varying from one (totally erect) to five (totally prostrate). Means of two evaluators were used in the analysis of variance for each trait.

The reaction to anthracnose was evaluated through inoculations of each pathogen race (2047, 1545 and 81) derived from monospore culture, in the seedling stage (Pereira and Santos 2004). The SCAR marker linked to allele *Co-5* by primer SAB03 was also used (Vallejo and Kelly 2001). For this purpose, approximately 2g of young leaves were collected from every ten plants per line for DNA extraction, using a procedure modified by Rogers and Bendich (1988). The SCAR marker analyses were performed in a similar procedure to the one used by Silva et al. (2003).

The data of three seasons at the different sites were subjected to individual analysis and then to the joint analysis of variance, using adjusted means of the 49 common treatments. Considering the treatment effect as fixed, the coefficient of genotypic determination (h^2) was estimated. This estimate was designated heritability (h^2), restricted to the set of lines used in this study, and was estimated by the expression

$h^2 = (MS_{lines} - MS_{mean\ error}) / MS_{lines}$ for the joint analyses. Confidence intervals of h^2 were also estimated, according to Knapp et al. (1985). The expected gain with selection (GS) was estimated by means of the expression $GS(\%) = ds \cdot h^2$ where ds: differential of selection, i.e., the difference between the mean of the selected lines and the general mean of the experiment. The partition of the interaction lines x environments in simple and complex parts (Ramalho et al. 1993) was realized. The Pearson phenotypic correlations were also estimated for the evaluated traits on software MStatC.

RESULTS AND DISCUSSION

The performance of the common 48 lines indicated significant genetic differences among them, corroborating the results of the individual analyses of variance (data not shown). This genetic divergence can be confirmed by the estimates of heritability, which were higher than those obtained by other authors (Ramalho et al. 1993, Pereira et al. 2004) and indicate success with selection for all traits (Table 1).

The performance of the lines was not coincident in the different environments for all evaluated traits, except

for grain type. This indicates that grain type is little influenced by the environment, which represents a favorable situation for selection. Based on this presupposition, selection in this study was in a first stage only based on the commercially favorable grain type, aiming to evaluate the other traits in the lines most promising for grain type. The reaction to angular leaf spot presented interaction lines x environments, and may be an indicative of different races at the two locations.

In the decomposition of the interaction lines x environments predominance of the complex part was observed for grain yield as much as for plant type. For grain yield it corresponded to 85.7% of the interaction, while for the plant type it was 81.35%. This implies in difficulty of selecting lines common to different environments. The mean estimates of the correlation of Pearson (0.85**) and Spearman (0.84**) however indicate that the classifications of the best lines regarding the plant type should be similar in the different environments. According to Cruz and Castoldi (1991), the adopted procedure for the decomposition of the interaction tends to overestimate the complex part. This overrating must have been significant since from among the ten lines with best plant type, the four environments had two in common and six occurred in three environments.

Considering the selection of the five best lines (approximately 10%) based on the adjusted means of the joint analyses individually for grain yield, weight of 100 seeds, grain type, plant type or reaction to angular leaf spot, expressive gains were observed for most of the traits (Table 2). However, if the five best lines (D1, D7, E5, E9 and E38) were selected considering all traits together (Table 2), notable gains with selection were still attained for yield

Table 1. Summary of the joint analysis of variance for the grades (1-9) of angular leaf spot grades (1-5) of plant type, grain yield ($kg\ ha^{-1}$), grades (1-5) of grain type, weight of 100 seeds, and estimates of heritability (h^2) and respective inferior (h^2_{LI}) and superior (h^2_{LS}) limits

Sources of variation	df	MS		MS		MS		
		Angular leaf spot	df	Plant type	Grain yield	df	Grain type	Weight of 100 seeds
Environments (E)	1	3.123*	3	15.357**	159135819.70**	1	9.078 **	3380.505 **
Lines (L)	48	2.892**	48	1.902**	929599.62**	48	0.885 **	41.694**
L x E	48	1.230 *	144	0.219 **	617366.99 **	48	0.162	11.4**
Pooled error	420	0.845	576	0.139	209930.99	288	0.126	0.4502
Mean	-	5.612	-	2.725	2238.06	-	2.40	22.841
CV (%)	-	15.98	-	13.71	20.27	-	14.81	2.90
h^2	-	72.16	-	92.66	77.41	-	85.76	98.92
h^2_{LI}	-	58.78	-	88.41	64.30	-	77.30	98.28
h^2_{LS}	-	75.58	-	95.00	84.60	-	90.48	99.28

** significant at the level of 1% of probability by the F test

and grain type. Although these two traits had been focused on throughout the successive generations of selection, there is still broad genetic variability for both and the efficiency of the performed selection is confirmed. The gains for plant type and angular leaf spot-resistance were somewhat reduced but still considerable. Even for the weight of 100 seeds the lines presented ideal sizes, considering the adopted criterion. Considering only yield as selection criterion, a reduction was also observed in the expected gain for all traits, except for weight of 100 seeds since the five most productive lines had smaller seeds (Table 2).

When one takes various traits into consideration for the selection process, the gains for each one are smaller, as shown in Table 2. One of the causes of the reduction of the gain with selection of an individual trait, compared to the selection in multiple traits, could be the unfavorable correlation between the evaluated traits. Significant correlation were only observed between grain type and weight of 100 seeds and between grain type and plant type (Table 3), in other words, the lines that presented the best grain type were also the best lines for weight of 100 seeds, although tending to an inferior plant type. The magnitudes of the correlations were however low and intermediate with the possibility of selection of favorable recombinant lines. For the other estimates the correlation indicated the possibility of selecting superior lines for all traits, in agreement with Pereira et al. (2004).

To verify the genetic constitution regarding the resistance alleles to anthracnose all lines evaluated in the 2003- winter season were initially inoculated with race 2047, and 36 resistant ones were identified. However, when the selection was based on other traits, mainly grain type, only 11 remained among the 48 selected lines (Table 4). Race 2047 is only incompatible to lines that carry the resistance allele *Co-4²*, derived from the differentiating cultivar G2333. These 11 lines would therefore be susceptible to any race equal or superior to 2048, yet to date not identified in Brazil. Consequently, inoculation does not permit to verify whether these lines are carriers of the alleles *Co-5* and/or *Co-7*, derived from the donor parent G2333, making the use of molecular markers necessary.

To detect the pyramid *Co-4²* and *Co-5* the SCAR marker linked to allele *Co-5* and amplified by primer SAB03 was used. This marker had 400pb and was not amplified in any of the 11 lines, only in cultivars G2333 and TU, indicating that they do not have allele *Co-5*. It is worth mentioning, however, that this marker can recombine at a frequency of 12.98% (Vallejo and Kelly 2001). It is therefore possible that there are lines with *Co-4²* as well as with *Co-5*.

Young et al. (1998) used populations derived from a crossing of G2333 with a cultivar susceptible to race 1545 ($2^{10}+2^9+2^3+2^0$) to show that anthracnose-resistance is controlled by two genes. Since this race is compatible with lines carrying allele *Co-5*, due to the presence of 512 (2^9) in their constitution, the resistance, in this crossing, is evidently conditioned by the alleles *Co-4²* and *Co-7*. The conclusion may therefore be drawn that the resistance observed in the race 1545-inoculated lines is due to allele *Co-7*. These lines, in turn, can be carriers of *Co-5* or not. To verify the presence of allele *Co-5*, the lines were also inoculated with race 81, since allele *Co-7* does not confer resistance to a race that has 2^6 (64) in its constitution, as in the case of 81 (Alzate-Marín et al. 2001). However, by inoculating race 2047-susceptible lines with race 81 ($2^6+2^4+2^0$), it was possible to identify lines carrying allele *Co-5* and with the pyramid *Co-5*, *Co-7* (Table 4).

With the intention of confirming the validity of the SCAR marker to select carrier lines of the allele *Co-5* and of *Co-5* and *Co-7* (Table 4), they were also used in the PCR with primer SAB03. The marker was only amplified in line A1 and in the resistant control TU (Figure 1). Lines identified with letter C are derived from only one backcrossing and both parents are carriers of allele *Co-5*, so consequently, the lines are homozygous for this allele. Nevertheless, the SCAR marker was not observed. We therefore concluded that parent ESAL 696 despite resistant does not have the marker. Given the distance of the marker to the resistance allele there is the possibility of separating the two, especially because parent G2333, carrier of the marker, was only used in the first crossing. Among the descendant lines of family A only one is carrier of allele *Co-5* as well as of the SCAR marker (Figure 1). This allowed the conclusion that the allele is derived from donor parent G2333. Interestingly all lines selected in family B were carriers of allele *Co-5* but none had presented the SCAR marker, so the possibility that the allele had been inherited from the recurrent parent ESAL 696 was greater. In the case of the lines selected in family D, none was observed with allele *Co-5*, but in seven of these lines we find allele *Co-4²*, where there is a smaller possibility of occurring allele *Co-5*. Most lines selected from family E are allele *Co-5* carriers and did not present the SCAR marker either (Figure 1). We emphasize that the two lines of this family with *Co-4²* have a greater chance of being carriers of *Co-5* as well, compared to the lines derived from family D.

It was not possible to identify the presence of allele *Co-7* in the carrier lines of allele *Co-4²*, since no marker has yet

been found for this allele. Carrier lines of this allele can only be identified by inoculation with race 1545 (Table 4).

It is important to point out that during the backcrossings and conduction of the segregating populations selections for agronomical phenotypes, principally grain type and anthracnose-resistance were realized. Consequently, with the elimination of the unfavorable phenotypes derived from the donor parent, it was inferred that great part of the genome had been eliminated from most of the carrier lines of allele *Co-5*, including the SCAR marker.

Among the 48 selected lines, five stood out (D1, D7, E5, E9, E38) for their mean performance (Table 4). Lines E5, E9, E38 are carriers of the pyramid of alleles *Co-7* and *Co-5*. Line D2 is also promising, since it has reasonable yield and grain type, besides carrying allele *Co-4*². The fact that most lines with allele *Co-4*² have lower yields and inferior grain type suggests that this allele could be associated with unfavorable alleles derived from donor parent G2333.

Of the 48 lines 69.39% performed better than the control mean and 44.89% presented higher grain yield than the general mean (Table 4). We further observed that 61.22% of the evaluated lines presented a more upright plant type than that

of the control Talismã, a very favorable result for the selection of lines with this trait. Regarding grain type, which is fundamental for the acceptance of the cultivar, 32.65% of the selected lines outmatched the Talismã cultivar, and can therefore be considered commercially promising. For the reaction to angular leaf spot the general mean was 5.61, indicating susceptibility to this disease as in the control Talismã. One of the recurrent parents (ESAL 696), besides carrying the anthracnose-resistance allele *Co-5* is also carrier of a dominant allele of angular leaf spot-resistance. However, pathogen races compatible with the lines that carry this allele have probably appeared. Finally, for the weight of 100 seeds, 20.41% of the lines were clustered by Scott-Knott' test of into the same group as control cultivar Talismã (Table 4).

When taking all traits into consideration it was possible to select lines that combined a high yield, Carioca-like grain type, a more upright bush habit, and anthracnose-resistance owing to the *Co-5* and *Co-7* allele pyramid, as well as some carrier lines of the alleles *Co-4*², *Co-5* and *Co-7*. No lines with high resistance to angular leaf spot were identified. The SCAR marker was not effective for selecting allele *Co-5*-carrier genotypes in this set of lines.

Table 2. Estimates of expected selection gain of the five lines of highest expression for grain yield, weight of 100 seeds, grain type, plant type and, reaction to angular leaf spot

Estimate	Grain yield	Plant type	Grain type	Weight of 100 seeds	Angular leaf spot
GS ¹	408.5 (18%)	-0.57 (21%)	-0.43 (18%)	0.34 (1.5%)	-0.94 (17%)
GS ²	329.9 (15%)	0.25 (9.4%)	-0.38 (16%)	-0.21 (0.9%)	-0.48 (8.6%)
GS ³	408.5 (18%)	0.11 (4.1%)	-0.18 (7.4%)	-0.76 (3.3%)	-0.42 (7.6%)

¹ Selection gain considering the five most promising lines for each trait separately

² Selection gain considering all traits together

³ Selection gain considering the five most productive lines

Table 3. Phenotypic correlations between the evaluated traits

Trait	Correlation
Weight of 100 seeds x Grain yield	0.053
Weight of 100 seeds x Angular leaf spot	-0.24
Weight of 100 seeds x Grain type	0.34*
Weight of 100 seeds x Plant type	-0.23
Grain yield x Angular leaf spot	-0.26
Grain yield x Grain type	-0.27
Grain yield x Plant type	0.28
Angular leaf spot x Grain type	0.23
Angular leaf spot x Plant type	-0.06
Grain type x Plant type	-0.62**

*, ** significant at the level of 5% and 1% of probability by the t test

Table 4. Means for grain yield, 100 seeds weight, plant type and angular leaf spot, and anthracnose resistance alleles of the lines

Line	Grain yield (kg ha ⁻¹)	100 seedweight (g)	Grain type grade (1-5)	Plant type grade (1-5)	Angular leaf spot grade (1-9)	Allele combinations Co
A1	2208.80c	28.22c	2.54b	3.05b	6.02b	Co-7 and Co-5
A5	2195.39c	19.87l	2.32c	2.96b	6.13b	None
A6	2188.86c	21.16j	2.09d	2.89b	6.67a	None
A11	2128.63c	20.38k	2.46c	3.01b	5.98b	None
A17	1849.28c	21.04j	2.60b	2.84b	5.73b	Co-4 ²
A20	1884.78c	21.79i	2.28c	2.73c	5.82b	None
A21	1823.51c	20.45k	2.20c	2.61c	6.14b	None
A35	2057.22c	21.33j	2.41c	2.48c	6.54a	None
B10	1865.83c	22.77g	2.24c	2.30d	5.30c	Co-5
B20	2366.63b	20.50k	2.68b	2.01d	6.18b	Co-7 and Co-5
B21	1875.46c	23.02g	2.62b	2.29d	4.88c	Co-5
B22	1643.32c	22.39h	2.54b	2.31d	4.72d	Co-5
B24	2169.09c	21.76i	2.46c	2.47c	5.30c	Co-5
C2	2026.46c	20.70k	2.56b	2.42c	6.78a	Co-5
C19	2004.87c	21.96i	2.48c	2.26d	6.04b	Co-5
D1	3099.25a	22.83g	1.95d	2.84b	3.46e	None
D2	2336.64b	27.98c	2.22c	2.52c	4.61d	Co-4 ²
D3	2134.05c	23.77f	2.13d	2.53c	5.49c	None
D4	2460.68b	22.29h	2.19c	2.57c	5.63b	None
D6	2354.31b	26.49d	2.71b	2.40c	5.80b	Co-4 ²
D7	2746.91a	23.51f	1.98d	2.69c	4.59d	None
D11	2100.63c	23.13g	3.22a	2.03d	5.37c	Co-4 ²
D12	2201.70c	30.23b	3.18a	2.64c	5.00c	Co-4 ²
D13	2500.62b	23.98f	3.35a	2.19d	6.05b	Co-4 ²
D15	2062.85c	22.06i	2.99a	2.02d	5.31c	Co-4 ²
D16	2533.46b	24.21e	2.37c	2.47c	6.33a	None
D19	2132.51c	24.23e	2.09d	2.49c	5.24c	None
D22	2361.27b	24.63e	2.08d	2.53c	5.99b	None
D23	2333.72b	32.13a	3.17a	2.39c	5.82b	Co-4 ²
E5	2524.65b	22.26h	1.98d	3.01b	5.95b	Co-7 and Co-5
E9	2448.13b	21.60i	1.82d	3.16a	5.15c	Co-7 and Co-5
E10	2332.77b	21.52i	2.15d	3.27a	5.51c	Co-7 and Co-5
E11	2667.40a	19.67l	2.37c	2.95b	4.79d	Co-7
E12	2309.29b	24.47e	2.76b	2.68c	5.84b	Co-4 ²
E14	2461.69b	18.68m	2.05d	3.26a	5.06c	Co-5
E15	2253.53c	24.74e	2.14d	3.40a	4.70d	Co-5
E16	2417.78b	19.69l	1.75d	3.22a	6.22a	Co-7 and Co-5
E17	2795.85a	20.19k	2.30c	3.30a	5.94b	Co-7 and Co-5
E18	2380.80b	21.45i	2.07d	3.29a	5.80b	Co-5
E19	2296.01b	20.77j	2.50c	3.13a	6.52a	Co-5
E21	2061.14c	23.37g	2.01d	3.13a	5.79b	Co-5
E22	1846.66c	21.44i	2.25c	3.36a	5.49c	None
E23	2064.27c	22.18h	3.05a	2.30d	6.54a	Co-4 ²
E28	2221.12c	24.35e	1.91d	3.28a	4.18d	Co-5
E38	2513.92b	22.94g	2.02d	3.31a	5.59b	Co-7 and Co-5
M3	2230.58c	23.90f	2.60b	2.25d	4.88c	None
M19	2162.61c	23.83f	2.42c	2.64c	5.34c	None
M31	1939.37c	21.03j	2.87b	2.80b	6.59a	Co-4 ²
TALISMÄ	2090.63c	22.44h	2.44c	2.88b	6.33a	None

Means followed by different letters in the columns are of different groups according by Scott-Knott' test at the level of % probability. Lines denominated with the letters A, B, D, E and M are originated from F₁₄ BC₂ families and lines with letter C from a F_{4,7} BC₁ family

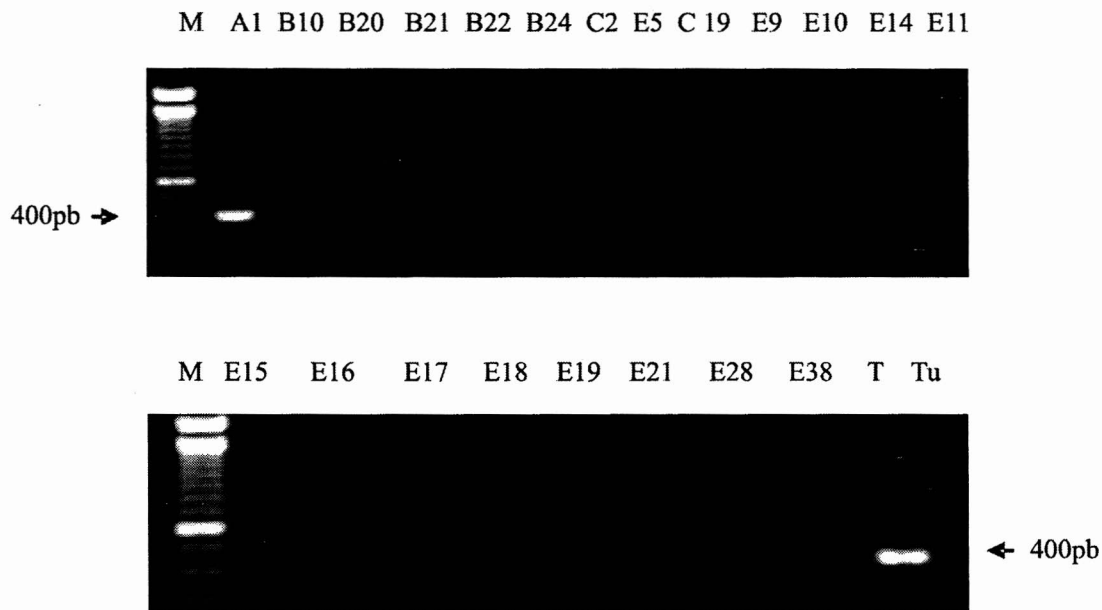


Figure 1. Band profiles of standard size marker in column M and band of 400pb in Column A1 and Column Tu (line Tu). Columns A1 to E38 are race 81-resistant lines. Columns E11 and T (Talismã) are race 81-susceptible

Seleção de linhagens de feijão tipo carioca com resistência à antracnose e à mancha angular

RESUMO - O objetivo do presente trabalho foi identificar linhagens que reúnam além da resistência a antracnose, alta produtividade, grãos tipo Carioca, resistência à mancha angular e porte arbustivo. Utilizaram-se 153 linhagens selecionadas dentro de cinco famílias provenientes de retrocruzamentos entre G2333 (genitor doador) e ESAL 696 e CI 140 (genitores recorrentes). As linhagens foram avaliadas em três safras. A resistência à antracnose foi verificada por meio de inoculações com as raças 2047, 1545 e 81 e também com o uso de um marcador molecular SCAR. Foi possível selecionar linhagens que reuniram alta produtividade, tipo de grão semelhante ao da cultivar Carioca, porte mais arbustivo e resistência à antracnose. O marcador SCAR não foi eficiente para realizar seleção de genótipos portadores do alelo *Co-5* para o conjunto de linhagens avaliado. Não foi possível identificar linhagens resistentes à mancha angular.

Palavras-chave: *Phaseolus vulgaris*, linhagens tipo carioca, *Colletotrichum lindemuthianum*, *Phaeoisariopsis griseola*.

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