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Genetic diversity in Carioca and Pérola cultivars of common bean based on RAPD markers

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ABSTRACT - Common bean is a self-pollinated species and as such has a low cross-pollination rate (around 0.5%). Besides the low crossing rate, mixtures of cultivars and lines and mutations on large planted areas contribute to the variability within cultivars, which is selectable in common bean breeding. Then, the objective of this study was a preliminary evaluation of variability by means of the genetic similarity based on RAPD (Random Amplified Polimorphic DNA) markers. Ninety-eight common bean lines of Carioca and Pérola cultivars were sampled on farms in Sete Lagoas, State of Minas Gerais. The genetic similarity based on eighty-one polymorphic RAPD bands were estimated by Nei Li's method, and grouped by the UPGMA procedure to generate a dendrogram. The lines presented wide genetic variability, mainly those selected from the Carioca cultivar. The Carioca cultivar farmers use is therefore substantially different from the original one. A smaller variability was observed among Pérola cultivar lines, which was approved for sale more recently than "Carioca".

Key words: RAPD, lines, genetic similarity, Phaseolus vulgaris.

INTRODUCTION

Common bean is a self-pollinated species and as such has a low cross-pollination rate (less than 3%) (Junqueira Netto and Lasmar Filho 1971, Marques Júnior and Ramalho, 1995). This occurs due to peculiarities of the plant's reproductive system, which is already self-fertilized at the time of anthesis. Owing to this characteristic, most farmers prefer to save seed from the previous crop or obtain it from their neighbors instead of buying seed. This behavior, associated with the cultivar mixtures, mutations, mechanical mixture, and the great amount of lines in use contribute to the variability within cultivars.

When the Carioca cultivar became popular at the beginning of the 1980s, it was adopted as standard, mainly due to its great amount of desirable phenotypes. Its long-

standing use has accumulated a great genetic variability in many traits such as pest and disease resistance. One of the forms of evaluating variability is by means of molecular markers like RAPD-Random Amplified Polymorphic DNA (Williams et al. 1990), which provide relationship information (Hagiwara et al. 2001). The information may suggest the possibility of selection of new cultivars, as occurred with Carioca bean itself. There is also the possibility of verifying if variation among the lines was caused by the mixture of other previously utilized cultivars.

On this background the objective of this work was to evaluate the genetic similarity of different lines selected out of Carioca and Pérola cultivars utilized by farmers of the region of Sete Lagoas, a county in the State of Minas Gerais, by means of RAPD.

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MATERIALS AND METHODS

One hundred and three genetic materials were used, 88 lines selected in the Carioca cultivar and 10 in the Pérola, used by farmers of Sete Lagoas in winter 1999, plus five checks (Table 1).

DNA was extracted from young leaves from 12 seedlings of each line or cultivar. The DNA was extracted and amplified by the RAPD method according to the procedures reported by Nienhuis et al. (1995), with some modifications.

The reaction products were separated by 1% agarose gel using TBE buffer at 65 volts for 4 hours. DNA fragments were stained with ethidium bromide at a concentration of 0.5 mg ml⁻¹ viewed in ultraviolet transilluminator and photographed with 667 Polaroid film.

The bands were classified visually as intense, medium, and faint based on the resolution and amplification degree. Only the intense and medium bands were used for analysis. A matrix of 0 and 1 was built, where 1 denotes the presence of band and 0 the absence, and it was used to obtain the estimates of genetic similarity among every combination pair of lines.

The genetic similarity (sg_{ij}) was estimated by the procedure of Nei and Li (Rohlf, 1992), by means of the expression $sg_{ij} = 2a/(2a + b + c)$, "a" is the presence of a particular band in individuals i and j; "b" the presence of the band in i and absence in j; and "c" the absence of the band in i and presence in j. The error associated with each value of genetic similarity (sg_{ij}) was estimated by an expression similar to that suggested by Skroch, et al. (1992): $s_{ij} = [sg_{ij}(1-sg_{ij})/(n-1)]^{1/2}$, where n is the total number of band combinations a, b, and c utilized in each sg_{ij} estimate.

The grouping analysis of similarities was performed by means of UPGMA algorithm, giving rise to a dendrogram, established with software NTSY-PC 2.0 (Rohlf, 1992). Genetically different genotypes were identified in the dendrogram using the estimate of the minimum similarity value (sg_m) above which the families are similar. The sg_m was estimated by means of the t test by the expression (Hagiwara et al. 2001): $sg_m = 1 - (t.\overline{s}_{ij})$, where t is the tabulated value of t with n-2 degrees of freedom and \overline{S}_{ij} is the error mean s_{ij} . Spearman's correlation coefficient (Steel and Torrie, 1980) was estimated among the medium values of similarity of each line and its average grain yield obtained by Ramalho et al. (2001). The average similarity value of each line $(\overline{s}_{i,})$ was obtained by the expression: $\overline{sg}_{i,} = \sum_{i=1}^{102} sg_{ij}/102$ and the correlation coefficient checked by the t test.

RESULTS AND DISCUSSION

Eighteen random primers identified polymorphism in lines and controls and generated 81 polymorphic bands. The average number of bands per primers was 4.5, ranging from two to eight. This number of polymorphic bands may be regarded as adequate to identify the diversity of the common bean genome, and is in line with the numbers used by other authors in related studies (Vasconcelos et al. 1996, Johns et al. 1997, Duarte et al. 1999, Machado et al. 2000, Hagiwara et al. 2001).

Among the 98 lines and five controls, 5253 genetic similarity estimates, ranging from 0.51 to 0.94, were obtained. Based on the estimates, line and cultivar relationships were represented in the dendrogram in (Figure 1). The range of variation of the similarity estimates was small due to the greater relationship among the genotypes in use (Vasconcelos et al. 1996, Duarte et al. 1999, Machado et al. 2000).

Out of the 98 selected lines within crops, 18 farms used the Carioca cultivar and only one the Pérola cultivar (Table 1).

Line ¹	Farm/Farmer	Line	Farm/Farmer	Line	Farm/Farmer
1	Barreiras	20 to 25	Fortuna	81 to 85	Carlos
2	M. Flora	26 to 34	Nilson	86 to 87	Ubaldo
3	J. Souza	35	Bárbara	89 to 98	Pérola cultivar
4	Nicodemos	36 to 45	Waldir	99	Carioca 1
5	NovaUnião	46 to 50	Domenico	100	Carioca 2
6,7	D. Joaquim	51 to 70	Lourenço	101	Carioca MG
8,9	Jaime	71 to 74	J. Carvalho	102	Aporé
10 to 19	Damásio	75 to 80	Raimundo	103	Pérola

Table 1. Identification of lines and their respective origins

¹1 to 98 are lines, and 99 to 103 are cultivars used as check

Among the 10 lines selected within the Pérola, seven were genetically identical or very similar to the Pérola cultivar used as control, taking the minimum significant value of similarity (sg_m = 0.78) at a level of 0.1% probability into account. Only three were different, 92 and 93 were equal, and 97 different from the others. Therefore, only 30% of the lines selected within the Pérola cultivar proved to be genetically different.

The lines selected on the farms where the Carioca cultivar was utilized were genetically more variable, by contrast to the lines selected from Pérola cultivar (see Figure 1 and Table 2)

Such results were to be expected in view of the long time the Carioca cultivar had been cultivated in great part of Brazil and throughout the State of Minas Gerais. Previously,



Figura 1. UPGMA dendrogram of the common bean lines and checks based on RAPD markers

this variation had already been observed in evaluation trials based on grain yield and pathogen reaction (Ramalho et al. 2001). It is worth mentioning that there was no correlation between the average similarities of lines and their respective grain yields, as evaluated by Ramalho et al. (2001).

Nevertheless, these results had already been expected because the diversity detected by RAPD includes the total genetic variation, positively correlated with the joint variation only of many morpho-agronomic common bean characters (Duarte et al. 1999; Machado et al. 2000).

A cause of non-correlation of genetic similarity, particularly with grain yield, is because this stands for a very small portion of the morphogenetic variation, less than 2% among the cultivars evaluated by Abreu et al. (2002).

Considering the relationship among the lines presented in Figure 1, a higher diversity was observed among lines that came from a lower number per farmer. There was an incidence of a higher number of identical lines on farms where a greater number was selected. For example, 20 lines were selected on Lourenço's farm and grouped into 12 groups. Only one of the groups with three lines was equal to the Carioca cultivar. Therefore, the cultivar he used had only 15% similar seeds to the true Carioca cultivar. However, it is important to notice that 11 other lines from the same farm were genetically little divergent from Carioca (Figure 1). Given the nature of RAPD (Nienhuis et al. 1995), these lines can be considered as of the Carioca cultivar and have probably appeared by mutation, which alters only tiny part of the genome. In this case, about 67% of the seeds utilized by the farmer Lourenço would belong to the Carioca cultivar, while 33% of the lines are substantially different from Carioca. Among them, 59 and 61 are similar to each other while the others are distinct. Origin of these lines are certainly crosses with other quite different cultivars. Such crosses occurred naturally or were probably artificial crosses of one of the several breeding programs that have been conducted in Brazil for a long time. In this latter case, there would have been a mixture of lines with the Carioca cultivar obtained in such programs.

Among the remaining 68 lines, most are also drastically different from the Carioca cultivar and might stem from crosses with genetically contrasting cultivars. Only one, line 78, is equal to Carioca, selected in the crop of farmer Raimundo. In addition, eight lines differ slightly from Carioca and may have had their origin in spontaneous mutations (Figure 1).

The genetic similarity among the controls Carioca (99 and 100), Pérola (103), and Carioca MG (101) were

Farm/Farmer	Number of lines	Numbe	Number of groups	
		1%	0.1%	
Barreiras	1	1	1	
Sogra	and the second second in the second se	1	1	
J. Souza	the of the sectors the representation of the dependence	Service of the servic	1	
Nicodemos	1	1	1	
Nova União	the second s	1	1	
Bárbara	L and the state of	1	1	
D. Joaquim	2	2	2	
Jaime	2	2	2	
Ubaldo	3	3	3	
J. Carvalho	4	4	4	
Domenico	5	3	2	
Carlos	5	5	5	
Fortuna	6	4	3	
Raimundo	6 here a balance the strength of the state o	4	4	
Nilson	9	- 8	6	
Damásio	10	7	6	
Waldir	10	10	8	
Pérola	10	7	6	
Lourenço	20	16	12	
Total	08	81	69	

Table 2. Number of lines per farm/farmer of 'Carioca' and "Pérola" respective groups with those genetically similar

unexpected results (Figure 1). Regardless, they differ in several features such as height, grain type, and resistance to some *C. olletotrichum lindemuthianum* and *Phaeoisariopsis griesola* races. However, Pérola and Carioca MG cultivars are descendents from crosses in which the Carioca cultivar participated as a common parent. This means they should have most of the alleles and genomic regions in common. It is important to mention that the three controls were also utilized by Machado et al. (2000), who confirmed they are genetically similar based on the use of 137 polymorphic RAPD bands.

An unexpected result was the elevated divergence of the control Aporé, despite selected from a cross in which the Carioca cultivar was a parent (Machado et al. 2000). Additionally, the Pérola cultivar is a line selected within the Aporé cultivar. The other parents of the Aporé cultivar were México 168 and BAT 76, which have certainly contributed to its genetic diversity. In accordance to the present result, Machado et al. (2000) also confirmed the genetic divergence of Aporé in relation to Carioca and Pérola. Finally, it is worth mentioning that corn genotypes with 88 to 89% of genetic similarity based on random markers exhibited marked differences in grain yield and other agronomic characters (Troyer and Rocheford 2002). These authors recommend that a similarity of 90% or more should be established for corn lines to be considered essentially derived cultivars for protection purposes. Such results confirm the broad diversity among the lines utilized in the present study.

CONCLUSIONS

1. RAPD markers identified a broad genetic divergence within common bean cultivars.

2. Genetic divergence was greater among the lines from the Carioca cultivar than among those selected in the Pérola cultivar.

3. The Carioca cultivar farmers use is different from the original cultivar.

Diversidade genética dentro das cultivares Carioca e Pérola de feijão baseada em marcadores RAPD

RESUMO - O feijão é uma espécie autógama e, como tal, possui baixa taxa de fecundação cruzada, no mínimo de 0,5%. Além disso, as misturas de cultivares e as mutações nas extensas áreas plantadas, contribuem para gerar variabilidade dentro das cultivares, que pode ser utilizada no melhoramento. Então, o objetivo do estudo foi avaliar previamente essa variabilidade, por meio da similaridade genética estimada a partir de marcadores RAPD. Foram utilizadas 98 linhagens de feijão amostradas dentro das cultivares Carioca e Pérola, na região de Sete Lagoas – MG. Foram obtidas 81 bandas polimórficas e estimada a similaridade genética por meio do coeficiente de Nei e Li. Utilizou-se o algoritmo UPGMA na análise de agrupamento e constatou-se ampla variabilidade genética das linhagens, principalmente entre aquelas selecionadas na cultivar Carioca. A acentuada divergência genética de mais de 50% das linhagens indica que a cultivar Carioca utilizada pelos agricultores é completamente diferente da cultivar original.

Palavras-chave: RAPD, linhagens, similaridade genética, Phaseolus vulgaris.

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