Genetic diversity of common bean genotypes of Carioca commercial group using RAPD markers

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ABSTRACT - *The objective of this research was to characterize the genetic diversity of bean genotypes (*Phaseolus vulgaris *L.), from Carioca commercial group, utilizing RAPD markers to discriminate and to support breeders to choose genetic material and to develop breeding methods to use for obtaining new cultivars with Carioca type seeds. Twenty-eight genotypes, fifteen cultivars, and thirteen lines were evaluated. The genetic distances were achieved utilizing the Euclidian's method modified by Roger's (1972) and the grouping analysis were by UPGMA method, showed by a dendogram. Through 89 RAPD loci, obtained from 12 primers, 65.17% were polymorphics. We have verified that the RAPD markers were efficient in discrimination and grouping of genotypes, showing to have large genetic variability. Although Carioca cultivar has a common ancestor from all studied material, it has shown low similarity with genotypes derived from it, corroborating to exist sufficient genetic variability for production of new cultivars, maintaining the original coloration of seeds.*

Key words: *Phaseolus vulgaris*, molecular markers, RAPD, genetic distances.

INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) is one of food source with high level of protein for Brazilian diet, being Brazil the biggest world bean producer from genus *Phaseolus*, and the majority of grain yield are consumed in Brazil, specially from Carioca type bean. The grains are eatten for majority of Latin America and Africa populations being as one of the unique protein source from them (Center of Tropical Agriculture 1992).

The total area in Brazil cultivated with commercial group of Carioca bean has after thirty years since the begining from pure line approximately 2 million of hectars, (Almeida et al. 1971).

Certainty, throughout the time, has occurred changing in the genetic structure of original Carioca cultivar, creating higher variability because of segregation passing by the continuous use from the same seeds yielded by the farmers, exposed from mutations, and mainly by production of new cultivars in breeding programs. However, those new cultivars maintained as pattern of Carioca grain type, still phenotypically similar for the most

of characters, making difficult it identification, being necessary the utilization of modern techniques to characterize different genotypes.

Thus, the knowledge of genetic diversity among commercial cultivars and elite line programs of plant breeding is the vital importance for the breeders to allow identification and organization of available genetic resources, targeting future breeding programs and to produce some new promising cultivars.

Since the 90's, the RAPD (Randon Amplified Polimorfhic DNA) technique has been the most utilized in studies of genetic and breeding programs, because of it simplicity, ability to detect differences among related genotypes, utilization of an universal group of primers, use of low amounts of genomic DNA, reduced costs, and easy execution, among other advantages (Ferreira and Grattapaglia 1998).

The RAPD markers have shown to be efficient tool to discriminate bean cultivars, considering: the domestication center (Nienhuis et al. 1995), the genetic diversity, between and within cultivars (Emydio et al. 2003), and origin of genotype (Alzate-Marin et al. 2003).

Recently, Menezes et al. (2004) studying the genetic diversity within Carioca and Perola cultivars used in large scale through the main bean producer region, verified high genetic variability, within Carioca cultivar, showing to be different from the original Carioca.

The breeding programs in Brazil are creating progressively bean cultivars with seeds of Carioca type, because of the preference and most acceptance from the customers. Therefore, the breeders always have no knowledge of the line pedigree to be used for the development of a new cultivar, being necessary to characterize the cultivars by molecular procedures to verify the genetic potential for crossing and to select pure lines to replace with advantages, the current cultivar.

Due to the importance of bean crop in Brazil, the objective of the study was to evaluate different genotypes of Carioca commercial bean group, using RAPD markers, targeting to know the genetic variability.

MATERIAL AND METHODS

In this study we used twenty-eight genotypes of Carioca commercial group, considering fifteen cultivars and thirteen lines. The lines are originally from Empresa Brasileira de Pesquisa Agropecuária-Embrapa Arroz e Feijão, from the groupings of trial evaluation of Carioca bean, presented in Table 1.

The genomic DNA was obtained from tissues of just germinated seedling in laboratory, approximately 200 mg of tissues. The procedure was based on the DNAzol protocol (Ausubel et al. 1990).

The following phases were done for amplification: 94 ºC for 3 minutes (denaturation); after 40 cycles of 94 ºC for 1 minute, 35 ºC for 1 minute (annealing), and 72 ºC for 1.5 minute (elongation) were used, and an additional elongation cycle of 72 ºC during 6 minutes have concluded the reaction.

The products of amplification have been separated by electrophoresis, in agarose gels, with 1.5% of concentration, running through 80 V, for 3 hours. The gels were developed using 2 ml of ethidium bromide 0.2%, in 1.5 L of water, for 1 hour. The DNA bands were visualized by ultraviolet light and photographed.

A total of 49 RAPD primers (Operon Technologies) were utilized to separate those; they have presented better resolution and polymorphism of bands. After tests, were selected the best 12 with highest polymorphism and appropriated pattern of bands to analyze the genetic diversity of genotypes.

The evaluation was based on presence (1) and absence (0) of DNA fragments building a matrix of binary data. The dendrogram of Roger´s (1972) genetic distances was obtained by UPGMA (Unweighted Pair-Group Method with Arithmetic Averages) method. The used software was TFPGA 1.3 version (Miller 2000).

RESULTS AND DISCUSSION

The 12 selected primers have shown a total of 89 bands of RAPD, with an average of 7.5 bands for primer, which 65.17% were polymorphic. Similar results were obtained by Franco et al. (2001), studying other bean cultivars, obtained 64.8% of polymorphic loci, using 15 primers. Emydio et al. (2003) found a high number of polymorphic loci from 7 primers, and obtained a total of 104 bands, which 85.6% were polymorphic.

The literature presents different number of bands to characterize bean genotypes, not existing an adequate pattern of band numbers which shows more efficiency to estimate genetic parameters. Thus, Nienhuis et al. (1995) studying beans found that up to 100 or superior numbers of RAPD bands, the coefficient of variation of genetic distances have not presented variation, and indicating tendency for stabilization. However, in another study of bean cultivars originating from Chile, Johns et al. (1997), demonstrated that samples of 50 RAPD bands selected by

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random events, presented similar patterns of grouping when compared with 106 bands.

Observing the dendogram elaborated by UPGMA algorithm (Figure 1), the analysis of grouping showed two groups, one established just by IAC-Carioca Aruã cultivar and the others formed for a large group distributed by smaller subgroups, being hard to establish a clear relation of similarity between different genotypes and their provenances. That polymorphism showed by RAPD markers were expected, due to the utilization of different ancestors for formation of genotypes creation and from the same provenance, contributing even more for that variability (Table 1).

Thus, in the Figure 1, the grouping analysis by UPGMA method, shows the smallest Roger´s (1972) genetic distance occurred between CNFC8012, CNFC 8013,

CNFC 8156, and RELAV 37-19 genotypes. The first three are originally from EMBRAPA and the forth from UFLA-Lavras, MG, Brazil. The largest genetic distance was presented between IAPAR 14 and IAC Carioca Aruã, both from different genitors and origins explained, in part, for that divergence.

We can verify that IAC Carioca Aruã cultivar was different from the other materials, presenting lower similarity when compared with other studied genotypes. It could be because besides there are different genitors, also presents morphological and chemical different characters which become them distinct from the others, like the reniform seeds form and low rates of lignina and phenolic components which confer resistance to the darkeness of grain tissue (integument) (Whitehead and Swardt 1982), inherited characters possibly from a different

Table 1. Cultivars of bean (*Phaseolus vulgaris* L.) of commercial Carioca group utilized for diversity studies

	Cultivars	Pedigree	Provenance
1	Carioca	Massal selection from local cultivar (Palmital-SP)	IAC
2	IAC-Carioca	Carioca x Cornell 49-242	IAC
3	IAC-Carioca Eté	L 933 x LM 30630	IAC
4	IAC–Carioca Pyatã	Dor 41 x (10-3-1. TU1B1-2.10-9-1)	IAC
5	IAC-Carioca Aruã	(10771.122) x [(H5380-41.A156) x (H5380-41.AB136)]	IAC
6	FT-Bonito	IAPAR 14 x Carioca 80	FT-sementes
7	Porto Real	FT 85-75	FT-sementes
8	Rudá	Carioca x Rio Tibagi	CIAT
9	Princesa	A-252 x BAT 1550	CIAT
10	IAPAR 14	Carioca 99 x GN Nebraska 1 sel. 27 x 77 EP 173-CV 173	IAPAR
11	IAPAR 80	BAT 93/2/ Carioca sel. 99/ GN Nebraska 1	IAPAR
12	IAPAR 81	{BAT 93 x [(Carioca 99 x GN Nebraska 1# sel. 27) x sel. Aroana]} x (A 248 x EMP 117)	IAPAR
13	Pérola	Selection in the cultivar Aporé	Embrapa
14	Carioca Precoce	-	Embrapa
15	Aporé	A 445 (Carioca x México 168) x A246 (Carioca x BAT 76)	Embrapa
16	CNFC 8005	-	Embrapa
17	CNFC 8006	-	Embrapa
18	CNFC 8007	-	Embrapa
19	CNFC 8008	-	Embrapa
20	CNFC 8009	-	Embrapa
21	CNFC 8010	-	Embrapa
22	CNFC 8011	-	Embrapa
23	CNFC 8012	-	Embrapa
24	CNFC 8013	-	Embrapa
25	CNFC 8156	-	Embrapa
26	MA 534534	-	Embrapa
27	EL 49	-	Embrapa
28	RELAV 37-19	-	UFLA

genitor, because the most of bean genotypes, with Carioca seed type, has the elliptical format and dark color after short time of storage (Esteves et al. 2002).

For lines deriving from EMBRAPA we observed a little tendency for grouping, between almost half of them, whose CNFC 8156 and CNFC 8008 presented lowest genetic distance with 0.27. For Perola and Carioca Precoce cultivar, however, showed the lowest distances (0.15). We can also observed the genotypes proceeding from EMBRAPA, rather cultivars and lines, had tendency to set up independent groups. That fact can be because of the utilization of different genitors for each group, considering the lines are in process of selection to be recommended later. However, the cultivars have already been used by the farmers for some years and the tendency of grouping have had origin through the time and the genetic material.

We can verify in Table 1 that Perola cultivar was obtained by selection from the Aporé cultivar, and high degree of similarity was expected, therefore, that was not observed. Similar results was found by Menezes et al. (2004).

Carioca cultivar nowadays cultivated, did not show any similarities with other cultivars, but presented close similarities to Pérola, IAC-Carioca Eté, Carioca Precose, and Rudá, old cultivars and broadly used in Brazil. By the fact they are old cultivars, increase the possibilities they are closely related to Carioca cultivar, however, they distinguish by their genitor, and through the time, by each generation, the genetic variability was amplified, increasing the genetic divergence, as showed in Figure 1, that the modern lines show higher genetic distance than old cultivars.

Carioca and IAC Carioca Aruã cultivars showed similarity around 0.43, considered low, because they have the same type of grains. They have different pedigree, becoming important for breeding programs, pointing the existence of genetic variability between populations, with advantage to present the same type of seeds.

According to the dendrogram built by Euclidian´s distance method, modified by Roger (1972), in the Figure 1, the classification of different genotypes was not distributed in a defined groups, having just a little tendency by some developed genotypes from EMBRAPA, considering normal, through the majority of representatives, which in general do not present similarity between those genotypes, and show the existence of sufficient genetic variability to continue getting new cultivars, with Carioca type of grains.
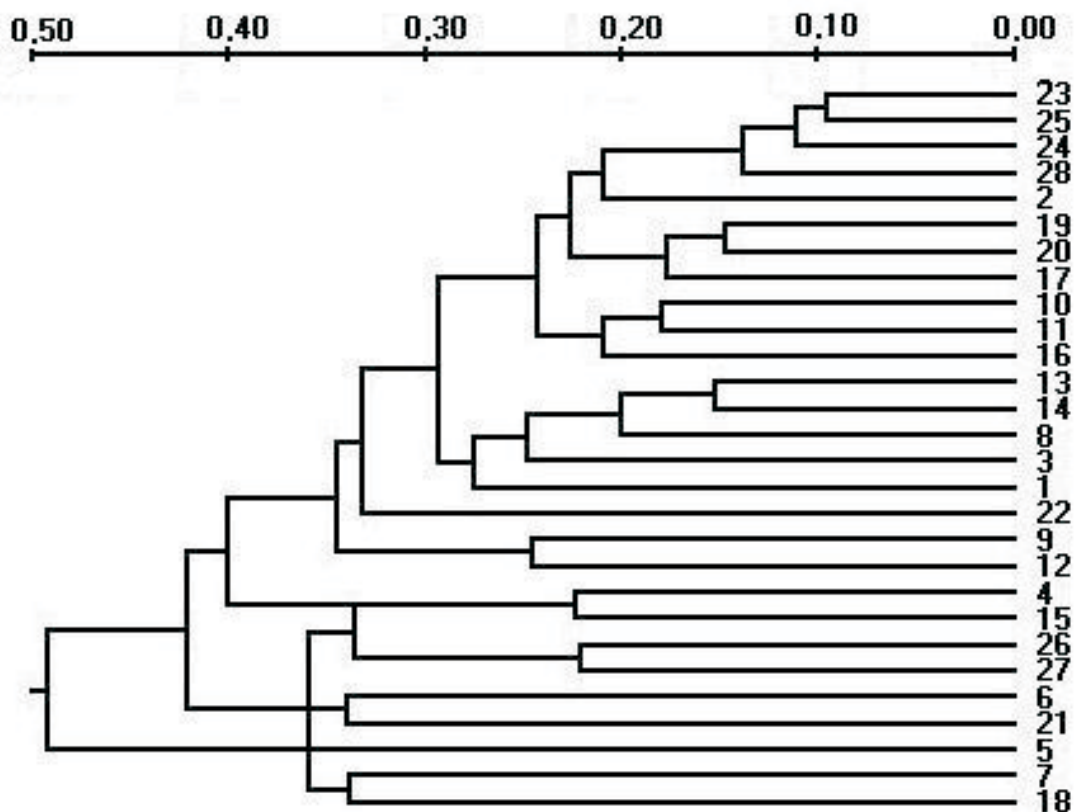

Figure 1: Dendogram by UPGMA method of genotypes of common bean Carioca type, based on genetic distances of Roges

It is important to emphasize that the found genetic diversity was expected, although Carioca cultivar be a common ancestor, there were changes on the genetic structure of the autogamous species (Menezes et al. 2004) that studying the genetic diversity between Perola and Carioca cultivars, verified the Carioca cultivar used nowadays is completely different from the origin. Therefore, the results show the existence of enough genetic variability within group of beans with Carioca seed type for the future breeding programs.

CONCLUSION

The RAPD markers were efficient to discriminate and group genotypes, giving important and useful information from the genome of beans.

The genetic variability within group of seed with genotypes of Carioca type was developed through the time with the introduction of new genes, thus, there is no restriction to continue obtaining new cultivars maintaining the same seed pattern.

Diversidade genética de genótipos de feijão com sementes tipo Carioca utilizando marcadores RAPD

RESUMO - *O objetivo da pesquisa foi caracterizar a diversidade genética de genótipos de feijão (*Phaseolus vulgaris *L), do grupo comercial Carioca, utilizando marcadores RAPD para a discriminação e fornecer subsídios aos melhoristas na escolha do material e dos métodos de melhoramento a serem adotados na obtenção de novas cultivares com sementes tipo Carioca. Avaliaram-se 28 genótipos, quinze cultivares e treze linhagens. As distâncias genéticas foram obtidas utilizando o método da distância euclidiana modificada por Roger (1972) e as análises de agrupamento pelo método UPGMA, representadas por um dendograma. Dos 89 locos de RAPD, obtidos a partir de 12 primers, 65,17% foram polimórficos. Verificou-se que os marcadores RAPD foram eficientes na separação e agrupamento dos genótipos, mostrando ampla variabilidade genética. Embora a Carioca seja o ancestral comum de todos, apresentou pouca similaridade com os genótipos que dela se derivaram, confirmando existir suficiente variabilidade genética para a criação de novas cultivares, mantendo a coloração original das sementes.*

Palavras-chave: *Phaseolus vulgaris*, marcadores moleculares, RAPD, distância genética.

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