Ciência Rural, Santa Maria, v.41, n.6, p.996-1002, jun, 2011
ISSN 0103-8478

Genetic variability of *Passiflora* spp. from commercial fields in the Federal District, Brazil

Variabilidade genética de *Passiflora* spp. em plantios comerciais do Distrito Federal, Brasil

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ABSTRACT

This study aimed to characterize the genetic variability in commercial accessions of passion fruit from the Federal District, Brazil, by RAPD markers. Genetic analyses were done with leaf samples of 30 accessions. DNA samples were amplified by RAPD technique, and respective markers converted into a binary matrix, from which the genetic distances between the accessions were estimated. Clustering analyis based on genetic distances allowed to detect a wide range of genetic variabillity among the accessions of sour passion fruit, and to separate them from the two sweet passion fruit. The graphical positioning of 'BRS Ouro Vermelho' confirms its potential to improve the genetic variability of commercial varieties of sour passion fruit. Dispersal of genetic distances among commercial accessions of sour passion fruit supports evidence for different genetic origins of the materials planted in the Federal District. The verified genetic variability indicates the potential success of future breeding programs for this region.

Key words: *diversity, passion fruit, plant breeding, RAPD analysis.*

RESUMO

Este estudo teve como objetivo caracterizar a variabilidade genética de acessos de maracujá comerciais no Distrito Federal por meio de marcadores RAPD. Análises genéticas foram feitas com amostras foliares de 30 acessos. As amostras de DNA foram amplificadas pela técnica de RAPD e os respectivos marcadores convertidos em uma matriz binária, a partir da qual as distâncias genéticas entre os acessos foram estimadas. Análises de agrupamento baseadas em distâncias genéticas permitiram detectar uma ampla gama de variabilidade genética entre os acessos de maracujazeiro-azedo, bem como para separá-los dos dois de maracujazeiro-doce. O posicionamento gráfico de 'BRS Ouro Vermelho' confirma a sua importante contribuição para aumentar a variabilidade genética das atuais variedades comerciais de maracujazeiro-azedo. A dispersão das distâncias genéticas entre os acessos comerciais de maracujazeiro-azedo suportam as evidências de diferentes origens genéticas para os materiais plantados no Distrito Federal. A variabilidade genética verificada evidencia o potencial de sucesso de futuros programas de melhoramento para essa região.

Palavras-chave: *diversidade, maracujá, melhoramento vegetal, RAPD.*

INTRODUCTION

Despite the yield decrease of sour passion fruit in Brazil, this country is the most important world producer and consumer (FALEIRO et al., 2008). The lack of high yield genotypes is considered as major factor related to the production decrease (GONÇALVES et al., 2007). Brazil is known as a diversity center of passion fruit, and possessing a wide genetic variability of ***Passiflora***, which is the starting point for genetic breeding programs (GANGA et al., 2004). As revised by BELLON et al. (2007), due to market advantages for the pulp industry and spread consumption, genotypes of ***Passiflora edulis*** Sims. have been employed by breeding programs in Brazil and Australia. On the other side, ***Passiflora alata*** Curtis is relatively unknown among consumers, its pulp is sweet but it is not processed by the industries (MELETTI et al., 2003).

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Received 12.18.10 Approved 05.04.11 Returned by the author 06.07.11
CR-4533

Pest and diseases are causes for major economic losses in commercial orchards of passion fruit in Brazilian Federal District (FALEIRO et al., 2006). The authors consider that these issues are related to poor genetic variability in the commercial varieties currently cultivated, as supported by the results of PIO VIANA et al. (2003), using RAPD markers that revealed no expressive genetic variability among genotypes of sour passion fruit. The improvement of genetic resources has been established as priority to increase genetic variability in ***Passiflora*** collections for breeding resistant varieties (AUKAR et al., 2002), as an alternative for controlling pests and diseases, and to prevent food and environment contamination with toxic residues.

RAPD markers have been regarded to detect DNA polymorphism, allowing characterization of variability among and within species (FAJARDO et al., 1998), resulting in a complementary tool for characterization of genetic diversity in Brazilian ***Passiflora*** species for breeding programs (CROCHEMORE et al., 2003). Therefore, the objective of this study was to characterize the genetic variability in commercial accessions of passion fruit from Brazilian Federal District by RAPD markers.

MATERIALS AND METHODS

Plant material

Thirty accessions of passion fruit, comprising 24 of sour passion fruit from 13 commercial orchards in the Brazilian Federal District, three hybrids of sour passion fruit from Empresa Brasileira de Pesquisa Agropecuária - Embrapa (BRS Sol do Cerrado, BRS Ouro Vermelho, and BRS Gigante Amarelo), one accession from the local market, and two of sweet passion fruit (***Passiflora alata*** Dryander) considered as outgroup were used (Figure 1 and Table 1).

Young leaves were collected in the tips of vines of adult plants cultivated under field conditions, and its genomic DNA extracted by the CTAB (cetyltrimethylamonium bromide) modified method (FALEIRO et al., 2003).

DNA amplification (RAPD)

The amplification reactions were done with 13µL of total volume, comprising 10mM tris-HCl at pH 8.3, 50mM KCl, 3mM $MgCl_2$, 100µM of each dNTP, 0.4µM *primer* (Operon Technologies Inc., Alameda, CA, USA), 1U *Taq* polymerase, and 15ng of DNA. Ten decamer *primers* were used to obtain RAPD markers: OPD (03), OPE (5 and 16), OPF (02, 04 and 7), OPG (02 and 03), OPH (05 and 10). The amplifications were performed in a thermocycler (MJ Research, Inc.). Each amplification cycle comprised the following sequence: 15s at 94ºC, 30s at 35ºC and 90s at 72ºC. After 40 cycles, as described above, and a final extension of 6min at 72ºC, reduced the temperature to 4ºC. Subsequently, 3µl of an aqueous solution (0.25% of bromophenol blue and 60% of glycerol) was added to the each sample, which were analyzed by electrophoresis (4h at 90V) in agarose gel (1.2%). The gel was stained with ethidium bromide (0.5µg mL^{-1}), submersed in TBE buffer (tris-borate 90mM, EDTA 1.0mM) and photographed under ultraviolet light.

Statistical analysis

The RAPD markers obtained were converted into a binary matrix, from wich genetic distances among the accesses were estimated on the

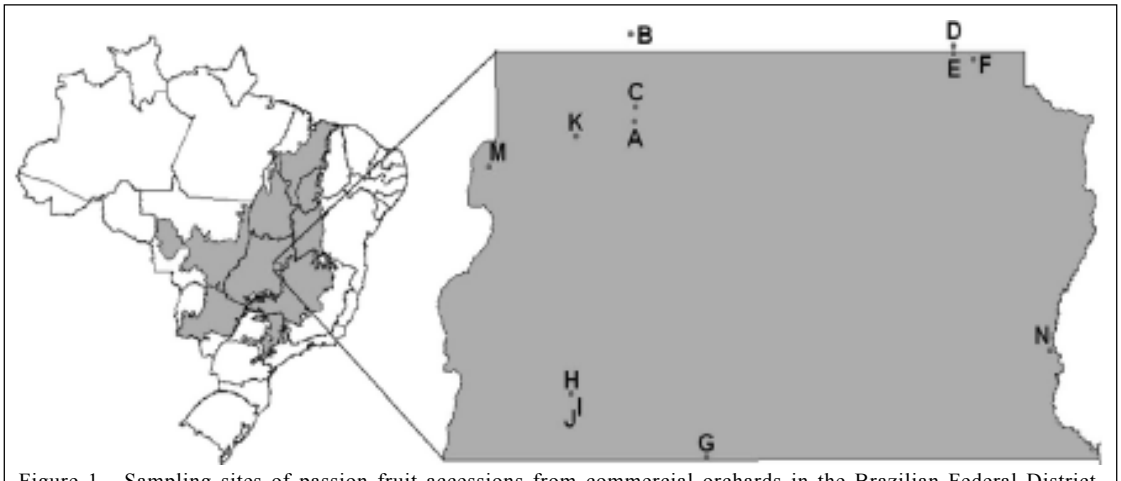

Figure 1 - Sampling sites of passion fruit accessions from commercial orchards in the Brazilian Federal District. Properties and accessions form each point are shown in table 1.

Table 1 - Sampling sites of accessions of passion fruit in the Brazilian Federal District.

Accession number	Locality, Map figure 1	Property
1		
2		
3		
4[1]	A	NR[2] Lago Oeste – Rua 18
5		
6[1]		
7		
8	B	NR Lago Oeste – Chácara 480
9	C	NR Lago Oeste – Chácara 14
10	D	Fazenda Santa Maria
11	E	Pipiripau – Chácara Dois Irmãos
12		
13	F	Pipiripau – Fazenda Paraná
14		
15		
16	G	Gama – Fazenda Cerradinho
17	H	Gama – Chácara Ipê Amarelo
18	I	NR Taguatinga
19	J	Gama – Chácara Paloma
20	K	Brazlândia– Fazenda Manga Rosa
21		Brazlândia – NR Alexandre Gusmão
22	M	Brazlândia – MOA
23		
24	N	Paranoá – Sítio Vale dos Pássaros
25		
26		
27		Maracujá Com.[3]
28		BRS Gigante Amarelo[4]
29	Embrapa Cerrados	BRS Ouro Vermelho[4]
30		BRS Sol do Cerrado[4]

[1]*Passiflora alata*; [2]Núcleo Rural; [3]Commercial accession, seeds obtained from local market; [4]Hybrid launched by Embrapa Cerrados.

basis of Nei and Li similarity coefficient, by the use of Genes software (CRUZ, 1997). A dendrogram was generated by the Hierarchic Clustering Method UPGMA (Unweighted Pair Group Method with Arithmetic Mean) through the software Statistica (STATSOFT INC., 1999). The matrix of genetic distances was displayed in a scatter plot based on the multidimensional scaling, using the principal coordinate analysis method. The software SAS (SAS INSTITUTE INC., 1989) and Statistica (STATSOFT INC., 1999) were used for the analysis and graphic construction. Cluster stability was measured by bootstrap analysis (YAP & NELSON, 1996) with 1,000 replications using the Genes software (CRUZ, 1997).

RESULTS AND DISCUSSION

Ten decamer primers were able to generate 91RAPD markers (9.1 markers primer^{-1}). Among 91 markers observed in the accessions of passion fruit, 18 (19.78%) were monomorphic (Table 2), while the other ones were polymorphic.

Table 2 - *Primers* with their respective sequences and generated number of polymorphic and monomorphic fragments for genomic DNA of 30 accessions of passion fruit collected in the Brazilian Federal District.

Primer	Sequence (5´ 3´)	Number of polymorphic fragments	Number of monomorphic fragments
OPD-03	GTCGCCGTCA	11	1
OPE-05	TCAGGGAGGT	11	0
OPE-16	GGTGACTGTG	12	0
OPF-02	GAGGATCCCT	4	1
OPF-04	GGTGATCAGG	10	3
OPF-07	CCGATATCCC	5	3
OPG-02	GGCACTGAGG	3	2
OPG-03	GAGCCCTCCA	1	5
OPH-05	AGTCGTCCCC	10	2
OPH-10	CCTACGTCAG	6	1
Total		73	18

Major and minor genetic distances among the 30 accesses of passion fruit were 0.438 and 0.033, respectively (Figure 2). Higher genetic distances were observed between the hybrid BRS Ouro Vermelho and the other accessions. A pair of accessions of ***P. alata*** labeled as NR Lago Oeste 4 and 6 (outgroup) were grouped side by side in the dendrogram, and simultaneously, several clusters of accessions were established (Figure 2). Repeatability (%) of clustering above 50% for 1,000 bootstrap cycles occurred in some groups, revealing that such clusters are the most consistent. The repeatability of groups involving accessions from the same locations, in general were higher and from different locations, in general were lower, showing a certain regionalization of genetic variability (Figure 2). In figure 2, this fact can be observed for accessions from NR *Lago Oeste* and *Sitio Vale dos Passaros*.

The dispersion diagram constructed based on the data of the binary matrix grouped the two sweet passion fruit accessions (outgroup) separated from the others accessions. 'BRS Ouro Vermelho' was also

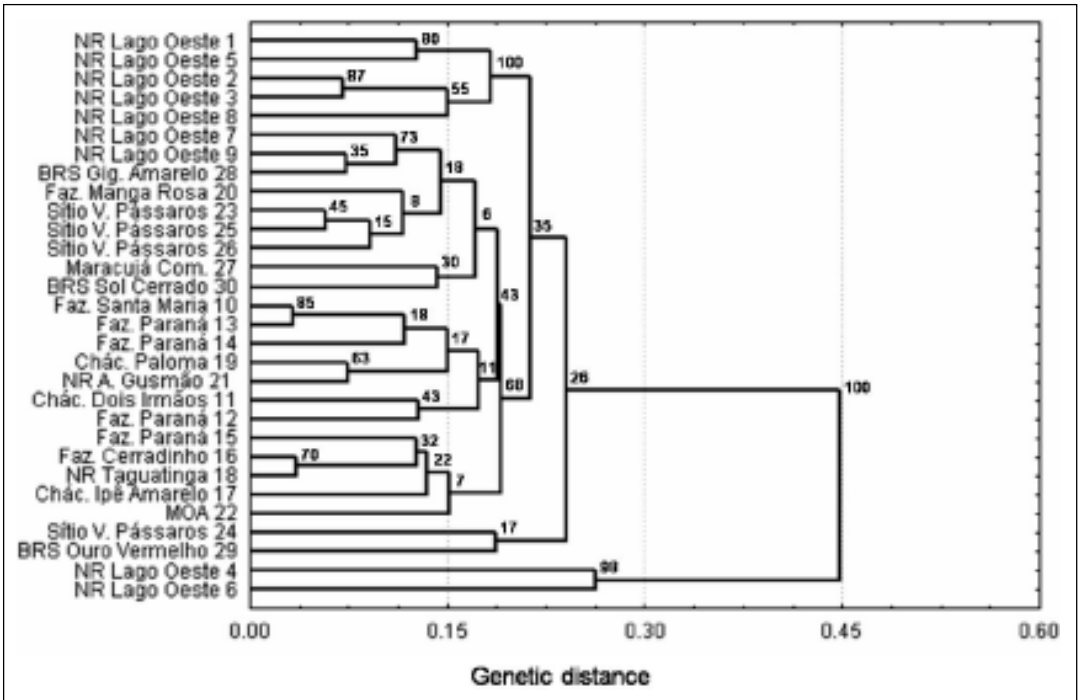

Figure 2 - Dendrogram of 30 accessions of passion fruit collected in the Brazilian Federal District, based on 91 RAPD markers with arguments of UPGMA. The values in each group are the repeatability (%) of clustering for 1,000 bootstrap cycles.

separated from the others accessions (Figure 3). The percentage of total variance explained by the coordinates (Figure 3) estimated by the fit between genetic distances and graphic distances, was 90.8 per cent and the value of stress was 9.2 per cent.

As predicted by FALEIRO et al. (2005), the genetic variability within the studied accessions was confirmed by the high ratio of markers generated per *primer*, and the low percentage of monomorphic markers. Furthermore, it was observed that among the analysed accessions, 'BRS Ouro Vermelho' placed away from the other accessions. A possible explanation for this result could rely on the involvement of wild species of ***Passiflora*** in the genealogy of this hybrid. In a similar study, BELLON et al. (2007) analyzing commercial and wild accessions of ***P. edulis*** verified that mostly, the wild accessions were responsible for the amplification of the genetic basis. Clustering of the two accessions of ***P. alata***, used as outgroup is common to occur between related genotypes (AUKAR et al., 2002). According to MELETTI et al. (2003), this species is tolerant to soil-borne pathogens, and potentially can to be used in breeding programs, or as rootstock.

As it was expected, genetic diversity among the passion fruit accessions was detected, since the majority of ***Passiflora*** species are alogames, with a genetic system of auto-incompatibility favoring cross pollination, and consequently the gene flow among distinct genotypes, and within species (GANGA et al., 2004).

It is also worth mentioning about the relationship between the hybrid BRS Ouro Vermelho (accession 29) and the accession 24 collected in the farm Sítio Vale dos Pássaros that clustered in the same group in the dendogram. This fact is justified by the location in that farm of experimental fields with hybrids of passion fruit produced by Embrapa, including BRS Ouro Vermelho. Thus, these accessions may share a common genetic background, considering that accession 24 may have origined from BRS Ouro Vermelho. The distinct position of the hybrid BRS Ouro Vermelho in the graphic of dispersion, is an indication of its potential to improve the genetic variability of commercial varieties of sour passion fruit. This hybrid possesses the advantages of high fruit yield and physical-chemical qualities, as well as, tolerance and resistance to diseases (JUNQUEIRA et al., 2008).

Studies have been done using RAPD markers to verify the level of DNA polymorphism in some species of fruit plants, including passion fruit.

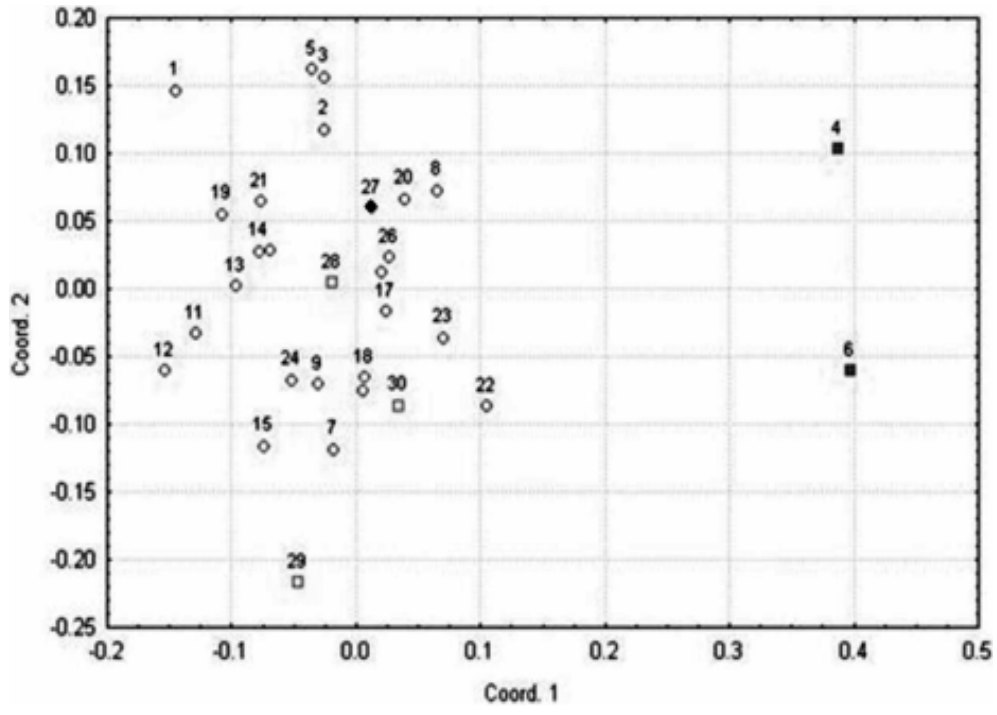

Figure 3 - Graphic dispersion of 30 accessions of passion fruit collected in the Brazilian Federal District (DF), based on 91RAPD markers. The numbers are corresponding to the accessions of figure 2 as follow: Hybrid launched by Embrapa (□), local market commercial accession (♦), accessions of sour passion fruit from growers in DF (○) and sweet passion fruit (■). Total variance explained by the coordinates was 90.8%. The stress of genetic and graphic distances was 9.2%.

PIO VIANA et al. (2003) detected a broad genetic basis among several species of ***Passiflora***. These authors estimated the genetic distance between several ***Passiflora*** genotypes, with the highest one occurring between ***P. edulis*** *f. flavicarpa* and ***P. gibertii*** N.E.Brown. High genetic distances among sour and sweet passion fruit also were verified by BELLON et al. (2007). Athough, RAPD is known as a reliable technique to verify genetic variability in passion fruit, AUKAR et al. (2002) suggested the f-AFLP technique to confirm the large genetic variability within the genus ***Passiflora***, as well as, for a possible taxonomic re-evaluation. In this research, we are confident that RAPD technique allowed satisfactory achievements of the scientific goals of the study. It was used a high number of markers (91), in addition, analyses of correlation and stress (data not shown) in the estimation of genetic distances proved that even with a reduced number of 60 bands there would be less than 10 per cent stress and over 84 per cent correlation with the genetic distance matrix calculated with total bands. The genetic distinction between the two accessions of ***P. alata*** in relation to those of ***P. edulis*** also shows the efficiency of this technique on the estimation of genetic distances between the genotypes evaluated.

CONCLUSIONS

The data presented confirmed RAPD markers as a powerful tool to analyze and to quantify genetic variability among accessions of passion fruit in commercial orchards. Present results also represents a contribution to knwoledge on the genetic background of passion fruit genotypes grown in the Brazilian Federal District. Upon this knowledge, passion fruit breeding programs can draw up further researchs to select accessions and to breed high yield varieties with tolerance and resistance to diseases.

ACKNOWLEDGMENTS

This work was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

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