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Genetic diversity in yellow passion fruit (*Passiflora edulis* Sims) based on RAPD

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ABSTRACT – *This study aimed to evaluate the genetic diversity by RAPD markers in 20 genotypes of 'yellow' passion fruit (*Passiflora edulis *Sims). The 16 primers generated 92 markers, 57 (62%) of which were polymorphic. The genetic distance (gd_{ij}) estimated by the complement of the Dice index (gd_{ij} = 0.19) and genotype grouping based on UPGMA algorithm showed low variability among genotypes. These results show a narrower genetic basis than reported for other Passiflora populations and the need to increase this variability by germplasm introduction. Divergent genotypes were also identified for the choice of parents for crosses for genetic gains in traits previously selected within the population studied.*

Key words: *Molecular markers, yellow passion fruit, genetic improvement, genetic variability.*

INTRODUCTION

The genus *Passiflora* comprises at least 400 species, of which approximately 120 are native and dispersed across the Brazilian territory (Bernacci et al. 2005). This is the reason why Brazil is considered one of the major centers of genetic diversity of the group (Faleiro et al. 2005). However, the survival of this diversity of both economic and ecological interest is threatened by a significant reduction in forest areas, caused by human actions, eg., the high rates of deforestation that widely affect tropical regions (Bernacci et al. 2005).

The interest in *Passiflora* is focused primarily on edible fruit species such as the passion fruit (*Passiflora edulis* Sims), which is prevalent in the passion fruit production fields across Brazil (Borges et al. 2005). Currently, Brazil stands out as the largest passion fruit producer and consumer, accounting for about 70% of the world production (Ferreira 2005, Bellon et al. 2007). However, the increased passion fruit production in Brazil in recent years is a consequence of the extension of cultivated area in the country. Among the factors that explain the low productivity of passion fruit is the irregularity of the orchards, mainly due to a lack of improved genotypes available (Meletti et al. 2000).

Actions of screening to estimate the genetic variability of *Passiflora* spp. by physicochemical descriptors (Cardoso-Silva et al. 2007, Araújo et al. 2008, Cerqueira-

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Silva et al. 2009a) to characterize the disease reaction (Junqueira et al. 2003, Leão et al. 2006, Cerqueira-Silva et al. 2008) and to conduct intra-and interspecific crosses (Junqueira et al. 2008, Fonseca et al. 2009) have shown an increase in the number of studies associated with the improvement of the crop in Brazil.

The access to molecular polymorphism at the DNA level has been an important tool in the generation of useful knowledge for different stages of breeding programs of *Passiflora* spp. Different molecular techniques have been applied to estimate the genetic variability of passion fruit, eg., studies using restriction enzymes of cpDNA sites (Sánchez et al. 1999), isozymes (Segura et al. 2003) and amplified fragment length polymorphism (AFLP) markers (Segura et al. 2002), although the genetic variability of *Passiflora* spp. is mostly estimated by random amplified polymorphic DNA (RAPD) (Fajardo et al. 1998, Viana et al. 2003, Bellon et al. 2007, Bellon et al. 2009).

Although markers may be defective in terms of repeatability of the results (Borém and Caixeta 2006), strategies to reduce the occurrence of experimental errors are available, such as the use of DNA repeats of a same genotype in all amplification cycles and the assessment (gel reading) by more than one observer. Such measures reduce the possibility of experimental errors (Leal et al. 2008).

The application of RAPD markers is not restricted to the characterization of the genus *Passiflora* and the use efficiency of this technique in different genetic approaches is demonstrated by recent results in solving problems in different plant species, generating useful information for conservation and genetic improvement of plants (Juchum et al. 2007, Leal et al. 2008, Ferrão et al. 2009). In these studies a variable number of polymorphic bands was observed, for example, 30 polymorphic bands by Leal et al. (2008) and 231 polymorphic bands obtained and evaluated by Ferrão et al. (2009). Variation in the number of RAPD markers used in diversity studies is also demonstrated and discussed by Dias et al. (2004). The objective of this study was to characterize a *Passiflora edulis* Sims genebank by RAPD markers, based on estimates of genetic dissimilarity.

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MATERIAL AND METHODS

Plant material

The 20 genotypes of yellow passion fruit (*Passiflora edulis* Sims; *Pe*-G1 to *Pe*-G20) evaluated in this study had been naturally pollinated and are part of the *Passiflora* Genbank of the Universidade Estadual do Sudoeste da Bahia (UESB), campus Vitória da Conquista (CAGT-*Passiflora*/UESB) (lat 14º 53' S, long 40º 47' W, and alt 900 m asl). These 20 genotypes are represented together with the populations that were characterized for resistance to the Passion-fruit woodiness virus (Cerqueira-Silva et al. 2008) and that are being characterized in relation to the physicochemical fruit descriptors (Nonato el at. 2008).

Molecular analysis

Leaf tissue samples of passion fruit were collected and stored in an ultrafreezer (-80 ºC) until DNA extraction using the protocol of Doyle and Doyle (1990). The amplification reactions (PCR) were performed using standard RAPD procedures (Williams et al. 1990), with 16 primers of the series OPD (D-03, -05, -07, -11, -13, -16, -18, and -20) and OPE (E-01, -02, -03, -04, -07, -14, -15, and -18) of Operon Technology. These primers were selected in advance for their high repeatability among the 40 primers that constitute these series (OPD and OPE). The PCR consisted of 40 cycles of 30 sec at 94 °C, 40 sec at 50 °C and 1 min at 72 °C, with an initial denaturation step (5 min at 94 °C) and after the cycles a final extension step at 72 °C for 7 min, followed by temperature reduction to 15 °C.

The amplification products were electrophoresed on 1.6% agarose gel stained with ethidium bromide and submerged in 1X TBE (buffer consisting of Tris-borate-EDTA). After electrophoresis, the gels were photographed under ultraviolet light in an EDAS 290 gel doc system (Kodak). The banding pattern observed was used to construct a binary data matrix (0 for absence and 1 for presence of bands), including all monomorphic and polymorphic markers obtained based on the criteria of clarity and repeatability.

The RAPD technique is sensitive to small variations in experimental conditions, which can cause problems of data consistency (Borém and Caixeta 2006). To ensure data reliability, three replications of one sample per primer were used, so that in each amplification cycle DNA of the same genotype was used, considered as control. Thus, only primers that produced clearly visible bands of the control

in the evaluations were considered in the final analysis. Moreover, the electrophoretic patterns were evaluated by two researchers, based on the criterion of agreement of the raters for the analysis.

Statistical analyses

The following multivariate analysis procedures were carried out: *i*) estimation of the genetic similarity ($gd_{ij} = 1 - gs_{ij}$, where similarity = gs_{ij} and dissimilarity = gd_{ij}), based on the coefficient of Dice (1945); *ii*) genotype grouping by the unweighted pair-group method with arithmetic mean (UPGMA), selected for the lowest values of distortion and stress, as well as the highest cophenetic correlation for the species, as applied by Cerqueira-Silva et al. (2009b); *iii*) two-dimensional data projection and; *iv*) evaluation of cluster quality and two-dimensional data projection based on distortion, stress and correlation estimates.

The efficiency of cluster matrices and two-dimensional data projection was evaluated using a classification proposed by Kruskal et al. (1964). Statistical analyses were performed using software Genes (Cruz 2001).

RESULTS AND DISCUSSION

The amplification reactions performed with 16 RAPD primers produced a total of 92 bands, 57 polymorphic and 35 monomorphic, representing 62 and 38%, respectively (Table 1). The mean number of bands observed per primer was 5.7 and ranged from one for OPE-4 to 11 for OPE-1. The number of polymorphic bands and the mean ratio of these bands per primer, observed between the genotypes of *P. edulis*, can be considered low when compared with data available for *P. edulis*, with 75.03% polymorphic bands and a mean of 14.4 bands per primer (Bellon et al. 2007) and *P. alata*, with 62.12% polymorphic bands and a mean of 12.7 bands per primer (Bellon et al. 2009).

These results can be explained, at least partially, by the origin of the germplasm evaluated. The *P. edulis* genotypes used in this study were originated from open-pollinated genotypes of field production, and despite indirectly, they were the result of participatory plant breeding or local selections performed by the producers themselves. On the other hand, Bellon et al. (2007) and Bellon et al. (2009) assessed plants from wild and commercial accessions together, contributing to a greater variability between members of the population.

The dg_{ij} of 20 genotypes of *P. edulis* ranged from 0.12 (between *Pe*-G4 and *Pe*-G20) to 0.26 (between the pairs Pe and *Pe*-G3 and *Pe*-G7; *Pe*-G3 and *Pe*-G18; *Pe*-G4 and *Pe*-G16; *Pe*-G12 and *Pe*-G16); the mean gd_{ij} of all 380 pairs was 0.19. These dissimilarity values were lower than those observed in other variability studies involving *Passiflora* spp., such as *P. edulis* ($0.09 \leq gd_{ij} \leq 0.50$) (Bellon et al. 2007), *P. alata* ($0.086 \leq gd_{ij} \leq 0.32$) (Bellon et al. 2009) and *P. nítida* ($0.031 \leq gd_{ij} \leq 0.471$) (Junqueira et al. 2007) and evidence the narrowing of the genetic basis of the population evaluated in this study.

Despite the wide genetic and interspecific variability observed for the genus *Passiflora*, low dg_{ij} values were observed among *P. edulis* genotypes (Viana et al. 2003) as well as among commercial genotypes of *P. alata* (Bellon et

Table 1. Primers used to obtain RAPD data, with respective descriptions of the numbers of bands in genotypes of passion fruit (*Passiflora edulis* Sims)

Primers	Sequence 5' → 3'	No. of polymorphic bands	No. of monomorphic bands	No. of bands per *primer*
OPD-03	GTCGCCGTCA	6	2	8
OPD-05	TGAGCGGACA	2	1	3
OPD-07	TTGGCACGGG	2	2	4
OPD-11	AGCGCCATTG	6	1	7
OPD-13	GGGGTGACGA	8	0	8
OPD-16	AGGGCGTAAG	5	2	7
OPD-18	GAGAGCCAAC	1	2	3
OPD-20	ACCCGGTCAC	4	3	7
OPE-01	CCCAAGGTCC	6	5	11
OPE-02	GGTGCGGGAA	1	2	3
OPE-03	CCAGATGCAC	2	5	7
OPE-04	GTGACATGCC	1	0	1
OPE-07	AGATGCAGCC	1	5	6
OPE-14	TGCGGCTGAG	6	1	7
OPE-15	ACGCACAACC	3	0	3
OPE-18	GGACTGCAGA	3	4	7
Total	-	57	35	92

al. 2009). In this context, several studies show the reduction of variability among *Passiflora* genotypes on the market or found in a particular geographical region, compared to genotypes from different geographic regions or growing wild (Fajardo et al. 1998, Viana et al. 2003, Bellon et al. 2007, Junqueira et al. 2007, Bellon et al. 2009).

This is the first report on specific genetic variability of *P. edulis* genotypes grown in the state of Bahia, estimated by molecular markers. This study is of fundamental importance because it deals with a population drawn from participatory plant breeding that been characterized in terms of some agronomic features of regional significance, such as the resistance to cowpea aphid-borne mosaic virus (CABMV) (Cerqueira-Silva et al. 2008) and physicochemical fruit descriptors (Nonato et al. 2008).

The estimated cophenetic correlation coefficient calculated as the difference between the dissimilarity matrix and graphical representation was 0.46, while the correlation between the original distance and distance in two-dimensional projection was 0.28. The percentages of stress observed for the cluster matrix (13.8%) and distance projection in two-dimensional space (60.1%) were classified as good and inadequate, respectively, according to Kruskal et al. (1964). Thus, the variability observed between the genotypes of *P. edulis* was visually represented by a dendrogram and not by scatterplots. The results observed for the stress values and use of UPGMA agreed with the previous, related to the effectiveness of different methods of multivariate statistics in the characterization of variability in *P. edulis* (Cerqueira-Silva et al. 2009b).

By the cluster analysis based on genetic distances, the 20 *P. edulis* genotypes were divided into four genetically similar groups, considering the cut-off point at a relative genetic distance of 0.27 (Figure 1). These groups agreed with the data in the dissimilarity matrix, since the genotypes with highest dissimilarity (as the genotype pairs *Pe*-G3 and *Pe*-G7; *Pe*-G3 and *Pe*-G18; *Pe*-G4 and *Pe*-G16; *Pe*-G12 and *Pe*-G16) were represented in

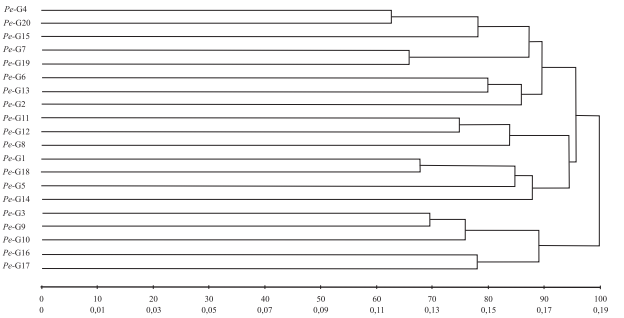

Figure 1. Clustering of 20 genotypes of passion fruit (*Passiflora edulis* Sims) obtained by UPGMA algorithm based on Dice' distance matrix from RAPD bands. *Pe*-G1 to *Pe*-G20 corresponds to the genotypes studied.

different groups in the dendrogram, while those with lowest dissimilarity (*Pe*-G4 and *Pe*-G20) were united in the same group with the shortest distance. Thus, the graphical representation in the dendrogram visualizes the variability of the divergent genotypes within the population studied.

The combination of past or future phenotypic characterizations with this analysis of the genetic variability of *P. edulis* can effectively contribute to genetic gain by crosses between pairs of genetically divergent plants at the DNA level (Figure 1) and controlled crosses in terms of their agronomic characteristics. The results indicate the need for introductions of exotic germplasm, at least grown in other regions of the country, into the work collections to be exploited in the early stages of genetic improvement with the goal of generating local selections and varieties. The increase of the number of accessions in banks is regarded as an efficient source for both species conservation and increased variability in breeding programs (Faleiro et al. 2005).

With the new knowledge on genetic variability it is believed that the study population in future crosses can be further exploited and the variability considerably increased in the active passion fruit bank CAGT-*Passiflora*/UESB, as a contribution to the conservation and use of this resource in genetic improvement programs of the species.

Diversidade genética de maracujazeiro 'amarelo' (*Passiflora edulis* Sims) com base em marcadores RAPD

Resumo - *Este estudo teve como objetivo avaliar a variabilidade genética, por meio de marcadores RAPD, de 20 genótipos de maracujazeiro 'amarelo' (*Passiflora edulis *Sims). Os 16 primers geraram 92 marcadores, dentre os quais 57 (62%) apresentaram-se polimórficos. A distância genética (dg_{ij}) estimada pelo complemento do índice de Dice (dg_{ij}=0,19) e o agrupamento dos genótipos com base no algoritmo UPGMA mostraram reduzida variabilidade entre os genótipos. Tais resultados evidenciam um estreitamento da base genética, em relação ao relatado para outras populações de Passiflora, e a necessidade de incremento desta variabilidade pela introdução de germoplasma. Os resultados permitem ainda identificar genótipos divergentes para escolha de genitores em cruzamentos visando ganhos genéticos em caracteres agronômicos previamente trabalhados dentro da população estudada.*

Palavras-chave: Marcadores moleculares, maracujá 'amarelo', melhoramento genético, variabilidade genética.

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REFERENCES

Araújo FP, Silva NS and Queiroz MA (2008) Divergência genética entre acessos de *Passiflora cincinnata* Mast com base em descritores morfoagronômicos. **Revista Brasileira de Fruticultura 30**: 723-730.

Bellon G, Faleiro FG, Junqueira KP, Junqueira NTV, Santos EC, Braga MF and Guimarães CT (2007) Variabilidade genética de acessos silvestres e comerciais de *Passiflora edulis* Sims com base em marcadores RAPD. **Revista Brasileira de Fruticultura 29**: 124-127.

Bellon G, Faleiro FG, Peixoto JR, Junqueira KP, Junqueira NTV, Fonseca KG and Braga MF (2009) Variabilidade genética de acessos obtidos de populações cultivadas e silvestres de maracujazeiro-doce com base em marcadores RAPD. **Revista Brasileira de Fruticultura 31**: 197-202.

Bernacci LC, Meletti LMM, Soares-Scott MD and Passos IRS (2005) Espécies de maracujá: caracterização e conservação da biodiversidade. In Faleiro FG, Junqueira NTV and Braga MF (eds.) **Maracujá: germoplasma e melhoramento genético**. Embrapa Cerrados, Planaltina, p.559-586.

Borém A and Caixeta ET (2006) **Marcadores moleculares.** UFV, Viçosa, 374p.

Borges RS, Scaranari C, Nicoli AM and Coelho RR (2005) Novas variedades: validação e transferência de tecnologia. In Faleiro FG, Junqueira NTV and Braga MF (eds.) **Maracujá: germoplasma e melhoramento genético**. Embrapa Cerrados, Planaltina, p. 618-640.

Cardoso-Silva CB, Melo JRF, Pereira AS, Cerqueira-Silva CBM, Oliveira AC (2007) Estudo da diversidade genética mediante caracterização físico-química de frutos de maracujazeiros-do-sono nativos do estado da Bahia. **Magistra 19**: 352-358.

Cerqueira-Silva CBM, Cardoso-Silva CB, Notato JVA, Corrêa RX and Oliveira AC (2009a) Genetic dissimilarity of 'yellow' and 'sleep' passion fruit accessions based on the fruits´ physical-chemical characteristics. **Crop Breeding and Applied Biotechnology 9**: 210-218.

Cerqueira-Silva CBM, Cardoso-Silva CB, Conceição LDHCS, Nonato JVA, Oliveira AC and Corrêa RX (2009b) Comparison of coefficients and distance measurements in passion fruit plants with molecular markers and physical-chemical descriptors. **Genetics and Molecular Research 8**: 870-879

Cerqueira-Silva CBM, Moreira CN, Figueira AR, Corrêa RX and Oliveira AC (2008) Detection of resistance gradient to the Passion fruit woodiness virus and selection of yellow passion fruit plants in field conditions. **Genetics and Molecular Research 7**: 1209-1216.

Cruz CD (2001) **Programa Genes: aplicativo computacional em genética e estatística**. Editora UFV, Viçosa, 648p.

Dias LAS, Picoli EAT, Rocha RBR and Alfenas AC (2004). A priori choice of hybrid parents in plants. **Genetics and Molecular Research 3**: 356-368.

Dice LR (1945) Measures of the amount of ecologic association between species. **Ecology 26**: 297-302.

Doyle JJ and Doyle JL (1990) Isolation of plant DNA from fresh tissue. **Focus 12:** 13-15.

Fajardo D, Angel F, Grum M, Tohme J, Lobo M, Roca WM and Sanchez I (1998) Genetic variation analysis of the genus *Passiflora* L. using RAPD markers. **Euphytica 101**: 341-347.

Faleiro FG, Junqueira NTV and Braga MF (2005) Germoplasma e melhoramento genético do maracujazeiro - desafios da pesquisa. In Faleiro FG, Junqueira NTV and Braga MF (eds.) **Maracujá: germoplasma e melhoramento genético**. Embrapa Cerrados, Planaltina, p. 187-210.

Ferrão FAG, Fonseca AFA, Ferrão RG, Barbosa WM and Souza LMR (2009) Genetic divergence in conilon coffee revealed by RAPD markers. **Crop Breeding and Applied Biotechnology 9**: 67-74.

Ferreira FR (2005) Recursos genéticos de *Passiflora*. In Faleiro FG, Junqueira NTV and Braga MF (eds.) **Maracujá: germoplasma e melhoramento genético**. Embrapa Cerrados, Planaltina, p. 41-50.

Fonseca KG, Faleiro FG, Peixoto JR, Junqueira NTV, Silva MS, Bellon G, Junqueira KP and Vaz CF (2009) Análise da recuperação do genitor recorrente em maracujazeiro-azedo por meio de marcadores RAPD. **Revista Brasileira de Fruticultura 31**: 145-153.

Juchum FS, Leal JB, Santos LM, Almeida MP, Ahnert D and Corrêa RX (2007) Evaluation of genetic diversity in a natural rosewood population (*Dalbergia nigra* Vell. Allemão ex Benth.) using RAPD markers. **Genetics and Molecular Research 6**: 543-553.

Junqueira KP, Faleiro FG, Junqueira NTV, Bellon G, Ramos JD, Braga MF and Souza LS (2008) Confirmação de híbridos interespecíficos artificiais no gênero *Passiflora* por meio de marcadores RAPD. **Revista Brasileira de Fruticultura 30**: 191-196.

Junqueira KP, Faleiro FG, Ramos JD, Bellon G, Junqueira NTV and Braga MF (2007) Variabilidade genética de acessos de maracujá-suspiro com base em marcadores moleculares. **Revista Brasileira de Fruticultura 29**: 571-575.

Junqueira NTV, Anjos JRN, Silva APO, Chaves RC and Gomes AC (2003) Reaction to diseases and yield of eleven cultivars of sour-passion fruit cultivated with no pesticides. **Pesquisa Agropecuária Brasileira 38**: 1005-1010.

Kruskal JB (1964) Multidimensional scaling by optimizing goodness of fit to a non-metric hypothesis. **Psychometrika 29**: 1-27.

Leal JB, Santos LM, Santos CAP, Pires JL, Ahnert D and Corrêa RX (2008) Diversidade genética entre acessos de cacau de fazendas e de banco de germoplasma na Bahia. **Pesquisa Agropecuária Brasileira 43**: 851-858.

Leão RMK, Peixoto JR, Junqueira NTV, Resende RO, Mattos JKA and Melo B (2006) Reação de progênies de maracujazeiro-azedo ao vírus do endurecimento do fruto (*cowpea aphid-borne mosaic virus* - CABMV) em casa de vegetação. **Bioscience Journal 22**: 87-92.

Meletti LMM, Santos RR and Minami K (2000) Melhoramento do maracujazeiro-amarelo: obtenção do cultivar 'composto IAC-27'1,2. **Scientia Agricola 57**: 491-498.

Nonato, JVA, Cerqueira-Silva CBM and Oliveira AC (2008) Avaliação biométrica e química de frutos de maracujazeiros amarelo (*Passiflora edulis* Sims f. *flavicarpa* O. Deg.) contrastantes quanto a prolificidade de produção de frutos. **Proceedings Interamerican Society for Tropical Horticulture 52**: 153-155.

Pham TD, Bui TM, Werlemark G, Bui TC, Merker A and Carlsson AS (2009) A study of genetic diversity of sesame (*Sesamum indicum* L.) in Vietnam and Cambodia estimated by RAPD markers. **Genetic Resources and Crop Evolution 56**: 679–690.

Sánchez I, Angel F, Grum M, Duque MC, Lobo M, Tohme J and Roca W (1999) Variability of chloroplast DNA in the genus *Passiflora* L. **Euphytica 106**: 15-26.

Segura SD, Coppens d'EG, Ocampo CH and Ollitrault P (2003) Isozyme variation in *Passiflora* subgenera Tacsonia and Manicata. Relationships between cultivated and wild species. **Genetic Resources and Crop Evolution 50:** 417-427.

Segura SD, Coppens D'EG, Bohorquez A, Ollitrault P and Tohmé J (2002) An AFLP diversity study of the genus *Passiflora* focusing on subgenus *Tacsonia*. **Genetic Resources and Crop Evolution 00:** 1-13.

Viana AP, Pereira TNS, Pereira MG, Souza MM, Maldonado JVM and Amaral Junior AT (2003) Genetic diversity among yellow passion fruit commercial genotypes and among *Passiflora* species using RAPD. **Revista Brasileira de Fruticultura 25**: 489-493.

Williams JG, Kubelik AR, Livak KJ, Rafalski LA and Tingel SV (1990) DNA polymorphism amplified by arbitrary initiator are useful as genetic markers. **Nucleic Acids Research 18**: 6531-6535.