Original Article

MOLECULAR CHARACTERIZATION OF YELLOW PASSION FRUIT GENOTYPES WITH DIFFERENT YIELD AND DISEASE RESISTANCE LEVELS

CARACTERIZAÇÃO MOLECULAR DE GENÓTIPOS DE MARACUJAZEIRO AZEDO COM DIFERENTES NÍVEIS DE PRODUTIVIDADE E RESISTÊNCIA A DOENÇAS

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ABSTRACT: Brazil is the largest passion fruit producer in the world. However, the yield is still considered low, and the cultivation of unsuitable varieties is one of the factors directly influencing this trait. As a consequence, breeding studies have been developed with the purpose of obtaining genetic materials with high yield, high fruit quality, and disease resistance. The objective of this study was to characterize and quantify the genetic variability in 18 genotypes of yellow passion fruit (*Passiflora edulis* Sims) with different levels of yield and disease resistance, using RAPD markers. The RAPD markers were obtained from 10 decamer primers and converted into a matrix of binary data. Estimations of the genetic dissimilarities between different genotypes and cluster analysis were performed. A total of 58 markers were generated, 63.80% of which were polymorphic. The genetic distances among genotypes varied from 0.040 to 0.354 and genotypes were subdivided into at least 5 groups of similarity. The dispersion graphs showed a low clustering tendency for yield and resistance to different diseases (septoriosis, anthracnose, scab, bacterial spot, and passion fruit woodiness disease). These results demonstrate a high genetic variability among the evaluated genotypes, which is valuable information when selecting promising materials to be used per se or as parents in genetic breeding programs.

KEYWORDS: *Passiflora edulis* Sims. Breeding. Genetic variability.

INTRODUCTION

Brazil is the largest passion fruit (*Passiflora* spp) producer and consumer in the world. The crop is cultivated by small and medium producers and presents an average yield of 14,101 kg ha^{-1} (IBGE, 2016). However, Brazilian passion fruit yield is still considered low as compared to the reported yields from genetically improved varieties (FREITAS et al., 2011).

In Brazil, the crop is infected by many plant pathogens that cause several diseases, which can reduce fruit quality and yield, decrease the period of commercial exploitation, and shorten the plant´s life. Among them, septoriosis (*Septoria passiflorae*), anthracnose (*Colletotrichum gloeosporioides*), bacterial spot (*Xanthomonas axonopodis* pv. *passiflorae*), scab (*Cladosporium herbarum*), and passion fruit woodiness disease (PWD; *Cowpea aphid-borne mosaic virus* - CABMV) are of great importance to Brazilian passion fruit production (JUNQUEIRA et al., 2003; MACIEL et al., 2009).

Obtaining high-yielding cultivars with good fruit quality and pathogen resistance is an important goal for research programs (FALEIRO et al., 2008), especially those aiming at passion fruit breeding. Traits of agronomic importance are generally the goal of genetic breeding studies, which aim at developing superior materials and tend to use intra-specific hybridization to transfer genes of interest (BRUCKNER, 1997).

The use of resistant cultivars associated with other integrated management techniques is the most efficient, economical, and ecologically correct means for disease control. Such strategy is especially required for yellow passion fruit (*Passiflora edulis* Sims) due to its great susceptibility to the different diseases and low variability for disease resistance shown by the commercial cultivars (JUNQUEIRA et al., 2003). Some genotypes have been reported to be resistant to the bacterial scab (VIANA et al., 2014) and septoriosis (KOSOSKI, 2014), and moderately resistant to anthracnose (SOUSA et al., 2014) and scab (CASTRO, 2015). To date, there are no reports on yellow passion fruit genotypes showing long-term resistance to CABMV (SANTOS et al., 2015). Therefore, continued studies focused on the identification of disease resistance sources, both under protected cultivation and under field conditions, are an important strategy in a breeding program aiming at developing resistant cultivars (FALEIRO et al., 2005).

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The *Passiflora* genus is comprised of more than 450 species and over 150 species are native to Brazil. This places Brazil as one of the most important passion fruit diversity centers, presenting valuable sources of genes for genetic breeding (FALEIRO et al., 2011). There is great genetic variability to be studied, characterized, protected, conserved, and used commercially in breeding programs (FALEIRO et al., 2005a). In order to explore passion fruit's potential, genetic compatibility tests are performed aiming to subsidize the selection of materials to be used in such programs. However, environmental factors may limit genetic diversity studies in *Passiflora* spp. In this context, the use of molecular markers is a useful tool since it allows for a fast and accurate study of the existing variability, detecting variations directly in the DNA and obtaining numerous genetic polymorphisms with no environmental influence (FALEIRO, 2007).

According to Junqueira et al. (2008), Random Amplified Polymorphic DNA (RAPD) molecular markers are of great importance in breeding programs since they access the existence of cross-fertilization and genetic compatibility among *Passiflora* species. Moreover, RAPD markers are valuable for selecting compatible and superior genotypes, which may assist in the production of hybrids. RAPD markers have proved to be effective in identifying and quantifying genetic variability in different groups of plants. Consequently, such markers have been used as auxiliary tools in programs focused on characterization and genetic resources usage as well as in breeding programs (FALEIRO, 2007; FERREIRA et al., 2007). RAPD markers have been employed to assess molecular diversity in various *Passiflora* species, such as *P. edulis* Sims (FALEIRO et al., 2005b; BELLON et al., 2007), *P. alata* (BELLON et al., 2009), *P. trintae* (CERQUEIRA-SILVA et al., 2010), and *P. setaceae* (CERQUEIRA-SILVA et al., 2012).

Due to the numerous hybridizations performed in research studies, knowledge on the existing genetic variability in the group of plants studied is of great importance in order to continue with the selection of superior materials and breeding studies in passion fruit. Therefore, this study aimed at characterizing and quantifying the genetic variability in 18 genotypes of yellow passion fruit using RAPD markers, with the purpose to subsidize their use in genetic breeding programs.

MATERIAL AND METHODS

The experiment was conducted in the Laboratory of Genetic and Molecular Biology at Embrapa Cerrados, Planaltina, DF. The evaluated genotypes were obtained from a research program developed by the University of Brasilia (UnB) and Embrapa (Empresa Brasileira de Pesquisa Agropecuária – Brazilian Agricultural Research Corporation), which used yield, fruit quality, and disease resistance as selection criteria (Table 1). Genotypes MAR20#19, MAR20#34 F2, MAR20#34 Pl.7, MAR20#39, MAR20#41, MAR20#44, MAR20#46 Pl.1, MAR20#46, MAR20#49, MAR20#2005 Pl.1, and MAR20#2005 Pl.2 were originated from mass selection from nine superior genotypes: Maguary Mesa 1, Maguary Mesa 2, Havaiano, MSC (Marília Seleção Cerrado), Seleção DF, EC-2-0, F1 (Marília x Roxo Australiano), F1 (Roxo Fiji x Marília), and RC1 [F1 (Marília x Roxo Australiano) x Marília (recurrent parent)]. MSCA is derived from cultivar Marília Seleção Cerrado whereas EC-3-0 is a hybrid (RC1) obtained from controlled pollination between Marília x Roxo Australiano cultivars backcrossed with Marília (F1 x Marília). EC-L-7 is derived from cultivar Marília while MAR20#15 was originated from a mass selection of 32 genotypes at the University of Brasilia's (UnB) Água Limpa Farm. AP01 and Rosa Intenso Pl.1 were obtained from recurrent selection based on half-sib family, and AR02 was originated from an individual selection from anthracnose resistant plants of the Roxo Australiano family.

Genotypes were selected from previous studies, based on their agronomic performance and reaction to various diseases, which were assessed after four greenhouse evaluations and 32 field assessments, at four distinct harvest times, without the use of pesticides. Based on their performance, they were separated into genotypes with greater yield/disease resistance, intermediate yield/disease resistance, and lower yield/disease resistance, as described by Castro (2015).

Leaf samples were collected from each genotype and immediately used to extract the genomic DNA using a modified CTAB method (FALEIRO et al., 2003). Ten RAPD decamer oligonucleotide primers (OPD-04, OPD-07, OPD-08, OPD-10, OPE-16, OPF-01, OPF-17, OPG-05, OPH-04, OPH-12) (Operon Technologies Inc., Alameda, CA, USA) were utilized to obtain RAPD markers. The amplifications were performed in a thermocycler (MJ Research, Inc., Waltham, MA, USA) programmed to 40 cycles of denaturation (94 °C, 15 s), primer annealing (35 °C, 30 s), and primer extension (72 °C, 90 s). At the end of the 40 cycles,

an extension step of 6 min at 72 °C was added, followed by temperature reduction to 4 °C. The DNA amplification reaction volume was 13 µL and each reaction contained 15 ng of one DNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 3 mM $MgCl_2$, 100 µM each of the four dNTPs, 0.4 µm primer (Operon Technologies Inc., Alameda, CA, USA.), and 1U *Taq* polymerase (Invitrogen Corp., Carlsbad, CA, USA).

After amplification, 3 µl of a mixture of bromophenol blue (0.25%) and glycerol (60%) were added to each sample. The amplified fragments were separated in a 1.2% agarose gel in TBE (Tris-Borato 90 mM, EDTA 1 mM) buffer with ethidium bromide (0.5µg mL^{-1}). The electrophoresis process occurred at 90V and lasted for about four hours. Immediately after electrophoresis, the gel was visualized and photographed under ultraviolet light.

The reproducible RAPD markers were converted into a binary data matrix in which the presence of a band corresponded to value 1 and the absence to value 0. The genetic distance among genotypes was estimated based on the complement of Nei and Li's similarity coefficient (1979), using Genes software (CRUZ, 2013).

The matrix of genetic distance was used for genotype clustering based on the unweighted pair group method with arithmetic mean (UPGMA). In addition, a graphical dispersion was generated based on the Multidimensional Scale (MDS) using the principal coordinates method. Analyses were performed using the statistical analysis system (SAS, 2004) and Statistica (STATSOFT, 2000) softwares.

RESULTS AND DISCUSSION

The amplification reactions produced a total number of 58 RAPD bands and an average number of 5.8 bands per primer, with extreme values oscillating from 3 to 10 among the 10 primers used (Table 1). From this total, 37 (63.80%) RAPD markers were polymorphic, while 21 (36.20%) were monomorphic. OPD07 primer presented the greatest number of polymorphic and monomorphic bands, whereas OPD04 showed an equal number for both bands (Table 1).

A greater percentage of polymorphic markers demonstrated the existence of high genetic variability among genotypes. This pattern can be explained by the existing genetic variability within commercial genotypes, especially when plant materials with distinct origins are compared. According to Ganga et al. (2004), the high genetic diversity observed in passion fruit could be due to allogamy along with a self-incompatibility system, which favors cross-pollination and, consequently, gene flow among different genotypes.

Genetic dissimilarities varied from 0.040 to 0.354, characterizing the existence of an expressive diversity among the studied genotypes. The lowest genetic distance was verified between genotypes EC-3-0 and MAR20#39, whereas the greatest distance was observed between MAR20#34 Pl.7 and MAR20#46 (Table 2).

Table 1. Primers used for obtaining RAPD markers showing the number of polymorphic, monomorphic, and total bands in yellow passion fruit (*Passiflora edulis* Sims) genotypes.

Primers	Sequence 5´- 3´	Number of polymorphic bands	Number of monomorphic bands
OPD04	TCTGGTGAGG	3	3
OPD07	TTGGCACGGG	6	4
OPD08	GTGTGCCCCA	3	2
OPD10	GGTCTACACC	3	0
OPE16	GGTGACTGTG	4	1
OPF01	ACGGATCCTG	6	2
OPF17	AACCCGGGAA	5	2
OPG05	CTGAGACGGA	5	2
OPH04	GGAAGTCGCC	2	3
OPH12	ACGCGCATGT	3	2
TOTAL		**37**	**21**

Different studies using RAPD markers have described both high inter and intra-specific genetic variability in the *Passiflora* genus (JUNQUEIRA et al., 2007; BELLON et al., 2009; BELLON et al., 2014). Vilela (2013), for example, reported genetic distances varying from 0.080 to 0.390 for 32 yellow passion fruit progenies, which were subdivided into at least 7 groups of similarity at a relative genetic distance of 0.190. Pio Viana et al. (2003) also distinguished 3 groups of similarity in a study performed with commercial yellow passion fruit genotypes and native passion fruit species. In our study, genotypes were subdivided into at least 5 groups of similarity (Figure 1).

Genotypes were separated as demonstrated by the dispersion graphs (Figure 2), which were also created based on the genetic distance matrix. There was a tendency towards genotype clustering or dispersion according to the greater yield and resistance to septoriosis, anthracnose, scab, bacterial spot, and PWD. They were separated into three groups: green (greater yield/disease resistance), yellow (intermediate yield/disease resistance), and red (lower yield/disease resistance).

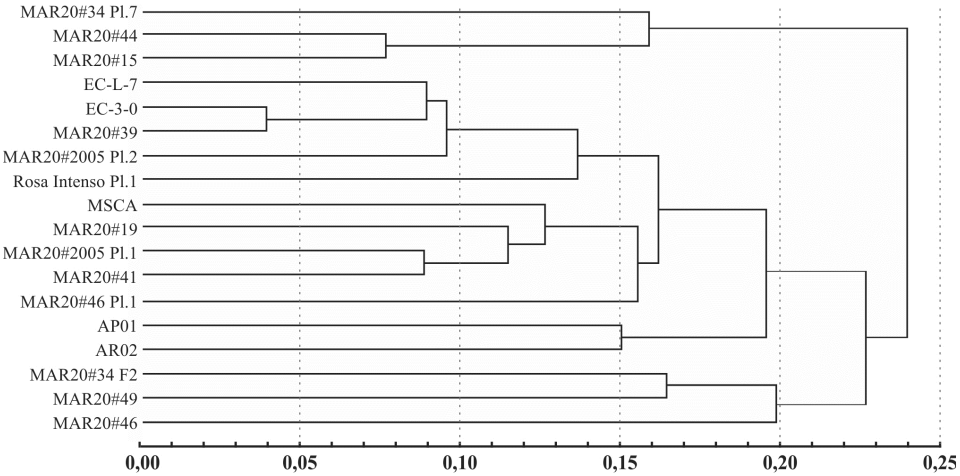

Figure 1. Dendrogram showing clustering of 18 yellow passion fruit (*Passiflora edulis* Sims) genotypes using the unweighted pair group method with arithmetic mean (UPGMA) of distances estimated for the Nei and Li coefficient from RAPD bands.

A slight clustering tendency was observed for genotypes with intermediate yield values (Figure 2A). Genotypes with greater resistance degree to septoriosis (Figure 2B) and anthracnose (Figure 2C) occupied various positions in the graphs, indicating that such genotypes probably present distinct genetic origins and genes for disease resistance. There was a clustering tendency for genotypes with greater resistance to scab (Figure 2D) and bacterial spot (Figure 2E), suggesting a possible common genetic origin for the different resistance genes. No clustering was observed for PWD (Figure 2F), indicating a greater genetic variability for the potential sources of disease resistance, as indicated by different studies (CERQUEIRA-SILVA et al., 2015; FARIAS, 2016).

Despite the low variability for disease resistance observed in yellow passion fruit and the difficulty for selection due to the probable polygenic inheritance of diseases, several studies have demonstrated that selection for resistant genotypes is possible (CASTRO, 2015; SOUSA et al., 2014; VIANA et al., 2014). The variability demonstrated by yellow passion fruit is an essential condition for the progress of a genetic breeding program (SANTOS et al., 2008) and it can be effectively explored to obtain resistant genotypes through gene incorporation in the actual elite cultivars or through the development of new cultivars (FALEIRO et al., 2005).

Our results reveal genotype phenotypic variability regarding yield and disease resistance. As an example, AR2 presented four greater yield/disease resistance levels (Figures 2C-F) from the six yield/disease resistance levels observed. Hence, these data confirm the existence of genetic variability among genotypes, which is valuable information in the selection of individuals with the largest possible intraspecific variability (CERQUEIRA-SILVA et al., 2010). Additionally, our findings could support breeders in selecting promising genotypes to be used per se or as parents in genetic breeding programs.

Table 2. Matrix of genetic distance based on the coefficient of Nei and Li among pairs of yellow passion fruit (*Passiflora edulis* Sims) genotypes, through RAPD markers. 1) MAR20#34 Pl.7; 2) MAR20#44; 3) MAR20#15; 4) EC-L-7; 5) EC-3-0; 6) MAR20#39; 7) MAR20#2005 Pl.2. 8) Rosa Intenso Pl.1; 9) MSCA; 10) MAR20#19; 11) MAR20#2005 Pl.1; 12) MAR20#41; 13) MAR20#46 Pl.1; 14) AP01; 15) AR02; 16) MAR20#34 F2; 17) MAR20#49; 18) MAR20#46.

Genotypes	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	0																	
2	0.171	0																
3	0.147	0.077	0															
4	0.268	0.185	0.19	0														
5	0.254	0.195	0.205	0.132	0													
6	0.27	0.14	0.146	0.048	0.04	0												
7	0.288	0.19	0.195	0.119	0.189	0.146	0											
8	0.268	0.22	0.205	0.139	0.143	0.14	0.153	0										
9	0.342	0.233	0.22	0.214	0.155	0.159	0.2	0.205	0									
10	0.254	0.237	0.233	0.211	0.13	0.225	0.225	0.179	0.215	0								
11	0.217	0.225	0.237	0.215	0.183	0.253	0.266	0.231	0.181	0.151	0							
12	0.289	0.169	0.2	0.093	0.108	0.087	0.124	0.126	0.165	0.221	0.15	0						
13	0.233	0.19	0.171	0.171	0.171	0.136	0.126	0.143	0.163	0.167	0.2	0.143	0					
14	0.315	0.214	0.229	0.157	0.2	0.149	0.14	0.205	0.149	0.151	0.205	0.204	0.129	0				
15	0.325	0.227	0.2	0.163	0.167	0.133	0.114	0.153	0.111	0.147	0.15	0.137	0.101	0.089	0			
16	0.353	0.275	0.273	0.266	0.273	0.268	0.21	0.273	0.157	0.217	0.227	0.287	0.215	0.157	0.172	0		
17	0.296	0.21	0.2	0.2	0.2	0.186	0.181	0.275	0.167	0.183	0.184	0.222	0.205	0.116	0.156	0.165	0	
18	0.354	0.342	0.297	0.307	0.365	0.291	0.215	0.253	0.231	0.292	0.314	0.301	0.221	0.19	0.19	0.178	0.221	0

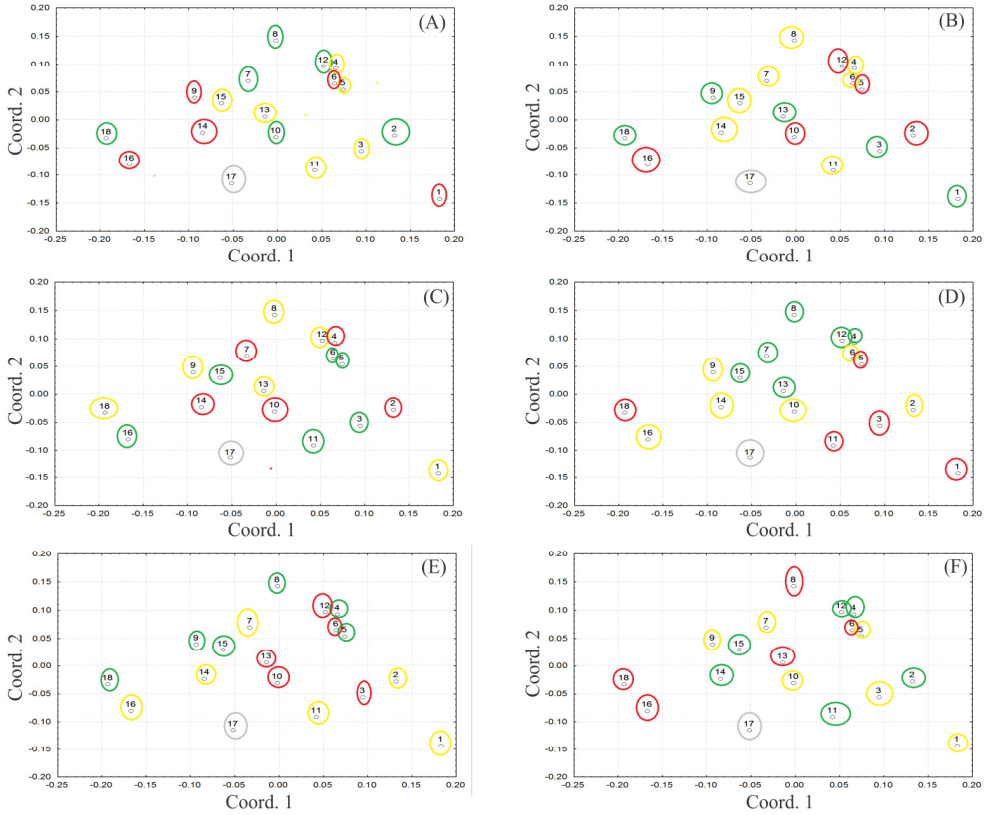

Figure 2. Graphical dispersion of 18 yellow passion fruit (*Passiflora edulis* Sims) genotypes according to yield (A) and resistance levels to septoriosis (B), anthracnose (C), scab (D), bacterial spot (E), and passion fruit woodiness disease (F). Green (greater yield/disease resistance), yellow (intermediate yield/disease resistance), and red (lower yield/disease resistance). 1) MAR20#34 Pl.7; 2) MAR20#44; 3) MAR20#15; 4) EC-L-7; 5) EC-3-0; 6) MAR20#39; 7) MAR20#2005 Pl.2. 8) Rosa Intenso Pl.1; 9) MSCA; 10) MAR20#19; 11) MAR20#2005 Pl.1; 12) MAR20#41; 13) MAR20#46 Pl.1; 14) AP01; 15) AR02; 16) MAR20#34 F2; 17) MAR20#49; 18) MAR20#46.

RESUMO: O Brasil é o maior produtor mundial de maracujá. Entretanto, a produtividade ainda é considerada baixa e o cultivo de variedades inadequadas é um dos fatores que influenciam diretamente esta característica. Como consequência, trabalhos de melhoramento genético tem sido desenvolvidos com a finalidade de obter materiais genéticos com alta produtividade, qualidade de frutos e resistência a doenças. Este trabalho objetivou caracterizar e quantificar a variabilidade genética em 18 genótipos de maracujazeiro azedo (*Passiflora edulis* Sims) com diferentes níveis de produtividade e resistência a doenças, utilizando marcadores moleculares RAPD. Os marcadores RAPD, obtidos por meio de 10 iniciadores decâmeros, foram convertidos em uma matriz de dados binários. Estimativas de dissimilaridades genéticas entre os diferentes genótipos e análises de agrupamento foram realizadas. Um total de 58 marcadores foram gerados, dos quais 63,80% foram polimórficos. As distâncias genéticas entre os genótipos variaram de 0,040 a 0,354 e os genótipos foram subdivididas em pelo menos 5 grupos de similaridade. Os gráficos de dispersão mostraram uma baixa tendência de agrupamento para produtividade e resistência à septoriose, antracnose, verrugose, bacteriose e virose do endurecimento dos frutos. Estes resultados demonstram uma alta variabilidade genética entre os genótipos estudados, que é uma informação valiosa para a seleção de materiais promissores para serem utilizados per se ou como parentais em programas de melhoramento genético.

PALAVRAS-CHAVE: *Passiflora edulis* Sims. Melhoramento. Variabilidade genética.

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