

Genetic variability in wild genotypes of Passiflora cincinnata based on RAPD markers

C.B.M. Cerqueira-Silva^{1,2}, L.D.H.C.S. Conceição³, E.S.L. Santos^{1,2}, C.B. Cardoso-Silva², A.S. Pereira⁴, A.C. Oliveira⁵ and R.X. Corrêa⁴

¹Departamento de Estudos Básicos e Instrumentais,

Universidade Estadual do Sudoeste da Bahia, Itapetinga, BA, Brasil ²Instituto de Biologia, Universidade Estadual de Campinas, Campinas, SP, Brasil ³Centro de Pesquisa Agropecuária dos Cerrados,

Empresa Brasileira de Pesquisa Agropecuária, Planaltina, DF, Brasil
⁴Departamento de Ciências Biológicas, Universidade Estadual de Santa Cruz, Ilhéus, BA, Brasil

⁵Departamento de Ciências Naturais, Universidade Estadual do Sudoeste da Bahia, Vitória da Conquista, BA, Brasil

Corresponding author: R.X. Corrêa

E-mail: ronanxc@uesb.br

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ABSTRACT. The genetic diversity and characteristics of commercial interest of *Passiflora* species make it useful to characterize wild germplasm, because of their potential use for fruit, ornamental and medicinal purposes. We evaluated genetic diversity, using RAPD markers, of 32 genotypes of *Passiflora cincinnata* collected from the wild in the region of Vitória da Conquista, Bahia, Brazil. Thirteen primers generated 95 polymorphic markers and only one monomorphic marker. The mean genetic distance between the genotypes estimated by the complement of the Dice index was 0.51 (ranging from 0.20-0.85), and genotype grouping based on the UPGMA algorithm showed wide variability among the genotypes. This type of information contributes

to identification and conservation of the biodiversity of this species and for the identification of pairs of divergent individuals for maximum exploitation of existing variability.

Key words: Genetic breeding; Genetic variability; Grouping analyses; Coefficient of similarity; Biodiversity conservation; Molecular marker

INTRODUCTION

The genus Passiflora L. consists of more than 400 species widely distributed in the tropics, mainly in Brazil, which is the birthplace of at least one third (Ganga et al., 2004). In general, wild species are used as sources of resistance to diseases or for the improvement of desirable characteristics. However, some species have high agronomic potential. Passiflora cincinnata Mast. is a native plant with reports of spontaneous occurrence in the northeast, southeast (Oliveira and Ruggiero, 2005) and midwest (Amorozo, 2002) regions of Brazil. This species is popularly known in Brazil as "maracujá mochila", "maracujá do-mato" or "maracujá tubarão". It is considered potentially important for use as rootstock, since it is tolerant to diseases and nematodes (Zucareli et al., 2007). In the region of Recôncavo, in the Brazilian State of Bahia, its fruits, which are used as food in the form of juice, are sold in street markets and grocery stores. The fruits of *P. cincinnata* Mast. are resistant to shipping and handling, and have greater durability compared to the yellow passion fruit (Oliveira and Ruggiero, 2005.) Total fruit mass is the most important aspect in total variability among morphological descriptors in accessions of *P. cincinnata* Mast. collected in northeastern Brazil (Araújo et al., 2008), thus highlighting the potential of this species for cultivation in order to produce juice. Other attributes related to P. cincinnata Mast. make this species suitable for ornamental or medicinal purposes.

Tropical flowers are a growing market in Brazil and worldwide (Pizano, 2005), while tropical fruits such as banana (Souza et al., 2008) and pineapple (Pasqual et al., 2008) have been used for this purpose. Eccentric flowers, lush foliage and continuous and plentiful bloom, make *Passiflora* suitable for the market of ornamental plants (Abreu et al., 2009). However, this potential is not exploited in Brazil. The potential use of species, such as *P. alata* and *P. edulis* Sims. in the southeast region, *P. coccinea* Aubl. in the north and *P. cincinnata* Mast. in the northeast, is limited to fences and arbors with the use of the fruits without the sole purpose for ornamentation (Peixoto, 2005).

Passion fruit was discovered as a medicinal plant in 1867, when passiflorine aroused great interest in medicine. Still, the therapeutic properties of passion fruit were already used by natives before the discovery of the Americas (Milward-de-Azevedo, 2008). The exploitation of genetic resources of medicinal plants in Brazil is largely related to the extensive collection and extraction of wild material (Rodrigues and Carvalho, 2001). Several ethnobotanical or ethnomedicinal surveys report the use of *P. cincinnata* Mast. in local communities in the midwest (Amorozo, 2002) and northeast (Silva et al., 2002; Dantas and Guimarães, 2007) regions of Brazil for therapeutic purposes. Agra et al. (2007) described the use of leaves of *P. cincinnata* Mast. in the form of tea, for the treatment of hemorrhoids and venereal diseases.

Thus, pre-breeding works aiming to evaluate and conserve the variability of this species in order to allow exploitation in a sustainable manner are essential. Molecular markers are currently a tool that can aid in estimating genetic diversity by determining the genetic relationships

among materials, hence defining the crossbreeding or selection of parents and the introgression of genes during the improvement process (Pereira et al., 2009). An enormous amount of information on genomes has been generated, thus providing numerous opportunities for exploitation of genetic variability in both agronomic species and wild germplasm (Ferreira and Grattapaglia, 1998).

In the present study, we quantified, by means of random amplified polymorphic DNA (RAPD) primers and the complement of the Dice index, the dissimilarity of wild genotypes of *Passiflora trintae* Sacco originating from the region of Vitória da Conquista, Bahia State, Brazil, belonging to the active collection of *Passiflora* of the Universidade Estadual do Sudoeste da Bahia (UESB). The results are discussed in terms of characterization and conservation.

MATERIAL AND METHODS

We used 32 specimens of *P. cincinnata* Mast. collected in fragments of a liana forest in Vitória da Conquista, Bahia State, Brazil (14°53' S and 40°47' W, elevation of 900 m; average annual precipitation of 700-800 mm, concentrated between November and March, average annual temperature of 20-22°C) (Instituto Nacional de Meteorologia/Ministério da Agricultura e Abastecimento), and belonging to the Active Collection of *Passiflora* Work Germplasm of the UESB, Vitória da Conquista campus (CAGT-*Passiflora*/UESB 'Planalto de Conquista'). For DNA extraction, we adopted the method described by Doyle and Doyle (1990). For amplifying reactions, the patterns described for the RAPD technique were adopted (Williams et al., 1990). Also, 13 RAPD primers from Operon® Technologies and the University of British Columbia were used (UBC). These primers were previously selected because they presented molecular polymorphism among the 47 primers tested, whose series were OPD (02, 08, 12, 18), OPE (03, 04, 05, 06, 07, 11, 12) and UBC (23 and 25).

The amplification products were separated by 1.6% agarose gel electrophoresis, stained with ethidium bromide, submerged in 1X TBE (composed of Tris-borate and EDTA buffer), and the results photographed with a Kodak transluminator (EDAS 290) under ultraviolet light. Molecular markers generated by different primers were analyzed for the presence or absence of bands. Only the bands with high sharpness and reproducibility were considered. To ensure reliability of data, the electrophoretic patterns were evaluated by two researchers. Moreover, only the consensus pattern was considered for analysis.

Multivariate statistical analysis was performed: i) estimate on the complement of genetic similarity ($dg_{ij} = 1 - sg_{ij}$; similarity = sg_{ij} and dissimilarity = dg_{ij}) based on the coefficient of Dice (1945); ii) grouping of genotypes by using the method of arithmetic averages (UPGMA), the latter being selected from other hierarchical methods (Ward, Gower, more distant neighbor, and nearest neighbor) for presenting the lowest distortion and stress values, as well as the highest values of cophenetic correlations for the species (Cerqueira-Silva et al., 2009); iii) the projection of data in the two-dimensional space, and iv) assessing the quality of clusterings and the projection into the two-dimensional space by estimating distortion values, stress and correlation.

To assess the efficiency of grouping matrices and the projection of data into the twodimensional plane, the classification proposed by Kruskal (1964) was used for stress levels, as follows: perfect (\leq 5%), excellent (\geq 5% and \leq 10%), good (\geq 10% and \leq 20%), regular (\geq 20% and \leq 40%), and poor (\geq 40%). Statistical analyses were performed using the Genes, Windows version software (Cruz, 2006).

RESULTS AND DISCUSSION

Among the 13 primers used, a total of 96 markers were amplified. An average of 7.4 marks per primer was generated while only one was found to be monomorphic at the OPE-03 primer. The number of different polymorphic traits ranged from 2 (OPE-12) to 19 (OPD-02) (Table 1). Characterization studies in *P. cincinnata* Mast. involving molecular markers are incipient. A reduced number of accessions are often used along with other species of the genus *Passiflora* L. (Aukar et al., 2002; Viana et al., 2003). Yet, the high degree of polymorphism detected is consistent with the observed variability based on morphoagronomic descriptors (Araújo et al., 2008). These results can be attributed to and are consistent with the reproductive system of this species. *P. cincinnata* Mast. is allogamous, with a natural fructification mean of 50%, and it has self-incompatible flowers (Oliveira and Ruggiero, 2005).

Table 1. Primers used to obtain RAPD data, with the respective descriptions of the numbers of bands (polymorphic, monomorphic and total) in genotypes of *Passiflora cincinnata* Mast.

Primers	Sequence 5' 3'	Polymorphic bands	Monomorphic bands	Bands for primer
OPD-02	GGACCCAACC	19	0	19
OPD-08	GTGTGCCCCA	7	0	7
OPD-12	CACCGTATCC	10	0	10
OPD-18	GAGAGCCAAC	7	0	7
OPE-03	CCAGATGCAC	3	1	4
OPE-04	GTGACATGCC	6	0	6
OPE-05	TCAGGGAGGT	5	0	5
OPE-06	AAGACCCCTC	4	0	4
OPE-07	AGATGCAGCC	7	0	7
OPE-11	GAGTCTCAGG	6	0	6
OPE-12	TTATCGCCCC	2	0	2
UBC-23	GTCCACACGG	10	0	10
UBC-25	ACCCCCGCCG	8	0	8
Total		95	1	96

The values found for the cophenetic correlation coefficient (0.76) and stress (14.3) are classified as "good" as per Kruskal (1964), suggesting a fit between the matrix of genetic dissimilarity and the graphical representation in the dendrogram, when compared with values obtained for two-dimensional projection (Table 2, Figure 1). The average distance revealed in the dissimilarity matrix generated from the molecular markers was 0.51. Among the comparisons made, maximum and minimum distances of 0.85 and 0.20 were found, respectively (Table 3). These values corroborate with the variability found in other native fruits such as the pitanga tree, from 0.26 to 0.75 (Nogueira et al., 2007) and cabbage palm fruit, from 0.06 to 0.67 (Oliveira et al., 2007).

Table 2. Efficiency of clustering matrices and the projection of distances in two-dimensional plane, based on the diversity observed among *Passiflora cincinnata* Mast. genotypes by accessing the polymorphism obtained by means of RAPD.

Criterions	Methods of	f viewing
	Grouping of genotypes	Projection of the distances
Distortion	2.05	55.6
Correlation*	0.76	0.49
Stress	14.3	61.1

^{*}The coefficient of cophenetic correlation was used for the clustering of genotypes, while the correlation between original distance and that projected for the two-dimensional plane was used for the projection of distances.

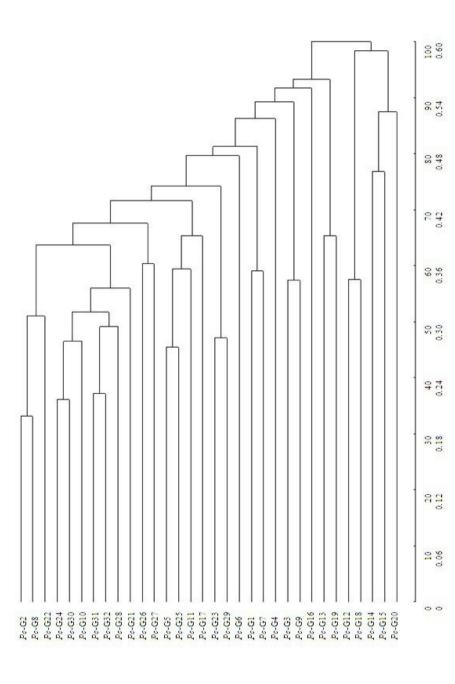


Figure 1. Dendrogram of 32 genotypes of *Passiflora cincinnata* Mast. obtained from the clustering method UPGMA (unweighted arithmetic average method) based on genetic distance matrix (Dice, 1945) calculated from the RAPD data. Pc-G1 to Pc-G32 correspond to the genotypes evaluated.

Table 3. Matrix of genetic distance obtained through the complement of Dice similarity index among genotypical pairs of *Passiflora cincinnata* Mast. by means of RAPD markers.

G31 G32	-												0.41	0.52	0.52	0.44	0.40	0.54	0.55	0.58		0.32	0.37	0.26	0.36	0.34					0.37 0.34 0.32 0.27 0.33 0.37 0.37 0.32
G29 G30 C	0.42	0.39	0.53	0.37	0.35	0.56	0.52	0.52	0.42	0.29	0.46	0.49	0.65	0.62	0.53	0.57	0.49	0.52	0.57	0.71	0.36	0.44	0.47	0.22	0.41	0.43	0	0.38	0.38		0.38 0.35 0.46
G28 G	1																				0.38 0.). - -		
G27	1		0.53			0.46				0.42									0.54			0.45	0.44	0.40	0.39	0.36	,				
G26	0.40	0.41	0.56	0.58	0.39	0.43	0.50	0.45	0.48	0.53	0.48	0.56	0.46	0.56	09.0	0.62	0.51	0.60	0.42	0.58	0.38	0.34	0.39	0.48	0.42						
G25	0.37	0.45	0.61	0.44	0.27	0.40	0.47	0.36	0.44	0.40	0.34	0.54	0.48	0.57	0.57	0.52	0.42	0.59	0.51	89.0	0.35	0.35	0.49	0.38							
G24	0.43	0.45	0.53	0.44	0.34	0.46	0.65	0.44	0.43	0.27	0.44	0.53	0.56	0.55	0.52	0.55	0.49	0.57	0.62	0.63	0.35	0.42	0.47								
G23	1																				_		٠								
G22	1																				0.34	٠									
0 G21	1			3 0.55																											
9 G20	1			57 0.73																											
8 G19	-			79.0 89												0.65 0.0		0.5	Ċ												
G17 G18	1			0.56 0.58										62 0.60		_	_	Ċ													
G16 G				0.57 0.												.0															
G15 G				0.64 0.									_	0.46 0.																	
4				0.63 0.									_	_																	
G13 G1	-			0.66 0.							0.55 0.		.0																		
2	-			0.55 0.							_	_																			
11 G1	-			0.46 0.3							0	·																			
G10 G1				0.36 0.							Ċ																				
G G				0.64 0.																											
G8 (0.57 0																											
G7 (1			0.77 0																											
99	45	27	28	28	51																										
G5 (1			0.52 0.																											
45			0.66																												
G3	0.56 (
, G1 G2	- 0.52	٠																													
otypes	31	32	33	75	35	95	37	85	39	310	EI	312	313	314	315	316	317	318	319	320	321	322	323	324	325	326	327		328	328	328 329 330

In other species of the genus *Passiflora* L. there is less variability characterizations based on RAPD markers. Variations between 0.03 to 0.42 were observed for *P. nitida* Kunth. (Junqueira et al., 2007), 0.09 to 0.50 for *P. edulis* Sims. (Bellon et al., 2007), 0.09 to 0.32 for *P. alata* (Bellon et al., 2009), 0.07 to 0.42 for *P. trintae* (Cerqueira-Silva et al., 2010a), and 0.12 to 0.19 for *P. edulis* Sims (Cerqueira-Silva et al., 2010b); values closer to those found in acerola (0.13 to 0.35), which is an introduced fruit species with a narrow genetic base (Salla et al., 2002). It is noteworthy that the collections studied in these species have to do with accessions of different regions of Brazil. However, except for *P. nitida* and *P. trintae*, cultivated populations were also used. These studies of divergence reinforce the importance of the conservation of native wild species for the sustainable exploitation of genetic resources and existing variability. The lowest variability found in other passiflora species, as compared to values obtained for *P. cincinnata* Mast., indicates a possible genetic erosion.

The genetic diversity-based markers can help the breeder determine crosses and selection of parents (Ferreira and Grattapaglia, 1998; Pereira et al., 2009). Jung et al. (2008) found that heritability estimates and genetic gain in fruit characters of *P. alata* are dependent on crossing. Authors suggest that this occurs because the progenitors present distinct genes controlling these characters ("genetic background" divergence). Crossings among genetically distant materials, such as *Pc*-G5 *vs Pc*-G7 and *Pc*-G20 *vs Pc*-G9 (Table 3), and with superior performance, are likely to promote the formation of populations that recover superior recombinants, whereas the need for a morphoagronomic characterization of similar genotypes was observed. Therefore, we must examine how this species should be exploited, either as an ornamental or medicinal plant, or for juice production, thus defining and testing which characters to evaluate for the chosen purpose. Thus, the most divergent genotypes within the population studied can be used for improvement, once the need to characterize them agronomically is established, according to the objectives of the program type (ornamental, medicinal or fruits).

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