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Genetic Diversity in Passion Fruit (*Passiflora* spp.) Evaluated by RAPD Markers

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ABSTRACT

The objective of this study was to characterize the genetic diversity within a Passiflora collection by PCR-RAPD markers. Genetic analysis was performed in 70 accessions, representing 11 species of the genus Passiflora. The use of only five primers produced 136 reproducible polymorphic bands. The hierarchical classification showed high levels of dissimilarities between and within the species studied. A clear separation was obtained among species and accessions of P. edulis and P. edulis f. flavicarpa, which were forms of the high commercial value species. The fingerprints produced from the studied genotypes would allow the identification of improved varieties/populations in an easy, fast and inexpensive manner.

Key words: *Passiflora* spp., germoplasm, genetic diversity, RAPD-PCR

INTRODUCTION

Passiflora is the most important genus in the family *Passifloriaceae* and it is distributed within tropical and sub-tropical regions. Approximately, 460 species of the genus *Passiflora* have already been described. From these, about 90% have originated from the Americas and close to 150 species occur in Brazil (Sousa and Meletti, 1997). The economical importance of some species is associated to the quality of the fruits for consumption and medicinal properties.

The passion fruit is an allogamous plant and the edible fruit-producing species are diploids (2n=18), namely, the purple (*P. edulis*) and the yellow passion fruit (*P. edulis* f. flavicarpa) (Martin and Nakasone, 1970), which is cultivated in Brazil. The high interspecific compatibility in natural and artificial crossings among the diploid species leads to a wide genetic diversity distributed in different agro-climatic regions. Morphological characters used for taxonomical description of passion fruit have allowed a classification up to the species level, but not to an intraspecific discrimination. Besides fruit color, flavor and resistance to diseases, *P. edulis* and *P. edulis* f. flavicarpa do not show contrasting differences (Martin and Nakasone, 1970). Although still not much used in passion fruit genetic variability studies, RAPD markers have been shown to be polymorphic, allowing the characterization of the variability among and within species as well as for the identification of hybrids and parentals (Fajardo et al., 1998, Cassiano, 1998; Otoni et al., 1995). The characterization of the genetic diversity within a Brazilian *Passiflora* spp. collection using RAPD markers may permit the discrimination of plant introductions, the analysis of inter- and intraspecific variability and the detection of

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duplicated accessions resulting in a complementary tool for selection of progenitors for breeding programs. Also, the identification of cultivars, which are forms of the high commercial value species by molecular markers, may be a valuable support in view of Cultivar Protection Laws (Jondle, 1992). Although these molecular descriptors are not accepted for protecting intellectual property by the UPOV convention (International Union for the Protection of New Varieties of Plants), these techniques have an enormous potential for cultivars discrimination and deserve further investigation. The objective of this study was to characterize the genetic diversity within a *Passiflora* collection by RAPD markers.

Table 1 - Codes, species and origin of the *Passiflora* spp. germplasm collection maintained at IAPAR.

Code	Species	Origin	Code	Species	Origin
LM 1	*P. edulis* f. flavicarpa	Brazil - SP	IA 83	*P. edulis* f. flavicarpa	Brazil - MG
LM 2	*P. edulis* f. flavicarpa	Brazil - SP	IA 81	*P. edulis* f. flavicarpa	Brazil - MG
LM 3	*P. edulis* f. flavicarpa	Brazil - SP	IA 80	*P. edulis* f. flavicarpa	Brazil - MG
LM 4	*P. edulis* f. flavicarpa	Brazil - PR	IA 65	*P. edulis* f. flavicarpa	Brazil - MG
LM 5	*P. edulis* f. flavicarpa	Brazil - PR	IA52	*P. edulis* f. flavicarpa	Brazil - ES
LM 6	*P. edulis* f. flavicarpa	Brazil - PR	IA 48	*P. edulis* f. flavicarpa	Brazil - PR
LM 7	*P. edulis* f. flavicarpa	Brazil - MG	IA 47	*P. edulis* f. flavicarpa	Brazil - MG
LM 8	*P. edulis* f. flavicarpa	Brazil - PR	IA 46	*P. edulis* f. flavicarpa	Brazil - PR
LM 9	*P. edulis* f. flavicarpa	Brazil - PR	IA 42	*P. edulis* f. flavicarpa	Brazil - MG
LM 10	*P. edulis* f. flavicarpa	Brazil - PR	IA 33	*P. edulis* f. flavicarpa	Brazil - SP
BG 15	*Passiflora coccinea*	Brazil - AM	IA 31	*P. edulis* f. flavicarpa	Brazil - MG
BG 01	*Passiflora macrocarpa*	Brazil - SP	IA 30	*P. edulis* f. flavicarpa	Brazil - SP
BG 10	*Passiflora caerulea*	Brazil - SP	IA 27	*P. edulis* f. flavicarpa	Brazil - SP
BG 11	*Passiflora suberosa*	Brazil - SP	IA 25	*P. edulis* f. flavicarpa	Brazil - SP
BG 17	*Passiflora giberti*	Brazil - SP	IA 24	*P. edulis* f. flavicarpa	Brazil - PR
BG 08	*P. serrato digitata*	Brazil - SP	IA 14	*P. edulis* f. flavicarpa	Brazil - ES
BG 04	*Passiflora edulis*	Kenya	IA 10	*P. edulis* f. flavicarpa	Brazil - PR
BG 26	*Passiflora edulis*	Chile	IA 28	*P. edulis* f. flavicarpa	Brazil - PR
BG 19	*Passiflora edulis*	Madeira Island	IA 59	*P. edulis* f. flavicarpa	Brazil - PR
BG 21	*Passiflora edulis*	Brazil - PR	IA 58a	*P. edulis* f. flavicarpa	Brazil - ES
BG 24	*Passiflora foetida*	Brazil - RJ	IA 12	*P. edulis* f. flavicarpa	Brazil - MG
BG 20	*Passiflora edulis*	Morocco	IA 60	*P. edulis* f. flavicarpa	Brazil - MG
BG 18	*P. edulis* x *P. edulis* f. flavicarpa	Brazil - SP	IA 45	*P. edulis* f. flavicarpa	Brazil - PR
BG 06	*Passiflora alata*	Brazil	IA 43	*P. edulis* f. flavicarpa	Brazil - SP
BG 02	*Passiflora edulis*	Brazil - MG	IA 91	*P. edulis* f. flavicarpa	Brazil - MG
BG 16	*Passiflora edulis*	Australia	IA 29	*P. edulis* f. flavicarpa	Brazil - SC
BG 25	*Passiflora alata*	Brazil	IA 20	*P. edulis* f. flavicarpa	Brazil - PR
BG 05	*P. alata* x *P. macrocarpa*	Brazil - SP	IA 49	*P. edulis* f. flavicarpa	Brazil - ES
BG 09	*Passiflora edulis*	Brazil - SP	IA 71	*P. edulis* f. flavicarpa	Brazil - PR
BG 23	*Passiflora edulis*	Brazil - SP	IA 54	*P. edulis* f. flavicarpa	Brazil - PR
BG 27	*Passiflora alata*	Peru	IA 37	*P. edulis* f. flavicarpa	Brazil - PR
BG 34	*Passiflora* sp. (1)	-----	IA 86	*P. edulis* f. flavicarpa	Brazil - MG
BG 14	*Passiflora edulis*	Brazil - SP	IA 11	*P. edulis* f. flavicarpa	Brazil - ES
BG 35	*Passiflora* sp. (2)	-----	IA 58b	*P. edulis* f. flavicarpa	Brazil - ES
IA 87	*P. edulis* f. flavicarpa	Brazil - MG	IA 62	*P. edulis* f. flavicarpa	Brazil - SC

MATERIALS AND METHODS

Plant Material

Seventy introductions of the IAPAR's *Passiflora* spp. Germplasm Collection, comprising 11 species and two not-classified introductions were used. Their origin and identification are shown in Table 1. Seedlings propagated by budding or grafting were grown under greenhouse facilities.

DNA extraction

Young leaf tissues (30-40 mg) from each introduction were harvested at the same plant growth stage. The leaves were oven-dried (37ºC) for 12 h and then macerated until a fine dust. DNA

extraction was performed as described by Molinari and Crochemore (2001). DNA concentration was estimated in a fluorimeter as described by the manufacturer (Hoefer Scientific Instruments).

RAPD analysis

RAPD reactions were performed according to Williams et al. (1990) in a final volume of 25 µl containing 25 ng genomic DNA, 2.5 µl buffer 10X [200 mM Tris (pH 8.4), 500 mM KCl), gelatin 0.01%, 1.5 mM $MgCl_2$, 0.25 mM of each dNTP, 0.2 µM primer and 1 U *Taq* polymerase. The amplifications were performed in a thermocycler (MJ Research, Inc.) using the following program: 1 cycle of 4 min at 94ºC followed by 37 cycles of 1 min at 93ºC, 1 min at 45ºC and 1 min at 72ºC, with a final extension of 6 min at 72ºC.

Amplification products were analyzed by electrophoresis at 5V/cm in agarose gel (1.4%). The gel was stained in 0.5 µg/ml ethidium bromide solution and photographed with the KODAK EDAS 120 system.

From a screening using 42 primers (Operon Technologies, Inc. Kits A, B, AE1 and C16 oligonucleotides), five primers were shown to be highly polymorphic and were used in this study (Table 2). Only amplified consistent DNA bands, in the approximate range of 0,1–3 kilobases (kb) were scored.

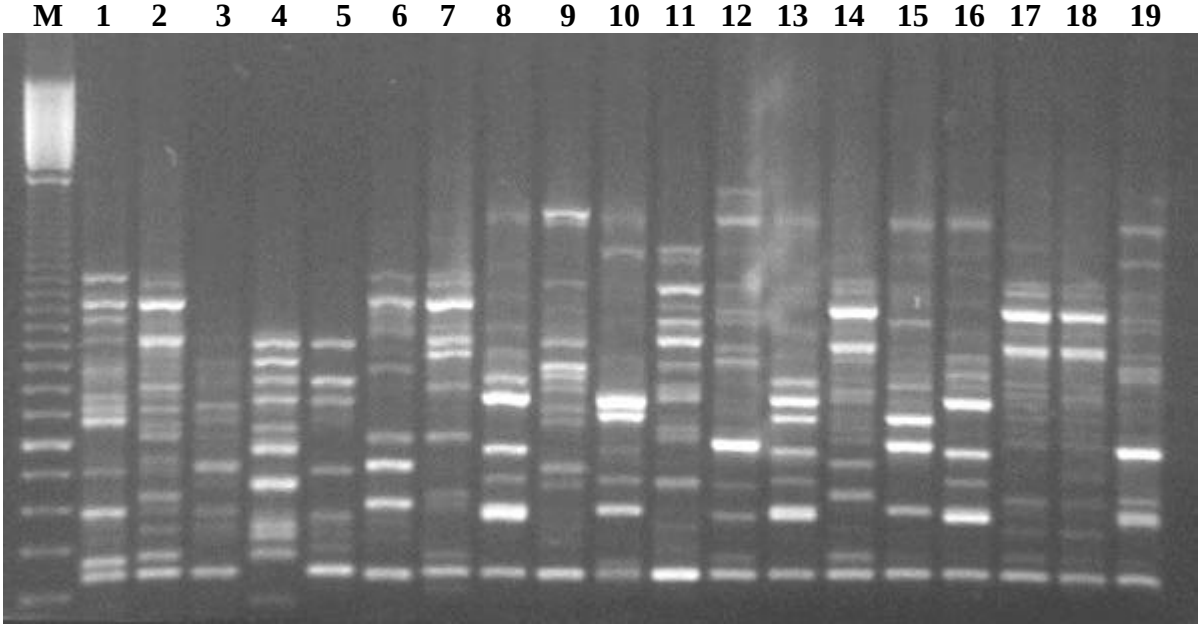

Figure 1 - Random amplified polymorphic DNA from 19 *Passiflora* spp. accessions using primer OPB-18. Lane M: 100bp molecular weigth. Lane (1) *P. coccinea*, (2) *P. macrocarpa*, (3) *P. caerulea*, (4) *P. suberosa*, (5) *P. giberti*, (6) *P. serrato digitata*, (7) *P. edulis* (Kenya), (8) *P. edulis* (Chile), (9) *P. edulis* (Madeira Island), (10) *P. edulis* (Brazil, Parana state), (11) *P. foetida*, (12) *P. edulis* (Morocco), (13) *P. edulis* x *P. edulis* f. *flavicarpa*, (14) *P. alata*, (15) *P. edulis* (Brazil, Sao Paulo state), (16) *P. edulis* (Australia), (17) *P. alata*, (18) *P. alata* x *P. macrocarpa*, (19) *P. edulis* (Brazil, Sao Paulo state).

Statistical analysis

Jaccard's similarity indexes (Jaccard, 1901) were calculated with data provided by the observation of the presence (1) or absence (0) of bands between pairs of accessions using the formula $D_{(ij)} = a/(a+b+c)$, where: **a** is the number of fragments shared by accessions **i** and **j**; **b** is the number of fragments present in **i** and absent in **j**; **c** is the number of fragments present in **j** and absent in **i**. A hierarchical classification (dendrogram) was obtained from the similarity matrix using the UPGMA (Unweighted Pair Group Mathematical Average) method. The mean similarities and variances detected in the hierarchical classification were calculated. All calculations were performed

using the SAS Statistical Program (SAS Institute, 1998).

RESULTS AND DISCUSSION

Table 2 summarizes the number, average and size range of amplified fragments obtained with the five primers used to compare the *Passiflora* collection. A total of 136 amplified fragments were selected. Depending on the introduction-primer combination, between 1 and 11 distinct RAPD products were amplified, ranging in size from approximately 120 to 3000 bp. Each RAPD amplification was prepared at least three times to determine reproducibility of the patterns.

Genetic diversity

High dissimilarity was observed among the introductions. The hierarchical classification obtained by the clustered method showed three large groups (Fig. 2). The values of mean dissimilarity, range and variances of all possible pairwise comparisons within these groups are shown on Table 3. As expected, the highest dissimilarity was observed among the species of Group I. This group is composed by 14 accessions, comprising the species: *P. cocinea*, *P. alata*, *P. macrocarpa*, *P. edulis*, *P. alata* x *P. macrocarpa* *P. caerulea*, *P. serrato-digitata*, *P. giberti* and *P. suberosa*, two accessions of *P. edulis* (Kenya and Madeira Island) and the accession *Passiflora* sp. (2). The lowest dissimilarity in Group I was found between accession BG25 (*P. alata*) and BG05 (*P. alata* x *P. macrocarpa*). On the other hand, the highest dissimilarity (0.97) was found between accession BG11 (*P. suberosa*) and BG23 (*P. edulis*) from Group II.

Group II was exclusively formed by eight introductions of *P. edulis*, a cross between *P. edulis* and the introduction *Passiflora* sp. (1). This group showed a mean dissimilarity of 0.67 (Table 3). This high variation observed within *P. edulis* was corroborated by Cassiano (1998) but was not detected in the studies done by Fajardo et al. (1998) probably due to the small number of accessions studied by these authors. Two introductions, originally classified as *P. edulis* - one from Kenya (BG04) and the other from Madeira Island (BG19) - were assigned to Group I. However, due to their close similarity to *alata-macrocarpa* and *P. caerulea* respectively, we inferred that these introductions were misclassified in the original germoplasm collection. The introduction named *Passiflora* sp. (1), was grouped very close to a wild Brazilian introduction (d=0.56) of *P. edulis*, and probably it was closed related to this species.

Group III was formed by 46 introductions of *P. edulis* f. flavicarpa, native of several states of Brazil (Table 1). The LM introductions had been selected according to their desirable agronomical characteristics. On the other hand, IA introductions were introduced genotypes, without any specific selected characteristic. Despite being formed only by *P. edulis* f. flavicarpa, this group presented a mean dissimilarity of 0.40 (Table 3).

Table 2 - Summary of data obtained by RAPD analysis for 70 *Passiflora* spp. introductions

Primer sequence	Number of amplified fragments	Average amplified fragments/plants (range)	Amplified fragment size range (bp)
OPA04 - AATCGGGCTG	33	7.46 (1-11)	120 - 2000
OPB08 - GTCCACACGG	26	4.91 (1-8)	350 - 2000
OPB18 - CCACAGCAGT	29	6.84 (2-10)	350 - 2100
OPB19 - ACCCCCGAAG	29	6.01 (1-10)	470 - 3000
OPB20 - GGACCCTTAC	19	4.81 (2-7)	420 – 2000
Total	136	6.00 (1-11)	120 – 3000

The grouping method (UPGMA) allowed a perfect distinction between the introductions LM and IA showing that the RAPD markers were useful even to discriminate closely related genotypes.

P. edulis f. *flavicarpa* was likely to be a population selected from *P. edulis* based on agronomical characteristics, like fruit color. The classification obtained evidences the genetic ascendancy of *P. edulis* over *P. edulis* f *flavicarp*a, supporting the origin of *P. edulis* f. *flavicarpa* as a mutation of *P. edulis* (Pope and Degener, cited by Martin and Nakasone, 1970) and not from a cross of *P. edulis* with any of the species herein studied. Our results have shown that could be possible to distinguish several *Passiflora* species as well as separate accessions of *P. edulis* and *P. edulis* f. *flavicarpa* with only five primers. Molecular markers are very useful in an early breeding program for allowing germoplasm screening at any developmental stage of the plants or when a large number of individuals need to be evaluated.

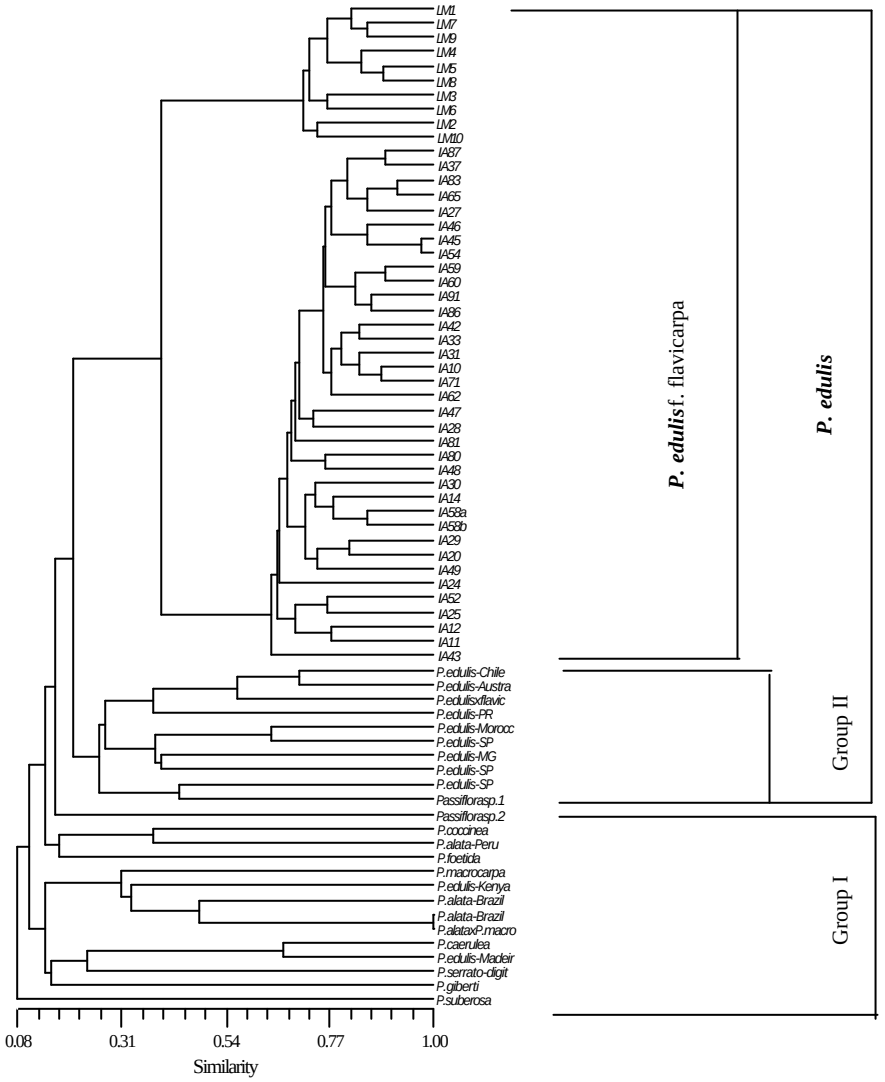

Figure 2 - Dendrogram showing the genetic relationships among 70 accessions representing 11 species of *Passiflora* clustered by UPGMA method based on bands generated using five primers. Scale value of 1 indicates 100% genetic similarity.

Table 3 - Mean dissimilarities and variances within the three groups formed by 70 accessions of *Passiflora* spp. based on RAPD markers.

Group	Number of introductions	Mean dissimilarity	Variance
Group I - *Passiflora* sp.	14	0,84	0,03
Group II - *P. edulis*	10	0,67	0,03
Group III - *P. edulis* f. *flavicarpa*	46	0,40	0,01
Total	70	0,64	0,07

Fingerprinting

The analysis of the RAPD products profile showed highly discriminative amplified fragments among the 70 introductions studied. An example of banding patterns given by DNA amplification products after agarose gel electrophoresis is presented in Fig. 1.

The primer OPA-04 showed the highest discriminative power and could be used to clearly discriminate the group flavicarpa from all other accessions. This primer revealed a specific band (700 bp) for all accessions of *P. alata* and *P. macrocarpa* and it also identified *P. foetida* by a 640 bp fragment. The primer OPB-08 separated *P. edulis* from *P. edulis* f. flavicarpa and also revealed one specific amplified fragment for *Passiflora* sp. (2) and one for *P. coccinea*. The lack of a OPB-18-440bp fragment, present in all introductions of the collection was characteristic for the *P. suberosa*. Moreover, three specific amplified fragments revealed with only primer OPB-18 were found in *Passiflora* sp. (2). The primer OPB-19 presented a specific amplified fragment (550bp) for *P. giberti* and the primer OPB-20 revealed four fragments that specifically discriminated *Passiflora* sp. (2) and *P. giberti*. Specific RAPD products were not found in *P. caerulea, P. serrato-digitata* and, therefore, it was not possible to discriminate these introductions from all others of the germoplasm collection.

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RESUMO

A diversidade genética de *Passiflora* spp. tem sido estudada basicamente através de características morfológicas e agronômicas, levando à uma classificação até espécie. A caracterização intraespecífica por meio destes descritores necessita de um ciclo integral de cultivo, e é pouco discriminante. O objetivo deste estudo foi caracterizar a variação genética de uma coleção de *Passiflora* através da técnica RAPD. Análises moleculares foram realizadas em 70 acessos, envolvendo 11 espécies do gênero *Passiflora*. A utlização de apenas cinco *primers* permitiu a obtenção de 136 fragmentos polimórficos. A classificação hierárquica obtida mostrou alto nível de dissimilaridade entre e dentro das espécies. Nítida separação foi obtida entre acessos de *P. edulis* e da forma *flavicarpa*, de alto interesse comercial, fracamente diferenciados pelas características morfológicas e agronômicas. A utilização desses marcadores na avaliação dos recursos genéticos de *Passiflora*, é atrativa para o melhorista e poderá muito auxiliar na identificação dos acessos, pela facilidade, rapidez e pelo baixo custo na condução das avaliações.

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