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ISSR Markers as a Tool for the Assessment of Genetic Diversity in *Passiflora*

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Abstract Genetic variation among sweet, purple, and yellow passion fruit accessions was assessed using inter-simple sequence repeat (ISSR) markers. Eighteen ISSR primers were used to evaluate 45 accessions. The number of polymorphic bands per primer varied from 4 to 22, with 12.4 bands per primer on average. Nei's genetic distance between accessions ranged from 0.04 to 0.35. Clustering using the neighbor-joining method resulted in the formation of 11 major clusters. It was not possible to classify the accessions according to their geographic origin, showing that there is no structure in the gene bank. The overall mean Shannon–Weaver diversity index was 0.32, indicating good resolution of genetic diversity in passion fruit germplasm using ISSR markers. Our results indicate that ISSR can be useful for genetic diversity studies, to provide practical information for parental selection and to assist breeding and conservation strategies.

Keywords Passion fruit · Genetic variability · Molecular markers · Genetic resources

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Introduction

According to Vanderplank (1996), the passion fruit belongs to the Passifloraceae family, which is composed of 18 genera and about 630 species distributed in the tropical regions of America, Asia, and Africa. The genus *Passiflora*, with its center of origin in South America and its center of geographic distribution in north and central Brazil, has the largest the number of species of the family, comprising ~520 species. Only about 60 species produce fruits with commercial value (Schultz 1968; Manica 1997), either for consumption or medicinal use. About 90% of the species originate from the Americas (Lopes 1991), and more than 120 of them are native to Brazil (Souza and Lorenzi 2005).

Despite the wide variety of species, more than 95% of the commercial passion fruit crop in Brazil consists of the yellow passion fruit (*Passiflora edulis* Sims.) because of the quality of its fruit, yield, and consumer preference (Meletti and Brückner 2001). The purple passion fruit and sweet passion fruit are grown in small areas for consumption by niche markets.

The passion fruit is an allogamous plant because it presents the phenomenon of self-incompatibility, which prevents self-fertilization and the crossing of different plants that contain the same incompatibility alleles. According to Bruckner et al. (1995), passion fruits present the sporophytic type of self-incompatibility. The same group of researchers reported that there is a gene with gametophytic effect associated with the sporophytic system (Suassuna et al. 2003). Because *Passiflora* is economically and socially important, many studies have been undertaken to develop varieties adapted to different cropping systems and climate conditions. Hence, activities related to the collection, conservation, characterization, and usage of *Passiflora* germplasm have been implemented to ensure that genetic variability is available to be exploited for breeding programs. Genetic variability can be used efficiently only if it is properly assessed and quantified. Thus, the germplasm must be characterized according to several morphological traits (Viana et al. 2010; Crochemore et al. 2003a; Plotze et al. 2005), agronomic behavior (Abreu et al. 2009; Cerqueira-Silva et al. 2008), and molecular diversity (Bellon et al. 2009; Cerqueira-Silva et al. 2009, 2010; Viana et al. 2010) to enable advances in the description of genetic divergence among accessions.

Molecular markers have been used in plant breeding and in activities related to the conservation of genetic resources. The most common markers are the random amplified polymorphic DNA (RAPD; Welsh and McClelland 1990; Williams et al. 1990), amplified fragment length polymorphism (AFLP; Vos et al. 1995), restriction fragment length polymorphism (RFLP; Botstein et al. 1980), diversity array technology (DART; Wenzl et al. 2004), sequence-tagged sites (STS; Bradshaw et al. 1994), single nucleotide polymorphism (SNP; Wang et al. 1998), single simple repeat (SSR; Tautz 1989; Weber and May 1989), and inter-simple single repeat (ISSR; Zietkiewicz et al. 1994). ISSR markers were developed from the need to explore microsatellite repeats without the use of DNA sequencing (Lagercrantz et al. 1993). The technique is based on the amplification of DNA segments between two microsatellite repeated regions (Zietkiewicz et al. 1994). ISSR is a simple, fast, and efficient technique that produces amplified products of 200–2000 bp in length.

The technique is highly reproducible due to the use of longer primers, which allow for high annealing temperatures (Reddy et al. 2002). The technique uses a primer containing only the repetition of a particular SSR (di-, tri-, tetra-, or penta-nucleotides), of 16–25 bp, either unanchored (Gupta et al. 1994; Meyer et al. 1993; Wu et al. 1994) or anchored in the 3′ or 5′ ends for up to four nucleotides, which are often degenerate (Zietkiewicz et al. 1994).

The ISSR combines the advantages of AFLP markers and SSR with the convenience of RAPD (Zietkiewicz et al. 1994). Therefore, this technique has been used successfully in diversity analyses of several fruit species, such as trifoliate orange (*Poncirus trifoliata*; Fang et al. 1997), citrus (*Citrus* spp.; Fang and Roose 1997), diploid banana (*Musa acuminata*; Godwin et al. 1997), poolasan hairy litchi (*Nephelium ramboutan-ake* Leenh.; Clyde et al. 2005), and grape (*Vitis* spp.; Moreno et al. 1998; Wu et al. 2009).

Studies on the genetic diversity of the genus *Passiflora* have been performed using RAPD markers (Fajardo et al. 1998; Crochemore et al. 2003b; Viana et al. 2003), restriction sites of cpDNA (Sánchez et al. 1999), isozymes (Segura et al. 2003), and AFLP (Segura et al. 2002). SSR markers have been developed for the yellow passion fruit (Oliveira et al. 2005) and the sweet passion fruit (Pádua et al. 2005). There are no reports of the use of ISSR markers in sweet, yellow, or purple passion fruit. The objective of this study was to evaluate the potential of this technique to detect the level of diversity present in samples of passion fruit collected in Brazil.

Materials and Methods

Plant Material

Thirty-four passion fruit accessions were used in this study, using one plant per accession (Table 1). In addition, five accessions obtained from controlled crosses and six others obtained by open pollination were analyzed using five plants per accession. Three *P. alata* and 42 *P. edulis* accessions (five of the purple type and 37 of the yellow type) were analyzed.

DNA Extraction

DNA was extracted from young leaves based on the procedure described by Doyle and Doyle (1990). A 1.0% agarose gel was used to estimate DNA concentrations by comparing the fluorescent signal from DNA stained with ethidium bromide (1.0 mg/ml) to a dilution series of commercial lambda DNA (Invitrogen, Carlsbad, CA).

PCR Amplification and Electrophoresis

Of 47 primers tested on phenotypically contrasting genotypes, 18 that revealed polymorphism and exhibited good resolution were selected for further analysis (Table 2).

Table 1 Passion fruit accessions from the Embrapa Mandioca e Fruticultura Tropical (CNPMF) genebank, Cruz das Almas, BA, Brazil

Accession	*Passiflora* species	Type	Origin	Plants	Open pollination
BGM004	*P. alata*	Sweet	São Paulo	5	
BGM007	*P. edulis*	Yellow	Minas Gerais	5	Yes
BGM008	*P. edulis*	Yellow	São Paulo	1	
BGM016	*P. edulis*	Yellow	São Paulo	1	
BGM017	*P. edulis*	Purple	São Paulo	1	
BGM018	*P. edulis*	Yellow	Bahia	5	Yes
BGM020	*P. edulis*	Yellow	São Paulo	5	Yes
BGM028	*P. edulis*	Yellow	Bahia	5	Yes
BGM037	*P. edulis*	Yellow	Bahia	1	
BGM038	*P. edulis*	Yellow	Bahia	1	
BGM044	*P. edulis*	Purple	Bahia	5	Yes
BGM049	*P. edulis*	Yellow	Brasília	5	Yes
BGM121	*P. edulis*	Yellow	São Paulo	1	
BGM123	*P. edulis*	Yellow	São Paulo	1	
BGM124	*P. edulis*	Yellow	São Paulo	1	
BGM129	*P. edulis*	Yellow	São Paulo	1	
BGM162	*P. alata*	Sweet	Bahia	5	
BGM163	*P. alata*	Sweet	Bahia	5	
BGM164	*P. edulis*	Yellow	Bahia	5	
BGM175	*P. edulis*	Yellow	Bahia	1	
BGM176	*P. edulis*	Yellow	Bahia	1	
BGM177	*P. edulis*	Yellow	Bahia	1	
BGM179	*P. edulis*	Yellow	Santa Catarina	1	
BGM180	*P. edulis*	Yellow	São Paulo	1	
BGM181	*P. edulis*	Yellow	São Paulo	1	
BGM182	*P. edulis*	Yellow	São Paulo	1	
BGM183	*P. edulis*	Yellow	São Paulo	1	
BGM184	*P. edulis*	Yellow	São Paulo	1	
BGM185	*P. edulis*	Yellow	São Paulo	1	
BGM186	*P. edulis*	Yellow	São Paulo	1	
BGM189	*P. edulis*	Yellow	São Paulo	1	
BGM190	*P. edulis*	Yellow	São Paulo	1	
BGM204	*P. edulis*	Yellow	São Paulo	1	
BGM205	*P. edulis*	Yellow	Rio Grande do Sul	1	
BGM207	*P. edulis*	Yellow	São Paulo	1	
BGM208	*P. edulis*	Purple	São Paulo	1	
BGM210	*P. edulis*	Yellow	Minas Gerais	1	
BGM221	*P. edulis*	Yellow	Bahia	1	
BGM222	*P. edulis*	Yellow	Bahia	1	

Table 1 continued

Accession	*Passiflora* species	Type	Origin	Plants	Open pollination
BGM223	*P. edulis*	Yellow	Bahia	1	
BGM224	*P. edulis*	Yellow	Bahia	1	
BGM225	*P. edulis*	Yellow	Bahia	1	
BGM227	*P. edulis*	Yellow	São Paulo	1	
BGMRB	*P. edulis*	Purple	Bahia	5	
BGMRJ	*P. edulis*	Purple	Bahia	1	

Table 2 Passion flower diversity obtained by primers used in ISSR polymorphism analysis

Marker	Sequence (5′–3′)[a]	Bands			Diversity		
		Total	Polymorphic	% Polymorphism	*h*	*I*	PIC
TriAAG3′RC	$(AAG)_5$	12	11	91.67	0.15	0.27	0.15
TriACA3′RC	$(ACA)_5$	14	14	100.00	0.20	0.32	0.20
TriCAA3′RC	$(CAA)_5$	15	14	93.33	0.15	0.26	0.15
TriAAC3′RC	$(AAC)_5$	16	16	100.00	0.26	0.41	0.26
TriAGC3′RC	$(AGC)_5$	23	22	95.65	0.22	0.36	0.22
TriAGG3′RC	$(AGG)_5$	15	14	93.33	0.27	0.41	0.27
TriCAG3′RC	$(CAG)_5$	16	16	100.00	0.13	0.23	0.13
DiGA5′C	$C(GA)_8$	8	8	100.00	0.37	0.55	0.37
DiCA3′YG	$(CA)_8YG$	7	7	100.00	0.26	0.38	0.26
DiCA5′CR	$CR(CA)_8$	4	4	100.00	0.10	0.18	0.10
DiGT3′YG	$(GT)_8YG$	11	11	100.00	0.15	0.26	0.15
DiCA3′G	$(CA)_8G$	13	13	100.00	0.17	0.28	0.17
DiGA3′C	$(GA)_8C$	18	18	100.00	0.11	0.19	0.11
DiGA5′CY	$CY(GA)_8$	10	10	100.00	0.18	0.30	0.18
DiGT5′CY	$CY(GT)_8$	8	8	100.00	0.19	0.34	0.21
DiGA3′YC	$(GA)_8YC$	18	18	100.00	0.13	0.23	0.13
DiGA3′T	$(GA)_8T$	8	8	100.00	0.18	0.31	0.18
DiCA3′RG	$(CA)_8RG$	11	11	100.00	0.28	0.43	0.28

[a] R = A or G; Y = C or T

h Mean gene diversity (Nei 1973), *I* mean Shannon–Weaver diversity index, *PIC* polymorphism information content

The PCR mix (25 µl) for ISSR analysis included 20 ng of passion fruit genomic DNA, 20 mM Tris–HCl (pH 8.4), 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM each dNTP, 0.3 µM each primer, and 1.0 U/µl *Taq* DNA polymerase (Invitrogen, Carlsbad, CA).

Amplification was performed in a PTC-100 thermal cycler (MJ Research, Watertown, MA) programmed with the following time and temperature profiles: initial denaturation at 94°C for 5 min; followed by 35 cycles of 94°C for 40 s, 48°C for 40 s, and 72°C for 1 min, with a final extension at 72°C for 2 min.

Following PCR, the samples were loaded onto a 2.0% agarose gel in 1 × TBE buffer and stained with ethidium bromide. In addition, a 100 bp ladder (New England Biolabs, Beverly, MA) was loaded. The gels were run at constant voltage (120 V) for 2 h, visualized by UV, and recorded using Vilber Lourmat equipment (Vilber Lourmat Bio-technology, Marne le Vallee, France).

Data Analysis

Each ISSR band was considered an independent locus, and only distinct, reproducible, and well-resolved fragments were scored visually, as absent (0) or present (1), for each of the 45 genotypes. A locus was considered polymorphic if a consistent band was present in one or more, but not all, individuals of the population. Qualitative differences in band intensity were not considered.

The marker index for ISSR markers was calculated to characterize the capacity of each primer to detect polymorphic loci among the genotypes. The marker index is the sum of the polymorphism information content (PIC) values of all the markers produced by a particular primer. PIC values were calculated using the formula

$$\mathrm{PIC} = 1 - \sum p_i^2,$$

where p_i is the frequency of the ith band (Powell et al. 1996). The discrimination power of each ISSR marker was evaluated by PIC. Shannon's information index (Lewontin 1972),

$$I = -\sum p_i \ln p_i,$$

was calculated for the genetic diversity, where p_i is the frequency of the ith band according to Kefyalew et al. (2000). Nei's genetic diversity (Nei 1973) was also calculated for each primer. All these genetic diversity parameters were estimated using Popgene version 1.32 (Yeh and Yang 1999). The pairwise genetic distance according to Nei (1978) was used to construct the dendrogram using the neighbor-joining cluster analysis on Mega version 4 (Tamura et al. 2007). Bootstrap analysis with 1,000 replicates was performed to obtain the confidence of the tree.

Results

ISSR Polymorphism Analysis

The 18 ISSR primers generated a total of 227 scorable bands, ranging from 4 to 23 bands per primer (average 12.61 bands per primer). A high percentage of the scored bands (98%, 223) were found to be polymorphic (Table 2; Fig. 1).

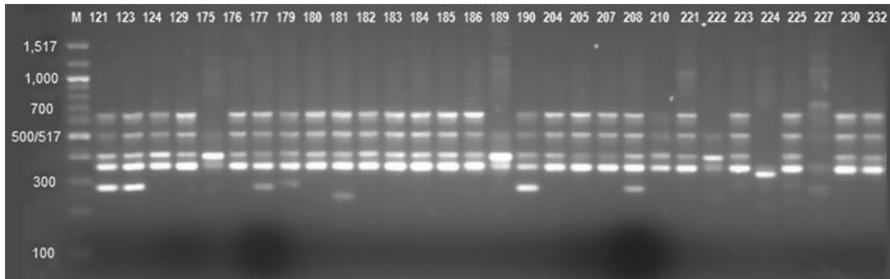

Fig. 1 ISSR profiles of passion fruit accessions. Agarose gel of PCR products amplified with the primer DiGA3'T. *Lane M* 100 bp ladder (New England Biolabs, Beverly, MA). *Lane numbers* refer to the accessions listed in Table 1

The number of polymorphic bands generated by a primer varied from 4 to 22 (Table 2). Based on the ISSR results, it was shown that of the 18 primers used, primer TriAGC3′RC, with the trinucleotide repeat $(AGC)_5$, produced the highest number of scorable bands, and the dinucleotide repeat DiCA5′CR [$CR(CA)_8$] showed the lowest number. On average, the trinucleotide ISSR was more polymorphic than dinucleotides, at 15.3 and 10.5 alleles per primer, respectively.

Nei's genetic distance ranged from 0.04 between the closest genotypes, BGM049-2 and BGM049-1, both from the same accession, to 0.35 between the most distant genotypes, BGM163-3 and BGM020-2, from *P. edulis* and *P. alata*, respectively.

The 18 ISSR primers enabled the separation of all 45 genotypes, including plants of the same accession. Evaluation of the band pattern among the most similar genotypes, BGM049-2 and BGM049-1, indicated that they differ in only 7 (3.2%) of the 223 bands, whereas the most distant, BGM163-3 and BGM020-2, presented 60 unshared bands (26.91%). The high level of polymorphism observed was consistent with other comparable studies (Gemas et al. 2004; Martins-Lopes et al. 2007; Gomes et al. 2009).

Genetic Diversity

Shannon's information index at the ISSR level ranged from 0.18 (DiCA5′CR) to 0.55 (DiGA5′C), with an average value of 0.32. These data are similar to previous studies of other allogamous species, such as 0.383 in *Nelumbo nucifera* (Han et al. 2007), 0.23 in *Cocos nucifera* (Manimekalai et al. 2007), 0.449 in *Coffea canephora*, and 0.3834 in *Stipa tenacissima* (Boussaid et al. 2010). Nei's gene diversity (h) at the ISSR level ranged from 0.10 (DiCA5′CR) to 0.37 (DiGA5′C), with an average value of 0.19. The PIC values varied from 0.10 for primer DiCA5′CR to 0.37 for primer DiGA5′C, and the mean PIC value was 0.20 (Table 2), which is similar to values published in the literature for ISSR primers (Bhardwaj et al. 2010; Tiwari et al. 2009; Zhao et al. 2007).

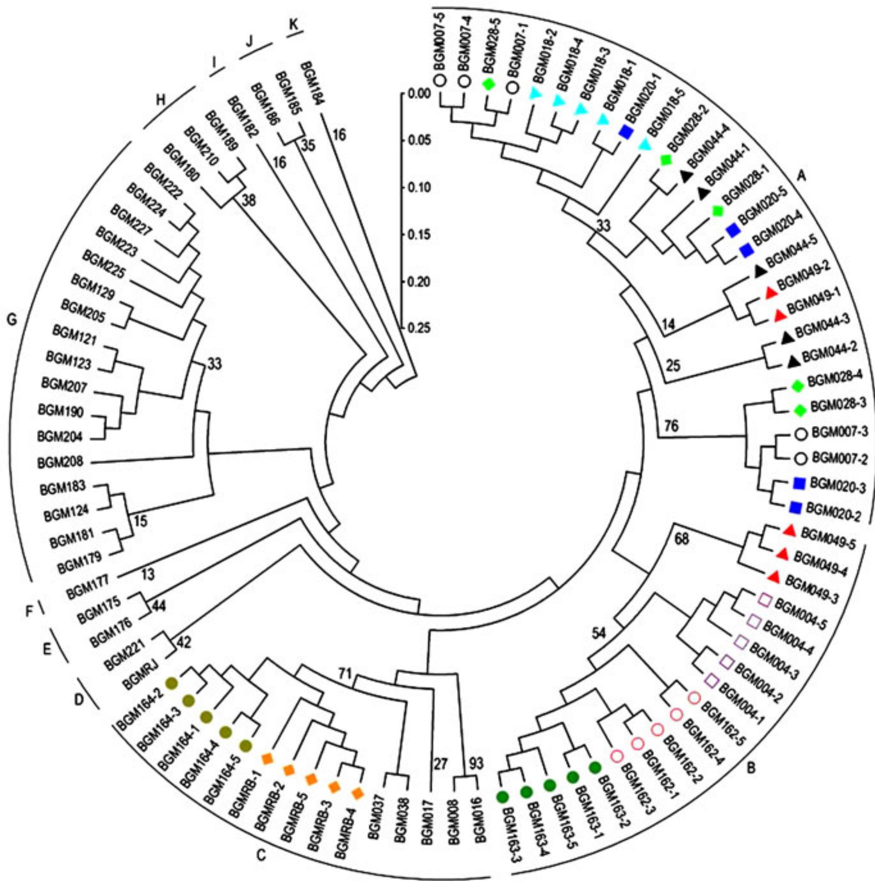

Fig. 2 Genetic relatedness among 45 passion flower germplasm accessions. Neighbor-joining dendrogram generated from Nei's distance, calculated from ISSR data set. *Letters* outside the dendrogram indicate the 11 clusters, A–K. Accession codes as listed in Table 1. Multiple plants in the same accession are marked with the same *symbol* (*circle*, *square*, *triangle*, etc.) and numbered (−1, −2, −3, etc.). Values at the branches are the stability of nodes estimated with a bootstrap procedure

Genetic Relatedness Among Passion Fruit Accessions

Inter-simple sequence repeat results revealed genetic relatedness among the 45 accessions; at about 16% genetic distance, the dendrogram generated using Nei's coefficient resulted in 11 groups (A to K; Fig. 2). A UPGMA-based dendrogram (not shown) of the 45 *Passiflora* accessions presented a clustering pattern identical to that obtained by the neighbor-joining method.

The accessions from Group A were composed of all five plants of the yellow passion fruit accessions BGM007, BGM018, BGM020, BGM028, two plants of the accession BGM049 (BGM049-1 and BGM049-2), and the five plants of the accession BGM044, a purple passion fruit. The remaining plants of the BGM049 accession were clustered in Group B, together with the sweet passion fruit (*P. alata*)

Table 3 Distribution of passion fruit germplasm accessions in clusters based on the neighbor-joining method

State of origin[a]	Cluster[b]										
	A	B	C	D	E	F	G	H	I	J	K
Bahia	3	2	4	2	2	1	4				
Brasília DF	1	1									
Minas Gerais	1							1			
Rio Grande do Sul							1				
Santa Catarina							1				
São Paulo	1	1	3				11	2	1	2	1

[a] Accessions listed by origin in Table 1

[b] Accessions listed by cluster in Fig. 2

accessions BGM004, BGM162, and BGM163. Group C was composed of the accessions BGM008, BGM016, BGM037, BGM038, and all plants of BGM164, plus the purple passion fruits BGM017 and BGMRB. All plants within the passion fruit accessions showed levels of molecular divergence because of the mating system of the species.

BGMRJ (a purple form) and BGM221 (yellow) were clustered into Group D. Group E contained the yellow accessions BGM175 and BGM176, and BGM177 was isolated in Group F.

Group G was composed of 17 accessions of yellow passion fruit: BGM179, BGM181, BGM124, BGM183, BGM208, BGM204, BGM190, BGM207, BGM123, BGM121, BGM205, BGM129, BGM225, BGM223, BGM227, BGM224, and BGM222.

Group H comprised three (BGM180, BGM210, and BGM189) and J comprised two (BGM185 and BGM186) yellow passion fruit accessions. Groups I and K included only one accession each, BGM182 and BGM184, respectively. Average genetic distances among the accessions were 0.14 in group A, 0.18 in B, 0.11 in C, 0.16 in D, 0.10 in E, 0.11 in G, 0.12 in H, and 0.06 in J.

Except for Santa Catarina and Rio Grande do Sul, which had only one accession per group (BGM179 and BGM205, respectively), the evaluation of the distribution among clusters did not result in comprehensively distinct clustering patterns based on the geographic collection sites (Table 3).

Discussion

ISSR Polymorphism

Compared with other agricultural crops, relatively little is known about the genetic diversity within and among species of *Passiflora*. The characterization of germplasm is an important step to assist breeding programs. Therefore, characterizing passion fruit germplasm using molecular markers such as ISSR is essential.

Comparative studies of *Passiflora* have successfully used RAPD, AFLP, and the nuclear-encoded chloroplast-expressed glutamine synthetase (*ncpGS*) gene (Fajardo et al. 1998; Sánchez et al. 1999; Crochemore et al. 2003b; Yockteng and Nadot 2004), but to date, no studies have been published using ISSR markers to characterize passion fruit germplasm.

During the characterization of passion fruit accessions from Brazil, we evaluated the discriminative power of primers for ISSR marker systems. Of the 18 ISSR primers used, six dinucleotide (DiGT3′YG, DiCA3′G, DiGA3′C, DiGA5′CY, DiGA3′YC, and DiCA3′RG) and seven trinucleotide primers (TriAAG3RC, TriACA3RC, TriCAA3RC, TriAAC3RC, TriAGC3RC, TriAGG3RC, and Tri-CAG3′RC) revealed more than 10 polymorphic bands. The 18 ISSR primers produced 227 markers, with a polymorphism level of 98%. Junqueira et al. (2007) detected 63.8% polymorphism in 17 *P. nitida* accessions with RAPD, whereas Ganga et al. (2004) reported 93.9% polymorphism using AFLP markers in 36 *P. edulis* accessions.

Inter-simple sequence repeat polymorphism is highly variable according to the species studied. Polymorphism levels of 95.93% were found for *Arabidopsis thaliana* (He et al. 2007), 42.53% for *Omphalogramma souliei* Franch (Huang et al. 2009), 56.05% for *Emmenopterys henryi* (Li and Jin 2008), and 81.82% for *Vernicia fordii* (Li et al. 2008). According to Sivaprakash et al. (2004), the ability to resolve genetic variation may be more directly related to the number of polymorphisms detected by the marker system.

The distribution of different microsatellite sequences in different plant genomes determines the utility of this method for DNA fingerprinting. ISSR PCR has proved successful for assessing genetic diversity within various plant groups (Weising et al. 1995; Cortesi et al. 2005; Luan et al. 2006), for gene mapping (Sankar and Moore 2001), and for germplasm identification (Blair et al. 1999).

Genetic Diversity and Clustering

The formation of 11 major clusters in this study is a good indication of the higher richness of this sample, compared with other studies of various passion fruit accessions, such as those evaluated by Ganga et al. (2004) and Crochemore et al. (2003b). The differences can be attributed to the higher genotypic variation and to the technique employed.

Group A was formed by one purple and four yellow passion fruits, all with five plants in each accession. Plants of the yellow passion fruit accession BGM049 were subdivided into groups A (BGM049-1 and BGM049-2) and B (BGM049-3, BGM049-4, and BGM049-5). Due to the allogamy system of *Passiflora*, in all of the cases, we found some divergence of plants within accessions. This was more pronounced in the accessions with seeds collected from fruits of open pollination, such as BGM007, BGM018, BGM020, BGM028, BGM044, and BGM049 (Fig. 1).

In contrast, Group B was composed of three accessions of *P. alata* and BGM049-3, BGM049-4, and BGM049-5. Because crosses between *P. alata* and *P. edulis* are uncommon in nature, the sharing of alleles among these species is probably due to random effects or to evolutionary aspects. In general, ISSR markers did not allow

the complete separation of different forms of *P. edulis* (yellow and purple), demonstrating high genetic similarity among these forms. The mean genetic distances within passion fruit accessions were 0.162 for sweet, 0.185 for yellow, and 0.164 for purple passion fruit. The genetic variation observed within *P. edulis* was corroborated by Cassiano (1998), but was not detected in the studies done by Fajardo et al. (1998) and Crochemore et al. (2003b), probably because of the small number of accessions studied and the class of markers employed by the authors.

The genetic diversity of a population in a species is affected by a number of evolutionary factors, including the mating system, seed dispersal, gene flow, natural selection, geographic range, and the diversity center (Hamrick and Godt 1989). Although broad genetic diversity is expected in *Passiflora* because of the geographic distribution of the genus (Lopes 1991), the divergence among collected genotypes at production areas is limited. This is due to the germplasm exchange within Brazil. In most cases, the passion fruit producers produce their own seedlings, either from seeds collected in their neighborhood or from fresh fruits purchased at the market. As a result, the close relationship observed among some of the accessions used in this study is presumably due to germplasm collection from similar locations or the similar origin (pedigree) of different landraces.

Implications for Conservation

Passiflora edulis and *P. alata* have been considered essential allogamous species because of their self-incompatibility (Bruckner et al. 1995; Suassuna et al. 2003). The mating system determines the level of heterozygosity, and it has an important impact on the performance of open-pollinated genotypes. The allogamy of *Passiflora* species results in high genetic variability within germplasm accessions, which impacts germplasm management. Hence, better estimates of genetic variability should be obtained using a pool of plants representing each accession.

In addition, there is a general necessity to establish isolation strategies between accessions, to minimize gene flow, and avoid risk of contamination in the multiplication or regeneration fields. Therefore, a continuous monitoring of the germplasm accession variability, including studies of allelic loss, effective population size, and the assessment of adaptive genetic variation, will be useful in the design of effective conservation strategies, given that the major objectives of a gene bank are preserving genetic variability and maintaining the genetic identity of the accessions as far as possible.

Implications for Breeding

Breeding practices for passion fruit represent one of the most important strategies to increase yield and fruit quality. The success of passion fruit breeding programs, however, is closely related to the appropriate choice of divergent parents, which when crossed must provide wide genetic variability in order to be used for selection among segregating populations. Therefore, the identification of parents with high genetic variability has been a goal of many breeding programs that aim to explore

the heterosis. Thus, germplasm characterization is necessary to provide information of gene sources for future use and to prevent the loss of these resources.

According to Kumar et al. (2009), DNA markers have become the markers of choice for the study of the genetic diversity of crop species because they are able to assess the genetic variation more precisely, quickly, and cheaply. In this study, the genetic diversity among Brazilian passion fruit accessions from different origins was assessed using ISSR. These allowed an accurate assessment of the genetic diversity among 45 accessions, which can be combined with the performance of these genotypes in field conditions to direct breeding programs. Using this information, crosses between accessions from different groups can be designed to maximize the chance of getting significant variation in the offspring.

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