ISSR markers in wild species of *Passiflora* L. (Passifloraceae) as a tool for taxon selection in ornamental breeding

A.G.R. Sousa[1], M.M. Souza[1], C.A.F. Melo[1] and G.A. Sodré[2]

[1]Departamento de Ciências Biológicas, Universidade Estadual de Santa Cruz, Ilhéus, BA, Brasil
[2]Departamento de Ciências Agrárias, Universidade Estadual de Santa Cruz, Ilhéus, BA, Brasil

Corresponding author: M.M. Souza
E-mail: souzamagg@yahoo.com.br

Genet. Mol. Res. 14 (4): 18534-18545 (2015)
Received December 19, 2014
Accepted September 30, 2015
Published December 23, 2015
DOI http://dx.doi.org/10.4238/2015.December.23.41

ABSTRACT. Inter simple sequence repeat (ISSR) molecular markers were developed and used to investigate interspecific genetic variation in 25 wild species of *Passiflora* preserved in an active germplasm bank (BAG-Passifloras); intraspecific diversity was also analyzed in *P. cincinnata* accessions. Of 31 primers tested, 20 identified polymorphic loci with a total of 331 bands, suggesting high polymorphism in the sample. Interspecific polymorphism was greater than intraspecific polymorphism. This is a common finding in studies of genetic variation using dominant markers. The ISSRs revealed species-specific amplification bands in 11 species; these bands ranged from 200 to 1000 bp, and they will be of use for developing SCAR markers for the identification of germplasm in further studies. The use of Jaccard's similarity coefficient to obtain a dendrogram by the UPGMA clustering method distributed the taxa into five major groups, with differences among grouping with respect to

principal coordinate analysis. Despite the high cophenetic correlation coefficient (r = 0.94) of the dendrogram, taxonomic inconsistencies were observed; similar irregularities have been reported previously in studies using dominant markers. Intraspecific analysis of *P. cincinnata* accessions revealed a larger genetic distance between those from Bahia (P2) and from Minas Gerais (P2), indicating that both accessions have considerable potential as parents in a genetic improvement program for this species.

Key words: Germplasm characterization; Molecular markers; Passion flower; Principal coordinate analysis

INTRODUCTION

The family Passifloraceae Juss. ex DC. is predominantly found in the tropical and subtropical areas of the Americas and Africa (Cervi and Imig, 2013), and consists of 17 genera and approximately 525 species. Brazil is the largest center of genetic diversity of the genus *Passiflora* L. and is also the center of origin of approximately 139 species (Bernacci et al., 2008). Numerically, *Passiflora* is the largest genus in the family with 83 endemic species that have been used as food, medicine, and also for ornamentation (Cervi et al., 2010). The considerable diversity within South America in terms of species largely accounts for this region being the main producer of passion fruit (Abreu et al., 2009).The Central North region of Brazil is regarded as the main center of the geographical distribution of *Passiflora*. However, anthropological activities have diminished the natural habitats of some species and, consequently, some taxa are now threatened (IAC, 2014). Moreover, in recent years, accessions of passion fruit vines in germplasm banks in Brazil, the United States, France, Australia, and elsewhere have notably declined (Faleiro et al., 2005). The conservation of this genetic resource for future germplasm characterization is important to enable the development of new lines in genetic breeding programs or in seedling rootstock production systems; germplasm conservation will also be vital to the diversification of production systems for new markets, such as for medicinal and ornamental plants. Because of their characteristics of exotic and beautiful flowers with strong and bright coloring and the fact that they bloom more than once per year, *Passiflora* species have been widely exploited as ornamental plants (Abreu et al., 2009). In the mid-18th century in Europe, interspecific crosses between *P*. *racemosa* Brot. and *P*. *caerulea* L. produced the first hybrids, *Passiflora* x violacea, for ornamental purposes (Vanderplank, 2000; Abreu et al., 2009). Currently, the interspecific hybrids of *Passiflora* represent a significant portion of the germplasm focused towards ornamentation (Abreu et al., 2009). Sexual hybridization in *Passiflora* is easily achieved between phylogenetically close species because of genetic compatibility and a low reproductive barrier (Abreu et al., 2009; Santos et al., 2012). In general, intraspecific hybridization can be an efficient method for obtaining improved plant lines because of increased hybrid vigor, especially in allogamous plants. For this reason, the use of molecular markers in the selection of parents can identify the optimal lines and, consequently, enable the generation of progeny with increased vigor and heterozygosity (Bueno et al., 2006; Borém and Miranda, 2005). Molecular markers have been used to identify genetic polymorphisms in population genetic studies, and are

also of value for germplasm identification (Xi et al., 2008), characterizing genetic diversity (Sawadogo et al., 2009), protection of plant varieties (Isshiki et al., 2008), monitoring of crosses, and for the construction of genetic maps (Reddy et al., 2002). The various molecular markers differ in their relative abilities to detect polymorphism with respect to dominance and co-dominance, specificity, reproducibility, cost, and detection method (Collard and Mackill, 2009). The Inter Simple Sequence Repeat (ISSR) technique uses *in vitro* amplification of sequences located between microsatellites, and enables multi-locus genomic analysis with a single primer based on Simple Sequence Repeat (SSR) regions. ISSRs have been shown to be informative in studies on genetic diversity (Bornet and Branchard, 2001), phylogenetic and evolutionary relations (Reddy et al., 2002), and in the characterization of accessions and cultivars. These markers can therefore enable the identification of promising parental plants in genetic improvement programs (Isshiki et al., 2008). Unlike SSR molecular markers, ISSRs act as a dominant marker and do not require prior knowledge of the primer sequence. The ISSR technique also has good reproducibility, is easy to use, and has a low cost compared to other types of molecular marker, e.g., amplified fragment length polymorphisms and SSRs. The aim of this work was to develop ISSRs to study interspecific genetic diversity in 25 wild species of *Passiflora* preserved in the active *Passiflora* germplasm bank of the Universidade Estadual de Santa Cruz (UESC), and also to characterize intraspecific diversity in accessions of *P. cincinnata* Mast. The information obtained will identify germplasm resources for use in *Passiflora* improvement programs, such as through identification of wild species for producing hybrids with ornamental potential.

MATERIAL AND METHODS

Genomic DNA was extracted from 25 species of *Passiflora* and their respective accessions (Table 1) in the *Passiflora* germplasm bank of the UESC in Ilhéus, Bahia, Brazil. The protocol proposed by Doyle and Doyle (1990) was used to obtain the genomic DNAs. Young leaves were macerated in liquid nitrogen in a 2% CTAB conditioned buffer (1.5 M NaCl, 20 mM EDTA, 100 mM Tris-HCl, and 0.2% β-mercaptoethanol) for 40 min at 65°C. DNA was precipitated with chloroform:isoamyl alcohol (24:1) and then resuspended in TE buffer (10 mM Tris-HCl, 1 mM EDTA). Genomic DNA concentrations were estimated by comparison with 100 ng of lambda DNA (Promega, Wisconsin, United States) after electrophoresis on a 1.2% agarose gel and stained with SYBR safe (Invitrogen). Each genomic DNA was diluted to 10 ng/µL in TE buffer.

The ISSR amplifications were performed using 31 UBC ISSR primers (Table 2) in a reaction mixture containing 30 ng genomic DNA solution, 10 mM reaction buffer 10 mM $MgCl_2$ buffer, 5 µm primer, 10 mM dNTP, and 1 U Taq DNA polymerase in a final volume of 20 µL. The amplifications were performed in a thermal cycler (Eppendorf Mastercycle) with the following schedule: initial denaturation at 95°C for 4 min; 31 cycles of denaturation for 30 s at 94°C, annealing for 45 s at 50-58°C depending on the primer (Table 2), extension for 2 min at 72°C, and a final extension at 72°C for 10 min. The amplified fragments in the ISSR reaction were stained with SYBR safe (Invitrogen) and separated by electrophoresis on a 1.8% agarose gel using 90V for 50 minutes and 1X SB buffer (100 mM H_3BO_3, 150 mM NaOH).

The data were assembled into a binary matrix with reference to the presence (1) or absence (0) of bands. NTSYS-pc software 2.1 was used for the analysis of similarity between species and accessions, (Rohlf, 2000), and Jaccard's similarity coefficient was calculated

(Jaccard, 1901). The unweighted pair group method with arithmetic mean (UPGMA) was used for dendrogram construction. The cophenetic correlation coefficient was calculated to compare the graphical representation against the original similarity matrix. Principal component analysis (PCO) was performed using NTSYS-pc software 2.1 (Rohlf, 2000) to group genotypes based on Jaccard's similarity coefficient (Jaccard, 1901). Corel DRAW X 5 (Corel) was used to produce the dendrogram and PCO graph.

Table 1. Species and accessions collected in Brazil and donated to other institutions, and preserved in the *Passiflora* active germplasm bank of the State University of Santa Cruz. Classification according to Ulmer and MacDougal (2004).

Species	Subgenus	Supersection	Section	Series	Code	Accessions
P. maliformis L.	*Passiflora*	*Laurifolia*	-	*Laurifoliae*	1	514 - P2
					2	514 - P1
P. filamentosa Cav.	*Passiflora*	*Passiflora*	-	*Passiflora*	3	513 - P1
					4	513 - P2
P. malacophylla Mast.	*Passiflora*	-	-	-	5	405 - P2 (Embrapa Cerrado)
P. eichleriana Mast.	*Passiflora*	*Stipulata*	*Granadillastrum*	-	6	488 - P1 (São Sebastião/RJ)
P. subrotunda Mast.	*Passiflora*	*Stipulata*	*Granadillastrum*	-	7	452 - P1 (Fortaleza/CE)
					8	452 - P2 (Fortaleza/CE)
P. actinia Hook	*Passiflora*	*Stipulata*	*Granadillastrum*	-	9	427 - P1 (UFPR)
					10	427 - P2 (UFPR)
P. tenuifila Killip	*Passiflora*	*Stipulata*	*Granadillastrum*	-	11	489 - P1 (Embrapa)
P. foetida L.	*Passiflora*	*Stipulata*	*Dysosmia*	-	12	490 - P1
P. vitifolia Kunth	*Passiflora*	*Coccinea*	-	-	13	481 - P2 (Miranda/MG)
P. sublanceolata Killip	*Passiflora*	*Stipulata*	*Dysosmia*	-	14	496 - P2 (Embrapa)
					15	496 - P1 (Embrapa)
P. elegans Mast.	*Passiflora*	*Stipulata*	*Granadillastrum*	-	16	491 - P2 (Embrapa)
					17	491 - P1 (Embrapa)
P. racemosa Brot.	*Passiflora*	*Stipulata*	*Calopathanthus*	-	18	475 - P1
					19	475 - P2
P. misera HBK	*Decaloba*	*Decaloba*	*Decaloba*	-	20	P1
					21	P2
P. hatschbachii Cervi	*Passiflora*	*Passiflora*	-	*Setaceae*	22	446 - P1
					23	446 - P2
P. gibertii N.E. Brown	*Passiflora*	*Stipulata*	*Granadillastrum*	-	24	P1
P. galbana Mast.	*Passiflora*	*Stipulata*	*Granadillastrum*	-	25	500 - P1
					26	500 - P2
P. suberosa L.	*Decaloba*	*Cieca*	-	-	27	P2 (UENF)
					28	P1 (UENF)
P. trintae Sacco	*Passiflora*	-	-	-	29	P1
P. morifolia Mast.	*Decaloba*	*Bryonioides*	-	-	30	P2 (UENF)
P. serratodigitata L.	*Passiflora*	*Laurifolia*	-	*Tiliifolia*	31	487 - P2 (Embrapa Cerrado)
					32	487 - P1 (Embrapa Cerrado)
P. mucronata Lam.	*Passiflora*	*Stipulata*	*Granadillastrum*	-	33	509- P1 (Canavieiras/BA)
					34	508- P2 (UESC)
					35	508- P1 (UESC)
P. galbana Mast.	*Passiflora*		*Granadillastrum*	-	36	507 - P2 (Oliveira/MG)
					37	507- P1 (Oliveira/MG)
P. micropetala Mast.	*Decaloba*	*Decaloba*	*Decaloba*	-	38	P1 (IAC)
P. cerradensis Sacco	*Passiflora*	*Passiflora*	-	*Passiflora*	39	511 - P1 (Embrapa)
		Passiflora			40	511 - P2 (Embrapa)
P. cincinnata Mast.	*Passiflora*		-	*Passiflora*	41	504 - P2 (Bahia)
					42	504 - P1 (Bahia)
					43	P1 (Campina de Moura /SP
					44	P2 (Campina de Moura /SP
					45	503 - P1 (Instituto Plantarum)
					46	503 - P2 (Instituto Plantarum)
					47	P1 (Minas Gerais)
					48	P2 (Minas Gerais)

(P1) Plant 1; (P2) Plant 2. (EMBRAPA) Empresa Brasileira de Pesquisa Agropecuária; (UFPR) Universidade Federal do Paraná; (UENF) Universidade Estadual do Norte Fluminense Darcy Ribeiro; (IAC) Instituto Agronômico/Campinas, SP.

RESULTS

Thirty-one ISSR primers were tested here and 20 (64%) gave satisfactory amplification in the population under study (see Figure 1). The number of amplified bands ranged from eight for primer $(GA)_8$YC to 28 for primer $(CA)_8$RC, demonstrating a clear ability to detect polymorphisms (Table 2). A total of 331 ISSR bands were amplified, giving an average number of 16 bands per primer. Approximately 99% of the bands were polymorphic and 19 of the primers only amplified polymorphic bands; the exception was $(GT)_8$A (UBC819) that amplified three monomorphic bands.

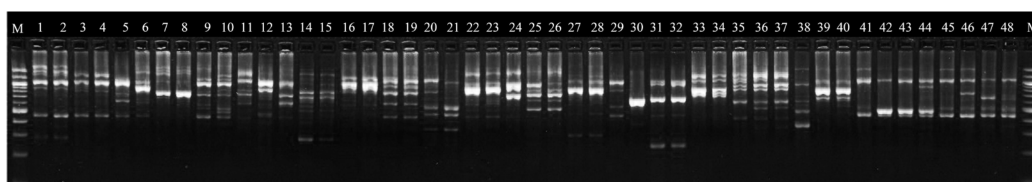

Figure 1. ISSR amplification products produced using the primer UBC826 and separated on a 1.8% agarose gel. (M) molecular weight marker 100 BP ladder, (1) *Passiflora maliformis* - P2; (2) *P. maliformis* - P1; (3) *P. filamentosa* - P1; (4) *P. filamentosa* - P2; (5) *P. malacophylla* - P2; (6) *P. eichleriana* - P1; (7) *P. subrotunda* - P1; (8) *P. subrotunda* - P2; (9) *P. actinia* - P1; (10) *P. actinia* - P2; (11) *P. tenuifila* - P1; (12) *P. foetida* - P1; (13) *P. vitifolia* - P2; (14) *P. sublanceolata* - P2; (15) *P. actinia* - P1; (16) *P. elegans* - P2; (17) *P. elegans* - P1; (18) *P. racemosa* - P1; (19) *P. racemosa* - P2; (20) *P. misera* - P1; (21) *P. misera* - P2; (22) *P. hatschbachii* - P1; (23) *P. hatschbachii* - P2; (24) *P. gibertii* - P1; (25) *P. galbana* - P1; (26) *P. galbana* - P2; (27) *P. suberosa* - P2; (28) *P. suberosa* - P1; (29) *P. trintae* - P1; (30) *P. morifolia* - P2; (31) *P. serratodigitata* - P2; (32) *P. serratodigitata* - P1; (33) *P. mucronata* - P1; (34) *P. mucronata* - P2; (35) *P. mucronata* - P1; (36) *P. galbana* - P2; (37) *P. galbana* - P1; (38) *P. micropetala* - P1; (39) *P. cerradensis* - P1; (40) *P. cerradensis* - P2; (41) *P. cincinnata* (Bahia - P2); (42) *P. cincinnata* (Bahia - P1); (43) *P. cincinnata* (Campina de Moura - P1); (44) *P. cincinnata* (Campina de Moura - P2); (45) *P. cincinnata* (Instituto Plantarum - P1); (46) *P. cincinnata* (Instituto Plantarum - P2); (47) *P. cincinnata* (Minas Gerais - P1); (48) *P. cincinnata* (Minas Gerais - P2).

Table 2. ISSR primers used to study genetic diversity.

Primer	Sequencies (5'-3')[1]	TM (°C)	Total bands	No. poly.
UBC 807	AGAGAGAGAGAGAGAGT	55.0	23	23
UBC 808	AGAGAGAGAGAGAGAGC	56.8	10	10
UBC 811	GAGAGAGAGAGAGAGAC	52.0	15	15
UBC 815	CTCTCTCTCTCTCTCTG	55.0	10	10
UBC 819	GTGTGTGTGTGTGTGTA	54.8	13	10
UBC 823	TCTCTCTCTCTCTCTCC	55.0	20	20
UBC 825	ACACACACACACACACT	54.8	12	12
UBC 826	ACACACACACACACACC	57.2	20	20
UBC 828	TGTGTGTGTGTGTGTGA	54.8	10	10
UBC 834	AGAGAGAGAGAGAGAGACYT	55.0	19	19
UBC 835	AGAGAGAGAGAGAGAGYC	55.0	08	08
UBC 847	CACACACACACACACARC	58.8	28	28
UBC 850	GTGTGTGTGTGTGTGTYC	58.8	22	22
UBC 855	ACACACACACACACACYT	56.5	13	13
UBC 857	ACACACACACACACACYG	58.8	14	14
UBC 858	TGTGTGTGTGTGTGTGRT	56.5	15	15
UBC 861	ACCACCACCACCACCACC	64.5	20	20
UBC 868	GAAGAAGAAGAAGAAGAA	50.8	24	24
UBC 884	HBHAGAGAGAGAGAGAG	55.6	15	15
UBC 889	DBDACACACACACACAC	55.6	20	20

[1]D, Y and V signify degenerate nucleotides: D = (A, G, T); Y = (C, T); and V = (A, C, G). TM, annealing temperature; total number of amplified bands per primer; No. poly., number of polymorphic bands per primer.

Table 3. Species-specific bands exclusively generated by ISSR primers.

Species	Primer (UBC)	Band (bp)
Passiflora cerradensis	884	910
P. elegans	850	260
P. elegans	857	420
P. elegans	884	310
P. galbana	868	510
P. hatschbachii	884	800
P. maliformis	807	900
P. maliformis	811	200
P. maliformis	826	950
P. misera	825	220
P. misera	826	320
P. misera	855	300
P. morifolia	811	1000
P. serratodigitata	847	780
P. serratodigitata	819	200
P. serratodigitata	858	210
P. suberosa	889	1000
P. sublanceolata	811	800
P. sublanceolata	826	270
P. subrotunda	828	700

bp = base pair.

Analysis of *P. cincinnata* accessions, the species with the largest number of samples, yielded 134 ISSR bands: of these, 102 (76.11%) were polymorphic. The presence of species-specific bands that were detectable in at least two accessions was observed for 11 species in the studied population (Table 3). Species-specific bands ranged from 200 to 1000 bp. *P. elegans, P. maliformis*, and *P. misera* showed the highest number of useful primers for the detection of species-specific bands, with three bands in each species.

The dendrogram produced using Jaccard's similarity coefficient and the UPGMA method identified five major groups (I, II, III, IV, and V) (Figure 2), with a high cophenetic correlation coefficient (r = 0.94). Group I could be subdivided into three subgroups, A, B, and C, although the latter only contained *P. trintae*. The closely related Group II had two subgroups, A and B. Group III had two subgroups, A and B, the first containing two species, *P. mucronata* and *P. galbana*, and latter containing only *P. micropetala*. Group IV contained two subgroups, A and B, the latter including two accessions of *P. cerradensis*. Group 5 was the least related to other groups, and contained only *P. suberosa* and *P. morifolia*.

The highest similarity coefficients were observed in the intraspecific analysis of *P. subrotunda* (0.8356), *P. racemosa* (0.8351), *P. hatschbachii* (0.8285), and *P. filamentosa* (0.8243). *P. cincinnata* accessions were found exclusively in Group IB (Figure 2). The similarity coefficients for *P. cincinnata* accessions revealed a greater genetic similarity between those from Bahia (0.6746), followed by those from Campina de Moura (0.6162) (Table 4). Less genetic similarity (0.4333) was observed in *P. cincinnata* accessions from Bahia (P2) and Minas Gerais (P2). *P. mucronata* accessions (P3) showed lower genetic similarity than did the other accessions of the same taxon (0.2637).

Jaccard's coefficient was used for the principal coordinate analysis (PCO) (Figure 3). In the PCO chart, two major groups could be distinguished, with one group exclusively containing the eight *P. cincinnata* accessions and other including the remaining species and accessions.

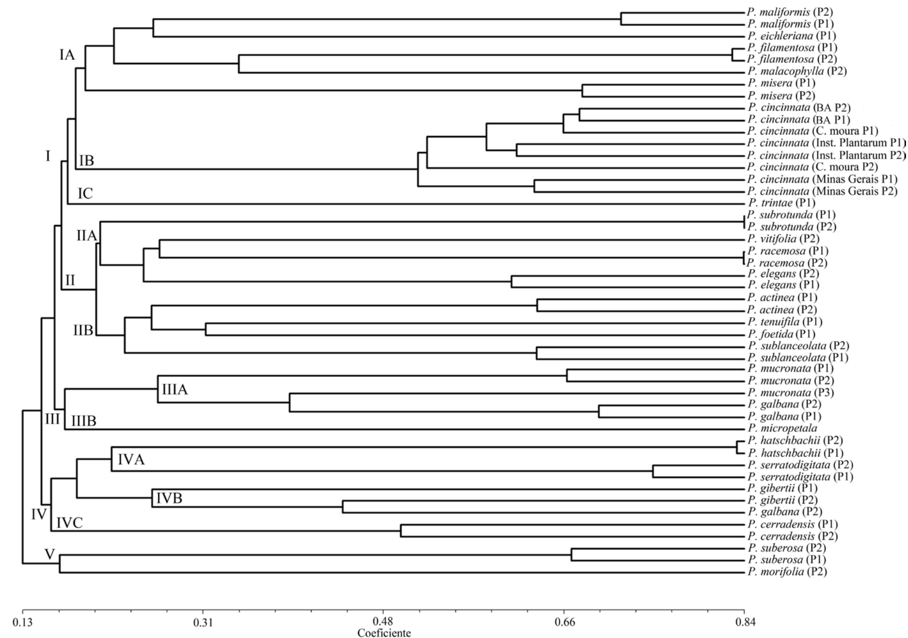

Figure 2. Dendrogram based on ISSR polymorphisms in species and accessions of *Passiflora*. The groups were generated using the UPGMA method with Jaccard's similarity coefficient. The value of the cophenetic correlation coefficient (r) is 0.94.

Table 4. Coefficients of genetic similarity between accessions of *Passiflora cincinnata*.

Access	41	42	43	44	45	46	47	48
41	1.00000							
42	0.67469	1.00000						
43	0.64772	0.67058	1.00000					
44	0.47826	0.49438	0.61627	1.00000				
45	0.56666	0.55056	0.63636	0.53409	1.00000			
46	0.55434	0.60919	0.58695	0.50549	0.61363	1.00000		
47	0.50000	0.59782	0.54545	0.50000	0.53608	0.62365	1.00000	
48	0.43333	0.44827	0.51724	0.53750	0.50588	0.46067	0.63095	1.00000

(41) *Passiflora cincinnata* (504 - Bahia) - Plant 2 (P2); (42) *P. cincinnata* (504 - Bahia) - P1; (43) *P. cincinnata* (Campina de Moura -Alegre/SP) - Plant 1 (P1); (44) *P. cincinnata* (Campina de Moura - Alegre/SP) - P2; (45) *P. cincinnata* (503 - Instituto Plantarum) - P1; (46) *P. cincinnata* (503 - Instituto Plantarum) - P2; (47) *P. cincinnata* (Minas Gerais) - P1; (48) *P. cincinnata* (Minas Gerais) - P2.

DISCUSSION

The development of ISSR molecular markers in agronomically important crops such as tomato (Tikunov et al., 2003), eggplant (Isshiki et al., 2008), and passion fruit (Santos et al., 2011) provided important insights into intraspecific genetic diversity; this information has proved invaluable for the use of these genetic resources in conservation and for genetic improvement. Although the use of ISSR markers is widespread in plants, relatively little has been reported in passifloras, except for wild species (Santos et al., 2011). However, genetic diversity in *Passiflora*

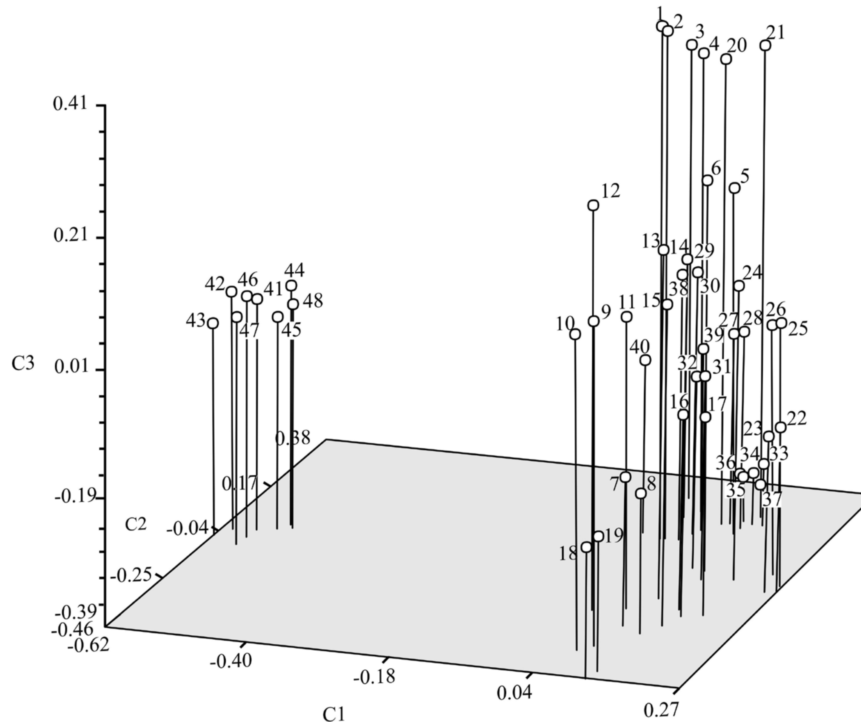

Figure 3. Principal component analysis of species of the genus *Passiflora* based on the Jaccard's similarity coefficient. The numbers on the chart show the species and accessions shown in Figure 1.

has been investigated using other dominant markers, such as AFLPs (Ganga et al., 2004) and random amplified polymorphic DNA (RAPD) markers (Fajardo et al., 1998), and co-dominant markers such as SSRs (Pádua et al., 2005; Oliveira et al., 2008; Silva et al., 2014). In the present study, genetic diversity was analyzed with 20 ISSR markers; most of the amplification primers were based on dinucleotides, suggesting a high rate of dinucleotide SSR repeats in the genus *Passiflora*. However, the genome of *P. edulis* contains many trinucleotide SSR repeats as evidenced by the amplification of ISSR primers based on trinucleotide sequences in commercial accessions (Santos et al., 2011). In general, the presence of SSR dinucleotides is the most common repeat in the genome of plants, such as in *Arabidopsis thaliana*, which has abundant dinucleotide sequences in the coding regions (Casacuberta et al., 2000).

Here, the presence of unique ISSR alleles in the *Passiflora* species was verified through the existence of species-specific bands. Unique bands amplified by ISSR primers have applications in both germplasm identification and genetic improvement programs (Isshiki et al., 2008; Lee et al., 2011). Additionally, species-specific bands and accession or cultivar restricted bands can be transformed into SCAR (sequenced characterized amplified region) type markers to increase specificity and reproducibility. In cultivars of medicinal *Panax ginseng*, the development of SCAR markers based on ISSR regions has enabled the differentiation of one of the six analyzed cultivars (Lee et al., 2011). In the present study, the species-specific bands could be used for confirmation of interspecific crosses or for germplasm protection.

By contrast to previous genetic diversity studies in *Passiflora*, the estimation of genomic variation was performed using a large number (331) of polymorphic loci (Santos et al., 2011). Generally speaking, the use of random primers in an interspecific analysis identifies a higher number of polymorphic bands in comparison to an intraspecific analysis (Fajardo et al., 1998; Santos et al., 2011). In agreement with this expectation, we observed greater interspecific polymorphism (99%) than intraspecific polymorphism (76%) in *Passiflora*. An intraspecific analysis performed using RAPD markers reported an average of 63.8% polymorphism in *P. nitida* (Ganga et al., 2004) and in *P. setacae* (Cerqueira-Silva et al., 2012). However, an intraspecific analysis with ISSR markers found higher polymorphism (98%) in *P. alata* (Santos et al., 2011). In interspecific analyses, a higher rate of polymorphism in combination with an increased average number of amplified bands per primer is indicative of high genetic variability (Cerqueira-Silva et al., 2012). Nevertheless, high intraspecific genetic variability was also observed here among the eight *P. cincinnata* accessions.

Although the high cophenetic correlation coefficient (r = 0.94) suggested fidelity of the graphical representation in the dendrogram, some taxa were distributed without taxonomic support. Thus, species of the same series were separated into different groups, for example, *P. misera* and *P. micropetala*, which both belong to the subgenus *Decaloba* and the *Decaloba* section ($2n$ = 12) were positioned in the distinct groups I and III, respectively. By contrast, *P. foetida* and *P. sublanceolata*, which belong to the subgenus *Passiflora*, *Dysosmia* Section, were positioned consistently with regard to their taxonomic category ($2n$ = 22) and in Group III. The study of genetic diversity in species of the genus *Passiflora* using RAPD markers distributed the taxa preferentially according to subgenus and section, indicating that these markers were a useful tool for taxonomic and phylogenetic studies; however, some species of the subgenus *Passiflora* and *Decaloba* did not show the expected relationship on their dendrogram (Fajardo et al., 1998). The species of Group V, *P. suberosa* and *P. morifolia*, showed greater diversity than other taxa; these were the only representatives of the subgenus *Decaloba*, from the *Cieca* supersection and *Bryonioides*, respectively. Fajardo et al. (1998) reported that *Passiflora coriacea*, from the Supersection *Cieca*, showed more dissimilarity than other species analyzed with RAPD markers. This conclusion reinforces the great distinction between RAPD and ISSR loci in taxa of the *Cieca* section compared to other botanical sections of this genus.

In this study, Jaccard's similarity coefficient and the UPGMA clustering method was used for verification of genetic diversity. Additional analyses using the Simple Matching similarity coefficient and S-Dice were also performed with the intention of decreasing collation errors based on phylogenetic and taxonomic relationships. In *Passiflora*, the test anticipated various similarity coefficients and was a strategy to minimize collation errors in relation to the taxonomy, making it possible to choose the most suitable method for the sample group (Fajardo et al., 1998; Crochemore et al., 2003; Viana et al., 2003). However, the choice of similarity or dissimilarity coefficients needs to be adequate for the studied population and for the particular marker used (Murguía and Villaseñor, 2003). The representation of genetic diversity in the PCO graph differed considerably among *P. cincinnata* accessions than in other species, in contrast to the dendrogram obtained by UPGMA grouping; this result indicated that *P. cincinnata* accessions were exclusively positioned in Group II. The PCO chart enabled the differentiation of the largest number of intraspecific taxon comparisons (eight accessions of *P. cincinnata*) from the other species examined. The differences observed in the two grouping methods indicates that principal coordinate analysis can generate a distancing of taxa that are classified as being closely related in UPGMA grouping (Silva et al., 2011).

The analysis of intraspecific diversity among the eight *P. cincinnata* accessions revealed

genetic dissimilarity among plants within the same accession, confirming their origin by crosses and not as clones. The least genetic similarity was observed between the *P. cincinnata* accessions from Bahia (P2) and Minas Gerais (P2), indicating that both accessions are potential progenitors for use in intraspecific interbreeding.

Heterosis, a hallmark of passifloras, is a result of the low self-incompatibility among species of the genus and can be exploited through crosses between heterozygous parents (Lippman and Zamir, 2007). Generally, F_1 hybrids exhibit superior heterosis than the parental species, resulting in better adaptation and an increased fitness in comparison to their parents. However, in just a few generations of crosses and backcrosses the high heterosis and its beneficial effects may be lost (Lippman and Zamir, 2007; Sanghera et al., 2011). Heterosis is characteristic of interspecific hybrids and the results in this study will be useful in selection of promising parents for conducting interspecific crosses. Thus, we suggest that species with lower levels of genetic similarity, as determined by their ISSR markers, could be used as parental plants in breeding programs for hybrid production.

Despite the weak reproductive barriers between species of the *Passiflora* genus, the artificial production of hybrids has only been reported for species that share the same diploid chromosome number; thus, this is an essential feature for the selection of genitors for hybridization (Conceição et al., 2011; Santos et al., 2012). Choice of parents for crosses can be made between taxa assigned to distinct groups by UPGMA clustering (dendrogram) to obtain progeny with higher fitness, for example, between *P. filamentosa* (group I) x *P. suberosa* (group V), which have $2n = 18$, or *P. misera* (group I) x *P. micropetala* (group III), which have $2n = 12$. Although it is possible to select parents within the same group, they would preferably be from different subgroups, for example, *P. subrotunda* (Group IIA) x *P. actinea* (Group IIB). However, the selection of parents to obtain hybrids with ornamental potential should be carried out with the inclusion of data on floral characteristics, such as color and flower size and also period and duration of anthesis. Thus, in addition to use of molecular parameters for genitor selection, phenotypic characteristics can also be important to obtain progeny suitable for the ornamental flower market.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

We thank UESC, CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) and FAPESB (Fundação de Amparo à Pesquisa do Estado da Bahia) for the financial support for research; CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) for the scholarships granted to the first author; CNPq for the scholarship awarded to the second author.

REFERENCES

Abreu PP, Souza MM, Santos EA, Pires MV, et al. (2009). Passion flower hybrids and their use in the ornamental plant market: perspectives for sustainable development with emphasis on Brazil. *Euphytica* 166: 307-315.

Bernacci LC, Soares-Scott MD, Junqueira NTV, Passos IRS, et al. (2008). *Passiflora edulis* Sims: the correct taxonomic way to cite the yellow passion fruit (and of others colors). *Rev. Bras. Frutic*. 30: 566-576.

Borém A and Miranda GV (2005). Melhoramento de Plantas. 4. ed. UFV, Viçosa.

Bornet B and Branchard M (2001). Nonanchored inter simple sequence repeat (ISSR) markers: reproducible and specific tool for genome fingerprinting. *Plant Mol. Biol. Rep*. 19: 209-215.

Bueno LCS, Mendes ANG and Carvalho SP (2006). Melhoramento de plantas: princípios e procedimentos. UFLA, Lavras.

Casacuberta E, Puigdomenech P and Monfort A (2000). Distribution of microsatellites in relation to coding sequences within the *Arabidopsis thaliana* genome. *Plant Sci*. 157: 97-104.

Cerqueira-Silva CBM, Santos ESL, Conceição LDHCS, Cardoso-Silva CB, et al. (2012). Genetic variation in a wild population of the 'sleep' passion fruit (*Passiflora setacea*) based on molecular markers. *Genet. Mol. Res.* 11: 731-738.

Cervi AC and Imig DC (2013). A new species of *Passiflora* (Passifloraceae) from Mato Grosso do Sul, Brazil. *Phytotaxa* 103: 46-50.

Cervi AC, Azevedo MAM and Bernacci LC (2010) Passifloraceae. In: Catálogo de plantas e fungos do Brasil. (Forzza RF, et al., ed.). Jardim Botânico do Rio de Janeiro, Rio de Janeiro, 2: 1432-1436.

Collard BCY and Mackill DJ (2009). Conserved DNA-derived polymorphism (CDDP): a simple and novel method for generating DNA markers in plants. *Plant Mol. Biol. Rep.* 27: 558-562.

Conceição LDHCS, Souza MM, Belo GO, Santos S, et al. (2011). Hybridization among wild passionflower species. *Rev. Bras. Bot.* 34: 237-240.

Crochemore ML, Molinari HBCand Vieira LGE (2003). Genetic diversity in passion fruit (*Passiflora* spp.) evaluated by RAPD markers. *Braz. Arch. Biol. Technol*. 46: 521-527.

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, et al. (1998). Genetic variation analysis of the genus *Passiflora* L. using RAPD markers. *Euphytica* 101: 341-347.

Faleiro FG, Junqueira NTV, Braga MF and Peixoto JR (2005). Germoplasma e melhoramento genético do maracujazeiro - Desafios da pesquisa. In: Maracujá: germoplasma e melhoramento genético (Faleiro FG, Junqueira NTV and Braga MF, eds.). Embrapa Cerrados, Planaltina, 187-209.

Ganga RMD, Ruggiero C, Lemos EGM, Grili GVG, et al. (2004). Diversidade genética em maracujazeiro-amarelo utilizando marcadores moleculares fAFLP. *Rev. Bras. Frutic* 26: 494-498.

Herbário Instituto Agronômico - IAC (2014). Available at [http://herbario.iac.sp.gov.br]. Accessed July 31, 2014.

Isshiki S, Iwata N and Khan MMR (2008). ISSR variations in eggplant (*Solanum melongena* L.) and related *Solanum* species. *Sci. Hortic.* 117: 186-190.

Jaccard P (1901). Étude comparative de la distribution florale dans une portion des Alpes et des Jura. *Bull. Soc. Vaudoise Sci. Nat.* 37: 547-579.

Lee JW, Kim YC, Jo IH, Seo AY, et al. (2011). Development of an ISSR-derived SCAR marker in Korean ginseng cultivars (*Panax ginseng* C. A. Meyer). *J. Ginseng Res.* 35: 52-59.

Lippman ZB and Zamir D (2007). Heterosis: revisiting the magic. *Trends Genet.* 23: 60-66.

Murguía M and Villaseñor M (2003). Estimating the effect of the similarity coefficient and the cluster algorithm on biogeographic classifications. *Ann. Bot. Fennici*. 40: 415-421.

Oliveira EJ, Vieira MLC, Garcia AAF, Munhoz CF, et al. (2008). An integrated molecular map of yellow passion fruit based on simultaneous maximum-likelihood estimation of linkage and linkage phases. *J. Am. Soc. Hortic. Sci.* 133: 35-41.

Pádua JG, Oliveira EJ, Zucchi MI, Oliveira GCX, et al. (2005). Isolation and characterization of microsatellite markers from the sweet passion fruit (*Passiflora alata* Curtis: Passifloraceae). *Mol. Ecol. Notes* 5: 863-865.

Reddy PM, Sarla N and Siddiq EA (2002). Inter simple sequence repeat (ISSR) polymorphism and its application in plant breeding. *Euphytica*. 128: 9-17.

Rohlf FJ (2000). NTSYS-pc: numerical taxonomy and multivariate analysis system, version 2.1. New York: Exeter Software 83.

Sawadogo M, Ouedraogo JT, Balma D, Ouedraogo M, et al. (2009). The use of cross species SSR primers to study genetic diversity of okra from Burkina Faso. *Afr. J. Biotechnol*. 11: 2476-2482.

Santos LF, Oliveira EJ, Santos AS, Carvalho FM, et al. (2011). ISSR markers as a tool for the assessment of genetic diversity in *Passiflora*. *Biochem. Genet*. 49: 540-554.

Santos EA, Souza MM, Abreu PP, Conceição LDHCS, et al. (2012). Confirmation and characterization of interspecific hybrids of *Passiflora* L. (Passifloraceae) for ornamental use. *Euphytica* 184: 389-399.

Sanghera GS, Wani SH, Hussain WS, Wajida HA, et al. (2011) The magic of heterosis: new tools and complexities. *Nat. Sci*. 9: 42-53.

Silva KVP, Alves AAC, Martins MIG, Melo CAF, et al. (2011). Variabilidade genética entre acessos do gênero *Manihot* por meio de marcadores moleculares ISSR. *Pesq. Agropec. Bras.* 46: 1082-1088.

Silva MAA, Souza MM, Silva GS, Melo CAF, et al. (2014). Analysis of transferability of microsatellite primers (SSR) in wild *Passiflora* species and intraspecific genetic diversity in *Passiflora alata. Genet. Mol. Res.* 13: 5908-5918.

Tikunov YM, Khrustaleva LI and Karlov G (2003). Application of ISSR markers in the genus *Lycopersicon*. *Euphytica* 131: 71-81.

Vanderplank J (2000) Passion flowers. The MIT Press, Cambridge.

Viana AP, Pereira TNS, Pereira MG, Souza MM, et al. (2003). Diversidade genética entre genótipos comerciais de Maracujazeiro-Amarelo (*Passiflora edulis* f. *flavicarpa*) e entre espécies de passifloras nativas determinadas por marcadores RAPD. *Rev. Bras. Frutic.* 25: 489-493.

Xi ZY, He FH, Zeng RZ, Zhang ZM, et al. (2008). Characterization of donor genome contents of backcross progenies detected by SSR markers in rice. *Euphytica* 160: 369-377.