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Genetic diversity in passion fruit plants at different altitudes

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Abstract

Passion fruit belongs to the Passifloraceae family and to the genus *Passiflora*, which is economically relevant. This genus is originated from Brazil, which has the greatest genetic diversity. The altitudinal gradient may influence the distribution of genetic variation in and between plant populations and genetic diversity may change according to the altitude. The objective of this study was to evaluate the genetic diversity in passion fruit in different altitudes of Espírito Santo, using microsatellite markers (SSR) and inter simple sequence repeat (ISSR). Five matrixes plants of each species of *Passiflora edulis* Sims f. *flavicarpa* Degener, *P. edulis* Sims and *P. alata* Curtis were studied at three altitudes (low, medium and high) in the state of Espírito Santo. For each sample, 13 microsatellite markers developed for *Passiflora edulis* and 14 ISSR primers were amplified according to the greatest number of polymorphic fragments and amplification quality. Low values of alleles were found (1-3), and low heterozygosity was expected and slight PIC values were found, while for the ISSR analysis a large number of bands per primer were detected and high polymorphism. The *P. alata* Curtis species was the most divergent to *P. edulis* Sims f. *flavicarpa* Deg. and *P. edulis* Sims. The SSR and ISSR markers enabled us to indicate differences among the species, but they did not always show variation between and within similar species. The populations of low altitude are different from the others, independent of the species and the marker used. The habitat has a highly important influence on genetic diversity.

Keywords: genetic dissimilarity, heterozygous, ISSR, Passiflora spp., SSR.

Abbreviation: A_amplitude of the fragments generated by the primers; DF_degree of freedom; Fm_maximum frequency of alleles; Fst and Φ ST_fixation index; He_expected heterozygosity; MS_mean square; NA_allele number; NBP_number of polymorphic bands; Nm_mean number of alleles; Nr_number of rare alleles; Nt_ total number of alleles; NTB_total number of bands; % P_percentage of polymorphism; Pa_allele proportion in each population; PIC_polymorphic information content; SQ_sum of square; SV_source of variation.

Introduction

The genus *Passiflora* (family *Passifloraceae*) is from Brazil, which has the greatest genetic diversity with approximately 139 species (Bernacci et al., 2013). The genus is widely distributed throughout the Americas, and has a great genetic variability to be studied and used in breeding programs. The species of this genus presents economic, medicinal, ornamental, and social value (Freitas et al., 2011). The yellow passion fruit (*Passiflora edulis* Sims *f. flavicarpa* Degener), the purple (*P. edulis* Sims) and the sweet (*P. alata* Curtis) commercially stand out. Yellow passion fruit is economically more interesting (Bellon et al., 2007) due to the fruit quality,

yield and consumer preference. The others are cultivated in small areas for consumption in restricted markets (Santos et al., 2011).

The most suitable regions for the planting passion fruit are those with altitudes between 100 and 1,000 m, and average temperature between 25 and 26 °C; with precipitation between 1,200 and 1,400 mm, well distributed throughout the year, low relative humidity and high luminosity. The plant needs 11 hours of light / day in order to flower and produce quality fruits for flavor and aroma (Fraife Filho et al., 2010). Altitude gradients bring together a set of environmental

variables that influence the genetic variation between and within populations and the distribution of plant species, being complex and variable between species (Ohsawa and Ide, 2008; Byars et al., 2009). The altitudinal gradient may influence the distribution of genetic variation in and between plant populations and genetic diversity may change according to the altitude.

Genetic diversity in the genus Passiflora has been studied by DNA molecular markers (Costa et al., 2012; Cerqueira Silva et al., 2014a; Cerqueira Silva et al., 2014b; Lougon et al., 2014). Between the markers, microsatellites are preferred because they are codominant and multi-allelic (Vieira et al., 2016). The occurrence of common microsatellites between Passiflora species (Castro et al., 2016) makes it possible to study the genetic diversity of different species of the genus with the same microsatellites. Therefore, this makes it possible to compare studies between species (Barbará et al., 2007). This maker is highly polymorphic in a intraspecific level, but it kept the locus conserved between close species, thus being necessary to compare the intraspecific discrimination level. One way to compare them is using neutral markers by distributing them in the genome, such as the inter simple sequence repeat (ISSR), which are dominant and very important in studies of interspecific genetic diversity (Santos et al., 2011) and intraspecific passion fruit (Melo et al., 2016).

The objective of this study was to evaluate the genetic diversity in passion fruit in different altitudes of *Espírito Santo* using the microsatellite markers (SSR) and inter simple sequence repeat (ISSR).

Results

Polymorphism screening of SSR loci

One to three alleles were detected by microsatellite locus in the three *Passiflora* species (Table 3), totaling 29 alleles in *P. edulis* Sims; 28 in *P. edulis* Sims f. *flavicarpa* Deg. and 24 in *P. alata*. Fixed alleles were found in the three species, but appeared more frequently in *P. alata* (78.6%), which also had the lowest values of expected heterozygosity, PIC and number of average alleles.

The highest values of expected heterozygosity and PIC were obtained for the locus PE 15 (He = 0.611; PIC = 0.535) in *P. edulis* Sims f. *flavicarpa* Deg., PE 18 (He = 0.631; PIC = 0.556) in *P. edulis* Sims and *P. Alata* Curtis (He = 0.650; PIC = 0.575). Most of the locus presented a maximum frequency parameter (mf) of alleles greater than 50%, indicating higher frequency of few alleles in the locus genotypes. Only the PE 08 and PE 18 locus in *P. edulis* Sims and PE 18 in *P. alata* Curtis had this parameter below 50% (Table 3).

Passiflora edulis Sims had a higher mean allele number (2.23), higher total allele number (29) and highest allele proportion in each population (0.73). *Passiflora edulis* Sims and *P. alata* Curtis presented a rare allele, each with 3.45 and 4.17%, respectively (Table 4).

Polymorphism screening of ISSR loci

The ISSR analysis detected a large number of bands per primer and a low percentage of monomorphic markers, therefore suggesting high interspecific genetic variability of the accessions. From the 14 ISSR markers evaluated in the *Passiflora* spp. species, 178 bands were observed, ranging in size between 110-2,200 bp, mean of 12.71 bands per primer, of which 96% were polymorphic. The ISSR markers, 854, 865, 878 and 886, presented a large number of bands (16) and 887 and 890 presented a small number of bands (9). All species showed a high number of polymorphic bands ranging from 94% for *P.alata*, 97% for *P. edulis* Sims f. *flavicarpa* Deg. and 98% for *P. edulis* Sims (Table 5).

AMOVA

The analysis of molecular variance (AMOVA) of the *Passiflora* species for both markers showed significant differences in the three species between and within populations, as well as between altitudes. The Fst values were higher for SSR data and *P. alata* Curtis presented the highest Fst value (0.81) with this marker. The Fst presented a higher value for the SSR data, in relation to the ISSR for the three altitudes. The highest value of Fst (0.87) was observed at a low altitude for SSR (Table 6).

There was a higher genetic difference in each species according to the altitude at which it was collected, and it was observed due to high genetic variation between altitudes for each species with SSR data. *P. edulis* Sims presented similar results with the use of both markers. However, for *P. edulis* Sims f. *flavicarpa* Deg. there was no significant variation within the altitudes, and for the *P. alata* Curtis species there was a higher variation within and between the altitudes, by the analysis with ISSR (Table 6).

The three species were studied together in each of the three altitudes (Table 6). High variation between species was observed by the SSR data, different from those obtained with ISSR, which revealed a higher difference within the species in a high altitude (62.85%) and medium altitude (62.95%). For the low altitude, with the ISSR data, the genetic diversity within (48.72) and among (51.28) the altitude was similar.

Clustering

Corroborating the variation data between and within the two markers, the clusters showed greater interpopulation genetic, similar to SSR and intrapopulation, with ISSR data (Figure 2). The population in low altitude differed from the others, regardless of the species and the marker used, and the medium and high altitude populations did not distinguish with SSR markers, but with ISSR markers.

The species *P. alata* Curtis showed a clear distinction between the individuals, depending on the altitude, and independent of the marker, with a formation of three well defined groups. The highest and the lowest genetic distances were between 0.46 and 0.05, respectively with SSR, and 0.70 and 0.26, respectively for ISSR (Figure 2 C).

Passiflora edulis Sims f. *flavicarpa* Deg. presented higher (0.75) and lower (0.11) genetic distances between SSR data accessions. The individuals at low altitude presented higher genetic dissimilarity in relation to the others and higher genetic similarity between them. With ISSR, (the highest and lowest dissimilarities between individuals were 0.77 and 0.45, respectively), and three groups were formed (Figure 2 A).

For *P. edulis* Sims. smaller and larger genetic distances between the 15 accessions, by SSR were 0.66 and 0.07,

Table 1. Place of collection of the plants of the species of *Passiflora* spp.

| Species | Locality | Latitude (South) | Longitude (West) | Altitude (m) |
|---------------------------|-----------------------|------------------|--------------------------|--------------|
| D adulis Sime f | Santa Maria de Jetibá | 19º 59' 37,68'' | 40º 40' 25 <i>,</i> 68'' | 674 |
| <i>flavicarpa</i> Degener | Alegre | 20º 46' 13,06'' | 41º 30' 27,96'' | 365 |
| | Marataízes | 21º 09' 32,58" | 40º 55' 07,56'' | 6 |
| | Santa Maria de Jetibá | 19º 59' 40,32'' | 40º 40' 21,66'' | 674 |
| P. edulis Sims | Jerônimo Monteiro | 20º 47' 43,86'' | 41º 30' 33 <i>,</i> 96'' | 210 |
| | Marataízes | 21º 09' 32,28" | 40º 55' 07,62'' | 6 |
| | Santa Maria de Jetibá | 20º 02' 34,20'' | 40º 43' 34,08'' | 655 |
| P. alata Curtis | Alegre | 20º 47' 44,94'' | 41º 30' 35,52'' | 350 |
| | Jerônimo Monteiro | 20º 46' 13,06'' | 41º 25' 33,06'' | 100 |



Fig 1. Passiflora spp sampling locations in the state of Espírito Santo, Brazil. The circles indicate the sampled localities: Alegre, Santa Maria de Jetibá, Marataízes e Jerônimo Monteiro.

| SSR** | Та | Primer "foward" (5 '- 3') | Primer "reverse" (5 '- 3') | ISSR | Sequence 5' \rightarrow 3' | - |
|-------|------|---------------------------|----------------------------|---------|------------------------------|---|
| | (°C) | | | | | |
| PE03 | 60 | GCAGCGAGGGAAGAAAAA | TGAGACATCGTGCGTGAA | UBC 807 | AGAGAGAGAGAGAGAGT | |
| PE08 | 56 | CCGGATACCCACGCATTA | TCTAATGAGCGGAGGAAAGC | UBC 808 | AGAGAGAGAGAG AGAGC | |
| PE15 | 56 | ACCGTTAAATCCAAGCAAGT | AAATGCAAAGAATGATATGTTA | UBC 810 | GAGAGAGAGAGAGAGAT | |
| PE18 | 56 | CCGTGAACCAACCATTTCTC | CCGTGAACCAACCATTTCTC | UBC 811 | GAGAGAGAGAGAGAGAC | |
| PE20 | 58 | AGGATCACCATAGAAAACCAT | GTTAGGTTGGCATTGCTCTT | UBC 812 | GAGAGAGAGAGAGAGAA | |
| PE23 | 60 | CAATCCCTTGACCCATAGA | CGTCCATCCTTCTCCTTT | UBC 815 | CTCTCTCTCTCTCTG | |
| PE27 | 58 | TTGCTCATTGCACTCATCCT | GCAGACATTTCCTGGAGCA | UBC 842 | GAGAGAGAGAGAGAGAYG | |
| PE37 | 60 | CAAAAGGATAGGCCTGATGTC | TGCTTGGTCATCCACTGAAG | UBC 854 | TCTCTCTCTCTCTCRG | |
| PE38 | 60 | GATCGGTCCTCGGTTAGAC | AGTCACACAGCATGAGAAATC | UBC 865 | CCGCCGCCGCCGCCGCCG | |
| PE41 | 56 | ATCGGGGTTCGCTTATTTG | CGTTCATCCTTTAGTGGGCTA | UBC 878 | GGATGGATGGATGGA | |
| PE66 | 60 | CCATAGTCCCAACAAGCATC | GCTGTGGACCCTAACTCAGTC | UBC 886 | VDVCTCTCTCTCTCT | |
| PE74 | 60 | CCCTCTTATCAATAGCGTTGG | GCACGAGCACGAGTATTTATT | UBC 887 | DVDTCTCTCTCTCTCTC | |
| PE90 | 60 | TCAGGAAGATTGCATGTTAGT | CTGGGTTTTGTTTATGTTGC | UBC 890 | VHVGTGTGTGTGTGTGT | |
| | | | | UBC 891 | HVHTGTGTGTGTGTGTG | |

Table 2. SSR and ISSR markers used in the amplification of Passiflora edulis Sims f. flavicarpa Degener, P. edulis Sims and P. alata Curtis collected at different altitudes.

**Oliveira et al. (2006).

Ta (PC) = Annealing temperature in degrees Celsius.

IUB nomenclature for degenerate bases "V" = A+C+G; "D" = A+T+G; "H" = A+C+T

| Table 3. Characterization of | f 13 SSRs used in the molecular characteriz | zation of <i>Passiflora</i> spp. |
|------------------------------|---|----------------------------------|
|------------------------------|---|----------------------------------|

| SSR | | Total | | | P. edulis S | P. edulis Sims f. flavicarpa Degener | | | | | P. edulis Sims | | | | P. alata Curtis | | | |
|------|-------|-------|------|-------|-------------|--------------------------------------|------|-------|-------|-------|----------------|-------|-------|-------|-----------------|-------|--|--|
| | He | PIC | NA | fm | He | PIC | NA | fm | He | PIC | NA | fm | He | PIC | NA | fm | | |
| PE03 | 0.092 | 0.079 | 1 | 0.944 | 0.000 | 0.000 | 1 | 1.000 | 0.000 | 0.000 | 1 | 1.000 | 0.277 | 0.239 | 2 | 0.833 | | |
| PE08 | 0.535 | 0.436 | 2 | 0.532 | 0.540 | 0.452 | 3 | 0.571 | 0.578 | 0.486 | 3 | 0.454 | 0.489 | 0.369 | 2 | 0.571 | | |
| PE15 | 0.567 | 0.494 | 3 | 0.555 | 0.611 | 0.535 | 3 | 0.500 | 0.480 | 0.412 | 3 | 0.666 | 0.611 | 0.535 | 3 | 0.500 | | |
| PE18 | 0.570 | 0.489 | 2 | 0.512 | 0.429 | 0.337 | 2 | 0.687 | 0.631 | 0.556 | 3 | 0.466 | 0.650 | 0.575 | 3 | 0.384 | | |
| PE20 | 0.000 | 0.000 | 1 | 1.000 | 0.000 | 0.000 | 1 | 1.000 | 0.000 | 0.000 | 1 | 1.000 | 0.000 | 0.000 | 1 | 1.000 | | |
| PE23 | 0.263 | 0.210 | 1 | 0.819 | 0.375 | 0.304 | 2 | 0.750 | 0.413 | 0.327 | 2 | 0.708 | 0.000 | 0.000 | 1 | 1.000 | | |
| PE27 | 0.310 | 0.254 | 2 | 0.786 | 0.152 | 0.141 | 2 | 0.916 | 0.459 | 0.353 | 2 | 0.642 | 0.320 | 0.268 | 2 | 0.800 | | |
| PE37 | 0.083 | 0.077 | 1 | 0.955 | 0.124 | 0.116 | 2 | 0.933 | 0.124 | 0.116 | 2 | 0.933 | 0.000 | 0.000 | 1 | 1.000 | | |
| PE38 | 0.083 | 0.077 | 1 | 0.955 | 0.124 | 0.116 | 2 | 0.933 | 0.124 | 0.116 | 2 | 0.933 | 0.000 | 0.000 | 1 | 1.000 | | |
| PE41 | 0.499 | 0.430 | 2 | 0.648 | 0.544 | 0.483 | 3 | 0.615 | 0.544 | 0.483 | 3 | 0.615 | 0.408 | 0.324 | 2 | 0.714 | | |
| PE66 | 0.534 | 0.426 | 2 | 0.511 | 0.606 | 0.529 | 3 | 0.500 | 0.500 | 0.375 | 2 | 0.500 | 0.497 | 0.373 | 2 | 0.533 | | |
| PE74 | 0.131 | 0.119 | 2 | 0.926 | 0.244 | 0.214 | 2 | 0.857 | 0.074 | 0.071 | 2 | 0.961 | 0.074 | 0.071 | 2 | 0.961 | | |
| PE90 | 0.325 | 0.277 | 2 | 0.776 | 0.320 | 0.268 | 2 | 0.800 | 0.524 | 0.440 | 3 | 0.600 | 0.132 | 0.123 | 2 | 0.928 | | |
| Mean | 0.307 | 0.259 | 1.69 | 0.763 | 0.313 | 0.269 | 2.15 | 0.774 | 0.342 | 0.287 | 2.23 | 0.729 | 0.266 | 0.221 | 1.85 | 0.786 | | |

Legend: He = expected heterozygosity; PIC = polymorphic information content; NA = allele number and fm = maximum frequency of alleles.



Fig 2. Dendrogram of the species of *Passiflora* spp., (5 individuals of each species at 3 altitudes) obtained by the UPGMA method. Legend: 1,2,3,4,5 = low altitude, 6,7,8,9,10 = average altitude, 11,12,13,14,15 = high altitude.



Fig 3. Grouping of individuals belonging to the three species of *Passiflora* spp. (5 individuals of each species at 3 altitudes), obtained by the UPGMA method.

Table 4. Mean number (Nm), total (Nt) of alleles, allele proportion in each population (Pa) and number of rare alleles (Nr) for SSR.

| Species | Nm | Nt | Ра | Nr (%) |
|--|------|----|------|--------|
| Passiflora edulis Sims f. flavicarpa Degener | 2.15 | 28 | 0.70 | 0.00 |
| Passiflora edulis Sims | 2.23 | 29 | 0.73 | 3.45 |
| Passiflora alata Curtis | 1.85 | 24 | 0.60 | 4.17 |

Table 5. Analysis of the ISSR primers of Passiflora spp.

| | | | Total | | <i>P. edulis</i> Sims f. <i>flavicarpa</i> Degener | | | | D pd | ulic Sin | nc | | P alata Curtis | | | |
|---------|-----|-----|-------|----------|---|-----|-----|----------|-------|----------|-----|----------|----------------|-----|-----|----------|
| ISSR | | | Total | | | | | | 7. cu | | 13 | | | | | |
| | NTB | NBP | % P | Α | NTB | NBP | % P | Α | NTB | NBP | % P | V | NTB | NBP | % P | А |
| UBC 807 | 12 | 12 | 100 | 1500-370 | 14 | 14 | 100 | 1500-370 | 13 | 13 | 100 | 1000-370 | 11 | 11 | 100 | 900-370 |
| UBC 808 | 10 | 10 | 100 | 1050-380 | 12 | 12 | 100 | 1010-390 | 12 | 12 | 100 | 1050-380 | 8 | 8 | 100 | 1010-490 |
| UBC 810 | 14 | 11 | 78 | 1000-110 | 18 | 14 | 78 | 1000-110 | 10 | 9 | 90 | 900-110 | 15 | 10 | 67 | 1000-150 |
| UBC 811 | 12 | 11 | 92 | 1500-290 | 11 | 10 | 91 | 1500-390 | 12 | 12 | 100 | 1500-490 | 14 | 12 | 86 | 1300-290 |
| UBC 812 | 10 | 10 | 100 | 1200-350 | 10 | 10 | 100 | 1200-350 | 9 | 9 | 100 | 850-350 | 11 | 11 | 100 | 1200-350 |
| UBC 815 | 13 | 13 | 100 | 1100-300 | 14 | 14 | 100 | 1100-300 | 16 | 16 | 100 | 1100-300 | 10 | 10 | 100 | 1050-380 |
| UBC 842 | 13 | 13 | 100 | 750-250 | 15 | 15 | 100 | 750-250 | 13 | 13 | 100 | 750-250 | 13 | 13 | 100 | 750-250 |
| UBC 854 | 16 | 16 | 100 | 2000-290 | 16 | 16 | 100 | 1800-290 | 18 | 18 | 100 | 2000-290 | 16 | 16 | 100 | 750-390 |
| UBC 865 | 16 | 16 | 98 | 1900-310 | 19 | 19 | 100 | 1800-390 | 13 | 13 | 100 | 1500-310 | 18 | 17 | 94 | 1900-410 |
| UBC 878 | 16 | 16 | 100 | 2000-390 | 17 | 17 | 100 | 2000-500 | 15 | 15 | 100 | 1800-390 | 17 | 17 | 100 | 2000-390 |
| UBC 886 | 16 | 15 | 98 | 2200-280 | 18 | 18 | 100 | 2200-300 | 13 | 13 | 100 | 1000-300 | 17 | 16 | 94 | 2100-280 |
| UBC 887 | 9 | 9 | 100 | 1080-480 | 9 | 9 | 100 | 1020-480 | 9 | 9 | 100 | 1050-500 | 9 | 9 | 100 | 1080-480 |
| UBC 890 | 9 | 9 | 100 | 1100-380 | 11 | 11 | 100 | 1100-380 | 9 | 9 | 100 | 1000-450 | 7 | 7 | 100 | 1050-380 |
| UBC 891 | 12 | 10 | 86 | 1100-300 | 13 | 12 | 92 | 1100-300 | 12 | 9 | 75 | 1000-300 | 12 | 11 | 92 | 1050-300 |
| Total | 178 | 171 | 96 | - | 197 | 191 | 97 | - | 174 | 170 | 98 | - | 178 | 168 | 94 | - |

Legend: NTB = total number of bands; NBP = number of polymorphic bands; % P = percentage of polymorphism; A = amplitude of the fragments generated by the primers.

respectively, forming two groups, one with low altitude individuals and the other with high and medium altitude individuals. Individuals at low altitude presented higher genetic dissimilarity in relation to high and medium altitude. With ISSR data, the highest and lowest genetic distances were 0.79 and 0.30, respectively, and and three groups were formed corresponding to the different altitudes, reflecting higher genetic dissimilarity between them (Figure 2 B).

Considering a general analysis of all the studied individuals of the three species, SSR data showed a higher interpopulation genetic similarity and ISSR data showed intrapopulational genetic similarity (Figure 3).

For the SSR data, (the smallest and largest genetic distances between the 45 accessions of *Passiflora* spp. were 0.67 and 0.06, respectively), two groups were formed: one grouping *P. edulis* Sims f. *flavicarpa* Deg. and *P. edulis* Sims at three altitudes and another grouping *P. alata* Curtis at three altitudes. Individuals of *P. edulis* Sims f. *flavicarpa* Deg. and *P. edulis* Sims presented higher genetic similarity at low altitude. Individuals 1; 3 and 4 (*P. edulis* Sims at low altitude) presented 100% similarity, as well as individuals 1 and 3 (*P. edulis* Sims f. *flavicarpa* Deg. at low altitude). Individuals of *P. edulis* Sims f. *flavicarpa* Deg. And *P. edulis* Sims in medium and high altitudes were grouped (Figure 3).

In the ISSR cluster analysis, (the highest and lowest genetic distances between the 45 accessions of *Passiflora* spp. were 0.78 and 0.26, respectively). There were three groups: I: *P. alata* Curtis at three different altitudes, together with *P. edulis* Sims f. *flavicarpa* Deg. in average altitude; II: *P. edulis* Sims f. *flavicarpa* Deg. and *P. edulis* Sims in high and low altitude and III: *P. edulis* Sims in average altitude. The individuals of *P. alata* were grouped in relation to the

altitudes. Individuals of *P. edulis* Sims f. *flavicarpa* Deg. And *P. edulis* Sims were closer at lower altitude, as were the individuals at higher altitude. However, *P. edulis* Sims in medium altitude showed higher genetic dissimilarity (Figure 3).

Discussion

The descriptive analysis of the SSR locus for expected heterozygosity in the three *Passiflora* spp. species presented low genetic variability in P. edulis Sims f. flavicarpa Deg. (He = 0,313), P. edulis Sims (He = 0.342) and P. alata Curtis (He = 0.266) locus and in general He = 0.307. Cerqueira-Silva et al. (2012), when evaluating P. cincinnata accessions found (He = 0.510), and Padua et al. (2005) found (He = 0.520) in P. alata accessions. Reis et al. (2011) when studying populations of two cycles of recurrent selection in P. edulis obtained He = 0.200. The low molecular variability can be attributed to the loss and fixation of the alleles by the selecting agronomically favorable genotypes. The variability found in the genus may be associated with the allogamy and self-incompatibility of the species (Pérez-Almeida et al., 2010). Genetic diversity in plants is influenced by the breeding system, seed dispersal, genetic drift and evolutionary history. The habitat has a highly important influence on genetic diversity (Hu et al., 2014).

Regarding the polymorphic information content (PIC), Botstein et al. (1980) defined this parameter as highly informative (PIC greater than 0.5); reasonably informative (PIC between 0.25 and 0.50); and slightly informative (PIC values less than 0.24).

| | | SSR | | | | | ISSR | | | | | | |
|-----------|--------|-------------------|-----------|-----------------------|---------|--------|--------|----|--------|--------|-----------------------|---------------------|--------|
| SV | DF | SQ | MS | Variance component | % | Fst | SV | DF | SQ | MS | Variance component | % | ФST |
| Passiflor | a edu | <i>lis</i> Sims f | . flavica | <i>arpa</i> Degener | | | | | | | | | |
| Among | 2 | 6.14 | 3.07 | 0.57 | 70.26** | 0.70** | Among | 2 | 159.00 | 79.50 | 10.61 | 28.63** | 0.28** |
| Within | 12 | 2.88 | 0.24 | 0.24 | 29.74** | | Within | 12 | 317.40 | 26.45 | 26.46 | 71.37 ^{ns} | |
| Total | 14 | 9.02 | 0.64 | 0.81 | 100.00 | | Total | 14 | 476.40 | 34.03 | 37.06 | 100.00 | |
| Passiflor | a edu | <i>lis</i> Sims | | | | | | | | | | | |
| Among | 2 | 3.13 | 1.56 | 0.25 | 43.33** | 0.43** | Among | 2 | 188.40 | 94.20 | 15,04 | 44.21** | 0.44** |
| Within | 12 | 3.89 | 0.32 | 0.32 | 56.67** | | Within | 12 | 227.80 | 18.90 | 18.90 | 55.79** | |
| Total | 14 | 7.02 | 0.50 | 0.57 | 100.00 | | Total | 14 | 416.20 | 29.72 | 34.03 | 100.00 | |
| Passiflor | a alat | a Curtis | | | | | | | | | | | |
| Among | 2 | 4.53 | 2.26 | 0.43 | 80.65** | 0.81** | Among | 2 | 207.20 | 103.60 | 16.70 | 45.38** | 0.45** |
| Within | 12 | 1.24 | 0.10 | 0.10 | 19.35** | | Within | 12 | 241.20 | 20.10 | 20.10 | 54.62** | |
| Total | 14 | 5.77 | 0.41 | 0.53 | 100.00 | | Total | 14 | 448.40 | 32.03 | 36.80 | 100.00 | |
| High Alti | tude | | | | | | | | | | | | |
| Among | 2 | 9.86 | 4.93 | 0.94 | 81.31** | 0.81** | Among | 2 | 165.87 | 82.93 | 12.39 | 37.15** | 0.37** |
| Within | 12 | 2.60 | 0.22 | 0.22 | 18.69** | | Within | 12 | 251.60 | 20.97 | 20.97 | 62.85** | |
| Total | 14 | 12.46 | 0.89 | 1.16 | 100.00 | | Total | 14 | 417.47 | 29.82 | 33.36 | 100.00 | |
| Average | Altitu | de | | | | | | | | | | | |
| Among | 2 | 10.12 | 5.06 | 0.94 | 71.34** | 0.71** | Among | 2 | 200.80 | 100.40 | 14.99 | 37.05** | 0.37** |
| Within | 12 | 4.52 | 0.38 | 0.38 | 28.66** | | Within | 12 | 305.60 | 25.47 | 25.47 | 62.95** | |
| Total | 14 | 14.64 | 1.05 | 1.31 | 100.00 | | Total | 14 | 506.40 | 36.17 | 40.45 | 100.00 | |
| Low Alti | tude | | | | | | | | | | | | |
| Among | 2 | 7.40 | 3.70 | 0.72 | 87.66** | 0.87** | Among | 2 | 239.20 | 119.60 | 20.10 | 51.28** | 0.51** |
| Within | 12 | 1.22 | 0.10 | 0.10 | 12.34** | | Within | 12 | 229.20 | 19.10 | 19.10 | 48.72** | |
| Total | 14 | 8.62 | 0.62 | 0.82 | 100.00 | | Total | 14 | 468.40 | 33.45 | 39.20 | 100.00 | |

Table 6. Analysis of molecular variance (AMOVA) of three species of *Passiflora* spp. collected at three altitudes, from SSR and ISSR data.

^{ns} not significant; ^{**} significant at 1% probability by the F test, respectively.

Legend: SV = source of variation, DF = degree of freedom, SQ = sum of square, MS = mean square, Fst and Φ ST = fixation index

The PE 18 locus in *P. alata* Curtis and *P. edulis* Sims and PE 15 in *P. edulis* Sims f. *flavicarpa* Deg. were highly informative (PIC: 0.575, 0.556 and 0.535, respectively). Overall, SSRs were classified as reasonably slightly informative for all species. Higher PIC values are related to the distribution and balance of allelic frequencies in the population in which the selected markers are reliable to detect genetic diversity (Missio et al., 2010).

In the three analyzed species, 22 alleles were found for the 13 SSR markers, ranging from 1 to 3 per locus. Paiva et al. (2014), when characterizing SSR locus, found 41 alleles, ranging from 2 to 5 alleles, with a mean of 3.42. Similar allelic variation was found by Cazé et al. (2012) with SSR locus, finding 42 alleles per locus and a variation from 2 to 9, with an average of 5. This shows a low number of alleles per locus and few polymorphic microsatellites have shown characteristic of this genus, therefore suggesting conserving the locus and small mutation rate (Cerqueira-Silva et al., 2014a).

Rare alleles are those with a frequency less than 0.05. The alleles are no longer rare in the population *P. edulis* Sims f. *flavicarpa* Deg., due to the increase of its frequency. Studies have reported that rare alleles may be linked to genes of agronomic interest (Reis et al., 2011).

PE03 and PE20 locus in *Passiflora edulis* Sims f. *flavicarpa* Deg and *P. edulis* Sims, and PE20, PE23, PE37 and PE38 in *P. alata* Curtis concentrated 1% of the maximum allele frequency. These frequencies explain the lower PIC values, and suggesting that the locus generate the lowest

information content in the analyzed accessions in this study and lower genetic diversity and frequency of alleles.

In the ISSR analysis, there was a high number of bands per primer and a low percentage of monomorphic markers, thus suggesting high interspecific genetic variability of the accessions. In other studies with *Passiflora*, similarly high levels of polymorphism were observed with this marker, varying 70.5-98% (Santos et al., 2011; Costa et al., 2012).

The AMOVA in each species revealed that the greatest genetic diversity occurred within and not between populations in the ISSR marker and similar results were found for *Passiflora setacea* (Pereira et al., 2015). These data suggest high genetic variability within plant populations, which may be due to sexual reproduction, somatic cell mutation, selection, genetic flow, genetic drift, and environmental change (Gao and Yang, 2006). The analyzed populations of each species are genetically structured by Φ ST values above 0.25 and they are considered indicators of a high level of structure population (Matioli, 2001).

In general, ISSR marker may be a valuable tool for studying intraspecific genetic diversity in *Passiflora*, mainly by grouping accessions according to genetic origin (Costa et al., 2012).

P. edulis Sims f. *flavicarpa* Deg. and *P. edulis* Sims individuals located in the middle and high altitudes were closer, and presented higher genetic similarity. Populations tend to cluster when they are geographically restricted and close (Hou and Lou, 2011).

The results showed that P. alata Curtis presented higher genetic dissimilarity in relation to the other species. Larger genetic distances between P. edulis Sims f. flavicarpa Deg. and P. alata Curtis were also observed by Castro et al. (2011). Populations in low altitude differed from the others. Geographic distribution may affect the level of genetic diversity of/in a species, and species in general may have a higher level of genetic variability than those distributed in a isolated way (Hamrick and Godt, 1996). The genetic variability found in this study can be explained by the geographic difference evaluated by passion fruit accessions, pollen flow between the different genotypes and the introduction of materials from other sites by farmers. Genetic polymorphism may be associated with the nature of the allogeneic species, which tends to favor the conservation of a high percentage of heterozygous genotypes (Bernal-Parra et al., 2014). The genus Passiflora spp. has variable agronomic characteristics, in which environmental influences are largely responsible for the changes in passion fruit.

In medium altitudes, related to high and low altitudes, there was genetic diversity in *Poa hiemata* (Byars et al., 2009). The diversity loss in low and high altitude populations can be due to being geographically isolated from the middle part, therefore, with reduced gene flow and genetic drift (Ohsawa et al., 2007).

The results contribute to the knowledge of inter and intraspecific variation of the analyzed species and can be used in breeding and conservation programs.

Material and methods

Plant materials

Plant samples collected in the field were composed of young leaves of plants of natural occurrence in different localities, in three altitudes (0 to 100 - low,> 100 to 600 - medium and> 600 m - high) in the state of *Espírito Santo* (Figure 1) (Table 1). From each site, five matrix plants of the *Passiflora edulis* Sims *f. flavicarpa* Degener (yellow passion fruit) species, *P. edulis* Sims (purple passion fruit) and *P. alata* Curtis (sweet passion fruit) were sampled.

Genotyping

Genomic DNA was obtained from about 300 mg of healthy young plant leaf tissue macerated in liquid nitrogen according to the Doyle and Doyle (1990) protocol. DNA integrity was verified in 0.8% agarose gel, stained with ethidium bromide and the concentration was determined in a spectrophotometer. DNA quality was established by the absorbance ratio A260/A280.

For each sample, 13 microsatellite markers developed for *Passiflora edulis* (Oliveira, 2006) were amplified (Table 2). The PCR amplification reaction consisted of: 30 ng of DNA; 0.1 μ M of the primers; 1 U of Taq DNA Polymerase; 0.2 mM of dNTP; 1.5 mM of magnesium chloride and IO PCR buffer (1X - Phoneutria) in 15 μ l reaction. The amplification program used was 4 minutes at 94 °C, followed by 35 cycles; at 94 °C for 1 minute; 56-60 °C (depending on the first Table 2) for 1 minute; 72 °C for 3 minutes, and a final extension at 72 °C for 7 minutes. The amplified fragments were separated in 6% polyacrylamide gel, with running TAE buffer (tris-base, acetic acid and EDTA), at 90 volts for approximately 3.5

hours, stained with ethidium bromide and subjected to UV light in order to be visualized.

For the amplifications with the ISSR, 14 primers were chosen (Table 2), according to the largest number of polymorphic fragments and amplification quality. The reactions were done using 30 ng of DNA; 0.5 µM of the primer; 1 U of Taq DNA Polymerase; 0.3 mM of dNTP; 1.5 mM of magnesium chloride and 10% PCR buffer (1X - Phoneutria), with ultrapure water being added to complete 20 µL reaction volume. The amplification conditions were: 5 minutes at 94 °C; followed by 40 cycles for 1 minute at 94 °C; 1 minute at 50 °C and 2 minutes at 72 °C, with an extension of 10 minutes at 72 °C. The fragments were separated by 1.5% agarose gel electrophoresis, stained with GelRed and 1X TBE runner at 90 volts for approximately 1.5 hours. The gels were photographed with ultraviolet light using the Photodocumentation System. All amplifications were performed in a Thermal Cycler (Veriti 384-well Thermal Cycler Applied Biosystems).

Diversity analysis

After the microsatellite alleles genotyping, the descriptive parameters of the locus were estimated by the expected heterozygosity (He), polymorphic information content (PIC), allele number in the population (AN), maximum allele frequency (mf), number of alleles per locus (Nm), total number of alleles (Tn), allele proportion in each population (Ap) and rare allele number (Nr). Estimates of genetic diversity in the population were obtained according to the weighted index and UPGMA cluster analysis (Unweighted Pair Group Method Using Arithmethic Average).

In the analysis, using the ISSR data, the polymorphic bands in the individuals were coded as the presence of bands (1) and absence (0). Only bands presenting consistent amplification were considered. Estimates of genetic dissimilarity were performed according to Jaccarde, and UPGMA clustering was done. The difference between and within the populations was evaluated by molecular variance analysis AMOVA. All analyzes of genetic difference and clustering were performed with the aid of the Genes program (Cruz, 2016).

Conclusion

The *P. Alata* Curtis species was more divergent in relation to *P. edulis* Sims f. *flavicarpa* Deg. and *P. edulis* Sims. SSR and ISSR markers make it possible to indicate dissimilarity between species, but they did not always show variation results between and within similar species. The populations in low altitude are different from the others, independent of the species and the marker used.

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