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EFFECT OF AGRONOMIC AND MOLECULAR INFORMATION ON THE GENETIC DIVERSITY OF PASSION FRUIT

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Abstract: Genetic divergence study enables a better decision making for the choice of promising and more divergent genotypes for advancing selection cycles. As such, the goals of this study were to evaluate genetic diversity by means of agronomic and molecular traits and to investigate the genetic structure of a population of sour passion fruit, making it possible to select superior and contrasting genotypes. A total of 95 genotypes belonging to 20 half-sib families of sour passion fruit were selected. For molecular traits, 170 microsatellite (SSR) primers and 58 Inter Simple Sequence Reapeats (ISSR) primers were used. On the basis of this information, estimates of genetic diversity parameters, genetic distances, and grouping of genotypes were determined. Lastly, a joint analysis was performed with the groupings of agronomic and molecular information. A differentiated structuring between phenotypic and molecular groupings was found. It was noticed the formation of five groups for phenotypic traits and three others for SSR and ISSR. The heterozygosity verified was greater than the expected, suggesting a significant number of heterozygous individuals. With respect to the joint analysis, it was verified an entanglement of 0.57 and 0.58, pointing out the divergence in the distribution of genotypes among the dendrograms. There was a greater genetic variability via phenotypic rather than molecular traits. With a view to continuing the breeding program, it is recommended the crossing of genotypes of groups 1, 2, and 5, which show good productivity, with genotypes via ISSR (23, 35, and 75) and SSR (6, 21, 26, 33, 38, 39, 52, 59, and 68).

Keywords: SSR and ISSR markers, genetic breeding, *Passiflora edulis*, recurrent selection.

Introduction

Genetic breeding of passion fruit has led to significant advances in terms of yield and fruit quality (Santos et al. 2015; Silva et al. 2016; Ribeiro et al. 2019). Even so, considering the economic and social importance of the crop, genetic breeding pro-

grams should enhance their research in order to develop new cultivars (Silva et al. 2016).

The recurrent selection methodology has been used for the release of new passion fruit cultivars (Viana et al. 2016). A successful outcome of any recurrent selection program, however, necessarily depends on the genetic variability of the population in question. Genetic variability is of essential importance for the breeder, given that, without it, it would not be possible to identify superior genotypes (Ramalho et al. 2012).To analyze genetic diversity by means of agronomic traits, multivariate analysis techniques are applied, evaluating the interrelation among descriptors, making it possible, in this way, to point out which are the most promising genotypes to be included in breeding programs (Cruz et al. 2012).

For sour passion fruit, by being a semi-perennial species, estimates of reduction of genetic variability, based on agronomic information, demand a lot of time, and experience strong environmental influence. In search of more efficient selection methods, molecular markers may provide some advantages in relation to the selective process of plants, saving time (Jiang 2013).

Among the classes of molecular markers available, ISSR (Inter Simple Sequence Repeats) markers can be used in the discrimination and molecular traits of individuals. The ISSR method has the advantage of being a reproducible marker with no further need for sequence information for genetic analysis (Kumar et al. 2011). Another type of molecular marker that has stood out for having codominance, multialelism, being numerous, having good reproducibility and wide coverage of the genome, is the SSR (Moe et al. 2010). Studies on population genetic structure are generally estimated by apply in gmicrosatel lite markers (SSR). A Bayesian approach has recently been used in genetic diversity and population structure studies, implementing the Structure program, a model based on attributing individuals to subgroups (Pritchard et al., 2000). Some studies can be mentioned using this strategy in fruit trees, sour passion fruit (Cerqueira-Silva et al., 2014a) and papaya (Matos et al., 2013).

Hence, this study intends to i) estimate the genetic variability in the fourth cycle of recurrent selection of sour passion fruit using phenotypic traits and DNA markers; (ii) estimate genotypic indexes to quantify and structure genetic variability among genotypes; and (iii) select superior and contrasting genotypes to enhance the crop breeding program.

Material and methods

Plant material

The experiment was conducted between January 2016 and January 2017 in the municipality of Itaocara, state of Rio de Janeiro, Brazil. Twenty-three half-sib progenies from the fourth cycle of recurrent selection were evaluated. An experimental randomized block design with four replicates and individual evaluation of three plants per plot was adopted. For DNA extraction, 95 genotypes were selected from the 20 best half-sib families considering the most productive individuals as criteria.

Agronomic traits

The 95 genotypes were characterized according to the following quantitative traits:number of fruits per plant (NF), evaluated by the harvests throughout the experiment; yield per plant in kilograms (YIE), obtained by the quantity, in kilograms, harvested during the experiment; average weight of fruit per plant in grams (WF), obtained from dividing the total yield per plant by the total number of fruits; length and average diameter of fruits in millimeters (LENG and DIAM), calculated by measuring a sample of five fruits per plant of the longitudinal and transverse dimensions of the fruits using a digital caliper; average husk thickness in millimeters (HT), determined using a digital caliper, measuring the median portion of the five fruits, cut transversally, in the direction of the largest diameter; total soluble solids content in brix degree (TSS), performed by refractometry, with a digital refractometer (model RTD-

45), with reading between 0 and 95° of Brix. Readings were taken by placing drops of fruit juice individually.

Based on the information on agronomic traits, a numerical matrix was obtained on the basis of the mean Euclidean distance, using the GENES software (Cruz, 2013). The cluster analysis of individuals via dendrogram was conducted using the UPGMA method (Unweighted Pair-Group Method Average) assisted by the R software.

Extraction and quantification of genomic DNA

Samples of young leaves were collected individually from each genotype and macerated in liquid nitrogen. Genomic DNA was extracted using the standard CTAB method (Doyle and Doyle 1990), with modifications described below.

In the tubes containing the macerated samples were added 900 µL of extraction buffer containing 2% CTAB, 2.0 mol L^{-1}NaCl, 20 mmol L^{-1} EDTA, 100 mmol L^{-1} Tris-HCl (pH 8.0), 2% PVP and 2.0% - mercaptoethanol. The material was incubated at 65°C for 40 minutes and gently homogenized by inversion every 10 minutes.

After the samples reached room temperature, the tubes were centrifuged for 8 minutes at 14,000 rpm and poured into a new 2.0 mL tube. 800 µL chloroform: isoamylalcohol (24:1) were added to carry out the deproteinization.

The organic phase was separated by centrifugation at 14,000 rpm for 8 minutes. The supernatant was transferred to a properly identified 2.0 mL tube, these steps were repeated three more times, and the add edchloroform must be 100 uL more than the volume of the supernatant in each step. Nucleic acids were precipitated by the addition of two-thirds (500 µL) of the volume of ice-cold isopropanol, and incubated for 30 minutes at -70°C or for 3 hours at -20°C.

The precipitate was sedimented by centrifugation at 14,000 rpm for 10 minutes. The supernatant was discarded and the precipitate washed twice with 500 µL of ice-cold 70% ethanol to remove the salt present and twice more with 500 uL of ice-cold 70% ethanol, after each wash the material was centrifuged for 5 minutes at 14,000 rpm.

After discarding the last supernatant, the material was taken to the dry bath equipment until all ethanol was removed. Then, the material was resuspended in 200 µL of TE buffer (Tris-EDTA - 10 mmol L^{-1} Tris-HCl, 1 mmol L^{-1} EDTA, pH 8.0) with RNa seat a final concentration of 10 µgmL^{-1} and incubated in a water bath at 37°C for 40 minutes. The material was then stored at 20°C until use.

After extraction, the DNA quantification was proceeded via analysis in 1% agarose gel with 1X TAE buffer (Tris, Sodium Acetate, EDTA, pH 8.0). DNA samples were stained using a mixture of GelRedTM and Blue Juice (1:1). The aliquots of each DNA were applied to the gel wells next to a 100 bp (100 ng/ μL^{-1}) Lambda marker (λ) (Invitrogen, USA) and submitted to electrophoresis (80W for 120 min), and the image was captured by the Mini Bis Pro photo documentation system (Bio-Imaging Systems). Sample concentration was estimated by means of a visual comparison of the fluorescence intensity of the DNA bands in relation to the Lambda marker (λ). From this result, all samples were diluted to the working concentration of 5ng μL^{-1}.

Primer screening

One hundred and seven microsatellite initiators were utilized, developed for *P. edulis* (Oliveira et al. 2005), to identify SSR loci that can distinguish parents. After screening performed in order to selecton lypolymorphic primers, 9 SSR primers were selected: six developed by Oliveira et al. (2005), two developed by Cerqueira-Silva et al. (2014b) and a primer developed by Padua et al. (2005). For the AGR-type markers of the 14 primers used, only two were polymorphic (Table 1).

With regard to the 58 ISSR primers utilized, 14 detected polymorphism in the genotypes under analysis (Table 2).

Table 1. Molecular markers, microsatellites, and regions similar to resistance genes (Rga) used in the genotyping of 95 genotypes of *Passiflora edulis.*

Loco	Primer forward	Primer reverse	Motive	At (ºC)	Reference
PE08	CCGGATACC CACGCATTA	TCTAATGAGC GGAGGAAAGC	$(GTTGTG)_4$	60	Oliveira et al. (2005)
PE10	AACCTTGATC TCCAGCCTAT	GTTTTCGCC CGCGTATT	$(GA)_{34}$	60	Oliveira et al. (2005)
PE23	CAATCCCTTG ACCCATAGA	CGTCCATCC TTCTCCTTT	$(GA)_{19}$	58	Oliveira et al. (2005)
PE46	CATTTCTCAG TCACCCGATA	GTCAATGCAG TCATTCACAA	$(AT)_{28}(TG)_{16}$	61	Oliveira et al. (2005)
PE58	GCAATTTCAC CATCTTCTGCT	CCACGGTCA TGGATGTTC	$(AC)_{11}$	62	Oliveira et al. (2005)
PE90	TCAGGAAGAT TGCATGTTAGT	CTGGGTTTTG TTTATGTTGC	$(AGC)_5$	56	Oliveira et al. (2005)
mPs-UNICAMP06	GTTGGATCAA AGGGTCACA	CAACTACTGGA TCGAACTGGTA	$(CGTG)_3$ $(ATGA)_3$	63	Cerqueira-Silva et al. (2014b)
mPs-UNICAMP17	CATCCAACCT CCGAACCTTA	TACCCAGTCC GGTCCATTAG	$(AC)_5$	58	Cerqueira-Silva et al. (2014b)
A08FP1	CACATTTGC CGTCACTGG	CGGCATACGA TAAATCTCCTG	$(TG)_9$	60	Pádua et al. (2005)
GlusRga	RYNGGNGT WTGYTAYGG	A01 (GACTGCG TACGAATTAA)	-	47	Pereira et al. (2013)
Rghdr2 Rga	CARMGCYAA WGGYAADCC	A01 (GACTGCG TACGAATTAA)	-	45	Pereira et al. (2013)

For Rga degenerate code: K = G / T; M = A / C; N = A / C / G / T; R = A / G; S = C / G;W = A / T; Y = C / T. Annealing temperature At (°C).

Table 2. Sequence of ISSR primers selected, annealing temperature (At), total number of bands (TNB), number of polymorphic bands (NPB), and percentage of polymorphism per marker used in the genotyping of 95 genotypes of *Passiflora edulis*.

Primer	Sequence*	At (ºC)	TNB	NPB	Polymorphism (%)
1	$(GA)_8C$	54	6	2	33.33
2	$CY(GT)_8$	58	9	7	77.77
3	$(GA)_8YC$	50	8	3	37.50
4	$(GA)_8T$	50	6	3	50.00
5	$(CA)_8RG$	56	7	7	100.00
6	$(AG)_8T$	48	4	1	25.00
7	$(CA)_8G$	52	4	3	75.00
8	$(GA)_8YT$	46	6	3	50.00
9	$(TC)_8A$	47	4	2	50.00
10	$(CT)_8G$	47	2	1	50.00
11	$(GA)_8A$	47	6	2	33.33
12	$(CT)_8T$	47	5	4	80.00
13	$(AG)_8YT$	47	3	2	66.66
14	$(AG)_8YA$	47	3	2	66.66
Mean		**-**	**5.21**	**3**	**56.80**

* R = A or G; Y = C or T

Polymerase chain reaction (PCR) for SSR and ISSR markers

PCR reactions for SSR markers were carried out in Applied Biosystems/Veriti 96 well thermal cyclers at the following temperatures and times: one cycle of 94 ºC for four minutes (initial denaturation) followed by 35 cycles of 94 ºC for one minute (cyclic dena-

turation), specific temperature of each primer in ºC for one minute (primer annealing), 72 ºC for three minutes (extension by taq-DNA polymerase and nucleotide incorporation). After the 35 cycles, a final extension step of 72 ºC was performed for seven minutes; subsequently, the temperature was reduced to 4 ºC for an indefinite time.

For ISSR markers, the same previous procedures were used, with change only in the number of cycles (38).

The reaction had a final volume of 13 µL for each sample, containing 5.38 µL ultrapure water; 2µL of DNA (5 ng/µL); 1.5 µL of 10X buffer (NH4SO4); 1.5 µL of MgCl2 (25 mM); 1.5 µL of dNTPs (2 mM); 1 µL of primer (F+R) (5 µM); and 0.12 µL of Taq-DNA polymerase (5 U/µL) (Invitrogen, Carlsbad, California, USA).

After amplification, 3 µL of Blue Juice and 3 µL of GelRedTM dye were added to each sample. These samples were applied in Metaphor agarose gel (4%) immersed in TAE buffer 1X. The electrophoretic separation was of approximately four hours, at 80 volts. The DNA Scada 100 pb ladder was used as a molecular weight marker. At the end of the electrophoresis, the gels were photo-documented by means of the MiniBis Pro device (Bio-Imaging Systems).

For ISSR markers, the separation of the fragments was carried out in 2% agarose gel. The processes were similar for the other steps.

Statistical analysis of SSR markers

The variables achieved by amplification of the nine SSR primers were converted into numerical code for each allele per locus. This numerical matrix was developed according to the following description: for a locus with three alleles, there are the representation 11, 22, and 33, for the homozygous forms (A1A1, A2A2, and A3A3), and 12, 13, and 23, for the heterozygous ones (A1A2, A1A3, and A2A3). From the numerical matrix, the genetic distance of Smouse and Peakall (1999) was calculated for 95 genotypes. The cluster analysis of individuals via dendrogram was carried out using the UPGMA method, with the assistance of the R program (*http://www.r-project.org*).

The genetic variability of the 95 genotypes was estimated by the Genalex 6.5 software (Peakall and Smouse, 2012), on the basis of the following parameters: number of alleles per polymorphic locus (NA);observed heterozygosity (Ho); expected heterozygosity (He); information index (I); and fixation index (F).

Statistical analysis of ISSR markers

The polymorphic bands obtained were converted into binary variables, in which (1) is presence and (0), absence of the band. For the analysis of genetic variability, the Jaccard similarity coefficient was estimated according to the GENES software (Cruz, 2013). The result was the generation of a genetic dissimilarity matrix, based on which the cluster analysis (UPGMA) were performed using the R software.

Analysis of population genetic structure by SSR markers

The genetic structure of the 95 genotypes analyzed was verified using the method based on Bayesian clustering algorithms by means of the "Structure 2.3.4" program (Pritchard et al. 2000). The "no admixture model" model and independent allele frequencies were adopted, using a "Burnin Period" of 100,000, followed by an extension (Markov Chain Monte Carlo) of 1,000,000 replicates, with the number of subpopulations (k) varying from 1 to 10. A probability threshold of 0.75 was used as the maximum probability of adherence among the groups. Given that the likelihood distribution L(k) quite frequently does not indicate a true value for K, the proposal of Evanno et al. (2005) was chosen to determine the true value of K for the present population, using SSR markers.

Joint analysis of agronomic and molecular variables

The matrices of relative distance among the seven agronomic variables were compared with the matrices obtained the based on SSR and ISSR markers, using the Dendextend package in the R program.

Results and discussion

From the 14 ISSR (Inter Simple Sequence Repeats) primers selected, 73 bands were generated, and 42 of them, are polymorphic, with a mean of three bands per primer.The number of polymorphic bands generated per primer ranged from 1 to 7 with a minimum polymorphism of 33.33 to 100% (Table 2).

When using ISSR primers in sour passion fruit, Costa et al. (2012) identified high polymorphism; from the 23 ISSR primers tested, 22 detected polymorphism among the accessions.The markers generated 266 bands (mean of 11.56 bands per primer), in which 260 (97.74%) were polymorphic fragments measuring from 220-2300 bp.These authors inferred that ISSR markers should be a useful tool to study genetic diversity in sour passion fruit, since they enable the grouping of accessions according to their genetic origin.

The analysis of agronomic variables divided the 95 genotypes into five groups, in which the cut-off point in the dendrogram was determined by the Mojena method, according to the relative size of the fusion levels (distances) (Figure 1).

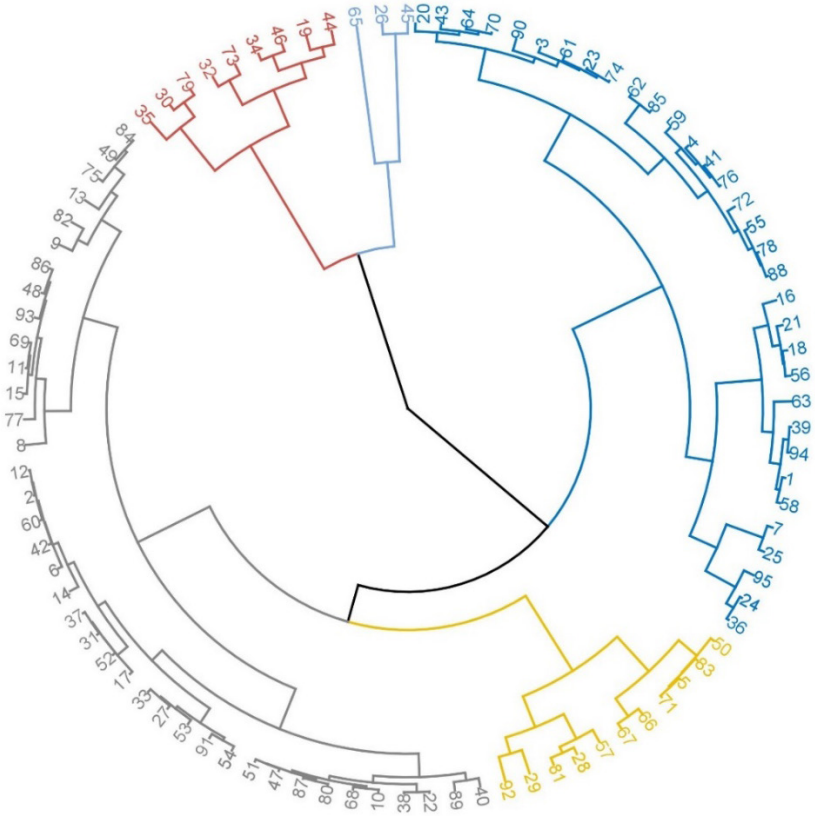

Figure 1. Dendrogram of genetic dissimilarity among 95 genotypes of sour passion fruit, obtained by the UPGMA method, based on mean Euclidean distance via quantitative phenotypic traits.

Group 1 was formed by genotypes 26, 45, and 65.These individuals presented the highest means for yield traits (20 kg per plant); number of fruits (114); and length and diameter of fruits (95 and 79.44 mm). For the trait of total soluble solids (10.54), however, they obtained the lowest mean compared to the other groups (Figure 1).This may be because of the effect of competition between fruits per photo-assimilates and a dilution effect with the increase in the amount of pulp (Morgado et al. 2010).

Group 2, comprised of genotypes 19, 30, 32, 34, 35, 44, 46, 73, and 79, was the second most productive group, with means close to the ones in Group 1.Group 4, formed by individuals 92, 29, 81, 28, 57, 67, 66, 71, 5, 83, and 50, had the lowest mean yield (7.4 kg), number of fruits (50), and weight of fruits (153 grams) (Figure 1).

The genotypes with the highest similarities were 49 with 84 (4.92), belonging to group 3. These genotypes had the second lowest mean for the fruit yield trait (10 kg per plant). Genotypes with greater dissimilarity were 65, group 1 more productive, and 29, group 4 less productive, with a distance of (5841.54). This genotype from group 4 should not be indicated for future crosses, as it has low productive potential (Figure 1). As passion fruit is an allogamous plant species, the adequate choice of parents and the design of crosses can maximize the use of favorable genes or explore heterosis by means of crosses among individuals with good agronomic attributes and with a certain degree of genetic divergence, enabling the obtaining of superior varieties (Negreiros et al. 2008).

Group 5 consisted of 33 genotypes that presented good agronomic performance, being the third most productive of them (12.9 kg) (Figure 1). It should be considered, for future crosses, the use of individuals from groups 1 and 2 (more productive) with genotypes from group 5, which presented greater phenotypic distance and good productive traits and quality of fruits.

Taking into account the divergence for ISSR markers, 3 groups were formed (Figure 2). Group 1 comprised most of the genotypes (96.84%). Group 2 allocated genotypes 23 and 35 and group 3, only genotype 75. Findings suggest low variability among accessions, considering the regions accessed by these markers are conserved among the genotypes.

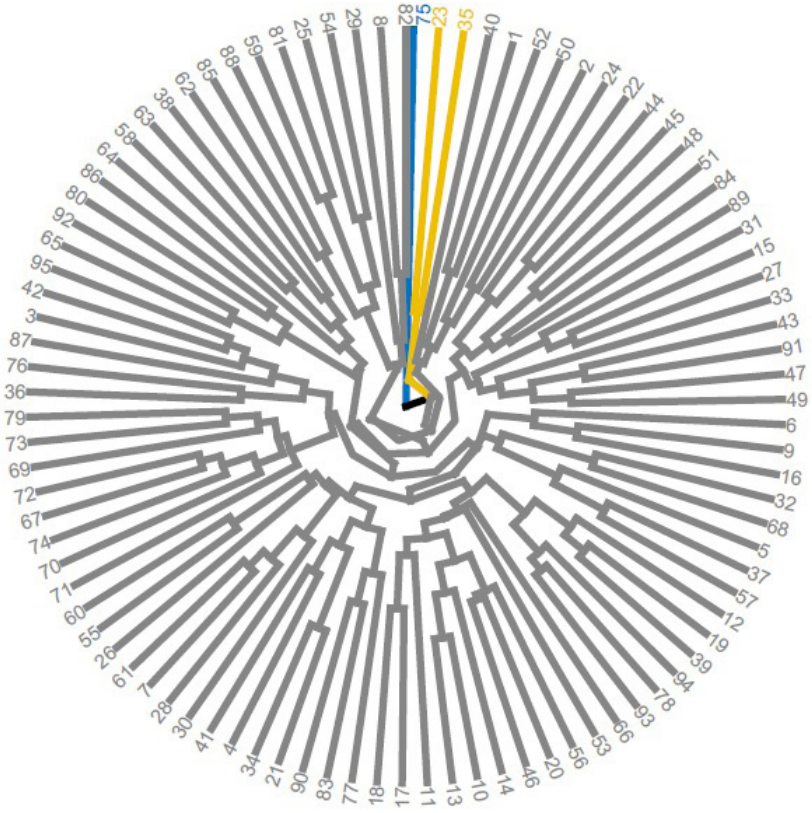

Figure 2. Dendrogram of genetic similarity among 95 genotypes of sour passion fruit, obtained by the UPGMA method, based on Jaccard distance via ISSR markers.

In contrast to this study, Costa et al. (2012), evaluating genetic diversity in improved (IG) and unimproved (UG) germplasm of sour passion fruit (*Passifloraedulis*) with the use of ISSR type markers, observed a high number of polymorphic bands (260 bands), regardless of the genotype, besides a number of specific fragments of groups, according to the breeding level. Unimproved genotypes had a higher number of polymorphic fragments. This study showed the potential use of the ISSR in determining the molecular polymorphism in passion fruit, as well as that the breeding has reduced the genetic variability.

Regarding the SSR markers, the genetic distance among the accessions ranged from 0 to 0.8. Three groups were composed, with the greatest distance between genotype 33 and 58. Group 1 was the one that grouped the greatest number of genotypes (86), accounting for a percentage of 90.52% (Figure 3).

It is recommended to use individuals from group 2 (26, 21, 52, and 68) and group 3 (6, 39, 33, 38, and 59) for future crosses for the maintenance of genetic variability in the population, since it contributes to delay the increase in population inbreeding, with the consequent maintenance of genetic diversity (Figure 3).

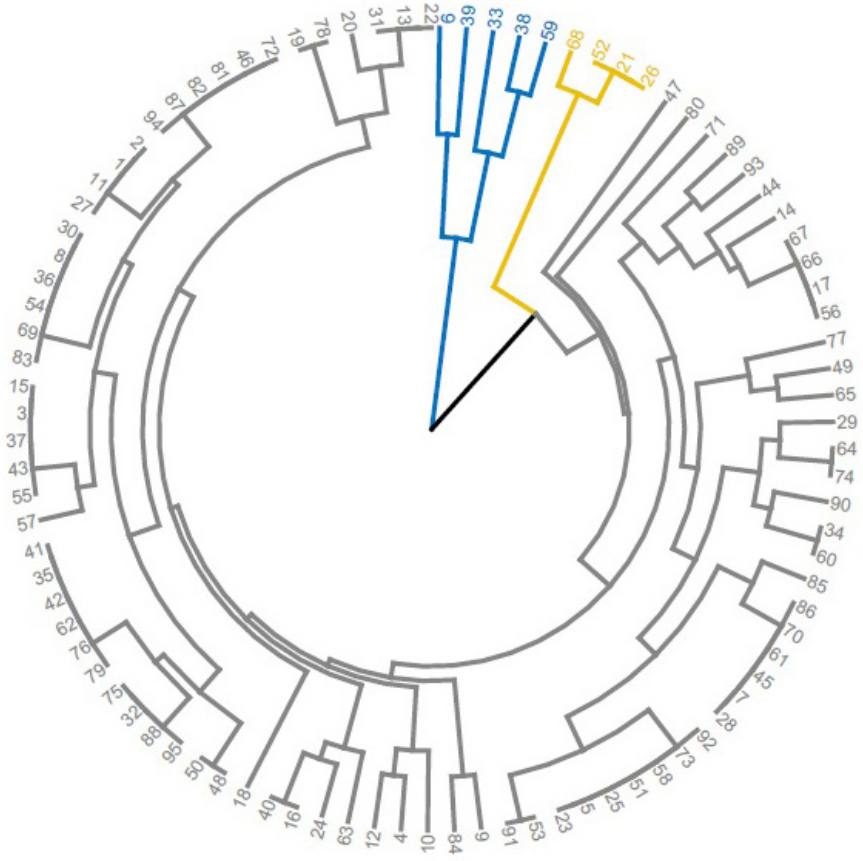

Figure 3. Dendrogram of genetic dissimilarity among 95 genotypes of sour passion fruit, obtained by the UPGMA method, based on Smouse and Peakall distance via SSR markers.

In this study, the number of alleles per locus varied from 2 to 4 in the fourth cycle of recurrent selection, with a mean equal to 2.22, totaling 20 alleles for the nine loci evaluated (Table 3). Silva et al. (2016) evaluated the third cycle of recurrent selection of the sour passion fruit from UENF, and observed that, in 10 SSR loci, they amplified a total of 29 alleles, with a minimum of two alleles (PE01, PE09, PE18, and PE64) and a maximum of six (PE04), with a mean of 2.9 alleles per locus. Studies suggest a reduction in genetic variability after some cycles of recurrent selection, generally because of the reduction in population size (Hallauer, 1971).

When using SSR markers to evaluate the intrapopulation genetic variability in popcorn (CMS-43), Franzoni et al. (2012) identified a reduction in the number of alleles and a change in the frequency of the allele throughout the cycles, causing the genetic narrowing of populations; on the other hand, the total genetic diversity found made it possible to perform later cycles with possible genetic gains for the main traits. This is one of the primary concerns of breeders, that is, to maintain variability without narrowing the genetic basis of the study population. A significant indication to avoid such narrowing is to verify whether the effective size of the population (Ne), which will compose the recombination step of the recurrent selection, is enough to guarantee a progress with the selection, thereby avoiding crosses between

related individuals, which may favor inbreeding in the population (Falconer, 1981).

The information index varied from 0.058 (UN06) to 1.075 (PE46) with a mean value of 0.369, which shows low variability in this population, but enough for this breeding program to continue (Table 3). The closer to 1 is the value of the information index, the more diverse the population. During the evaluation of genetic diversity by means of SSR microsatellites molecular markers in segregating populations of table grapes, Santos et al. (2019a) observed an index of information that ranged from 0.30 to 1.25 with a mean of 0.93, thereby indicating a high variability of the genotypes evaluated.

Table 3. Estimate of genetic parameters in 95 genotypes of sour passion fruit, with NA: number of alleles; I: information index; He: expected heterozygosity; Ho: observed heterozygosity; and F: fixation index.

Locus	NA	I	He	Ho	F
PE08	2.000	0.511	0.329	0.415	-0.262
PE10	2.000	0.655	0.463	0.614	-0.326
PE23	2.000	0.158	0.071	0.074	-0.038
PE46	4.000	1.075	0.586	0.753	-0.284
PE58	2.000	0.192	0.091	0.096	-0.050
PE90	2.000	0.102	0.041	0.042	-0.022
UN06	2.000	0.058	0.021	0.021	-0.011
UN17	2.000	0.361	0.207	0.234	-0.133
A08	2.000	0.209	0.102	0.000	1.000
Mean	**2.222**	**0.369**	**0.212**	**0.250**	**-0.014**

The Ho ranged from 0 to 0.753, with a mean of 0.250, and the He, from 0.102 to 0.586, with a mean of 0.212 (Table 3). Values of He and Ho presented close means, although the heterozygosity observed was higher than the expected, which suggests a high number of heterozygous individuals. This can be explained because these plants are self-incompatible, a mechanism that induces allogamy and maintains a high degree of heterozygosity (Bruckner et al., 1995).

Values for fixation index (F) were negative for the PE74, PE66, PE18, PE38, PE42, PE90, PE75, and PE58 loci, suggesting heterozygosity (Table 3). Negative values occur because of the higher values of Ho in relation to He. Positive values of F were seen in the locus (A08), which indicates the existence of inbreeding for this locus. The F estimated in the whole population showed a mean value of -0.014 and ranged from -0.011 to 1.000 among the loci.

The Bayesian approach indicated the passion fruit population was grouped into three genetic groups. Following the criterion of Evanno et al. (2005), the optimal ΔK was obtained when K=3, indicating that the maximum structuring was verified when the sample was divided into three groups (Figure 4).

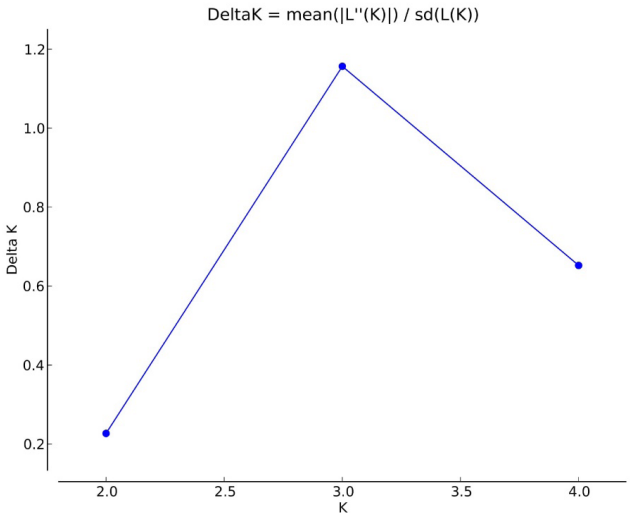

Figure 4. Delta K values (ΔK) for the respective numbers of groups (K).

The Bayesian clustering stated that there was no clear structuring among the 95 genotypes, suggesting that all individuals share the same alleles, with a slight alteration in frequency for some genotypes. Such results show low variability among the loci under study (Figure 5).

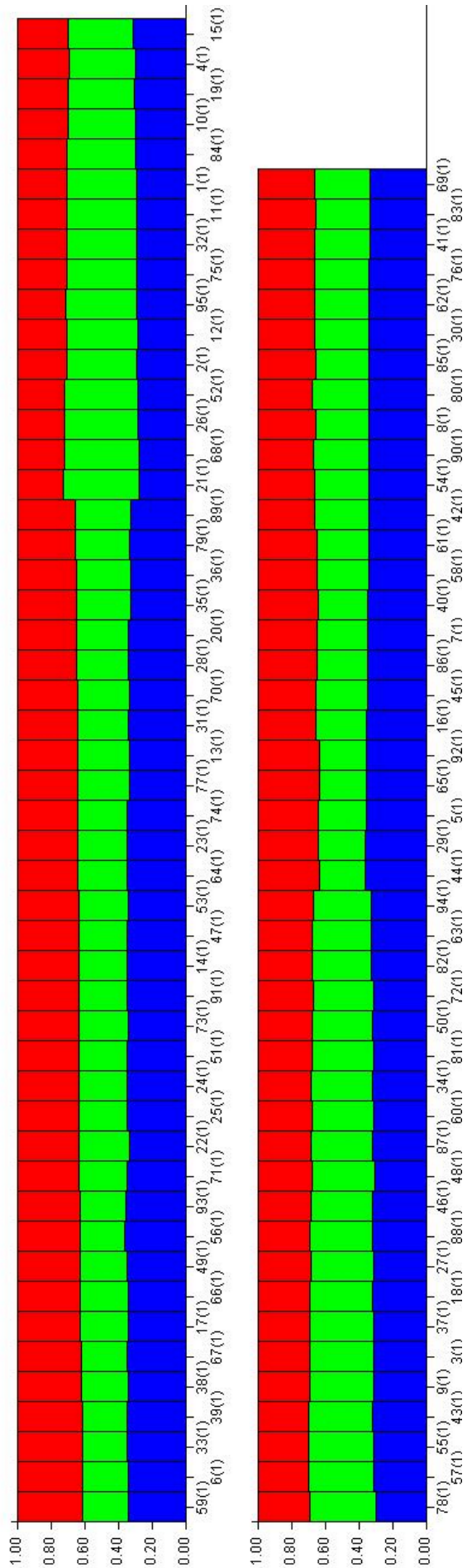

Figure 5. Grouping, by Bayesian inference, of 95 genotypes of sour passion fruit (*k*=3).

On the basis of the joint analysis, five groups were formed for phenotypic variables and three for molecular variables (SSR and ISSR), but the arrangement among the genotypes was different in the analyses (Figures 6 and 7).

The value of entanglement measures the correspondence of genotypes among different dendrograms ranging from 0 to 1, in which 0 means fully corresponding dendrograms and 1, dendrogram without any correspondence. The entanglement was 0.57, comparing phenotypic traits and SSR markers, and 0.58 for phenotypic variables and ISSR markers. This arrangement difference may be caused because the molecular markers used are not specific for particular phenotypic traits. They amplify more conserved regions of the genome, making it impossible to assimilate a molecular mark to a specific phenotypic trait. Santos et al. (2019b), using the Dendextend package to compare 38 morphoagronomic descriptors and dendrograms with only fruit, flower, and leaf descriptors in passion fruit, verified entanglement of (0.51, 0.44, and 0.43, respectively) corroborating divergence in the genotype distribution among the dendrograms.

It was found a greater genetic variability via phenotypic rather than molecular traits, enabling the continuity of the sour passion fruit breeding program.

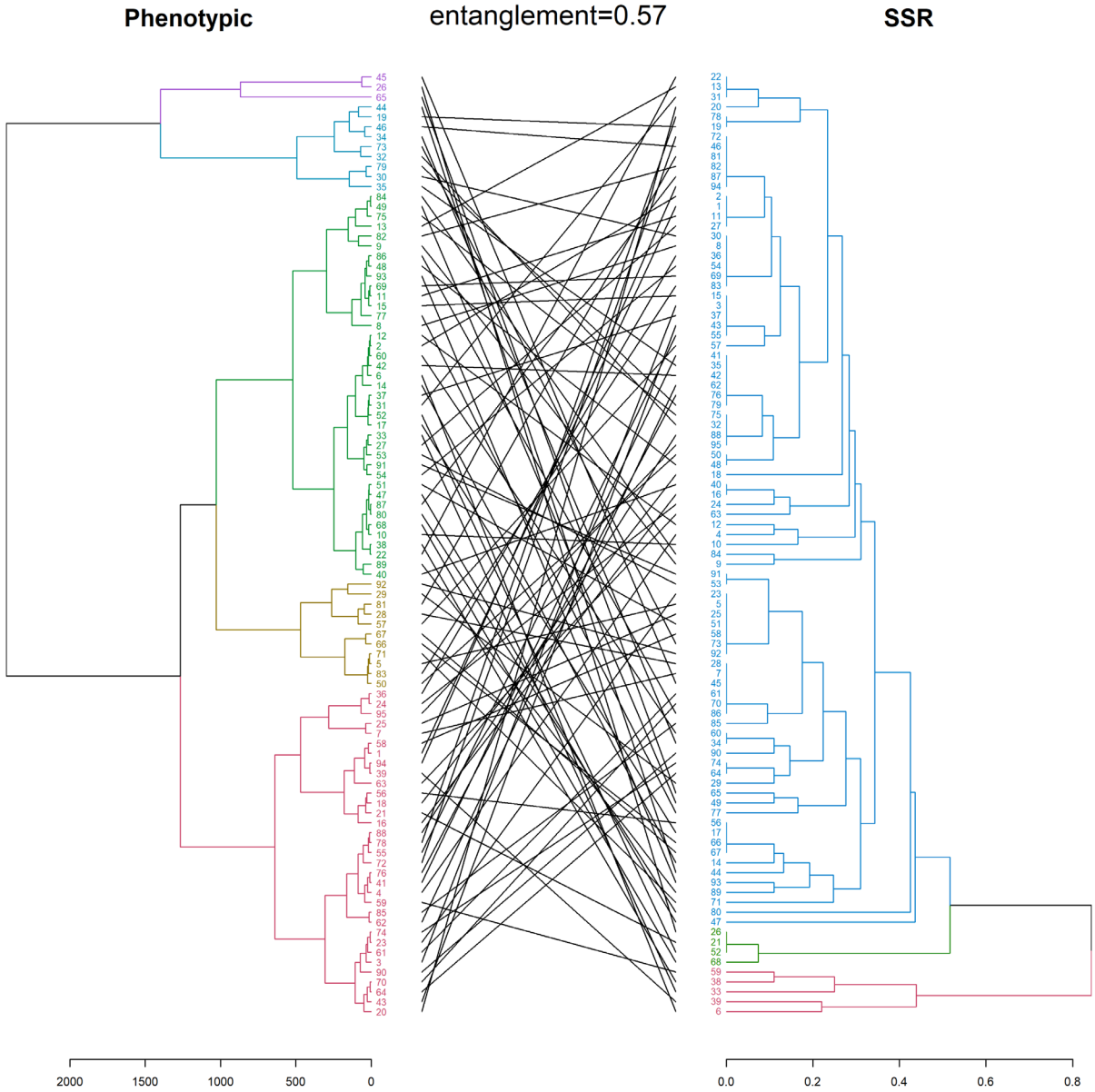

Figure 6. Entanglement among 95 genotypes of sour passion fruit, based on the mean Euclidean distance for quantitative phenotypic traits and Smouse and Peakall for molecular variables of SSR.

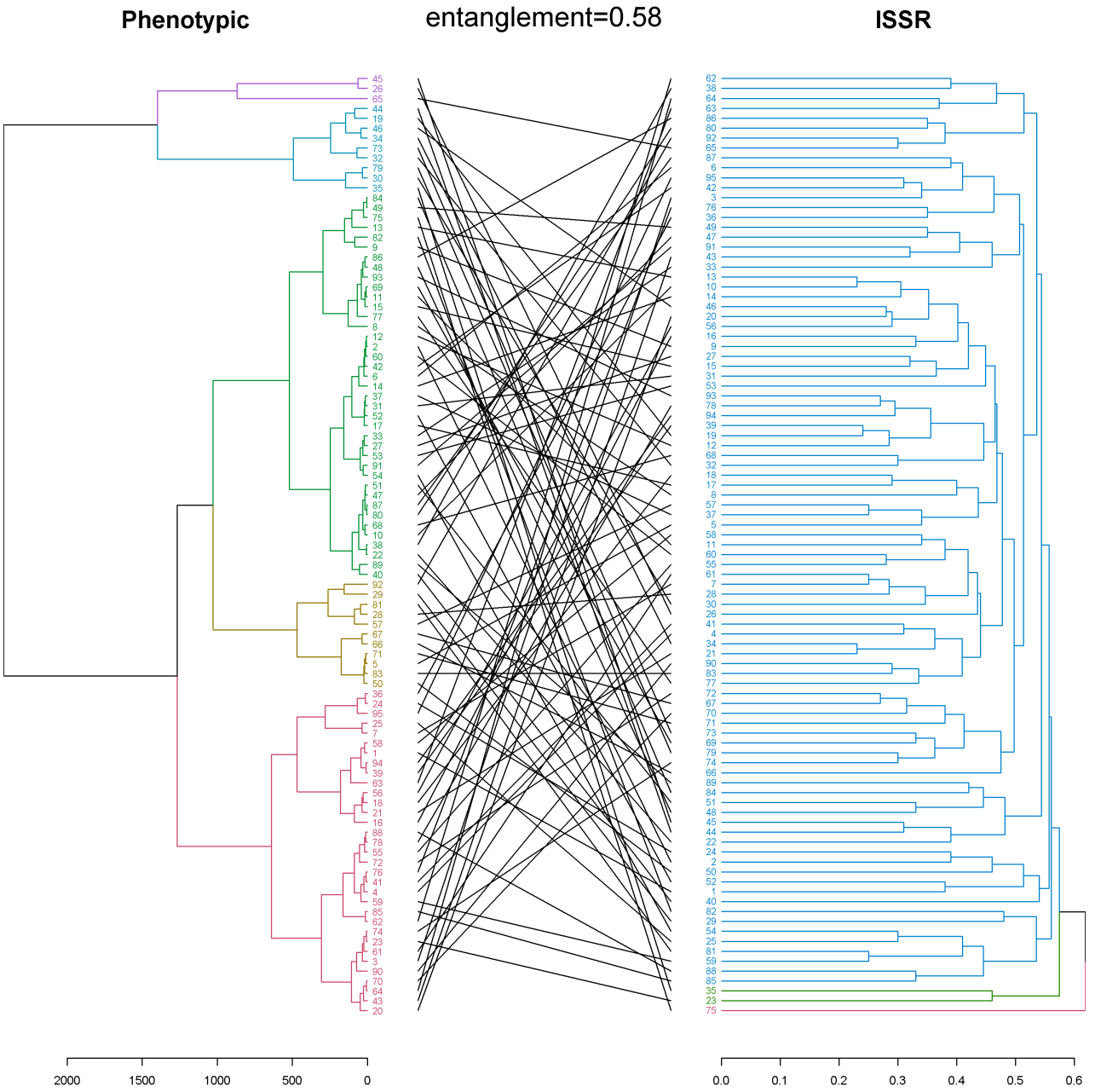

Figure 7. Entanglement among 95 genotypes of sour passion fruit, based on the mean Euclidean distance, for quantitative phenotypic traits and Jaccard for molecular variables of ISSR.

As the best strategy, it is advantageous to perform crosses among genotypes of groups 1, 2, and 5, which offered good productive potential with more divergent genotypes via ISSR markers (23.35 and 75) and genotypes (6, 21, 26, 33, 38, 39, 52, 59, and 68) via SSR markers.

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