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Characterization of pepper accessions using molecular markers linked to pungency and SSR

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ABSTRACT

Peppers of the genus *Capsicum* are of great socioeconomic importance, being pungency trait their main attraction. Pungency characterization, genetic distance estimates and population structure analysis of the accessions belonging to germplasm banks are important for parent selection which allows to obtain superior progenies. Therefore, the aims of this study were: i) evaluate 81 accessions of the *Capsicum* spp. Germplasm Bank of Universidade Federal de São Carlos (BGC-UFSCar) with molecular markers linked to pungency; ii) estimate the genetic diversity among accessions of the BGC-UFSCar using microsatellite markers (SSR); and iii) evaluate the efficiency of these markers in the distinction among species of *Capsicum* spp. We noticed that pun1[1] and SNP molecular markers were efficient in predicting the pungent phenotype of BGC-UFSCar accessions in 84.85% and 95.59%, respectively. From a total of 13 amplified microsatellite markers, seven were polymorphic and efficient to discriminate species of *Capsicum* genus, both through genetic diversity analysis and population structure analysis, which showed three subpopulations. The molecular markers used in this study are useful tools for breeding programs since they were able to characterize and discriminate *Capsicum* spp. species at DNA level. Information obtained with molecular markers can assist in the selection of contrasting parents for future breeding programs.

Keywords: *Capsicum* spp., SSR, germplasm, dissimilarity, polimorphism.

RESUMO

Caracterização de acessos de pimenta através de marcadores moleculares associados com pungência e SSR

Pimentas do gênero *Capsicum* possuem grande importância socioeconômica, sendo a pungência seu principal atrativo. A caracterização da pungência, as estimativas de distâncias genéticas e a análise da estrutura populacional entre os acessos pertencentes a Bancos de Germoplasma são importantes para seleção de genitores que permitam obtenção de progênies superiores. Assim, os objetivos deste estudo foram: i) avaliar 81 acessos do Banco de Germoplasma de *Capsicum* spp. da Universidade Federal de São Carlos (BGC-UFSCar) com marcadores moleculares relacionados a pungência; ii) estimar a diversidade genética entre os acessos do BGC-UFSCar por meio de marcadores microssatélites (SSR) e iii) avaliar a eficiência desses marcadores na distinção das espécies do gênero *Capsicum*. Os marcadores moleculares pun1[1] e SNP foram eficientes em predizer o fenótipo pungente dos acessos do BGC-UFSCar em 84,85% e 95,59%, respectivamente. Do total de 13 marcadores microssatélites amplificados, sete foram polimórficos e eficientes para distinguir as espécies, tanto através da análise de diversidade genética como da análise de estrutura populacional, as quais indicaram três subpopulações. Os marcadores moleculares utilizados no presente estudo são ferramentas úteis para programas de melhoramento, pois foram capazes de caracterizar e discriminar a nível de DNA as espécies de *Capsicum* spp. A informação obtida com os marcadores moleculares pode auxiliar na seleção de genitores contrastantes para futuros programas de melhoramento.

Palavras-chave: *Capsicum* spp., SSR, germoplasma, dissimilaridade, polimorfismo.

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Peppers of *Capsicum* genus belong to Solanaceae family, which also includes other vegetables such as potatoes, tomatoes and eggplants, being of great socioeconomic importance (Finger & Pereira, 2016). Pepper fruits show great diversity of color, aroma and flavor, being the pungency its main attractiveness (or spicy effect). Capsaicinoid (an alkaloid) is produced, mainly, in the placenta of the fruit and when this placenta suffers any kind of physical damage, these capsaicinoids are released. Pepper has great versatility of uses, for fresh or processed consumption (sauces, condiments, paprika) or as dyes (Rufino & Penteado, 2006).

The *Capsicum* genus consists of over 35 taxa, being five domesticated, ten semi-domesticated and 20 wild species (Bianchetti & Carvalho, 2005; Barboza *et al.*, 2011). The five most used domesticated species are *Capsicum annuum*, *C. frutescens*, *C. pubescens*, *C. chinense* and *C. baccatum*, considering that *C. chinense* and *C. baccatum* are the most popular in Brazil, due to adaption to the weather conditions of this country (Finger & Pereira, 2016). Peppers of *Capsicum* genus are diploids, and most members of the family have the same chromosome number (2n = 2x = 24), but it is possible to find some wild species

with 2n = 2x = 26 (Ahn *et al*., 2018; Souza *et al.*, 2011).

In addition to different pungency levels, the market demands cultivars with higher productivity and quality, especially in relation to resistance to pests and diseases, since pepper plants are susceptible to diseases caused by virus, fungi, bacteria and nematodes, resulting in significant losses (Bianchetti & Carvalho, 2005). Using some tools from molecular biology, aiming to relate DNA to phenotype, may contribute to develop new pepper cultivars which present higher productivity indexes and characteristics of economic valuation. So, molecular markers are of great importance in researches which subsidize pepper genetic improvement programs (Lee *et al*., 2016a). Molecular markers directly access genetic information and are useful to estimate genetic diversity, assisted screening, genetic mapping, and parental identification, among others (Rodrigues *et al*., 2016; Ahn *et al*., 2018). Among available molecular markers, microsatellite markers, also found in literature as SSR (*Simple Sequence Repeat*) or STR (*Short Tandem Repeat*), are widely used in genetic analyses of plants, since they show considerable advantages such as codominance, multiallelism, high frequency and random distribution in the genome, and high level of polymorphism (Rai *et al*., 2013; Buso *et al*., 2016). SSR markers are characterized by sequences, also called motifs, from one to six nucleotides, which are repeated in tandem, considering that the polymorphism produced consists of the difference in the number of replicates of motifs in each allele (Buso *et al*., 2016). Molecular markers associated with pungency in *Capsicum* spp. had already been described in literature. The pun1[1] locus is the main gene related to biosynthesis of capsaicinoids and the only one known so far, which shows quantitative effect on pungency accumulation (Stellari *et al*., 2010; Wyatt *et al*., 2012).

Molecular characterization of the accessions which are part of the Germplasm Banks, as well as the determination of genetic diversity and/or distances between them, is an essential activity in the management of these collections aiming to identify and select individuals with characteristics of economic interest, since this characterization consists of evaluating data to describe, identifying and differentiating accessions within species, classes or categories (Sudré *et al.,* 2010). In molecular terms, the characterization consists of identifying variations in DNA or specific gene sequences (Buso *et al*., 2016). Thus, the aims of this study were: i) evaluate accessions of *Capsicum* spp. Germplasm Bank of Universidade Federal de São Carlos (BGC-UFSCar) with molecular markers related to pungency; ii) analyze the genetic diversity in accessions using SSR markers and iii) evaluate the efficiency of these markers in the distinction among the species of *Capsicum* spp.

MATERIAL AND METHODS

Plant material

The authors used 81 accessions of *Capsicum* spp., consisting of 38 *C. annuum*, 18 *C. baccatum* var. *pendulum*, one *C. baccatum* var. *praetermissum* and 24 *C. chinense* (Table 1). The accessions are part of the *Capsicum* spp. Germplasm Bank of Universidade Federal de São Carlos (BGC-UFSCar), located at Centro de CiênciasAgrárias, in Araras-SP. BGC-UFSCar has accessions from different regions of Brazil. The accessions used in this study were obtained through self-fertilization during maintenance of BGC-UFSCar. To produce seedlings, each accession was sown in trays with 200 cells, filled with Bioplant® substrate. After 45 days, seedlings were transplanted into 2-L pots and kept for five months in a greenhouse under fertigation system.

DNA extraction and quantification

DNA was extracted from *Capsicum* spp. Accession's leaf tissue following the methodology described by Al-Janabi *et al*. (1999), with minor modification. Four grams of leaf tissue was macerated in 5 mL homogenization buffer (Tris HCl, pH 8, 200 mM; EDTA, pH 8, 50 mM; NaCl 2.2 M; CTAB 2%; Na_2SO_3 0.06%). We transferred approximately 3.5 mL macerated material into a Falcon tube and added the same amount of extraction mix (PVP 10%; N-Lauroyl-sarcosine 5%; CTAB 20%) previously heated at 65°C for 1 h. The materials were suspended with a vortex stirrer, and heated in water bath at 65°C for 90 minutes. Then, 7 mL phenol + CIA (25 phenol: 24 chloroform: 1 isoamyl alcohol) was added and vigorously mixed for 2 minutes. The samples were centrifuged at 3,000 rcf, at 4°C, for 10 min. About 7 mL supernatant was removed and 5.6 mL ice cold isopropanol and 1.4 mL 5 M NaCl was added. Samples were lightly shaken and taken to the freezer at -20°C for 1 h. Afterwards, they were centrifugated at 3,000 RPM, 4°C, for 10 minutes. Supernatant was removed and pellet washed twice with 500 µL 70% ice-cold ethanol. Samples were resuspended in TE (1x) with RNAse (10 mg/mL), put in water bath at 37°C for 1 h and, then, transferred into microtubes identified and stored in a freezer at -20°C.

DNA was quantified by agarose gel electrophoresis 1% (100 V for 1 h), using, for comparative purposes, known concentrations of phage lambda DNA (λ). Samples were stained with ethidium bromide (10 mg/mL) and extracted DNA visualized under ultraviolet light. After quantification, samples were kept in the freezer at -20ºC.

Amplification of microsatellite markers

First, the authors selected 25 pairs of primers (CaES0089, CaES0425, CaES1003, CaES1027, CaES1112, CaES1711, CaES1811, CaES2027,CaES2332, CaES2489, CaES2505, CaES2655, CaES2865, CaES2930, CaES3538, CaES3862, CaES3958, CaES4192, CaES4410, CaES4584, CaES4597, CaES4666, CaES4787, CaES5253, CaES5392), developed by Shirasawa *et al*. (2013), using *C. annuum* microsatellite regions. These primers were selected according to Polymorphism Information Content (PIC), with values varying from 0.50 to 0.89. Polimerase Chain Reaction was done in a 15 µL final volume containing PCR buffer (1X), 0.2 mM of each dNTP, 3 mM $MgCl_2$, 0.8 µM of each primer, 2 units Taq DNA polymerase

(Invitrogen®), 50 ng genomic DNA and ultrapure water. Amplification conditions were performed according to Sato *et al*. (2005). The amplified products were separated in 6% polyacrylamide gel at 65 W for 3 h. To estimate the size of amplified fragments, a 10 bp molecular size standard (Invitrogen®) was used.

Analysis of DP and PIC values of microsatellite markers

Discriminatory Power (DP) was estimated according to Tessier *et al*. (1999). PIC was calculated using PIC calculator (Jan, 2002), according to the equation:

$$PIC = 1 - \sum(p_i)^2$$

Where pi is the frequency of each allele per locus.

Pungency analysis: sensory evaluation and molecular markers

Sensory analyses of pungency in *Capsicum* spp. fruits were done according to Pereira *et al*. (2015). Two specific molecular markers to determine pungency were evaluated: pun1[1] (specific for *C. annuum*) and SNP (identifies pungency in several *Capsicum* species). Marker amplifications (reagent concentrations and thermocycler programming) and the analysis of the obtained fragments were performed according to Pereira *et al*. (2015). As a negative control of pungency, tomato (Santa Clara), eggplant (F_1 Ciça) and okra (Santa Cruz 47) samples were used.

Analysis of genetic diversity and population structure

A binary matrix was built, considering genotyping data of SSR, pun1[1] and SNP markers, in order to evaluate genetic diversity and population structure. Jaccard coefficient was used to estimate genetic distance between the used accessions. This coefficient was calculated using *vegan* package, *vegedist* function (Oksanen *et al*., 2018), available R software (R Development Core Team, 2013). Clustering was carried out through UPGMA (Unweighted Pair-Group Method with Arithmetic Mean) through the *hclust* function of the standard package stats in the R software, which allows visualize clustering through a dendrogram. We also analyzed 1000 bootstrap replicates using the *boot*. *phylo* function in the *ape* package (Paradis *et al*., 2004) and the number of subpopulations was verified using Mojena method (1977), both in software R. Population structure analysis was performed using Bayesiano model with the aid of Structure software version 2.3.4 (Pritchard *et al*., 2000). MCMC method (Markov Chain Monte Carlo) was used and a model with mixture of correlated alleles to evaluate the number of subpopulations (*K*), which varied from 2 to 10. The best probability for K was determined after five independent races. Each race consisted of one burn-in period of 50,000 steps followed by 100,000 MCMC replicates. We used Structure Harvester software, following the methodology proposed by Evanno *et al.* (2005), to compare the results associated to each K value obtained by using Structure and also the maximum value of Δk to identify the number of subpopulations which better describe data. Structure Plot software version 2.0 (Ramasamy *et al*., 2014) was used to build a bar graphic, which shows the division of accessions into subpopulations obtained with the aid of Structure software.

RESULTS AND DISCUSSION

Amplification and polymorphism of microsatellite markers

A total of 25 SSR markers was selected in literature, 13 were amplified, of which seven were polymorphic. The seven polymorphic SSR markers indentified a total of 17 alleles in pepper accessions, considering that for each locus the number of alleles ranged from two (CaES0425, CaES1811, CaES2027, CaES2865 and CaES4192) to four (CaES2332), with an average of 2.43 alleles per locus (Table 2).

According to the classification described by Botstein *et al*. (1980), markers with PIC values above 0.50 are considered to be highly informative, from 0.25 to 0.50 can be considered medially informative and below 0.25 are essentially not informative. Considering the seven polymorphic SSR markers, two were considered "not informative" (CaES4192 and CaES0425), four were considered medially informative (CaES1811, CaES2027, CaES2505 and CaES2865), and one showed to be highly informative (CaES2332), being 0.34 the average value (Table 2). In addition, DP ranged from 0.06 (CaES4192) to 0.57 (CaES2332), with an average value of 0.40 (Table 2). The PIC and DP values observed in this study are close to values reported in literature (Zhang *et al*., 2016; Lee *et al*., 2016a).

Sensory and molecular analysis of pungency

No discordance among the three panelists to determine pungency for evaluated accessions was noticed. Sensory analysis of the fruits showed that 83.95% (68) and 16.05% (13) of all the accessions were pungent and non-pungent, respectively (Table 1). Taking into consideration the representative accessions of *C. annuum*, *C. chinense* and *C. baccatum* var. *pendulum,* including Cumari pepper (*C. baccatum* var. *praetermissum*) were pungent 86.80%, 66.70% and 100%, respectively. These results show the sets of evaluated accessions and, a comparison with other collections may lead to biased results. However, these varying levels of pungency for accessions of *C. annuum*, high and extremely high levels for accessions of *C. chinense* and high level for *C. baccatum* var. *pendulum* (Aji) were reported by Guzmán & Bosland (2017), showing that besides presence or absence, pungency can also be evaluated by intensity.

In molecular evaluation using SNP marker, observed by tetra-primer ARMS-PCR method, three fragments (191 pb, 134 pb and 108 pb) were amplified. Fragment 108 pb corresponded to allele T and the fragment 134 pb represented allele G in pungent and non-pungent accessions, respectively. Finally, fragment 191 pb was common to all accessions. In *C. annuum*, the species-specific locus pun1[1], presented two fragments, 1063 pb and 746 pb, which showed pungency and no pungency, respectively (Table 3).

SNP marker showed high efficiency in predicting pungency (95.59%). Of

Table 1. Evaluation of pungency of 81 *Capsicum* accessions belonging to *Capsicum* spp. Germplasm Bank of Universidade Federal de São Carlos (BGC-UFSCar), using sensory method, and pun1[1] and SNP molecular markers (tetra-primer ARMS-PCR). Araras, UFSCar, 2018.

Accession	Species	Origin	**SNP	*pun 1[1]	Sensory analysis
CCA 1	*C. annuum*	Philipines	P (T)	P	P
CCA 2	*C. annuum*	Philipines	P (T)	P	P
CCA 8	*C. annuum*	Brazil	P (T)	P	P
CCA 13	*C. annuum*	Brazil	NP (G)	NP	NP
CCA 17	*C. annuum*	Brazil	NP (G)	NP	NP
CCA 20	*C. annuum*	Brazil	NP (G)	NP	NP
CCA 29	*C. annuum*	Brazil	NP (G)	P	NP
CCA 34	*C. annuum*	Mexico	NP (G)	NP	P
CCA 36	*C. annuum*	Brazil	P (T)	P	P
CCA 99	*C. annuum*	Brazil	P (T)	P	P
CCA 105	*C. annuum*	Brazil	NP (G)	NP	P
CCA 3	*C. annuum*	Philipines	NP (G)	NP	P
CCA 5	*C. annuum*	Brazil	P (T)	P	P
CCA 11	*C. annuum*	USA	P (T)	P	P
CCA 19	*C. annuum*	Italy	NP (G)	NP	P
CCA 23	*C. annuum*	Peru	NP (G)	P	P
CCA 27	*C. annuum*	Bolivia	NP (G)	P	P
CCA 39	*C. annuum*	Brazil	P (T)	P	P
CCA 40	*C. annuum*	Brazil	P (T)	P	P
CCA 50	*C. annuum*	Brazil	NP (G)	P	P
CCA 71	*C. annuum*	Brazil	P (T)	P	P
CCA 74	*C. annuum*	Brazil	P (T)	P	P
CCA 77	*C. annuum*	Brazil	NP (G)	P	P
CCA 90	*C. annuum*	Colombia	NP (G)	P	P
CCA 102	*C. annuum*	Mexico	P (T)	P	P
CCA 134	*C. annuum*	Brazil	P (T)	P	P
CCA 338	*C. annuum*	Argentina	NP (G)	NP	P
Mini Pimentão	*C. annuum*	USA	NP (G)	NP	NP
Criollo de Morelos	*C. annuum*	Mexico	P (T)	P	P
CCA 535	*C. annuum*	Brazil	P (T)	P	P
CCA 560	*C. annuum*	Brazil	P (T)	P	P
F1 48	*C. annuum*	China	P (T)	P	P
F1 49	*C. annuum*	China	P (T)	P	P
F1 53	*C. annuum*	China	NP (G)	NP	P
F1 60	*C. annuum*	China	P (T)	P	P
F1 63	*C. annuum*	China	P (T)	P	P
F1 67	*C. annuum*	China	P (T)	P	P
F1 68	*C. annuum*	China	P (T)	P	P
CCA 528	*C. baccatum*	Colombia	P (T)	-	P
CCA 544	*C. baccatum*	Brazil	P (T)	-	P
CCA 424	*C. baccatum*	Brazil	P (T)	-	P
CCA 113	*C. baccatum*	Brazil	P (T)	-	P
CCA 530	*C. baccatum*	Brazil	P (T)	-	P
CCA 112	*C. baccatum*	Peru	P (T)	-	P
CCA 415	*C. baccatum*	Brazil	P (T)	-	P
CCA 471B	*C. baccatum*	Brazil	P (T)	-	P

Table 1. Continued

Accession	Species	Origin	**SNP	*pun 1[1]	Sensory analysis
CCA 471B	*C. baccatum*	Brazil	P (T)	-	P
CCA 109	*C. baccatum*	Peru	P (T)	-	P
CCA 110	*C. baccatum*	Brazil	P (T)	-	P
CCA 114	*C. baccatum*	Brazil	P (T)	-	P
CCA 115	*C. baccatum*	Brazil	P (T)	-	P
CCA 122	*C. baccatum*	Peru	P (T)	-	P
CCA 175	*C. baccatum*	Colombia	P (T)	-	P
CCA 181	*C. baccatum*	USA	P (T)	-	P
CCA 404	*C. baccatum*	Brazil	P (T)	-	P
CCA 527	*C. baccatum*	USA	P (T)	-	P
CCA 548	*C. baccatum*	USA	P (T)	-	P
Cumari	*C. baccatum*var. *praetermissum*	Brazil	P (T)	-	P
Aji Cristal	*C. baccatum*	Brazil	P (T)	-	P
ButhJolokoia	*C. chinense*	Brazil	P (T)	-	P
Murupi	*C. chinense*	Brazil	P (T)	-	P
CCA 124	*C. chinense*	Brazil	P (T)	-	P
CCA 144	*C. chinense*	Brazil	P (T)	-	P
CCA 504	*C. chinense*	Brazil	P (T)	-	P
CCA 518	*C. chinense*	Brazil	P (T)	-	P
Biquinho	*C. chinense*	Brazil	P (T)	-	NP
CCA 561	*C. chinense*	Brazil	P (T)	-	P
CCA 563	*C. chinense*	Brazil	P (T)	-	P
CCA 507	*C. chinense*	Brazil	P (T)	-	P
CCA 177	*C. chinense*	Brazil	P (T)	-	P
CCA 151	*C. chinense*	Brazil	P (T)	-	P
CCA 150	*C. chinense*	Brazil	P (T)	-	P
CCA 148	*C. chinense*	Brazil	P (T)	-	P
CCA 140	*C. chinense*	Brazil	P (T)	-	P
313605	*C. chinense*	Brazil	P (T)	-	NP
37701	*C. chinense*	Brazil	P (T)	-	P
313603	*C. chinense*	Brazil	P (T)	-	NP
312804	*C. chinense*	Brazil	P (T)	-	NP
3SN01	*C. chinense*	Brazil	P (T)	-	NP
F1 10	*C. chinense*	Brazil	P (T)	-	NP
F1 12	*C. chinense*	Brazil	P (T)	-	P
F1 23	*C. chinense*	Brazil	P (T)	-	P

*Specific marker for *Capsicum annuum*; **SNP marker (tetraprimer ARMS-PCR) suitable for all species of *Capsicum* genus: pungent (P) and non-pungent (NP).

68 pungent accessions, in sensorial analysis, 65 had allele T identified using SNP marker (Table 3). The pun1[1] marker, specific for *C. annuum*, showed efficiency in predicting pungent phenotype of 84.85% (Table 3). On the other hand, the non-pungent phenotype prediction was 81.25% and for SNP and pun1[1] marker 50%, respectively (Table 3). Therefore, two evaluated molecular markers had high association between pungent phenotype and pungent alleles, being SNP marker also efficient in predicting non-pungent phenotype. These results are in accordance with the ones observed by Pereira *et al*. (2015).

All *C. baccatum* var. *pendulum* accessions and Cumari pepper (*C. baccatum* var. *praetermissum*) were characterized as pungent using sensory analysis and molecular evaluation through SNP marker (Table 1). On the other hand, all the 23 *C. chinense* accessions were pungent for SNP marker, six accessions (Biquinho, 313605, 313603, 312804, 3SN01 and F1 10) did not obtain pungency in

sensory analysis, though (Table 1). This result possibly shows that, despite the presence of SNP marker linked to pungency, the expression level is low, making pungency imperceptible in sensory analysis. According to Stellari *et al*. (2010), pungency is only detectable via sensory analysis when levels are higher than 10 ppm. In addition, some *Quantitative Trait Loci* studies are attributed to the existence of a polygenic complex which would regulate pungency in *Capsicum* spp. (Yarnes *et al*., 2013; Lee *et al*., 2016b).

In *C. annuum*, the relation between the results of molecular markers and sensory analysis varied according to the marker used in the study (Table 1). Considering the associations between SNP marker and sensory analysis, and between pun1[1] marker and sensory analysis, the same response pattern was obtained for 65.78% and 78.94% of the accessions, respectively. This result shows that, for *C. annuum,* pun1[1] marker was the most efficient in association between marker and phenotype. Despite this, SNP and pun1[1] markers showed the same molecular response pattern for 84.21% of *C. annuum* accessions (Table 1). In cases of discordance between phenotype and molecular information, when pungency is identified by the panelist but no allele marker associated with pungency is verified (for instance, in accessions CCA 34, CCA 105, CCA 3, CCA 19, CCA 338, and F1 53 for both markers, and in accessions CCA 23, CCA 27, CCA 50, CCA 77, CCA 90 exclusively for SNP marker), genes of small effect related to metabolic pathways for pungency may cause the pungency identified by the panelist or, some mistake during the sensory analyses conduction. Thus, the authors point out that biochemical analyses, for determining capsaicionoids concentration, should be done and mapped in *Capsicum* genome in future studies.

Genetic diversity and population structure

Genetic diversity among the accessions, estimated using Jaccard coefficient, was represented by the dendogram (Figure 1), which indicated the formation of three subpopulations according to Mojena method (1977). The three subpopulations were composed of accessions of *C. annuum*, *C. chinense* and *C. baccatum*, respectively.

The average value of genetic distances between *C. baccatum* var. *pendulum* and other two evaluated species, 0.68 with *C. annuum* and 0.64 with *C. chinense*, were greater than the average value of genetic distance between *C. annuum* and *C. chinense* (0.59). These results corroborate Pickersgill (1997) who divides *Capsicum* gender peppers, according to cross breeding among species, in three complexes: *C. annuum* complex, *C. baccatum* complex and *C. pubescens* complex, considering that *C. annuum* complex also comprises *C. chinense* species, justifying the greater proximity between these species than when it is related to *C. baccatum.* According to Martins *et al.* (2015) and Lee *et al*. (2016a), among the domesticated species, *C. chinense* shows better crossing ability with *C. annuum* and is used as a bridge between *C. annuum* and other species. In addition, according to Tong & Bosland (1999), *C. baccatum* var. *pendulum* cultivars only cross

Table 2. Polymorphic information content (PIC), discriminatory power (DP) and number of alleles of the seven SSR loci analyzed in 81 *Capsicum* accessions belonging to *Capsicum* spp. Germplasm Bank of Universidade Federal de São Carlos (BGC-UFSCar). Araras, UFSCar, 2018.

SSR markers	PIC	DP	Number of alleles
CaES0425	0.12	0.13	2
CaES1811	0.46	0.56	2
CaES2027	0.44	0.53	2
CaES2332	0.50	0.57	4
CaES2505	0.35	0.42	3
CaES2865	0.46	0.55	2
CaES4192	0.06	0.06	2
Total			17
Average	0.34	0.40	2.43
Min - Max	0.06 – 0.50	0.06 – 0.57	2 – 4

Table 3. Association between sensory analysis and molecular markers for determining pungency in 81 *Capsicum* accessions belonging to *Capsicum* spp. Germplasm Bank of Universidade Federal de São Carlos (BGC-UFSCar). Araras, UFSCar, 2018.

SNP marker (Tetra-primer ARMS PCR) (*Capsicum* spp.)					
Pungent accessions (P)			Non-pungent accessions (NP)		
Sensory analysis	Molecular analysis[1]	Marker prediction*	Sensory analysis	Molecular analysis[3]	Marker prediction*
68	65	95.59%	13	16	81.25%
pun1[1] marker (*Capsicum annuum*)					
33	28	84.85%	5	10	50.00%

*Percentage of association between sensory analysis and molecular markers; [1]Presence of 108 bp fragments related to SNP marker (tetra-primer ARMS-PCR); [2]Presence of 1063 bp fragments related to pun11 marker; [3]Presence of 134 bp fragments related to SNP marker (tetra-primer ARMS-PCR);[4]Presence of 746 bp fragments related to pun11 marker.

among each other or with *C. tovarii*, highlighting the greater genetic distance between *C. baccatum* var. *pendulum* in relation to other species.

Considering intraspecific genetic distances, the authors observed a greater diversity among *C. annuum* accessions, whereas *C. baccatum* var. p*endulum* was the species which presented minor distinction between its accessions (Figure 1). The greatest diversity among *C. annuum* accessions observed in this study, combined with the low diversity of *C. chinense* and *C. baccatum,* may be due to some factors, such as: i) the SSR markers used in this study were developed for *C. annuum* (Shirasawa *et al*., 2013) and/or ii) the small quantity of markers used in this study (Table 3) made greater interspecific distinction of *C. chinense* and *C. baccatum* impossible.

Population structure analysis showed that the best genetic structure of the 81 *Capsicum* spp. accessions was the division into three subpopulations (K= 3). The distribution of the accessions of BGC-UFSCar in each subpopulation can be verified in Figure 2. Evaluating estimated ancestry of each accession in each subpopulation, using the y-axis, the authors verified that *C. baccatum* var. *pendulum* accessions do not present significant contribution from other species, corroborating the analysis of dendogram (Figure 1), which shows *C. baccatum* var. *pendulum* accessions in a group isolated from the others. Few accessions showed overlapping; partially belonging to two or three subpopulations, as inferred by the proportion of their genomes assigned to each subpopulation. In addition, as well as in dendrogram, population structure analysis also showed an accession grouping according to the species which they belong. We observed that *C. baccatum*, *C. annuum* and *C. chinense* accessions formed three subpopulations (Figure 2). An only exception to this pattern was verified for CCA 102 accession, which belongs to *C. annuum* but was grouped with *C. Chinense* accessions. Genetic proximity between *C. annuum* and *C. Chinense* was observed in other studies (Martins *et al*., 2015; Lee *et al*., 2016a) and overlapping among subpopulations shows that an exchange of genetic material among accessions through breeding or natural recombination may have happened (Zhang *et al*., 2016).

In conclusion, in this study, we observed the efficiency of SSR markers in differentiating species of *Capsicum* gender, but little ability to detect intraspecific variability when *C. chinense* and *C. baccatum* species are taken into consideration. Molecular markers linked to pungency, pun1[1] and SNP, efficiently predicted pungent phenotype

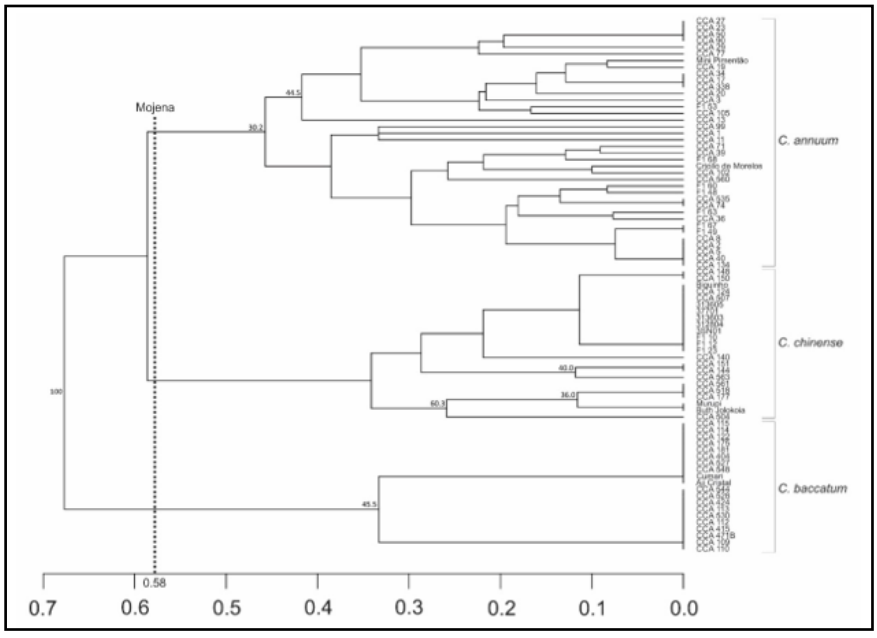

Figure 1. Dendrogram obtained by the UPGMA method representing genetic distances, estimated by Jaccard coefficient, among 81 *Capsicum* accessions belonging to *Capsicum* spp. Germplasm Bank of Universidade Federal de São Carlos (BGC-UFSCar), based on SSR, SNP and pun1[1] molecular markers. In the figure: i) the subpopulations through Mojena method (1977) and; ii) bootstrap values above 30% at corresponding nodes. The accessions of species *C. annuum*, *C. chinense* and *C. baccatum* var. *pendulum* are indicated by brackets. Araras, UFSCar, 2018.

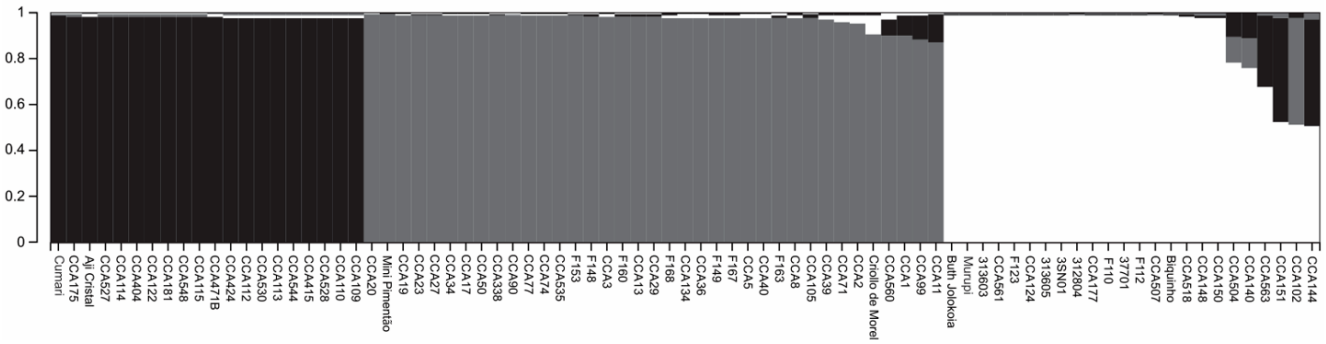

Figure 2. Population structure of 81 *Capsicum* accessions belonging to *Capsicum* spp. Germplasm Bank of Universidade Federal de São Carlos (BGC-UFSCar), evaluated through Structure software based on SSR, SNP and pun1[1] molecular markers. The three subpopulations detected in the study are represented by different colors. Araras, UFSCar, 2018.

of accessions of BGC-UFSCar in 84.85% and 95.59%, respectively, considering these markers useful tools for *Capsicum* spp. breeding programs, which aim to develop cultivars in the presence or absence of pungency. This study is able to help pepper breeding programs, since characterizing pungency, knowing population structure and genetic distances among the accessions of BGC-UFSCar making it possible to select discrepant parents which may provide the generation of more productive cultivars.

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