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 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, polymorphism.

**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 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.

**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 progêniuses 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 microsatélites (SSR) e iii) avaliar a eficiência desses marcadores na distinção das espécies do gênero *Capsicum*. Os marcadores moleculares pun1 e SNP foram mais eficientes em predizer o fenótipo pungente dos acessos do BGC-UFSCar em 84,85% e 95,59%, respectivamente. Do total de 13 marcadores microsatélites amplificados, sete foram polimórficos e eficientes para distinguir as espécies, tanto através da análise de diversidade genética como da análise da 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.
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., 2016). 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 pun$^1$ 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 Ciências Agrárias, 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$_2$SO$_4$ 0.06%). We transferred approximately 3.5 mL macerated material into a Falcon tube and added the same amount of extraction mix (PV 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 isomyl 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 -20°C.

**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.
Considering genotyping data of SSR, population structure of okra (Santa Cruz 47) samples were used. Two specific molecular markers to identify pungency in several Capsicum species were used. Marker amplifications were performed according to Pereira et al. (2015). As a negative control of pungency, tomato (Santa Clara), eggplant (F. Ciça) and okra (Santa Cruz 47) samples were 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:

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

Where $p_i$ 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: pun1¹ (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. 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, pun1¹ 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, vegenist 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 Structure software. Population structure analysis was performed using Bayesian 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 $ak$ 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 identified 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). 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 pun1¹, 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<sup>1</sup> and SNP molecular markers (tetra-primer ARMS-PCR). Araras, UFSCar, 2018.

| Accession | Species | Origin | **SNP** | *pun 1<sup>1</sup> | Sensory analysis |
|-----------|---------|--------|---------|------------------|-----------------|
| CCA 1     | *C. annuum* | Philippines | P (T) | P | P |
| CCA 2     | *C. annuum* | Philippines | 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* | Philippines | 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 |
68 pungent accessions, in sensorial analysis, 65 had allele T identified using SNP marker (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

| Accession | Species | Origin | **SNP** | *pun* | 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).*
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 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, pun11 marker was the most efficient in association between marker and phenotype. Despite this, SNP and pun1 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 capsaicinoids 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.

| 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.

| Molecular analysis¹ | Marker prediction* |
|---------------------|-------------------|
| 68                  | 65                |
| 95.59%              |                   |

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

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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. *pendulum* 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 dendrogram (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 and SNP, efficiently predicted pungent phenotype

![Figure 1](image1.png)

**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 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](image2.png)

**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 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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