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Transferability of microsatellite markers of *Capsicum annuum* L. to *C. frutescens* L. and *C. chinense* Jacq.

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ABSTRACT. In order to support further genetic, diversity, and phylogeny studies of *Capsicum* species, the transferability of a *Capsicum annuum* L. simple sequence repeat (SSR) microsatellite set was analyzed for *C. frutescens* L. ("malagueta" and "tabasco" peppers) and *C. chinense* Jacq. (smell peppers, among other types). A total of 185 SSR primers were evaluated in 12 accessions from 115 *C. frutescens* L. and 480 *C. chinense* Jacq, representing different types within each species. Transferability to *C. frutescens* L. and *C. chinense* Jacq. occurred for 116 primers (62.7%). Nineteen (16.37%) were polymorphic in *C. frutescens* L. and 36 (31.03%) in *C. chinense* Jacq., 17 of which were coincident and could be used to analyze samples obtained for the 2 species. Among these primers, CA49 showed a different amplitude range of alleles between the 2 species (130-132 base pairs for *C. frutescens* L. and

120-128 base pairs for *C. chinense* Jacq.), and could differentiate the species. A total of 55 alleles were identified among the 19 polymorphic SSR loci among accessions of *C. frutescens* L., with the number of alleles per locus ranging from 2 to 5, a mean of 2.89, and the polymorphic information content ranging from 0.30 to 0.65. The number of alleles identified in *C. chinense* Jacq. was 119, ranging from 2 to 5 alleles per locus, an average of 3.30, and polymorphic information content from 0.19 to 0.68. The *C. annuum* L. SSR primers were most often transferable and polymorphic for *C. frutescens* L. and *C. chinense* Jacq., and we present a set of SSR for each species.

Key words: Breeding; Germplasm bank; Pepper; Polymorphism; Simple sequence repeat

INTRODUCTION

Peppers (*Capsicum*) are part of the cultural wealth and valuable genetic heritage of the Brazilian biodiversity (Reifschneider et al., 2000). They are grown throughout the country and they include a variety of types, names, sizes, colors, flavors, and pungency. Peppers are cultivated by small, medium, and large producers by family farmers and smallholder agribusiness as an alternative income source. The annual cultivated area for peppers in Brazil is approximately 5000 hectares, yielding approximately 75,000 Mg. The average yield depends on the type of pepper, ranging from 10-30 Mg/ha (Ribeiro et al., 2008).

The genus *Capsicum* includes approximately 35 *taxa* (species and varieties) that are grouped into 3 categories according to the level of domestication, corresponding to 5 domesticated and 30 wild taxa (Bianchetti and Carvalho, 2005). Domesticated species include *Capsicum chinense* Jacq., *C. frutescens* L., *C. annuum* L., *C. pubescens* Ruiz et Pav, and *C. baccatum* L. (Moses et al., 2014). Among these, *C. annuum* L., *C. chinense* Jacq. and *C. frutescens* L. have traditionally been treated as a species complex because of the high genetic and morphological closeness and they are often treated as the same species based on immediate ancestor identification (Pickersgill, 1988). Thus, *C. chinense* Jacq. is easily confused with *C. frutescens* L. accessions; the main morphological distinction between them is the presence of calyx annular constriction, found only in the fruits of *C. chinense* Jacq. (Carvalho et al., 2006).

The fruits of *C. frutescens* L. are generally small, upright, spindle-shaped, red-colored at the mature stage, have very thin walls, are spicy, and the calyx does not have annular constriction. *C. frutescens* L. also presents much lower morphological variability than other domesticated species (DeWitt and Bosland, 2009). The most common types of this species include the "malaguetas" in Brazil and tabascos in the United States.

C. chinense Jacq. presents great variability expressed in the diversity of shapes and colors of fruits, which ranges from light yellow, strong yellow, orange, salmon, to red and are typically very spicy and aromatic. There are many types in this species, with the best known including the smell peppers, Murupi, goat, cumari of Pará, small beak, and Habanero (Ribeiro et al., 2008).

In the characterization of the *Capsicum* Germplasm Bank (BG *Capsicum*) of Embrapa, some *C. chinense* Jacq. taxonomic classifications were unresolved, as they presented fruit traits typical of *C. frutescens* L., despite presenting the calyx annular constriction, a major distinctive trait of *C. chinense* Jacq. Thus, molecular tools should be used to achieve more accurate classification.

Among the different molecular markers, simple sequence repeat (SSR) microsatellites

are suitable for detailed genomic analysis. However, for the amplification of SSR loci, knowledge of its DNA sequence is required, implying that the development of specific primers for this type of analysis is a costly and time-consuming process (Zucchi et al., 2003). However, several studies indicate similarity in DNA sequences located in regions at the edges of repetitive sequences, constituents of SSRs, among different species (Ciampi et al., 2008), indicating that these SSR primers can be transferred between related species. This greatly reduces the cost and time required for research (Lorieux et al., 2000).

Several studies have shown high transferability of SSR markers for species within the same genus, including *Cucumis* (Ritschel et al., 2004), *Arachis* (Bravo et al., 2006), and even for *Capsicum* (Yi et al., 2006; Nagy et al., 2007; Ince et al., 2010a). However, the success of this approach depends on the species considered, as the probability of obtaining transferability is inversely proportional to the evolutionary distance among them (Cota et al., 2012).

In this study, we examined the transferability of a *C. annuum* L. set of primers (Buso et al., 2000) for *C. frutescens* L. and *C. chinense* Jacq. to correctly identify accessions from the 2 species using materials stored in the BG *Capsicum* of Embrapa to contribute to the conservation and use of this germplasm.

MATERIAL AND METHODS

Plant material

Twelve accessions representing different types within each species (Table 1) were selected from a collection of 115 accessions of *C. frutescens* L. and 480 *C. chinense* Jacq. of the BG *Capsicum* of Embrapa, based on morphological characterization made by applying 5 internationally recommended descriptors for *Capsicum* fruits (IPGRI, 1995): color at mature stage, shape, position, calyx annular constriction, and wall thickness. The genotypes studied included 4 accessions of *C. frutescens* L. and 8 accessions of *C. chinense* Jacq.

Table 1. Fruit traits of accessions representing different types within the species *Capsicum frutescens* L. and *C. chinense* Jacq. from the germplasm bank of Embrapa. Brasília, DF, 2014.

Accession CNPH nº	Species	Type	Fruit				
			CM[a]	Position	Format	EP[b]	PCA[c]
3891	*C. frutescens*	malagueta	orange	standing	oblong	0.4	no
4266	*C. frutescens*	tabasco	red	standing	oblong	0.3	no
4353	*C. frutescens*	malaguetinha	red	standing	oblong	0.5	no
4364	*C. frutescens*	malagueta	red	standing	oblong	0.6	no
4361	*C. chinense*	malagueta	orange	standing	oblong	0.9	yes
4325A	*C. chinense*	similar to malagueta	red	standing	oblong	0.8	yes
4315	*C. chinense*	olho de peixe	red	standing	campanulate	0.5	yes
4316	*C. chinense*	similar to malagueta	orange	pendulous	campanulate	1.8	yes
4327	*C. chinense*	similar to habanero	orange	pendulous	triangular	2.9	yes
4328A	*C. chinense*	similar to bode	red	pendulous	rectangular	3	yes
4332	*C. chinense*	similar to cayenne	red	pendulous	oblong	2.6	yes
4360	*C. chinense*	murupi	yellow	pendulous	oblong	1.2	yes

[a]Fruit color at maturity; [b]wall thickness (mm); [c]presence of annular constriction.

Leaves of each accession were collected and genomic DNA was extracted using the cetyltrimethylammonium bromide method, with some modifications (Ferreira and Grattapaglia, 1998). DNA concentration was estimated by electrophoresis on a 1.0% (w/v) agarose gel stained with ethidium bromide; fluorescence intensities between each DNA were compared

to different patterns of the lambda DNA sample. Each sample was then diluted to 3.0 ng/μL.

Isolation of genomic SSRs from *C. annuum* L.

Approximately 50 μg genomic DNA from an individual *C. annuum* L. plant was digested with *Tsp*509 I, and 280-800-bp fragments were recovered. Approximately 30 μg DNA fragments were ligated to adapters containing the *Tsp*509 I restriction site. The fragments containing SSR sequences were selected by hybridization with biotinylated oligonucleotides complementary to the repetitive sequence AG/CT, and they were recovered using magnetic beads linked to streptavidin. Recombinant clones in plasmid vectors pGEM-T (Stratagene, La Jolla, CA, USA) were transformed into *Escherichia coli* XL1-Blue cells, and colonies containing SSRs were identified by polyAG/TC probe hybridization and anchor polymerase chain reaction (PCR). Positive clones were picked and sequenced using an ABI 377 DNA sequencer (Applied Biosystems, Foster City, CA, USA) using dye terminator fluorescent chemistry. Primers annealing at the SSR flanking regions were designed using the software primer (Lincoln et al., 1991).

Primer screening and PCR

Microsatellite amplification was carried out in a 13-μL reaction mix containing 0.9 μM of each primer, 1 U *Taq* DNA polymerase, 200 μM of each dNTP, 1% (v/v) reaction buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$) 1.3 μL 2.5 mg/mL bovine serum albumin, and 10.0 ng template DNA. Amplification was performed using a PT-100 thermal controller (MJ Research, Waltham, MA, USA) under the following conditions: 2 min 96°C (1 cycle), 1 min 94°C, 1 min 56°C, 1 min 72°C (30 cycles); and 7 min 72°C (1 cycle). Each locus was checked for clear and robust DNA band amplification as well as for product polymorphism. Analysis of amplified fragments was carried out on 1.5% (w/v) agarose gels stained with ethidium bromide and sized by comparison to a 1-kb DNA ladder standard. Next, PCR with primers that amplified visible products were evaluated on a 5% (w/v) polyacrylamide gel stained with silver nitrate following the protocol of Creste et al. (2001).

Data analysis

A total of 185 microsatellite primers of *C. annuum* L. were evaluated. Band-size (alleles) evaluation was carried out with reference to the standard size 10-bp DNA ladder. Bands and their respective sizes were recorded in a spreadsheet and analyzed using the Summary Function of Power Marker statistics software (Liu and Muse, 2005), which accounted for sample size, number of observations (equivalent to the number of genotypes evaluated, subtracted from the number of missing genotypes), number of available genotypes (relative to samples not considered), allele number, frequency of the most abundant allele, gene diversity (probability that 2 randomly chosen alleles were different, also referred to as the index of expected heterozygosity), heterozygosity (observed heterozygosity; proportion of heterozygous individuals in a population), and polymorphic information content (PIC, a measure related to gene diversity) for each locus.

RESULTS

Transferability occurred to *C. frutescens* L. and *C. chinense* Jacq. for 116 (62.7%) of the 185 SSR primers of *C. annuum* L. (CA) analyzed. Microsatellite primers designed for *C.*

annuum L. (Buso et al., 2000) were, therefore, most often transferable to the 2 species. Among the transferable primers, 19 (16.3%) were polymorphic in *C. frutescens* L. (Table 2) and 36 (31.0%) showed polymorphisms for *C. chinense* Jacq. (Table 3).

Table 2. Primers transferred from *Capsicum annuum* L. to *C. frutescens* L. with their motif, sequences of forward and reverse primers, allele number, size range of amplified fragment in base pairs (bp), gene diversity or expected heterozygosity (H_E), observed heterozygosity (H_O), and polymorphism information content (PIC). Brasília, DF, 2014.

Primers (CA)	Motif	Forward primer/ reverse primer (5'-3')	Allele No.	Size range (bp)	Gene diversity (H_E)	Heterozygosity (H_O)	PIC
19	$(TC)_{12}$	CCGCAATGGCAGTATGATCT/ CGGCTCTATCTACAACGGTG	4	98-104	0.69	1.00	0.63
20	$(AG)_{27}$	CCGTAAAGAAATCAAACCAC/ GCATGCACACATAAACACTC	3	68-88	0.63	0.00	0.55
26	$(AG)_{23}$	CGCATATAGGCAGATCAAAT/ TGACTCAAATGCTCTCTGAA	5	100-124	0.69	0.75	0.65
27	$(CA)_{12}(CT)_{17}ATCG(CT)_9$	GCAGAGGACCAGTTAGCATA/ TGTTCTGAGTCCACGATGCT	3	128-132	0.63	0.50	0.55
29	$(AG)_{21}$	TGGGTAAAGGTACTTAGTAG/ GTTGTATTGCTTTAGCTCAG	2	114-126	0.50	0.00	0.38
39	$(CT)_{13}$	CATCCATATATCGATCGGCT/ TTTCGACCATGTTCAGATCC	3	88-98	0.63	0.00	0.55
41	$(AG)_{27}$	GACATCAGTGTTCTGCAAA/ ACACTGGGTATGTTGTTGTA	3	152-172	0.63	0.00	0.55
49	$(AG)_{21}$	CTATCTTCGCATATAGGCAG/ AATCTCTGTGGCTGACTCAA	2	130-132	0.38	0.00	0.30
52	$(GT)_{14}(AG)_{14}TAGC(GA)_{10}$	TAGCAGAGGACCAGTTAGCA/ ATGTTCTGAGTCCACGATGC	3	114-130	0.59	1.00	0.51
56	$(AG)_{23}$	CTTCGCATATAGGCAGATCA/ TCTCTGTGGCTGACTCAAAT	3	110-130	0.41	0.50	0.37
62	$(AG)_{22}$	CGCATATAGGCAGATCAAAT/ GGTCAGACTACGCTCTCTCA	4	78-84	0.66	0.50	0.60
79	$(TC)_{26}$	CACTGGGTATGTTGTTGTAA/ CCGTAAAGAAATCATACCAC	3	90-110	0.63	0.00	0.55
88	$(AG)_{22}$	AATGGATGTTCCCTTGCTTT/ CAACTGATCAACCATTCCGT	2	160-162	0.38	0.00	0.30
96	$(AG)_{23}$	CGCATATAGGCAGATCAAAT/ AATCTCTGTGGCTGACTCAA	3	108-116	0.59	1.00	0.51
159	$(AG)_{19}$	GCAGAAAATGGGTAAAGGTA/ ATTGCTTTAGCTCAGAATGG	3	110-120	0.63	0.50	0.55
167	$(TC)_{24}$	CATTCTCTTCTTCTCAAACG/ CACGTCAATCTGTTGTGAAA	2	80-84	0.38	0.00	0.30
172	$(TC)_9GCTA(TC)_{14}(CA)_{13}$	ATGTTCTGAGTCCACGATGC/ CTTAGCAGAGGGCCAGTTAG	3	120-132	0.59	0.25	0.51
174	$(CT)_{18}$	CCTGCATTCACCATCTAGGA/ GGAGCCTTGCCATAACAGAT	2	252-254	0.50	0.00	0.38
178	$(CT)_{25}$	CCCAACTCATTTAATTCCAC/ CACGTCAATCTGTTGTGAAA	2	160-162	0.38	0.00	0.30
Mean	-	-	2.89		0.55	0.32	0.48

Although the primers that detected polymorphisms between accessions were different within each species, 17 were coincident, meaning that they were polymorphic among the accessions of *C. frutescens* L. and among *C. chinense* Jacq. Thus, considering that the annealing temperature used in this study was standardized at 56°C, these 17 primers can be used to analyze samples obtained for the 2 species. CA49 showed the largest amplitude range of alleles between the 2 species (130-132 bp for *C. frutescens* L. and 120-128 bp for *C. chinense* Jacq.), and could be used to differentiate between these species. The size range of alleles was 68-254 and 68-260 bp for *C. frutescens* L. and *C. chinense* Jacq., respectively.

Table 3. Primers transferred from *Capsicum annuum* L. to *C. chinense* Jacq. with their motif, sequences of forward and reverse primers, allele number, size range of amplified fragment in base pairs (bp), gene diversity or expected heterozygosity (H_E), observed heterozygosity (H_O), and polymorphism information content (PIC). Brasília, DF, 2014.

Primers (CA)	Motif	Forward primer/ reverse primer (5'-3')	Allele No.	Size range (bp)	Gene diversity (H_E)	Heterozygosity (H_O)	PIC
1	$(AG)_{19}$	TGGCATGGTACTTCTTAGCA/ AGACACCAAGCCATCAATTA	4	110-116	0.65	0.88	0.58
11	$(CT)_{12}$	GTTGTTATCTCCTTTTCCCA/ AAATGTTAGGAACTCACCAG	5	78-94	0.66	0.25	0.62
19	$(TC)_{12}$	CCGCAATGGCAGTATGATCT/ CGGCTCTATCTACAACGGTG	2	98-100	0.22	0.25	0.19
20	$(AG)_{27}$	CCGTAAAGAAATCAAACCAC/ GCATGCACACATAAACACTC	3	68-90	0.32	0.13	0.29
24	$(TC)_{11}$	GTTATCTCCTTTTCCCAATC/ AAATGTTAGGAACTCACCAG	5	84-94	0.63	0.25	0.59
26	$(AG)_{23}$	CGCATATAGGCAGATCAAAT/ TGACTCAAATGCTCTCTGAA	4	100-120	0.73	0.63	0.68
27	$(CA)_{12}(CT)_{17}ATCG(CT)_9$	GCAGAGGACCAGTTAGCATA/ TGTTCTGAGTCCACGATGCT	5	120-130	0.68	0.80	0.64
29	$(AG)_{21}$	TGGGTAAAGGTACTTAGTAG/ GTTGTATTGCTTTAGCTCAG	3	114-128	0.40	0.25	0.35
41	$(AG)_{27}$	GACATCAGTGTTCTGCAAA/ ACACTGGGTATGTTGTTGTA	3	152-174	0.32	0.13	0.29
49	$(AG)_{21}$	CTATCTTCGCATATAGGCAG/ AATCTCTGTGGCTGACTCAA	3	120-128	0.61	0.86	0.53
54	$(AG)_{25}$	GCAGAACATACTGAGACAGA/ CTAAGTGGTCATTCGAAGAG	2	136-138	0.22	0.00	0.19
55	$(GT)_7(GA)_{23}$	CAGCGCTAAACAGAAGGAA/ CTCTCTAAACACAAACGGCT	2	114-140	0.38	0.00	0.30
56	$(AG)_{23}$	CTTCGCATATAGGCAGATCA/ TCTCTGTGGCTGACTCAAAT	3	110-120	0.54	0.13	0.45
62	$(AG)_{22}$	CGCATATAGGCAGATCAAAT/ GGTCAGACTACGCTCTCTCA	4	78-84	0.49	0.38	0.46
66	$(TC)_{18}$	CAACCCATACAAATCATCCA/ AGGGTTGAGCAATTCATAGA	2	122-124	0.38	0.00	0.30
67	$(GA)_{25}$	CCGAGAAAATGCACACAA/ TGACATACTCTTCCTACAGCTA	4	90-104	0.68	0.13	0.62
75	$(TC)_{26}$	CAACACTAAGTGGTCATTCG/ CTGAGACAGAAATTCTTGCT	2	136-138	0.38	0.00	0.30
79	$(TC)_{26}$	CACTGGGTATGTTGTTGTAA/ CCGTAAAGAAATCATACCAC	3	90-112	0.32	0.13	0.29
80	$(TC)_{20}(TG)_7$	CTCAAGTGTGCCAGGTGATT/ GAGAGACAGGAAGAGACGTACA	2	106-122	0.22	0.00	0.19
88	$(AG)_{22}$	AATGGATGTTCCCTTGCTTT/ CAACTGATCAACCATTCCGT	4	150-162	0.67	0.38	0.61
94	$(GT)_5(AG)_{22}$	ACACAAAATGTCCCCGAA/ CCATTGACAAGGACAATTCT	2	112-114	0.22	0.00	0.19
95	$(AG)_{20}$	CGCATATAGGCAGATCAAAT/ AATCTCTGTGGCTGACTCAA	4	108-120	0.66	0.43	0.62
96	$(AG)_{23}$	CGCATATAGGCAGATCAAAT/ AATCTCTGTGGCTGACTCAA	4	108-120	0.70	0.88	0.64
98	$(AG)_{20}$	CGAGCTAATGGCATGGTACT/ TCAGACACCAAGCCATCAAT	3	122-128	0.55	1.00	0.46
99	$(AG)_{23}$	TTCGCATATAGGCAGATCAA/ AATCTCTGTGGCTGACTCAA	3	120-130	0.54	0.13	0.45
101	$(AG)_{17}$	TGATTAGGGCGTAGGGTTTA/ TATTCCTCTTCACCTCCGCT	2	130-134	0.22	0.00	0.19
105	$(AG)_{20}$	CTTCGCATATAGGCAGATCA/ GGCTGACTCAAATGCTCTCT	3	112-120	0.54	0.13	0.45
130	$(AG)_{44}$	TGCATACGCTGGTGTGTC/ TTCAGCTGTGGTTATGGG	3	112-126	0.55	0.25	0.46
131	$(AG)_{26}$	AAATGCACACAAAAACAC/ AATATGACCACATTTGTG	3	114-130	0.66	0.14	0.59

Continued on next page

Table 3. Continued.

Primers (CA)	Motif	Forward primer/ reverse primer (5'-3')	Allele No.	Size range (bp)	Gene diversity (H_E)	Heterozygosity (H_O)	PIC
136	$(AG)_{16}$	GTGGACTAACAGACTCAACG/ CTGATGATGCAGAACATGAT	2	150-156	0.47	0.00	0.36
140	$(GA)_{12}$	GTGTGTCTGTGTGCATGAGC/ TTCAGCTGTGGTTATGGGA	3	90-114	0.55	0.38	0.46
159	$(AG)_{19}$	GCAGAAAATGGGTAAAGGTA/ ATTGCTTTAGCTCAGAATGG	4	110-120	0.55	0.25	0.51
167	$(TC)_{24}$	CATTCTCTTCTTCTCAAACG/ CACGTCAATCTGTTGTGAAA	5	80-130	0.66	0.63	0.62
172	$(TC)_9GCTA(TC)_{14}(CA)_{13}$	ATGTTCTGAGTCCACGATGC/ CTTAGCAGAGGGCCAGTTAG	3	88-132	0.36	0.14	0.33
174	(CT_{18})	CCTGCATTCACCATCTAGGA/ GGAGCCTTGCCATAACAGAT	5	160-260	0.62	0.29	0.59
178	$(CT)_{25}$	CCCAACTCATTTAATTCCAC/ CACGTCAATCTGTTGTGAAA	5	160-200	0.57	0.38	0.54
Mean	-	-	3.30		0.50	0.29	0.44

A total of 55 alleles were identified from the 19 polymorphic SSR loci among accessions of *C. frutescens* L. The number of alleles per locus was 2-5, with a mean of 2.89. PIC ranged from 0.30-0.65. Loci showing the highest PIC values included CA 62, CA 19, and CA 26 with PIC values of 0.60, 0.63, and 0.65, respectively. In 10 of the 19 loci (CA 20, CA 29, CA 39, CA 41, CA 49, CA 79, CA 88, CA 167, CA 174, and CA 178), the observed heterozygosity (H_O) was 0, corresponding to 100% homozygous individuals, and in 3 loci (CA 19, CA 52, and CA 96), H_O was equal to 1, corresponding to 100% heterozygous individuals.

The number of alleles identified among the sampled individuals of *C. chinense* Jacq. was 119, ranging from 2-5 alleles per locus and an average of 3.30. Only 8 of the 36 loci (CA54, CA 55, CA 66, CA 75, CA 80, CA 94, CA 101, and CA 136) presented H_O equal to 0 and 5 of the 36 loci (CA 1, CA 27, CA 49, CA 96, and CA 98) had values of H_O higher than the expected heterozygosity (H_E). The loci analyzed in *C. chinense* Jacq. showed PIC variation of 0.19-0.68, with high level (greater than 0.5) of polymorphic information, 0.62 for CA 11, CA 67, CA 95, and CA 167 and 0.64 for CA 27 and CA 96 and 0.68 for CA 26.

DISCUSSION

Transferability of microsatellite primers for species within the same genus has been reported previously, such as in *Euterpe*, *E. edulis* to *E. oleracea*, the 7 loci tested presenting 100% success (Oliveira et al., 2010) and *Anacardium*, *A. occidentale*, to *A. humile*, with transferability of all 11 loci analyzed (Cota et al., 2012). Furthermore, rates of polymorphism higher than those observed in this study were obtained by Ince et al. (2010a), who detected polymorphisms between the species *C. annuum* L. and *C. frutescens* L., with 34 microsatellite primer pairs among the 45 pairs tested. This corresponds to a rate of 75.5% of polymorphism and 2.5-4.6 times the rate of polymorphism obtained in the present study for *C. chinense* Jacq. and *C. frutescens* L., respectively.

The average PIC values obtained for *C. frutescens* L. (0.48) and *C. chinense* Jacq. (0.44) were higher compared to those observed by Nagy et al. (2007) in 14 accessions of *C. annuum* L. evaluated using 2 microsatellite markers (EPMS = 0.30 and GPMS = 0.27) and lower when the author evaluated 33 genotypes of 8 domesticated and wild species of *Capsicum* (EPMS = 0.67 and GPMS = 0.68). This higher level of interspecific polymorphism was

observed likely because the materials used were extremely diverse. Polymorphic microsatellite markers for mapping in *Capsicum* developed by Lee et al. (2004) from genomic libraries showed a high PIC value (0.75), which is twice the value obtained by the same authors using primers designed based on GenBank data (0.38).

The difference between the numbers of alleles in individuals sampled for *C. chinense* Jacq. and *C. frutescens* L. was clearly observed in this study. The smaller number of alleles for *C. frutescens* L., as well as the higher rate of homozygosity found, may reflect the relatively lower genetic variability shown by this species compared with other species in the genus. *Capsicum frutescens* L. showed the most conserved morphology, with fewer shapes, sizes, and colors of fruits compared to *C. annuum* L., *C. chinense* Jacq., and *C. baccatum* L. (DeWitt and Bosland, 2009). In contrast, *C. chinense* Jacq. can be distinguished based on its extensive morphological variability, expressed in diverse forms and colors of fruits, which are typically very spicy and aromatic (Ribeiro et al., 2008).

This study describes microsatellite primers showing acceptable amplification and polymorphism for the species *C. frutescens* L. and *C. chinense* Jacq. Countries in the "new world", including those in South America, regard these species as being economically and socially important (Moses et al., 2014), and may show the highest benefit from our results. In these countries, the planning and implementation of germplasm conservation and breeding programs are limited by the lack of knowledge regarding the genetic structure and diversity of *C. frutescens* L. and *C. chinense* Jacq. (Moses et al., 2014). Thus, the primers described in this study can be used in studies of diversity and phylogeny.

Other studies have reported a considerable number of microsatellite primers evaluated for different sets comprising *Capsicum* species, including *C. frutescens* L. and *C. chinense* Jacq. [Lee et al., 2004 (76 primers), Nagy et al., 2007 (157 primers), Ince et al., 2010b (45 primers)]. Our study offers additional primers, evaluated specifically for these 2 species (19 primers for *C. frutescens* L., assessed for 4 accessions, and 36 for *C. chinense* Jacq., analyzed for 8 accessions). These primers were reliable and polymorphic within accessions of a Brazilian collection, including accessions with typically wild characteristics such as CNPH 4353 (*C. frutescens* L.) and CNPH 4315 (*C. chinense* Jacq.), the latter collected in the Amazon region, a diversity center for *C. chinense* Jacq. (Moses et al., 2014).

Microsatellite primers showing polymorphism among wild and cultivated genotypes enable further studies on the structure of these species. Moreover, one of the primers described (CA49) was able to differentiate between *C. frutescens* L. and *C. chinense* Jacq., and may be useful to the classification of the Germplasm Bank of Embrapa and, possibly, in other studies of the Brazilian germplasm. The primers can also be used for diversity studies, allowing for the selection of the most divergent genotypes with potential for heterosis gains through hybridization (Moses et al., 2014). Recently, we used the same microsatellite primers to obtain a core collection of *C. frutescens* L. that included 13 accessions, representing 77 and 81% of the alleles and the morphologic categories, respectively, found in the complete collection (104 accessions) (Carvalho SIC, unpublished results). These data reinforce the potential impact of this study, particularly for further studies of the species *C. frutescens* L. and *C. chinense* Jacq.

In summary, the CA microsatellite markers developed for *C. annuum* L. were most often transferable and polymorphic for the species *C. frutescens* L. and *C. chinense* Jacq. A set of SSRs was presented for each of these species, which may be valuable for germplasm identification as well as for further genetic, diversity, and phylogeny studies.

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