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Construction of an integrated genetic map for *Capsicum baccatum* L.

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ABSTRACT. *Capsicum baccatum* L. is one of the five *Capsicum* domesticated species and has multiple uses in the food, pharmaceutical and cosmetic industries. This species is also a valuable source of genes for chili pepper breeding, especially genes for disease resistance and fruit quality. However, knowledge of the genetic structure of *C. baccatum* is limited. A reference map for *C. baccatum* (2n = 2x = 24) based on 42 microsatellite, 85 inter-simple sequence repeat, and 56 random amplified polymorphic DNA markers was constructed using an F_2 population consisting of 203 individuals. The map was generated using the JoinMap software (version 4.0) and the linkage groups were formed and ordered using a LOD score of 3.0 and maximum of 40% recombination. The genetic map consisted of 12 major and four minor linkage groups covering a total genome distance of 2547.5 cM with an average distance of 14.25 cM between markers. Of the 152 pairs

of microsatellite markers available for *Capsicum annuum*, 62 were successfully transferred to *C. baccatum*, generating polymorphism. Forty-two of these markers were mapped, allowing the introduction of *C. baccatum* in synteny studies with other species of the genus *Capsicum*.

Key words: Hot pepper; Linkage map; Segregation analyses; Molecular markers; Transferability of microsatellites

INTRODUCTION

Peppers of the genus *Capsicum* belong to the family Solanaceae, and are widely cultivated throughout the world (Wahyuni et al., 2013). They are considered to be one of the first spices used by mankind and they have been found along with other fossils of food dating back 6000 years (Perry et al., 2007; Hill et al., 2013). Apart from its use as a spice, the fruit and seeds of peppers are increasingly being recognized for their medicinal and antioxidant properties (Spiller et al., 2008; Dias et al., 2013; Taveira et al., 2014).

The genus *Capsicum* is native to South and Central America and it includes five domesticated species: *Capsicum annuum*, *Capsicum chinense*, *Capsicum frutescens*, *Capsicum baccatum*, and *Capsicum pubescens* (Pickersgill, 1991). Despite the economic and cultural importance of these species and their potential use in diverse markets, research concerning genetic resources and improvement of *Capsicum* has been developed primarily on *C. annuum* (Rodrigues et al., 2012).

The development of genetic maps, where wide coverage and complete analysis of the genome occurs, is considered to be one of the highest impact applications of molecular markers. Linkage maps represent a fundamental resource for the study of complex genetic traits in plant species (Chutimanitsakun et al., 2011), enabling the localization of regions that control them and the quantification of their effects on the expression of the phenotype. The first linkage map of *Capsicum* containing 85 random amplified polymorphic DNA (RAPD) markers was developed by Tanksley et al. (1988) using *C. annuum* cv. Doux des Landes crossed with *C. chinense* PI 159234. Since then, several genetic maps have been established for *C. annuum* (Lefebvre et al., 1995, 2002; Paran et al., 2004; Minamiyama et al., 2006; Barchi et al., 2007; Mimura et al., 2012; Sugita et al., 2005, 2013).

Several types of molecular markers have already been used for the development of high resolution linkage maps for *C. annuum*, including simple sequence repeats (SSRs) (Wu et al., 2009; Mimura et al., 2012; Sugita et al., 2013), RAPD (Paran et al., 2004; Barchi et al., 2007; Lee et al., 2009), restriction fragment length polymorphism (RFLP) (Sugita et al., 2005; Barchi et al., 2007; Lee et al., 2009), amplified fragment length polymorphism (AFLP) (Sugita et al., 2005; Barchi et al., 2007), and cleaved amplified polymorphic sequences (CAPS) (Lee et al., 2009). RFLPs and RAPDs are the most commonly used markers for the construction of these maps (Mimura et al., 2012). However, microsatellite markers are considered useful and the most reliable for the construction of genetic maps and QTL mapping in *Capsicum* populations (Sugita et al., 2013).

Lefebvre et al. (1995) developed the first integrated map for *C. annuum* using RAPD and RFLP markers, yielding a total of 85 markers arranged in 14 linkage groups and covering approximately 820 cM. Paran et al. (2004) constructed a fairly saturated map for *C. annuum* resulting from the integration of six genetic maps, consisting of 2262 markers (1528 AFLPs, 440 RFLPs, 288 RAPDs, and 6 TAGs). The markers were ordered in 13 linkage groups with a total

length of 1832 cM. Sugita et al. (2005) proposed a linkage map for pepper (*C. annuum*) in which 464 molecular markers were mapped (328 AFLPs, 122 RAPDs, 3 RFLPs, 7 SCARs, and 4 CAPS) in 11 major and 5 minor linkage groups, covering 1043 cM. Another map was produced by Minamiyama et al. (2006) using 106 SSR markers to map a progeny composed of 117 F_2 individuals of *C. annuum*. The map consisted of 13 linkage groups and was 1042 cM in length. Mimura et al. (2012) also developed a linkage map for the species *C. annuum* based on 253 markers (151 SSRs, 90 AFLPs, 10 CAPS, and 2 TAGs covering 1336 cM of the genome. This was the first map based on SSR markers and consisted of 12 linkage groups. However, Sugita et al. (2013) developed a map with greater coverage of the *C. annuum* genome based on 265 SSR markers, which were distributed in 12 linkage groups covering a total genetic distance of 2028 cM.

A few mapping studies have been conducted with the other cultivated species of *Capsicum*, which result from interspecific crossings within the *C. annuum* complex according to the compatibility and fertility of the hybrid progeny. Interspecific linkage maps originating from the crossing of *C. annuum* x *C. frutescens* (Wu et al., 2009), and of *C. annuum* x *C. Chinense* (Tanksley et al., 1988; Kang et al., 2001; Lee et al., 2009, 2011), have been constructed. For *C. baccatum* only one genetic map has been constructed based on populations derived from interspecific crosses between *C. baccatum* and *C. annuum* (Eggink et al., 2014). Nevertheless, genetic maps considering only the *C. baccatum* genetic background are no longer available in the current literature.

In this study, we construct a reference genetic map for *C. baccatum*, based on SSR, inter-SSR (ISSR), and RAPD molecular markers. Furthermore, we examine the transfer of microsatellite markers available for *C. annuum* to *C. baccatum*.

MATERIAL AND METHODS

Plant material and DNA extraction

The mapping population consisted of 203 F_2 individuals originating from the crossing of UENF 1616 (female parent) and UENF 1732 (male parent). The female parent produces pungent fruit of an orange color in the intermediate stage and of a red color in the mature stage and is susceptible to pepper yellow mosaic virus (PepYMV). The male parent is characterized as very pungent, with green colored fruit in the intermediate stage and red colored fruit in the mature stage, with a slightly pronounced aroma and is resistant to PepYMV (Bento et al., 2009).

Young leaves of the two parents, and of the F_1 and F_2 generations were collected for DNA extraction according to the protocol by Doyle and Doyle (1990). The quantity of DNA was determined using a spectrophotometer (NanoDrop ND-1000, Thermo Scientific) and DNA quality was evaluated on a 1% agarose gel.

Molecular markers

ISSRs and RAPD

The ISSR primers used were proposed for pepper by Kumar et al. (2001), Refaat and Hoda (2007), and Yao et al. (2008). The evaluated RAPD primers were from the respective OPAA, OPAB, OPAC, OPB, OPE, OPF, OPK, OPN, and OPW kits from Operon Technologies (Alameda, CA, USA). In order to detect polymorphisms between the parents, 61 ISSR and 19 RAPD primers (Table 1) were used.

Table 1. Number and sequence of ISSR and RAPD markers used in the linkage map of *Capsicum baccatum* var. *pendulum*.

Marker identification	Motif (5'-3')	Marker identification	Motif (5'-3')	Marker identification	Motif (5'-3')	Marker identification	Motif (5'-3')	Marker identification	Motif (5'-3')
70; 71	$(CT)_8TG$	123; 124	$(GA)_8YC$	161; 162	$(CCA)_5$	208#	$(CAC)_5GC$	OPAC 17	CCTGGAGCTT
72#; 73#; 74#	$(CT)_8AC$	132; 133	$(GA)_8T$	163; 164	$(GCC)_5$	216#; 218	$(AG)_8YT$	OPAC 20	ACGGAAGTGG
75; 77	$(CT)_8GC$	134; 135#; 136	$(GC)_6CC$	165#; 166	$(GA)_7RC$	225; 226	$(AC)_8CG$	OPB 17	AGGGAACGAG
78; 79	$(CA)_8AC$	137	$(CAG)_4TA$	167; 168	$(AG)_7C$	OPAA 04	AGGACTGCTC	OPE 06	AAGACCCCTC
80; 81#; 82	$(CA)_6AG$	141; 142#	$(AG)_8T$	169; 171	$(GTG)_4RC$	OPAA 11	ACCCGACCTG	OPE 07	AGATGCAGCC
83; 84; 85	$(CAC)_3GC$	143#; 144; 145	$(TG)_8A$	175; 176; 177#	$(GT)_7YG$	OPAA 16	GGAACCCACA	OPF 1	ACGGATCCTG
86#; 87	$(GAG)_3GC$	146; 147; 148	$(AAT)_4CT$	180; 181	$(GAA)_6AA$	OPAA 18	TGGTCCAGCC	OPK 16	GAGCGTCGAA
88; 90	$(AC)_8C$	149; 150	$(AC)_8YA$	186; 187; 188	$(AC)_8T$	OPAB 05	CCCGAAGCGA	OPN 06	GAGACGCACA
98; 99#; 100#	$(CT)_8RA$	152; 153	$(GT)_8YG$	189#	$(ATG)_6$	OPAB 09	GGGCGACTAC	OPN 08	ACCTCAGCTC
101; 104	$(CT)_8RG$	154#	$(CAA)_5GAC$	192	$(AC)_8YG$	OPAB 14	AAGTGCGACC	OPW 06	AGGCCCGATG
108#; 109; 112	$(CA)_8RG$	155; 156	$(AG)_8CG$	196; 199	$(GGGTG)_3$	OPAC 07	GTGGCCGATG		
118; 120	$(GA)_7C$	158#; 159#; 160	$(AG)_8TG$	201	$(GA)_8T$	OPAC 10	AGCAGCGAGG		

#Loci that show significant distortion from Mendelian segregation ($P < 0.05$).

Similar amplification reactions were conducted for both ISSR and RAPD markers. The final reaction volume was 21 μL and contained the following reagents: 5 ng genomic DNA, 0.75 μL Taq DNA polymerase, 10X buffer (500 mM KCl, 100 mM Tris-HCl, pH 8.3), 2.4 mM $MgCl_2$, 0.1 mM of each dNTP, and 1.0 μM of each primer. Two microliters of DNA was added and then 11 μL of the previously described master mix was added, being added for coloring 8 μL red/blue juice gel.

The PCR profile consisted of an initial denaturation step of 1 min at 94°C, followed by 40 cycles of 94°C for 1 min, primer annealing temperature for 1 min (40 to 55°C for ISSR primers and 37°C for RAPD primers), 72°C for 3 min, and a final extension at 72°C for 7 min. Amplification conditions were optimized for each ISSR primer to detect the most suitable temperature for the amplification. The PCR products were visualized on a 2% agarose gel stained with red/blue juice gel (1:1) and photographed under UV light for visualization (MiniBIS Pro, DNR Bio-Imaging Systems).

SSRs

Microsatellite markers were selected based on Minamiyama et al. (2006) for the mapping of *C. annuum*. One hundred and fifty-two pairs of microsatellite primers were tested. For the detection of polymorphism, six genotypes were chosen for testing (P_1, P_2, F_1, and the three F_2 individuals).

Amplification reactions were conducted in a final volume of 21 μL containing the following reagents: 5 ng genomic DNA, 0.75 μL Taq DNA polymerase, 10X buffer (500 mM KCl, 100 mM Tris-HCl, pH 8.3, Invitrogen), 2.4 mM $MgCl_2$, 0.1 mM of each dNTP, and 0.5 μM of each primer. Two microliters of DNA was added and then 11 μL of the mix previously described was added, being added for coloring 8 μL red/blue juice gel.

The amplification reactions (Veriti Thermocycler, Applied Biosystems) were conducted as follows: 3 min at 94 ° C for initial denaturation, followed by 35 cycles of 94°C for 1 min, 55°-63°C for 1 min (depending on the primer used), 72°C for 3 min, and a final extension at 72°C for 7 min. For preview, 8 μL red/blue juice gel was added at 1:1 ratio. The amplified fragments were then separated on a high resolution MetaPhor agarose gel (4%) and subjected to UV light for visualization (MiniBIS Pro, DNR Bio-Imaging Systems). Images of the gels were captured for subsequent analysis.

Segregation analysis and map construction

JoinMap (version 4.0) was used to construct the integrated linkage map using the methodology of van Ooijen (2006). Linkage groups were formed and sorted using a minimum LOD score of 3.0 and a maximum of 40% recombination. Linkage groups were determined using the Kosambi (1943) function for the translation of recombinatorial units to genetic distance. Markers within the resulting linkage groups were ordered relative to each other by automatic multipoint analyses using the default values of JoinMap (mapping threshold LOD >1, recombination frequency threshold <0.4). The chi-square test (χ^2; $P < 0.05$, d.f. = 1) was used to test the hypotheses of 1:2:1 Mendelian segregation for SSR markers and 3:1 for ISSR and RAPD markers.

RESULTS AND DISCUSSION

The linkage map consisted of 183 markers: 42 microsatellites (22.95%), 85 ISSRs (46.45%), and 56 RAPDs (30.60%). The generated map spanned 2547.5 cM across 12 major linkage groups (321.8 to 110.3 cM) and four minor linkage groups (80.8 to 54.2 cM) (Figure 1), each grouping from 5 to 23 markers, with an average of 159.2 cM (Table 2).

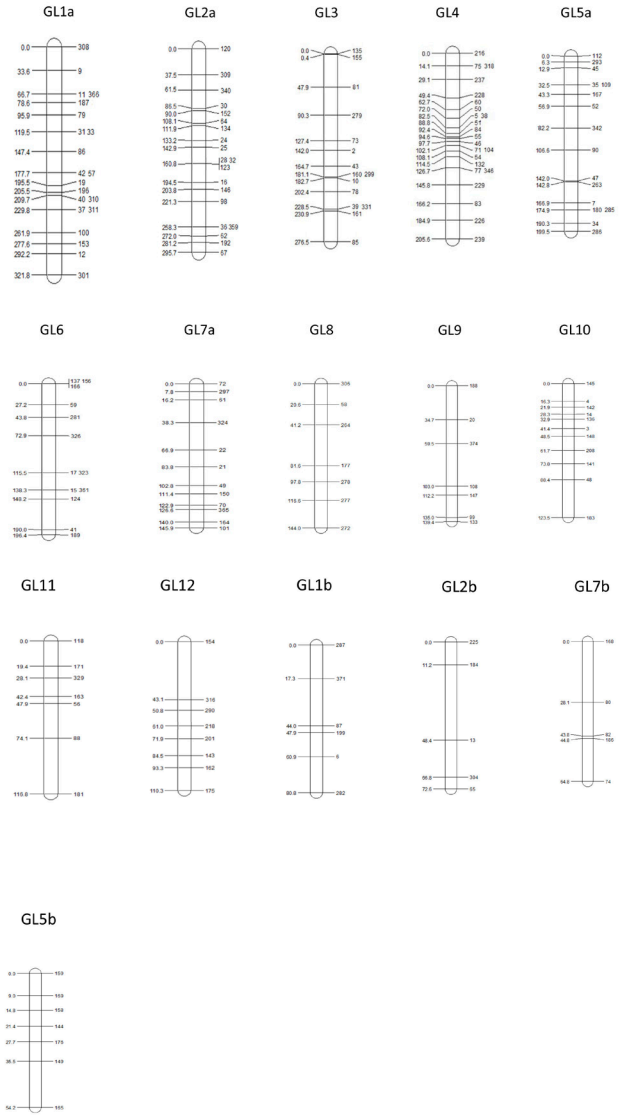

Figure 1. Genetic linkage map of *Capsicum baccatum* var. *pendulum* (LOD Score 3.0, JoinMap 4.0), showing linkage relationship of 183 molecular markers (42 SSR, 85 ISSR, and 56 RAPD) in 12 major and four minor linkage groups. The markers are indicated on the right side of each linkage group and the genetic distance (cM) on the left side. Loci that show significant distortion from Mendelian segregation are indicated by $^{\#}(P < 0.05)$.

Table 2. Number, type of marker, linkage group length (cM), and mean distance between markers (cM) in the *Capsicum baccatum* var. *pendulum* linkage map.

Linkage group	Number and type of markers	Size (cM)	Average distance between markers (cM)
GL1a	21 (10 RAPD; 6 ISSR; 5 SSR)	321.8	15.32
GL1b	6 (1 RAPD; 2 ISSR; 3 SSR)	80.8	13.47
GL2a	20 (10 RAPD; 7 ISSR; 3 SSR)	295.7	14.78
GL2b	5 (2 RAPD; 2 ISSR; 1 SSR)	72.6	14.52
GL3	15 (4 RAPD; 8 ISSR; 3 SSR)	276.5	18.43
GL4	23 (8 RAPD; 9 ISSR; 6 SSR)	205.6	8.94
GL5a	16 (6 RAPD; 5 ISSR; 5 SSR)	199.5	12.47
GL5b	7 (ISSR)	54.2	7.74
GL6	13 (4 RAPD; 5 ISSR; 4 SSR)	196.4	15.11
GL7a	12 (4 RAPD; 5 ISSR; 3 SSR)	145.9	12.16
GL7b	5 (ISSR)	64.8	12.96
GL8	7 (1 RAPD; 1 ISSR; 5 SSR)	144.0	20.57
GL9	7 (1 RAPD; 5 ISSR; 1 SSR)	139.4	19.91
GL10	11 (4 RAPD; 7 ISSR)	123.5	11.23
GL11	7 (1 RAPD; 5 ISSR; 1 SSR)	116.8	16.68
GL12	8 (6 ISSR; 2 SSR)	110.3	13.79
Mapped markers	183	2547.5	14.25
Unmapped markers	191		
Total	374		

The distance between the markers in the linkage groups ranged from 0 (i.e., two markers that co-segregate are completely linked) to 43.5 cM, with an average distance of 14.25 cM between markers. Smaller distances than those found in the present study have previously been reported, indicating that the map developed here has a higher saturation level than previous maps and may enable better detection of QTL.

Tanksley et al. (1988) developed a reference genetic map for *C. annuum* using approximately 85 RAPD markers obtained from a population composed of 61 individuals (Table 3). As for the other species of the family Solanaceae, the first genetic map described for tomato was developed by Bernatzky and Tanksley (1986), in which 112 isozyme markers were used to map a population comprising 46 individuals, covering 760 cM of the genome. Gebhardt et al. (1989) constructed a reference map for *Solanum tuberosum* using 141 RFLP markers, which covered 690 cM of the genome, and used a population composed of only 38 individuals. A reference map created for *Solanum melongena* originated from a population of 168 individuals using 181 RAPD and AFLP markers to map 779.2 cM of the genome (Nunome et al., 2001). In similar research, Priyamdha et al. (2012) reported the first genetic map for *Brassica carinata*, which was created by mapping an F_2 population consisting of 150 individuals using 69 markers (23 RAPDs, 29 ISSRs, and 17 SSRs), using a LOD score of 3.0, and covering of 2166 cM of the genome. The same types of markers were used by these authors and in the current study; however, the number of markers mapped for *C. baccatum* here was higher, indicating a higher saturation level for the constructed map. In addition, a larger population (203 individuals) was used, giving greater reliability to the data.

Kole et al. (2012) constructed the first genetic map for bitter melon (*Momordica charantia*) by mapping an F_2 population consisting of 146 individuals, solely based on 108 AFLP markers, covering 3060.7 cM of the genome. Lanteri et al. (2006) developed a reference genetic map for artichoke (*Cynara cardunculus*) in which a population consisting of 94 F_1 individuals was used, based on 59 different primers (SSRs, AFLPs, and SSAPs), and covering 2569.9 cM of the genome.

In the current study, an average of 2.61 useful markers were obtained using the domi-

nant primers (ISSR and RAPD), and one useful marker was obtained using the microsatellite primers, since they enable the mapping of only one locus for an F_2 population of diploid species.

Table 3. Comparing reference genetic maps from different sources with the *Capsicum baccatum* var. *pendulum* linkage map developed in the current study.

Species	Reference	Population size	Number of markers	Map length (cM)	Marker type
Solanum lycopersicum x *S. pennellii*	Bernatzky and Tanksley (1986)	46	112	760	Isozymes
Capsicum annuum x *C. chinense*	Tanksley et al. (1988)	46	85	-	RAPD
Solanum tuberosum	Gebhardt et al. (1989)	38	141	690	RFLP
Capsicum annuum	Lefebvre et al. (1995)	61	85	820	RAPD and RFLP
Solanum melongena	Nunome et al. (2001)	168	181	779.2	RAPD and AFLP
Cynara cardunculus	Lanteri et al. (2006)	94	59	2569.9	SSR, AFLP and SSAP
Brassica carinata	Priyamedha et al. (2012)	150	69	2166	RAPD, ISSR and SSR
Momordica charantia	Kole et al. (2012)	146	108	3060.7	AFLP
Capsicum baccatum	This study	203	183	2547.5	RAPD, ISSR and SSR

The size of the *Capsicum* genome is estimated to be between 3300 and 3600 cM (Moscone et al., 2003). The linkage map constructed in this study covered between 70.76 and 77.2% of the *C. baccatum* var. *pendulum* genome, which is greater than the coverage previously obtained for *C. annuum*. Lefebvre et al. (1995) developed the first integrated map for *C. annuum* based on 85 RAPD markers arranged in 14 linkage groups, covering approximately 820 cM of the genome, equivalent to 22.78 to 24.85% coverage. Subsequently, other studies were undertaken to map *C. annuum* in order to saturate the map (Lefebvre et al., 2002; Paran et al., 2004; Sugita et al., 2005; Minamiyama et al., 2006; Barchi et al., 2007; Mimura et al., 2012).

Kang et al. (2001) published a genetic map created using a population obtained by interspecific crossing of *C. annuum* x *C. chinense*, covering 1320 cM of the genome, equivalent to 36.7 to 40% coverage. Barchi et al. (2007) developed a map for *C. annuum* with a total length of 1857 cM, representing between 51.58 and 56.27% of the genome. In the most recently published linkage map for the species (Sugita et al., 2013), which was based on 265 SSR markers distributed within 12 linkage groups, a higher total genetic distance was covered, corresponding to 2028 cM, i.e., from 56.33 to 61.45% of the genome.

When constructing the map developed in this study, the first markers to be mapped were those that followed the segregation ratio of 1:2:1 for microsatellite markers and of 3:1 for ISSR and RAPD markers. Of the mapped ISSR markers, 22.35% were distorted ($P > 0.05$), whereas 14.28% of the mapped RAPD markers were distorted. For the final map, a total of 27 markers (14.75%) were distorted (Table 4). Deviations from the expected segregation can be attributed to a number of factors, such as structural rearrangements of chromosomes, deleterious recessive alleles, gametic selection, pre- or post-zygotic selection of allelic combinations, among others (Priyamedha et al., 2012).

Using the ISSR technique and the 61 primers tested, 26 primers did not detect polymorphisms in the progeny and 35 primers showed clear polymorphism in the population studied. A total number of 85 markers were obtained and the average number of markers mapped per primer was 2.43. Among the 35 primers used, a total of 201 loci were amplified, from which 85 were mapped. For the RAPD markers, 24 primers were tested, of which five did not detect polymorphism, and 19 were selected. A total of 56 markers were generated, with an average of 2.95 mapped markers per primer. For the 19 RAPD primers, a total of 111 loci were produced, of which 56 were polymorphic for the parents and were mapped.

From the 152 microsatellite markers available for *C. annuum* and tested in *C. baccatum* var. *pendulum*, 62 were successfully transferred and considered polymorphic and 42 were mapped, resulting in 40.8% of microsatellite markers being transferred. As information about the transferability and use of microsatellites for *C. baccatum* is limited, the construction of linkage groups incorporating these markers of a co-dominant nature represents a valuable strategy for genomic studies on the species.

Table 4. Molecular markers selected to construct the reference linkage map of *Capsicum baccatum* var. *pendulum*.

Type of marker	No. of tested primers	No. of polymorphic primers	Total No. of bands	Total No. of mapped bands	Total No. of unmapped bands	No. of loci distorted from Mendelian segregation	No. of bands per primer
SSR	152	62	62	42	20	-	1.00
ISSR	61	35	201	85	116	19	2.43
RAPD	24	19	111	56	55	8	2.95
Total	237	116	374	183	191	27	

There was good correspondence between the allocation of microsatellite markers in the linkage groups of *C. baccatum* var. *pendulum* obtained in this study and those from the linkage group of *C. annuum* obtained by Minamiyama et al. (2006). Of the microsatellite markers mapped here, 66.67% were common to the linkage groups constructed by Minamiyama et al. (2006). This high similarity is of great relevance to studies on synteny and comparative mapping with other species of *Capsicum*.

In the map developed here, 191 loci were not mapped, suggesting that over half (51.07%) of the markers were not linked. These unallocated markers show that the saturation level of the obtained map needs to be increased. These available, unlinked markers facilitate the saturation of the map with the addition of new markers obtained in further studies. Mimura et al. (2012) found that as the coverage of a genetic map increases, the number of linkage groups becomes closer to the haploid number of chromosomes of the species and the number of unlinked markers approaches zero. Barchi et al. (2007) observed that approximately 50% of the markers of *C. annuum* were considered unlinked in a test at the 1% level performed using the 304 Mapmaker software. Studies conducted by Lee et al. (2011) obtained better results than those obtained in this study, with only 31.5% of the markers not mapped compared to 51.07% in the current study. However, these authors worked with an interspecific crossing (*C. annuum* x *C. chinense*), which extends the generated polymorphism, as well as the number of linkage groups, allowing the allocation of most of the markers.

Subdivision of the linkage groups 1, 2, 5, and 7 was carried out, because although they contained gaps determined by mapped markers they were over 50 cM. To this end, we added a letter to the number of the original linkage group. Groups 1b, 2b, 5b, and 7b resulted from this subdivision, allowing 6, 5, 7, and 5 markers, respectively, to be maintained in the linkage groups. The subdivision indicates that the markers are within the same group; however, there are not enough markers to group them. According to Priyamedha et al. (2012), the existence of gaps in linkage groups is expected for species with a larger genome, even if a high number of maps have already been developed.

In maps constructed for *Capsicum* with different types of markers and different types of mapping populations, the presence of gaps is quite common due to the size of the genome. Kang et al. (2001) constructed a genetic map with 11 major linkage groups (60.3-206 cM),

and five minor linkage groups (10.3-32.6 cM). Similar results were obtained by Sugita et al. (2005), where the genetic map also consisted of 11 major linkage groups (56.7-118.5 cM), and five minor linkage groups (1.8-33.1 cM).

According to Paran et al. (2004), it is possible to obtain a number of linkage groups that differs from the haploid number of the species, indicating that a higher saturation is needed to achieve complete coverage of the pepper genome. Conversely, all markers found with a distance smaller than 50 cM should be retained as these are fundamental to the higher saturation of the map with the introduction of new markers.

With regards to the number of linkage groups, genetic maps developed by Mimura et al. (2012) and by Sugita et al. (2013) showed 12 groups, equivalent to the haploid number of the species, *C. annuum*, while Alimi et al. (2013) constructed a map for the same species with 17 linkage groups.

In the current study, a higher concentration of certain types of markers was found in certain linkage groups. The clustering of markers in specific areas of the genome is a common phenomenon in the development of genetic maps and it has been previously described in several species and for different types of markers (Qi et al., 2004). In order to explain this concentration, biological factors, such as the low rate of recombination in regions of centromeres and telomeres, the discontinuous distribution of levels of polymorphism and the variation in the number of copies of certain sequences throughout the genome, have been highlighted (Tanksley et al., 1992; Qi et al., 2004).

The map constructed in this study enables the inclusion of *C. baccatum* in genomic studies, such as comparative mapping, and it can be used as a starting point for selecting features of interest for plant improvement. Furthermore, the identification of polymorphic microsatellite markers represents a major breakthrough for the implementation of marker-assisted selection in programs for the improvement of *C. baccatum*, and it also allows the culture to benefit from advances in genetics and genomics of other most studied species, such as *C. annuum*. From this reference map, the inclusion of new markers can be made, enabling major, and improved, coverage of the genome, and also a better definition of the linkage groups.

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