RESEARCH

Molecular profiling of sweet cherry cultivars present in Chile using polymorphic microsatellite markers

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ABSTRACT

Sweet cherry (*Prunus avium* (L.) L.) is one of the most important fruit crops of temperate climates. In Chile, the actual planted area is over 42 000 ha that produce over 260 000 t yearly. The accurate identification of sweet cherry cultivars is key for processes involved both in breeding new cultivars and along the production chain. In this study, we performed the molecular characterization of 87 sweet cherry genotypes cultivated in Chile, using nine microsatellite markers originally described for both peaches and sweet cherries. The analysis showed that 69 of these genotypes corresponded to unique cultivars, each harboring a unique allelic pattern. They could be differentiated using only five markers (BPPCT-037, BPPCT-039, BPPCT-040, PMS-30 and UCD-CH18). The remaining 19 genotypes could correspond to misidentified, mutated or even synonyms of the studied genotypes, since they have allelic patterns identical to one or more of the 69 individualized genotypes. Between 3 and 8 alleles per marker were identified, with a mean of 6, while the expected heterozygosity over the nine polymorphic loci averaged 0.72, ranging from 0.59 in UDP96-001 to 0.78 in BPPCT-040. Phylogenetic and population structure analyses showed that most cultivars were grouped according to their country of origin or the breeding program from where they were released, being also coincident with their presumed pedigrees. These results are the basis for a fingerprinting protocol, based on microsatellite markers, for sweet cherry cultivars.

Key words: Cultivar identification, fingerprinting, germplasm, molecular markers, *Prunus avium,* SSR.

INTRODUCTION

The sweet cherry (*Prunus avium* (L.) L.) is one of the most important fruit crops of temperate climates, with a global yearly production (2019/2020) of almost 4 million metric tons, of which 260 000 are produced in Chile, in about 42 000 ha. This makes Chile the main exporter of this fruit to the world, with a year total export of 230 000 metric tons (https://gain.fas.usda.gov/).

The species is a member of the Rosaceae family, subfamily Amygdaloideae, tribe Amygdaleae. It is a diploid species with a basic chromosome number of 8 (x = 8) and the diploid genome organized in a $2n = 2x = 16$ chromosomes (Iezzoni et al., 2017). Although cherries have been cultivated for more than 2000 years, cherry breeding started around the early 1800s, so some modern cultivars are just a few generations away from their early ancestors (Iezzoni et al., 1990). Breeding programs in Europe initially began by selecting particular landraces, which were used afterwards as parents (Quero-García, 2019). Seeds transported from Europe to North America started the production of this fruit in the New World, where important cultivars have been developed since the 19th Century (Brown et al., 1996).

Corporative and private sweet cherry breeding programs have released numerous cultivars (Quero-García, 2019), which are planted at different locations, depending on their adaptation to particular sites. It has been suggested that only a

limited number of genotypes has been used in breeding this species and, consequently, modern cultivars harbor a narrow genetic base. For instance, Choi and Kappel (2004) indicate that five founding genotypes were most commonly used as parents in North American breeding programs.

The correct identification of existing and new cultivars is an important challenge both for fruit production and for breeding new cultivars. Germplasm repositories, breeding programs and the commercialization of fruits and plants, require unequivocal identifications of cultivars to avoid the use of incorrect genotypes in crossings, to protect registered or patented cultivars, and to assure the propagation of true-to-type genotypes.

The use of molecular markers complements the traditional cultivar characterization based on morphological and/or phenological data, providing a genetic profile or fingerprinting of each individual. The use of this complement, enables the clarification of the origin of plant cultivars (Krmpot et al., 2020), standardization of planting material, protection of new cultivars by breeders' rights (Ru et al., 2015), and the study of the genetic relatedness of cultivars to keep the heterozygosity of the breeding populations (Yaroslav and Volkov, 2018).

Microsatellites or simple sequence repeats (SSRs) are among the most widely used molecular markers, due to their hypervariability, multiallelic nature, codominant inheritance, and reproducibility, (Kalia et al., 2011). Microsatellite identification has been successfully used for the molecular characterization of sweet cherries (*P. avium* (L.) L.) and for the tetraploid sour cherry (Cantini et al., 2001, Dirlewanger et al., 2002; Struss et al., 2002; Wünsch and Hormaza, 2002; Clarke and Tobutt, 2003; Vaughan and Russell, 2004; Kacar et al., 2005; Pedersen, 2006; Fernández i Marti et al., 2012; Liu et al., 2018; Muccillo et al., 2019).

It has been shown that the genomes of the diploid *Prunus* species exhibit a high degree of synteny and that they exhibit chromosome collinearity (Shirasawa et al., 2017; Wang et al., 2020), and so the microsatellite markers developed from different species are theoretically useful to develop genetic studies in any *Prunus* species.

The aim of this study was to perform a molecular characterization of sweet cherry cultivars grown in Chile, defining a minimal set of microsatellite markers able to differentiate sweet cherry cultivars in a trustable fashion. The results presented here are the basis for the development of a fingerprinting protocol for the unequivocal identification of the sweet cherry cultivars grown in Chile.

MATERIALS AND METHODS

Plant material

A total of 87 sweet cherry (*Prunus avium* (L.) L.) cultivars and a wild *P. avium* seedling selection used as rootstock ('Mazzard F12/1') were used for this study. Three groups of cultivars where included: one group of 49 samples came from a private germplasm collection ("Paine" collection) located at the Metropolitan Region (33º48'13.4" S, 70º40'02.0" W), Chile. Another group of 19 samples came from another private collection ("Talca" collection), established at the Maule Region (34º58'53.9" S, 71º13'50.7" W), Chile, which contained mainly cultivars of Hungarian origin. The other 19 cultivars were collected in different regions throughout the Chilean cherry growing area (Table 1).

DNA extraction

Genomic DNA was extracted from young fresh leaves of each cultivar according to Varas et al. (2013). DNA integrity and concentration were both checked on 1% (w/v) agarose gels as well as with the Infinite 200 PRO NanoQuant spectrophotometer (Tecan Tradind AG, Männedorf, Switzerland).

Microsatellite markers amplification

Samples were PCR-amplified using nine microsatellite markers (Table 2). PCR reactions were carried out in a total volume of 12 µL, with 20 ng genomic DNA, 0.5 µM each forward and reverse primers, 0.2 mM dNTPs, 2.5 mM $MgCl_2$, 2.4 µL Colorless GoTaq reaction buffer (5×), and 0.25 U GoTaq DNA polymerase (Promega, Madison, Wisconsin, USA). PCR reactions were carried out on an XP Cycler thermocycler (Bioer Technology, Hi-tech [Binjiang] District Hangzhou, P.R. China) using the following temperature profile: 94 °C for 5 min, 35 cycles of 94 °C (30 s), 56 °C (30 s), 72 °C (30 s), and a final extension at 72 °C for 7 min. Polyacrylamide gel electrophoresis and silver-staining were done as was described by Narváez et al. (2001). Fragment sizes were estimated by comparison with known allelic patterns.

Table 1. Description of plant material used in this study.

Cultivar/Selection	Parentage (if known)	Origin
7_19[1]	Unknown	Unknown
Aida[2]	Moldvai fekete × H-236 (Germersdorfi × o.p.)	Hungary
Alex[2]	Van × JI2420	Hungary
Anita[2]	Trusenszkaja 2 × H-3 (Germersdorfi × o.p.)	Hungary
B54[1]	Unknown	Unknown
Badacsony[3]	Unknown	Hungary
Benton (Columbia)[3]	Stella × Beaulieu	USA
Bing[1]	Black Republican × Napoleo	USA
Bing 874[1]	Unknown	Unknown
Black Republican[1]	Eagle or Napoleon × Black Tartarian	USA
Black Tartarian[3]	Unknown	England
C15[3]	Unknown	Unknown
C78[1]	Unknown	Unknown
C82[3]	Unknown	USA
Carmen[2]	Sarga Dragan × H-203 (Germersdorfi × o.p.)	Hungary
Chamichoco[3]	Unknown	Unknown
Checa 9[1]	Unknown	Unknown
Chelan[3]	Stella × Beaulieu	USA
Compact Stella[1]	Irradiated Stella	Canada
Corazón de paloma[3]	Unknown	Unknown
Cristal champagne[3]	Unknown	USA
Duroni 3[3]	Unknown	Italy
Early Bing[3]	Unknown	Unknown
Early Burlat[1]	Unknown	USA
Emperor Francis[1]	Unknown	Austria
Enjidel (Bigalise)[4]	Delbard × Starking Hardy Giant	France
Garnet[1]	Starking Hardy Giant × Bing	USA
Germersdorfi 3[2]	Unknown	Hungary
Giorgia[1]	ISF 123 × Caccianese	Italy
Glenred (Sequoia)[1]	(Bing × Brooks) × Tulare	USA
Grace Star[1]	Burlat × o.p.	Italy
Hedelfingen[1]	Unknown	Germany
Karina[1]	Schneider × Rube	Germany
Katalin[2]	Germersdorfi × Podjebrad	Hungary
Kavics[2]	Germersdorfi × Budakalaszi	Hungary
Kordia (Attika)[1]	Seedling from Techlovice, North Bohemia	Czech Republic
Lambert[1]	Napoleon × Black Heart	USA
Lapins[1]	Van × Stella	Canada
Late Maria[3]	Unknown	USA
Linda[2]	Hedelfingen × Germersdorfi	Hungary
Margit[2]	Germersdorfi × o.p.	Hungary
Marvin (4-70)[3]	Unknown	USA
Mazzard F12/1[4]	*Prunus avium* selection	England
Moreau (Bigarreau)[1]	Unknown	France
Nadino[2]	Spansche Knorpel × o.p.	Germany
Newstar[1]	Van × Stella	Canada
NY1495[1]	Unknown	USA
Pal[2]	Burlat × Stella	Hungary
Peter[2]	Burlat × Stella	Hungary
PG[1]	Unknown	Unknown
Rainier[1]	Bing × Van	USA
Regina (USPP11530)[1]	Schneider × Rube	Germany
Reverchon[3]	Unknown	France
Rita[2]	Trusenszkaja 2 × H-2 (Germersdorfi × o.p.)	Hungary
Rivedel (Earlise)[1]	Starking Hardy Giant × Burlat	France
Royal Dawn (CE-14)[1]	Unknown	USA
Royal Rainier[1]	Stella o.p.	USA
Ruby[1]	Starking Hardy Giant × Bush Tartarian	USA
Sam[1]	V-160140 (Windsor × o.p.) × o.p.	Canada
Sandor[2]	Burlat × Stella	Hungary
Santina (13S-5-22)[1]	Stella × Summit	Canada

Continuation Table 1.

Cultivar/Selection	Parentage (if known)	Origin
Schmidt[1]	Schwarze knorpelkirsche × o.p.	Germany
Schneider[1]	Local cultivar, Guben	Germany
Solymari Gombolyu[2]	Local cultivar, Budavidek	Hungary
Sommerset[1]	Van × Vic	USA
Staccato (13S2009)[4]	Sweetheart × o.p.	Canada
Starking Hardy Giant[1]	Unknown	USA
Stella[1]	Lambert × JI 2420	Canada
Sumleta (Sonata)[1]	Lapins × 2N-39-5 (Van × Stella)	Canada
Summit[1]	Van × Sam	Canada
Sunana[3]	Unknown	USA
Sunburst[1]	Van × Stella	Canada
Sunset (Sunset Bing)[1]	Mutation of Bing	USA
Superior[3]	Unknown	Unknown
Sweet Georgia[1]	Mutation of Lapins	Australia
Sylvia (4C17-31)[1]	Van × Sam	Canada
Symphony (13S-25-25)[1]	Lapins × Bing	Canada
Szomolyai Fekete[2]	Unknown	Hungary
Techlovan[1]	Van × Kordia	Czech Republic
Toyama I[4]	Unknown	Unknown
Tulare[1]	(Bing × o.p.) × o.p.	USA
Tunde[2]	Drogans Gelbe × Burlat	Hungary
Utah Giant[1]	Unknown	USA
Valerij Tschkalov[2]	Rozornaya (Cherry Rose) × o.p.	Ukraine
Van[1]	Empress Eugenie × o.p.	Canada
Van Compact[1]	Irradiated Van	Unknown
Vanda[1]	Van × Kordia	Czech Republic
Vera[2]	Ljana (Trusenszkaja 6) × Van	Hungary

[1]Cultivars from private germplasm collection located in Paine, Metropolitan Region.
[2]Cultivars from private germplasm collection located in Talca, Maule Region.
[3]Cultivars from private germplasm collection located in Quillota, Valparaiso Region.
[4]Cultivars from diverse origin.

Table 2. Microsatellite primers information, linkage group (LG) and reference.

SSR locus	Species	Repeat motif	Primer sequences	LG	Start position-end position[a]	Reference
BPPCT-026	*Prunus persica*	(AG)8GG(AG)6	ATACCTTTGCCACTTGCG TGAGTTGGAAGAAAACGTAACA	5	4389437-4389454 4389578-4389557	Dirlewanger et al. (2002)
BPPCT-037	*P. persica*	(GA)25	CATGGAAGAGGATCAAGTGC CTTGAAGGTAGTGCCAAAGC	5	12306819-12306838 12306963-12306944	Dirlewanger et al. (2002)
BPPCT-039	*P. persica*	(GA)20	ATTACGTACCCTAAAGCTTCTGC GATGTCATGAAGATTGGAGAGG	3	6662508-6662529 6662654-6662632	Dirlewanger et al. (2002)
BPPCT-040	*P. persica*	(GA)14	ATGAGGACGTGTCTGAATGG AGCCAAACCCCTCTTATACG	4	6460766-6460785 6460902-6460883	Dirlewanger et al. (2002)
PMS-3	*P. avium*		TGGACTTCACTCATTTCAGAGA ACTGCAGAGAATTTCACAACCA	4		Cantini et al. (2001)
PMS-30	*P. avium*		CTGTCGAAAGTTTGCCTATGC ATGAATGCTGTGTACATGAGGC	3		Cantini et al. (2001)
PMS-67	*P. avium*		AGTCTCTCACAGTCAGTTTCT TTAACTTAACCCCTCTCCCTCC	1		Cantini et al. (2001)
UCD-CH18	*P. avium*	(CT)23	GATGGAAGGCCAAGGCAAC AATGTTCCCGGTTATATGC	4		Struss et al. (2002)
UDP96-001	*P. persica*	(CA)17	AGTTTGATTTTCTGATGCATCC TGCCATAAGGACCGGTATGT	6	7055429-7055450 7055550-7055531	Testolin et al. (2000)

[a]Physical position using the peach genome (Peach v2.0) as reference (Verde et al., 2017).

Genetic analyses

The Micro-Checker version 2.2.3 software (van Oosterhout et al., 2004) was used for checking microsatellite null alleles and scoring errors. The Popgene program version 1.32 (Yeh et al., 1999) was used for both genic variation and heterozygosity statistics. To compare the efficiency of the markers in cultivar identification, discrimination power (D), confusion probability (C_j) and theoretical number of indistinguishable genotypes (X_k) were estimated according to Tessier et al. (1999). The PICcalc program (Nagy et al., 2012) was used to calculate the polymorphic information content (PIC).

Phylogenetic and population structure analyses

A binary matrix was constructed based on the presence (1) and absence (0) of microsatellite alleles. DARwin version 6.0.17 software (Centre de Coopération Internationale en Recherche Agronomique Pour le Développement [CIRAD], Paris, France; https://darwin.cirad.fr) was used for calculating pairwise genetic distances and for constructing a dissimilarity matrix, which was subjected to cluster analysis using the weighted Neighbor-Joining analyses. Bootstrap support values were calculated over all the loci using 1000 repetitions. In order to identify population structure, microsatellite information was analyzed with the program STRUCTURE v2.3.4 (Evanno et al., 2005). To screen appropriate K-values, values of delta K and optimal K were computed using STRUCTURE Harvester (Earl and vonHoldt, 2012; http://taylor0.biology.ucla.edu/structureHarvester/). Genotypes were subdivided into different populations according to their maximum membership probability among the populations and the membership probabilities threshold of 0.80.

RESULTS AND DISCUSSION

Microsatellite informativeness

A total of 87 sweet cherry cultivars plus a *Prunus* rootstock ('Mazzard F12/1'), a wild *P. avium* seedling, were analyzed with nine informative microsatellite markers developed for peach and sweet cherry (Table 2; Figure 1). These markers had been previously selected after evaluating a set of 13 microsatellites on a group of 37 sweet cherry cultivars (results not shown); in addition, this set of markers were used previously in sweet cherry fingerprinting studies (Dirlewanger et al., 2002; Kacar et al., 2005; Pedersen, 2006). Micro-Checker showed no evidences for null alleles, scoring error due to stuttering or allelic dropout at any locus ($P > 0.05$). The allele size range determined for each microsatellite marker is shown in Table 3.

The statistical parameters obtained for microsatellite markers used in this study are presented in Table 3. A total of 56 alleles were detected, ranging from 3 in UDP96-001 to 8 in BPPCT-040, PMS-3 and PMS-30, with a mean of 6 alleles per locus. This mean is very similar to the one obtained by both Fernández i Marti et al. (2012) in a set of 99 sweet cherry accessions using seven microsatellite markers (7.0 alleles per locus) and by Liu et al. (2018) in a set of 95 sweet cherry accessions using 10 microsatellite markers (6.1 alleles per locus). On the other hand, the value 6 obtained in this study is higher than that obtained in other sweet cherry studies: 3.7 (Wünsch and Hormaza, 2002; 76 cultivars, nine microsatellites), 3.3 (Clarke and Tobutt, 2003; 14 cultivars, 19 microsatellites) and 4.1 (Kacar et al., 2005; 10 cultivars, nine microsatellites). These differences in information content per marker can be explained by the number of cultivars

Figure 1. Allelic pattern of a group of sweet cherry cultivars for the microsatellite marker PMS-30. Separation of the amplicons was done on a 6% PAGE-urea gel and bands were revealed by silver staining.

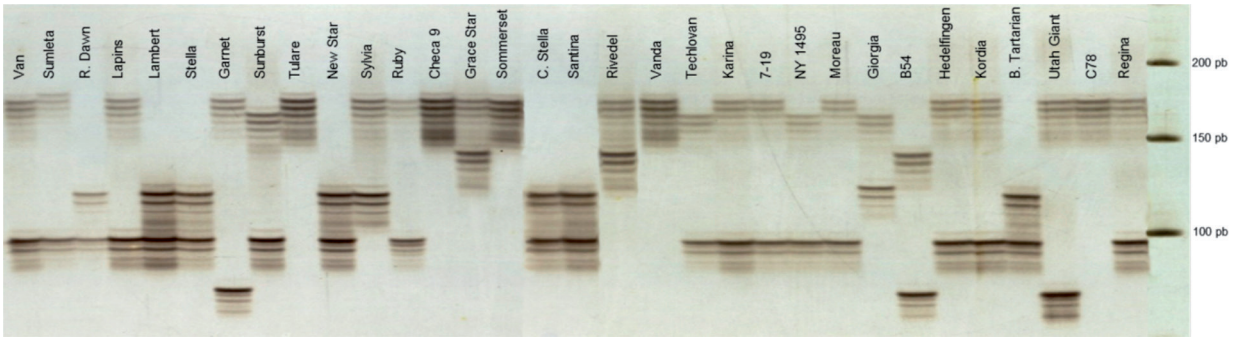


Table 3. Genetic parameters for nine microsatellite markers obtained by analyzing 87 sweet cherry cultivars and a *Prunus* rootstock.

Microsatellite marker	N	A	H_o	H_e	F	PIC	Allele size range (bp)	C_j	D	Nr of observed genotypes
BPPCT-026	88	7	0.74	0.72	-0.02	0.68	180-222	0.27	0.73	14
BPPCT-037	88	6	0.76	0.72	-0.06	0.68	150-166	0.27	0.73	17
BPPCT-039	88	4	0.73	0.74	0.02	0.70	144-160	0.25	0.75	11
BPPCT-040	88	8	0.89	0.78	-0.13	0.75	132-158	0.21	0.79	20
PMS-3	88	8	0.72	0.71	-0.01	0.68	206-232	0.28	0.72	18
PMS-30	88	8	0.76	0.76	0.00	0.73	158-200	0.23	0.77	19
PMS-67	88	6	0.80	0.67	-0.19	0.61	159-189	0.32	0.68	13
UCD-CH18	88	6	0.85	0.76	-0.12	0.73	200-218	0.23	0.77	17
UDP96-001	88	3	0.60	0.59	-0.01	0.55	122-140	0.39	0.61	7
Total	88	56								
Average		6	0.76	0.72	-0.06	0.68				

N: Number of scored cultivars; A: number of alleles; H_o: observed heterozygosity; H_e: expected heterozygosity; F: Wright's fixation index; PIC: polymorphism information content; C_j: confusion probability; D: discrimination power.

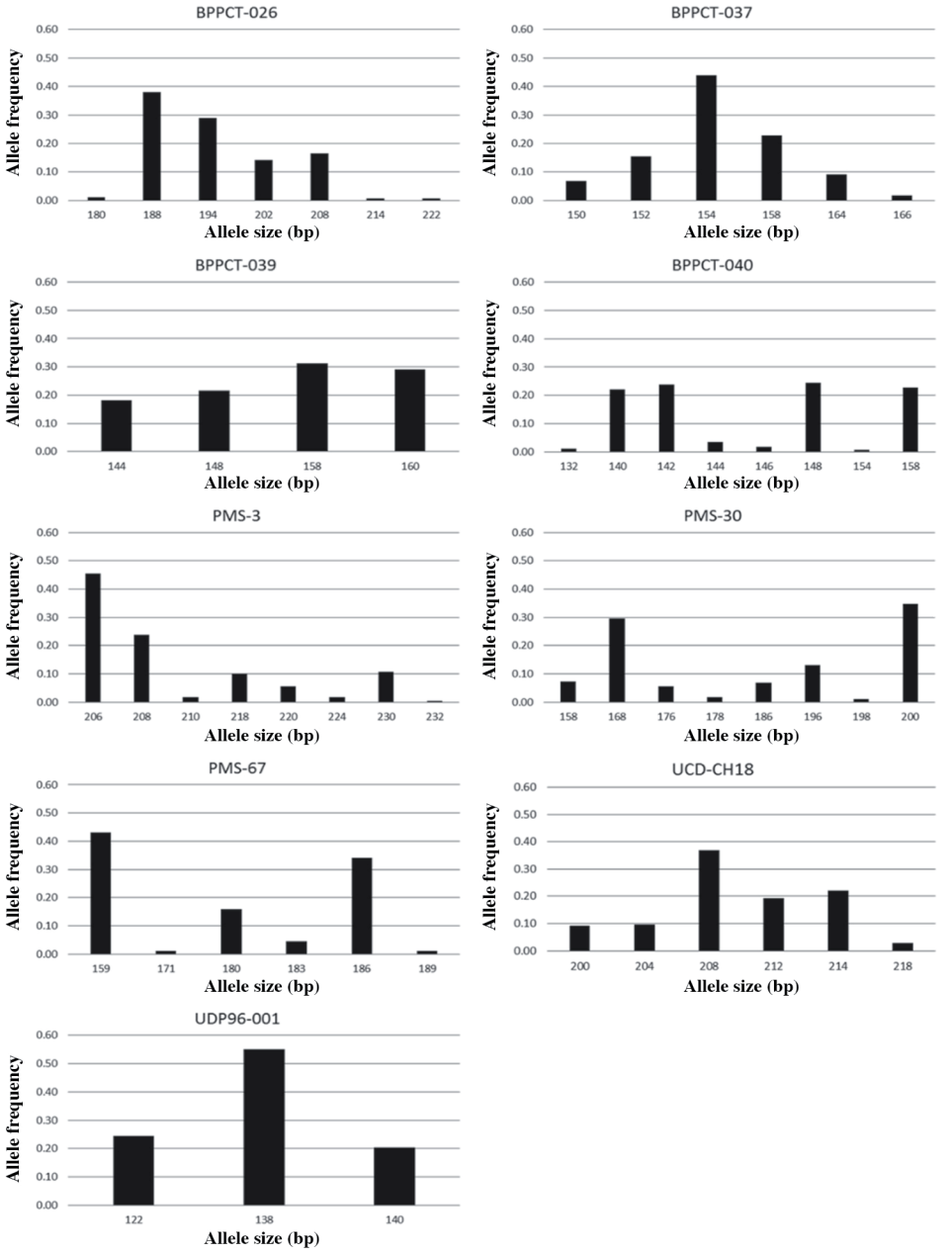
considered in every case, their genetic background and the set of microsatellites used in each study. Microsatellite marker PMS-30 was previously used by Liu et al. (2018) and it was the marker that produced the highest number of alleles, similar to results obtained in this study.

Allele frequencies (p_i) ranged from 0.006 to 0.545, with a mean of 0.141 (Figure 2). From 56 alleles detected, 23 of them were considered *rare alleles* ($p_i \leq 0.1$); this type of allele was observed in every microsatellite used in this work, except for BPPCT-039 and UDP96-001. These rare alleles were detected mainly in Hungarian cultivars such as 'Solymari gombolyu' and 'Katalin', among others, and from the rootstock 'Mazzard F12/1'. Among the markers, BPPCT-039 showed more uniform distribution of alleles, with no particular alleles overrepresented, while most of the markers showed one or two alleles with a largely higher frequency. Only UDP96-001 showed one over-represented allele with the highest value for p_i (0.545). Rojas et al. (2008) detected 59 alleles when they analyzed a group of 117 peach and nectarine cultivars using nine microsatellite markers, with allele frequency ranging from 0.004 to 0.846 (mean value = 0.152). They observed 41 rare alleles ($p_i \leq 0.1$) and six alleles with $p_i \geq 0.6$, both diminishing severely the heterozygosity of the harboring loci. By contrast, rare alleles detected in this study did not affect severely the overall heterozygosity. The mean observed heterozygosity (H_o) was 0.76 (ranging from 0.60 in UDP96-001 to 0.89 in BPPCT-040) and the mean expected heterozygosity (H_e) was 0.72 (ranging from 0.59 in UDP96-001 to 0.78 in BPPCT-040) (Table 3). When comparing these results with those obtained by Fernández i Marti et al. (2012) for a similar number of sweet cherry cultivars, the main difference was observed for H_o (0.44 *vs*. 0.76 in this study), while H_e had a similar value (0.68). Differences for H_o values between the two studies may be due to the use of Hungarian sweet cherry cultivars and *Prunus* rootstocks in this study, both of which contributed with a high number of heterozygous genotypes. When expected and observed heterozygosity values were compared with the Wright's fixation index (F), the value was 0 for PMS-30 ($H_o = H_e$) and it was positive for BPPCT-039 ($H_o < H_e$), while it was negative for all other markers, with a mean value of -0.06 indicating a subtle excess of heterozygosity in the material studied. Similar results were observed by Carrasco et al. (2012) in Japanese plum, proposing that excess of heterozygosity could be explained by negative assortative mating related to a self-incompatibility system such that parental lines carrying different alleles are favored. The polymorphic information content (PIC) mean value among the microsatellite markers was 0.68, with a minimum of 0.55 (UDP96-001, the least informative marker in this study) and a maximum of 0.75 (BPPCT-040, the most informative one).

Determination of the optimal microsatellite combination

With the aim of selecting the optimal combination of microsatellite markers necessary to identify the set of cultivars analyzed in this study, we estimated the risk of confusion using the C_j value (confusion probability, or the probability that two randomly chosen individuals from a sample of cultivars have identical banding patterns [Tessier et al., 1999]) of each microsatellite. The discrimination power (D) of each marker represents the probability that two randomly chosen individuals have different patterns and thus are distinguishable from one another, then $D = 1 - C_j$; D values closest to 1 indicates a higher level of polymorphism or variation. BPPCT-040 presented the highest number of observed

Figure 2. Allele frequency distribution of nine microsatellite markers in 87 sweet cherry cultivars grown in Chile.



genotypes (20) and the lowest value for C_j (0.21), while UDP96-001 showed the lowest number of observed genotypes (7) and the highest number of C_j (0.39) (Table 3). To determine the optimal microsatellite combination for cultivar identification, microsatellite markers were ranked according to the C_j value. When using the single marker BPPCT-040, 79 indistinguishable pairs were obtained (Table 4). As new microsatellite markers are added to the analysis, the theoretical number of indistinguishable genotypes (X_k) diminishes to finally reach a value of 0 by using the complete set of nine microsatellite. The first two markers were chosen on the basis of their discrimination power (Table 4), but the third marker (BPPCT-039) was selected because it allows differentiating 12 genotypes instead of eight for marker UCD-CH18 when the experimentally observed indistinguishable pairs of genotypes are considered. A similar case was reported by Tessier et al. (1999), when determining the optimal primers combination for the discrimination of a group of 224 cultivars of *Vitis vinifera* L. The choice of markers was done using the experimental values of the total number of non-differentiated

Table 4. Selection of the most efficient minimal set of microsatellite markers for identification of 87 sweet cherry cultivars analyzed in this study.

Microsatellite combination	Nr of indistinguishable pairs	
	Theoretical number (Xk)	Experimentally observed
BPPCT-040	804.0	79
BPPCT-040 + PMS-30	184.5	40
BPPCT-040 + PMS-30 + BPPCT-039	46.6	32
BPPCT-040 + PMS-30 + BPPCT-039 + UCD-CH18	10.8	23
BPPCT-040 + PMS-30 + BPPCT-039 + UCD-CH18 + BPPCT-037	3.0	19
BPPCT-040 + PMS-30 + BPPCT-039 + UCD-CH18 + BPPCT-037 + BPPCT-026	0.8	19
BPPCT-040 + PMS-30 + BPPCT-039 + UCD-CH18 + BPPCT-037 + BPPCT-026 + PMS-3	0.2	19
BPPCT-040 + PMS-30 + BPPCT-039 + UCD-CH18 + BPPCT-037 + BPPCT-026 + PMS-3 + PMS-67	0.1	19
BPPCT-040 + PMS-30 + BPPCT-039 + UCD-CH18 + BPPCT-037 + BPPCT-026 + PMS-3 + PMS-67 + UDP96-001	0.0	19

pairs, instead of the use of X_k, considering that the efficiency of a primer does not depend on its discrimination power alone, but also on its independence from the set of primers already selected. When the empirical results are analyzed, there was a group of 19 pairs of indistinguishable genotypes, corresponding to 21.6% of the genotypes analyzed in this study. This number was reached with just the five most informative markers (BPPCT-040, PMS-30, BPPCT-039, UCD-CH18 and BPPCT-037). The following pairs or group of genotypes could not be differentiated by any combination of the whole set of nine microsatellite markers tested: 'Lapins'/'Sweet Georgia', 'Schneider'/'Germersdorfi'/'Badacsony', 'Stella'/'Compact Stella', 'Sunana'/'PG', 'Symphony'/'Staccato'/'Early Bing', 'Van'/'Van Compact', 'Tulare'/'C78', and 'Bing WAB13'/'Bing 260'/'Sunset' (Figure 3). Similar results were observed by Wünsch and Hormaza (2002) and by Fernández i Marti (2012) by using microsatellites for analysis of sweet cherry genotypes derived from mutations of a single genotype. Only the use of single nucleotide polymorphisms (SNP) in the later study, allowed the differentiation of the sport 'Compact Stella' from the original cultivar.

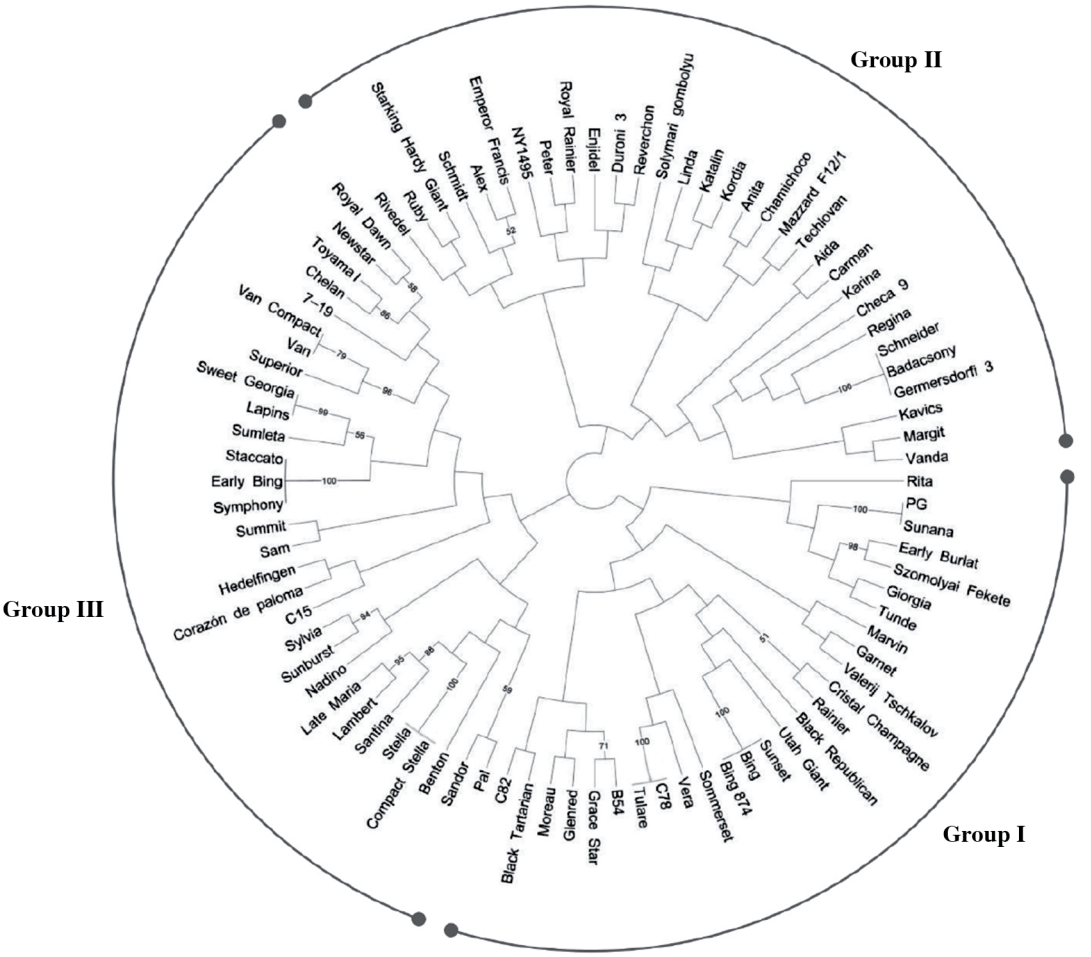
The use of this particular set of microsatellite markers allowed us to differentiate 69 out 88 genotypes (78.4%) of the analyzed cultivars. Five out of the nine microsatellite markers tested, showed high discrimination power, confirming the high efficiency of this type of marker for cultivar identification. Similar results were obtained by Wünsch and Hormaza (2002), who were able to differentiate 68 sweet cherry cultivars using nine microsatellite markers (89.5% of the studied population) and Liu et al. (2018), who completely differentiated 95 sweet cherry accessions using five microsatellite markers (100% of the studied population). Differentiation of all cultivars analyzed in this study was achieved with the same five microsatellite markers indicated previously, if one representative cultivar of each pair or group of cultivars with the same profile is considered (data not showed).

Sweet cherry diversity

A Neighbor-Joining dendrogram was constructed to assess the genetic diversity and establish the relationship amongst the sweet cherry accessions based on binary data collected for the nine microsatellite markers tested on the complete set of 88 genotypes (Figure 3). Three clusters were clearly observed and they were consistent with the presumed pedigree-based relationship of the cultivars: Group I included 27 cultivars, most of them from U.S. breeding programs; Group II included 31 cultivars, most of them with European origin; and Group III, included 30 cultivars mainly released by the Summerland Breeding Program in Canada. Using information compiled in Table 1 about pedigree relationships and cultivars origin, grouping was consistent for most of the cultivars. For example, 'Van' and 'Stella' are parent or grandparent of a series of other cultivars analyzed in this study and they appear grouped with most of their relatives in the same group. 'Bing' was grouped closely together with 'Black Republican', its maternal parent, and simultaneously, it was grouped together with 'Rainier', a descendent from the cross of 'Bing' × 'Van'.

When cultivar's origin was considered, 'Duroni 3' and 'Reverchon', that cluster together, where both taken to France from Italy (Wünsch and Hormaza, 2002); while 'Schneider', 'Germersdorfi' and 'Badacsony' that also clustered together, correspond to synonymous denominations of 'Ferrovia', an Italian cultivar spread in Central and Eastern Europe (Palasciano et al., 2006). 'Mazzard F12/1', a cherry rootstock, was grouped with European cultivars, probably because of its origin as a selected seedling at East Malling Research Station of England, which presents uncommon alleles compared to other cultivars in the same group.

Figure 3. Neighbor-Joining dendrogram of 87 sweet cherry cultivars based on their allelic variation at nine microsatellite loci.

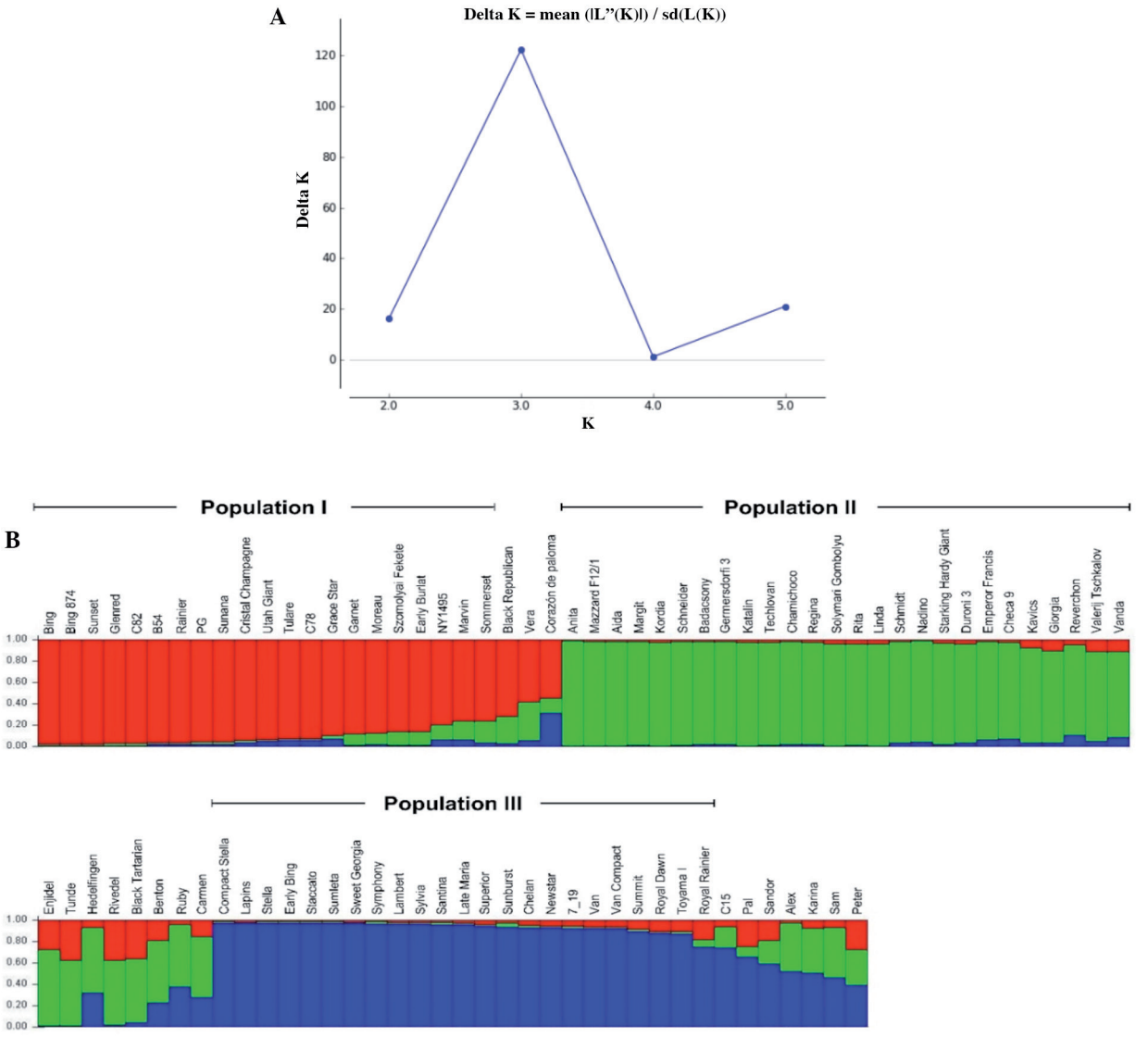


Respect to the cultivars that were not distinguishable using nine microsatellites, they exhibit in the dendrogram a high bootstrap value. 'Symphony', 'Staccato', and 'Early Bing' grouped together, but this is probably a misidentification of the samples, being necessary to use new certified samples from a germplasm repository to confirm their real identity. The pairs of cultivars 'Sunana'/'PG' and 'Tulare'/'C78' may correspond to the original cultivar and one clone with a code, while 'Van'/'Van Compact', 'Stella'/'Compact Stella', 'Lapins'/'Sweet Georgia' and 'Bing WAB13'/'Bing 260'/'Sunset' correspond to somaclonal mutants, or sports, from the original cultivar, respectively. The improbability of differentiating mutants that differ from the original genotype in one or a few genes is expected due to the intrinsic nature of this type of marker, since it is very unlikely that the microsatellite matches the mutated DNA region, considering they have been randomly chosen or selected from the whole genome (Wünsch and Hormaza, 2002). The use of SNPs has been demonstrated as a valuable tool for cultivars fingerprinting (Fernández i Marti et al., 2012), but microsatellite markers have demonstrated their usefulness for fingerprinting, pedigree and genetic analysis in different schemes, and probably they will continue being extensively used for diverse studies in the future.

Population structure analysis

Population genetic structure among the studied cultivars using STRUCTURE software suggested three populations (maximum ΔK-value of K = 3; Figure 4A). This is in accordance with the three groups found in the phylogenetic analysis (dendrogram in Figure 3). These three populations are shown in Figure 4B. Population I contains 20 cultivars present in Group I and one cultivar ('NY1495') present in Group II. Population II contains 22 cultivars present in Group II,

Figure 4. Estimation of the population structure for 87 sweet cherry cultivars grown in Chile. (A) Graph showing ΔK calculated according to STRUCTURE Harvester for the microsatellite dataset; (B) STRUCTURE plot obtained from the microsatellite dataset. The optimal population number was K = 3. Accessions with colored segments indicate their admixed origin, with a membership probabilities threshold of 0.80.



while three cultivars are from Group I ('Giorgia', 'Rita' and 'Valerij Tschkalov') and one is from Group III ('Nadino'). Population III is formed for 22 cultivars from Group III and one cultivar ('Royal Rainier') from Group II. Eighteen cultivars were classified as admixed. These results are substantially in agreement with previous published genetic studies in sweet cherry, where cultivars were grouped based on presumed pedigree-based relationships (Mariette et al., 2010; Fernández i Marti, 2012; Liu et al., 2018).

CONCLUSIONS

Molecular characterization and the study of genetic diversity of sweet cherry cultivars provide valuable information for germplasm managers such as breeders and industry specialists such as nurseries, growers, distributors and retailers. Our results demonstrated the usefulness of inter-specific transferability of microsatellite markers as a valuable tool for the molecular characterization of sweet cherry cultivars most planted in Chile and elsewhere. The information obtained in

this study is the basis for developing a fingerprinting protocol using microsatellite markers for cultivars identification, germplasm management and breeding of sweet cherry.

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