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Identification of a minimal microsatellite marker panel for the fingerprinting of peach and nectarine cultivars

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Abbreviations: AFLP: amplified length polymorphism
IP: intellectual property
PCR: polymerase chain reaction
S-SAP: sequence-specific a*mplification* polymorphism
SSR: simple sequence repeats

The genetic characterization of 117 peach and nectarine cultivars (*Prunus persica* (L.) Batsch) using microsatellite (SSR) markers is presented. Analyzed genotypes include the complete list of cultivars under intellectual property (IP) protection in Chile. One hundred and two out of the 117 cultivars under study could be identified using only 7 SSRs. Other 5 cultivars were differentiated using 3 additional markers, but 5

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pairs of genotypes were not differentiable. The average expected heterozygosity for the set of markers was 0.55, ranging from 0.28 in BPPCT-008 to 0.81 in CPPCT-022, with an F value of 0.37. A Neighbor-Joining dendrogram showed that, with few exceptions, peaches and nectarines clustered separately. These results are the basis for the development of a fingerprinting protocol for the unequivocal identification of most of the peach and nectarine cultivars officially registered in Chile.

Peaches and nectarines (*Prunus persica* (L.) Batsch) are among the most important fruit crops of temperate climates. Modern breeding of this species began in the USA towards the end of the 19th Century, based on a very limited number of genotypes. Because of this and because of its high degree of natural self-pollination, peach cultivars are known to have a quite narrow genetic base (Scorza et al. 1985; Scorza et al. 1988). In contrast, there are a larger number of peach and nectarine cultivars compared with other fruit crops, due to a very intense breeding activity developed worldwide during many decades. For example, almost 500 new cultivars were released around the world from 1990 to 1996 (Fideghelli et al. 1998).

To avoid misidentification of cultivars and to protect plant varieties owners' IP rights, efficient tools are needed such as DNA fingerprinting. The large number of cultivars and their very limited genetic diversity make cultivar differentiation and fingerprinting of this species particularly challenging. Microsatellites or simple sequence repeats (SSRs) are up to now the most powerful tool available for fingerprinting because of their characteristics of ubiquitous distribution along the eukaryotic genomes, high level of polymorphism, co-dominant inheritance, high discrimination power and easiness of detection (Morgante and Olivieri, 1993). A number of SSR markers for *P. persica* and other species of the same genus have been described until now, making hundreds of these markers available for different purposes (Cipriani et al. 1999; Sosinski et al. 2000; Testolin et al. 2000; Aranzana et al. 2002; Dirlewanger et al. 2002). These markers have been used for fingerprinting of peaches and nectarines (Cipriani et al. 1999; Aranzana et al. 2003a), apricots (Messina et al. 2004), plums (Decroocq et al. 2004; Mnejja et al. 2004), cherries (Wünsch and Hormaza, 2002; Vaughan and Rusell, 2004), and almonds (Testolin et al. 2004). Also, they have been used to construct inter- (Foolad et al. 1995; Dirlewanger et al. 2004) and intra-specific linkage maps (Howad et al. 2005), and their on the *Prunus* genetic map have been determined for most of them (Aranzana et al. 2003a). Some of the SSR markers isolated from peach have been used in other species of *Prunus* (Downey and Iezzoni, 2000) and *vice versa*, according to the close genetic relationships between *Prunus* species. This is very convenient because there exists a chance to apply the same set of markers to identify cultivars in different *Prunus* species, as well as in inter-specific hybrids used as rootstocks.

Aranzana et al. (2003b) characterized and differentiated over 200 cultivars of peaches and nectarines commonly grown in Spain, using a selected set of 16 microsatellite markers. Cultivars grown in Chile come mainly from North American breeding programs. So, when we considered the 100+ most planted cultivars in Chile it was found that less than 10% where shared by the set of Spanish cultivars previously characterized. Assuming that the Spanish varieties derived from a different genetic background respect of the American varieties, we hypothesized that the set of markers used by Aranzana et al. (2003b) would probably not have the best performance to identify the varieties registered in Chile. Therefore the purpose of this work was to determine to what extent the set of SSR markers used elsewhere can be useful to differentiate the main peach and nectarine cultivars grown in Chile and to find out the minimal set of markers that could afford this task. Also, in doing that we would be able to determine the genetic diversity of the of *P. persica* germplasm present in Chile.

MATERIALS AND METHODS

Plant material

A total of 117 *P. persica* cultivars, 64 peaches and 53

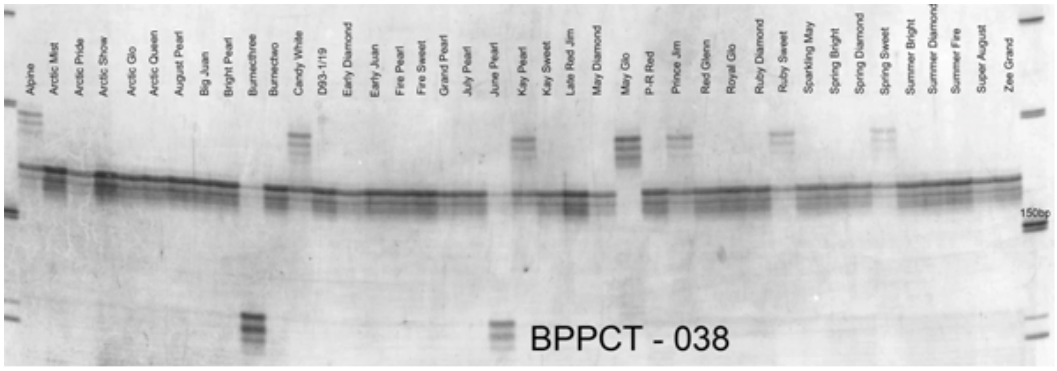

Figure 1. Microsatellite pattern of nectarine cultivars for the marker BPPCT-038.

Table 1. List of peach and nectarine cultivars used in this study.

Cultivar	Source	Fruit		Cultivar	Source	Fruit trait[a]
Andros	local nursery	P		Alpine-RVP	RVP-SAG	N
Andross-RVP	RVP-SAG[b]	P		Arctic Mist-RVP	RVP-SAG	N
August Lady-RVP	RVP-SAG	P		Arctic Pride-RVP	RVP-SAG	N
Autumn Flame-RVP	RVP-SAG	P		Arctic Show-RVP	RVP-SAG	N
Bowen	local nursery	P		Artic Glo-RVP	RVP-SAG	N
Bowen-RVP	RVP-SAG	P		Artic Queen-RVP	RVP-SAG	N
Burpeachfour-RVP	RVP-SAG	P		Artic Snow	local nursery	N
Burpeachone-RVP	RVP-SAG	P		August Pearl-RVP	RVP-SAG	N
Burpeachsix-RVP	RVP-SAG	P		August Red	RVP-SAG	N
Burpeachthree-RVP	RVP-SAG	P		Big Juan-RVP	RVP-SAG	N
Burpeachtwo-RVP	RVP-SAG	P		Bright Pearl-RVP	RVP-SAG	N
Cal Red	local nursery	P		Burnecthree-RVP	RVP-SAG	N
Carson	local nursery	P		Burnectwo-RVP	RVP-SAG	N
Carson-RVP	RVP-SAG	P		Candy White-RVP	RVP-SAG	N
Corona	local nursery	P		D 93-1/19-RVP	RVP-SAG	N
Corona-RVP	RVP-SAG	P		Early Diamond-RVP	RVP-SAG	N
Crown Princess-RVP	RVP-SAG	P		Early Juan-RVP	RVP-SAG	N
Dee Six - 15W-RVP	RVP-SAG	P		Fiesta	local nursery	N
Diamond Princess-RVP	RVP-SAG	P		Fire Pearl-RVP	RVP-SAG	N
Dixon	local nursery	P		Fire Sweet-RVP	RVP-SAG	N
Doctor Davis	local nursery	P		Grand Pearl-RVP	RVP-SAG	N
Dr Davis-RVP	RVP-SAG	P		July Pearl-RVP	RVP-SAG	N
Earlirich-RVP	RVP-SAG	P		June Pearl-RVP	RVP-SAG	N
Early Magestic	local nursery	P		Kay Pearl-RVP	RVP-SAG	N
Elberta-RVP	RVP-SAG	P		Kay Sweet-RVP	RVP-SAG	N
Elegant Lady	local nursery	P		Late Red Jim-RVP	RVP-SAG	N
Elegant lady-RVP	RVP-SAG	P		May Diamond-RVP	RVP-SAG	N
Everts-RVP	RVP-SAG	P		May glo-RVP	RVP-SAG	N
Flavor Crest	local nursery	P		Nectar Crest	local nursery	N
Flordagrand-RVP	RVP-SAG	P		P-R Red-RVP	RVP-SAG	N
Halford-RVP	RVP-SAG	P		Prince Jim-RVP	RVP-SAG	N
Ito Red-RVP	RVP-SAG	P		Red Diamond	local nursery	N
Ivory Princess-RVP	RVP-SAG	P		Red Glenn-RVP	RVP-SAG	N

Klamp	local nursery	P		Rio	local nursery	N
Klampt-RVP	RVP-SAG	P		Royal Delight	local nursery	N
Land Reth-RVP	RVP-SAG	P		Royal Glo-RVP	RVP-SAG	N
Lindo	local nursery	P		Ruby Diamond-RVP	RVP-SAG	N
Loadell	local nursery	P		Ruby Sweet-RVP	RVP-SAG	N
Loadell-RVP	RVP-SAG	P		September Lady	local nursery	N
Loadel-RVP	RVP-SAG	P		Sparkling May-RVP	RVP-SAG	N
Manon	local nursery	P		Spring Bright-RVP	RVP-SAG	N
Monaco-RVP	RVP-SAG	P		Spring Diamond-RVP	RVP-SAG	N
Pomona	local nursery	P		Spring Red	local nursery	N
Queen Crest -RVP	RVP-SAG	P		Spring Sweet-RVP	RVP-SAG	N
Reigels-RVP	RVP-SAG	P		Summer Bright-RVP	RVP-SAG	N
Rich Lady-RVP	RVP-SAG	P		Summer Diamond	local nursery	N
Rich May-RVP	RVP-SAG	P		Summer Diamond-RVP	RVP-SAG	N
Robin Neil-RVP	RVP-SAG	P		Summer Fire-RVP	RVP-SAG	N
Rome Star-RVP	RVP-SAG	P		Sun Grand	local nursery	N
Ross Peach	local nursery	P		Sun Rise	local nursery	N
Ross Peach-RVP	RVP-SAG	P		Super August-RVP	RVP-SAG	N
Ross-RVP	RVP-SAG	P		Super Queen	local nursery	N
Ryan Sun-RVP	RVP-SAG	P		Zee Grand-RVP	RVP-SAG	N
Scarlet Snow-RVP	RVP-SAG	P				
September Snow-RVP	RVP-SAG	P				
September Sun-RVP	RVP-SAG	P				
Snow King-RVP	RVP-SAG	P				
Snow Princess-RVP	RVP-SAG	P				
Sullivan #4-RVP	RVP-SAG	P				
Summer Lady-RVP	RVP-SAG	P				
Sweet September-RVP	RVP-SAG	P				
TUFTS-RVP	RVP-SAG	P				
Western Sun-RVP	RVP-SAG	P				
Wisser-RVP	RVP-SAG	P				

[a] P: peach, N: nectarine.
[b] RVP-SAG: *Registro de Variedades Protegidas* of SAG-Chile.

nectarines, were used in this study (Table 1). Most of them (88) came from the Chilean Registry of Protected Varieties, of the Ministry of Agriculture (RVP-SAG) and the rest were obtained from various nurseries. For plant DNA extraction, leaf samples were collected from actively growing shoots during late spring to early summer, transported to the laboratory in refrigerated containers and immediately stored at -80ºC.

Plant DNA extraction, PCR amplification and electrophoretic fragment separation

Genomic DNA was extracted following the method described by Lodhi et al. (1994). Extracted genomic DNA was PCR-amplified using 9 pairs of microsatellite primers (Table 2). PCR reactions were performed in 16 µl volumes containing 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 1.5 mM $MgCl_2$, 1 mM dNTPs, 50 pmol each primer, 20 ng genomic DNA, 0.5 µl *Taq* polymerase. Reactions were carried out on a Mastercycler Eppendorf thermocycler using the following temperature profile: an initial step of 5 min at 95ºC, 35 cycles of 30 sec at 94ºC, 30 sec at 56ºC and 30 sec at 72ºC, and a final step of 5 min at 72ºC. The PCR products were then denatured by the addition of 0.5 vol of 95% formamide/dye solution (loading dye: 95% deionized formamide, 10 mM EDTA, 0.1% xylene cyanol), heating for 5 min at 94ºC, chilled on ice and then 6 µl of the denatured preparation were loaded on a 6% polyacrylamide gels containing 7.5 M urea in 0.5 X TBE buffer (90 mM Tris, 90 mM boric acid, 2 mM EDTA). Gels were run for approximately 2 hrs at 85 W. Following electrophoresis, the gel was silver-stained according to the protocol described by Creste et al. (2001). Fragment sizes were estimated by comparison with known allelic patterns.

Data analysis

The parameters used to evaluate the information given by the 9 SSRs studied were the number of alleles (A) and the effective number of alleles (A_e) per locus ($A_e = 1/ \Sigma pi^2$, where *pi* is the frequency of the i^{th} allele), the observed heterozygosity (H_o = number of heterozygous individuals/number of individuals scored), the expected

Table 2. **Microsatellite primer pairs used for the analyses of peach and nectarines genotypes.**

Locus	Primer Sequence 5' – 3'	Allelic size range (pb)	T_a (ºC)	Reference
BPPCT-001	AAT TCC CAA AGG ATG TGT ATG AG	128 – 168	56	Dirlewanger et al. 2002
	CAG GTG AAT GAG CCA AAG C			
BPPCT-006	GCT TGT GGC ATG GAA GC	111 – 137	56	Dirlewanger et al. 2002
	CCC TGT TTC TCA TAG AAC TCA CAT			
BPPCT-007	TCA TTG CTC GTC ATC AGC	124 – 147	56	Dirlewanger et al. 2002
	CAG ATT TCT GAA GTT AGC GGT A			
BPPCT-008	ATG GTG TGT ATG GAC ATG ATG A	99 – 160	56	Dirlewanger et al. 2002
	CCT CAA CCT AAG ACA CCT TCA CT			
BPPCT-038	TAT ATT GTT GGC TTC TTG CAT G	135	56	Dirlewanger et al. 2002
	TGA AAG TGA AAC AAT GGA AGC			
CPPCT-022	CAA TTA GCT AGA GAG AAT TAT TG	249 – 297	56	Aranzana et al. 2002
	GAC AAG AAG CAA GTA GTT TG			
CPPCT-029	CCA AAT TCC AAA TCT CCT AAC A	170 – 194	52	Aranzana et al. 2002
	TGA TCA ACT TTG AGA TTT GTT GAA			
CPPCT-030	TGA ATA TTG TTC CTC AAT TC	170 – 200	52	Aranzana et al. 2002
	CTC TAG GCA AGA GAT GAG A			
PMS-67	AGT CTC TCA CAG TCA GTT TCT	144 - 191	56	Cantini et al. 2001
	TTA ACT TAA CCC CTC TCC CTC C			

[a] T_a: annealing temperature.

heterozygosity ($H_e = 1 - \Sigma pi^2$) and Wright's fixation index ($F = 1 - H_o/H_e$).

To compare the efficiency of the markers in varietal identification, we estimated the discrimination power (D) of each primer as in Tessier et al. (1999):

C_j (confusion probability for the j^{th} primer) is equal to the sum of the different c_i for all I patterns generated by the primer:

$$C_j = \sum_{i=1}^{1} c_i = \sum_{i=1}^{1} p_i \frac{(Np_i - 1)}{N - 1}$$

Where *pi* is the frequency of the i^{th} allele and N is the number of individuals scored.

Thus, the discriminating power of the j^{th} primer is equal to:

$D_j = 1 - C_j$

Total number of non-differentiated pairs of varieties for the j^{th} primer is given by:

$x_j = (N(N-1)/2)\ C_j$

For a given combination of k primers, Xk is equal to:

$$X_k = \frac{N(N-1)}{2} \prod_{j=1}^{k} C_j.$$

Abinary matrixwas constructed based on the presence/absence of microsatellite alleles. Phylogenetic trees were performed using the Neighbor Joining method with PAUP 4.0b software (Swofford, 1998).

RESULTS

Microsatellite polymorphism

A total of 117 peach cultivars were analyzed with 9 informative microsatellite markers. These 9 markers were previously selected as the most polymorphic from a larger set of SSRs, after evaluating a sub-sample of ca. 30 peach and nectarine cultivars (results not shown). Alleles were clearly differentiated by polyacrylamide gel electrophoresis. The separation of the amplicons for BPPCT-038 is shown as an example in Figure 1.

The statistical parameters obtained for the SSR markers used in this study are presented in Table 3. These SSRs amplified 59 alleles on this population of cultivars, with an average of 6.6 alleles per *locus* (ranging from 4 in BPPCT-038 to 9 in CPPCT-022). Allele frequencies ranged from 0.004 to 0.846, with an average of 0.152; more than half of these alleles (41) were *rare alleles* ($p_i \leq 0.1$). We found 5 markers exhibiting alleles with $p_i \geq 0.6$; this would explain why the H_e of those markers was lower than average (BPPCT-006, BPPCT-007, BPPCT-008, BPPCT-038 and CPPCT-029). For example, the marker BPPCT-008 amplified 6 alleles but one of them was over-represented with $p_i = 0.846$. Considering all *loci* under scrutiny, the average H_o was 0.345 (ranging from 0.145 in BPPCT-008 to 0.573 in PMS-67) and the average H_e was 0.552 (ranging from 0.276 in BPPCT-008 to 0.813 in CPPCT-022). Consequently, F values were positive with a mean value of 0.374.

Prunus persica diversity

A Neighbor Joining dendrogram (Figure 2) based on binary data collected for the 9 markers tested on the complete set of 117 genotypes, showed that peaches and nectarines clustered in 2 independent groups, with the exception of 5 nectarines that were included in the peaches cluster, and 13 peaches included among nectarines. 'September Lady' was classified as a nectarine by the local provider of the sample, but according to the California Department of Food and Agriculture this is a peach cultivar. According to our data, Septermbre Lady clustered with peaches. 'Ivory Princess' and 'Crown Princess', 2 of the peach cultivars found in the nectarines cluster have a nectarine as a parent. 'Ivory Princess' was developed as a first generation cross using 'Crown Princess' yellow flesh peach as the selected seed parent and 'June Pearl', a white flesh nectarine, as the selected pollen donor. 'Crown Princess' was the result of a seedling using 'Red Diamond' nectarine as the selected seed parent and an unknown peach seedling as the pollen parent.

The separation in 2 main clusters is consistent with an independent management of each genetic pool, driven by breeders during many decades. Most of the peaches used in this study were yellow fleshed, but they tend to group separately in clingstone/melting cultivars and freestone/non-melting cultivars. As with peaches, nectarine cultivars were mostly yellow fleshed, but the white fleshed ones tend to group together also. Based on this clustering, the diversity parameters were calculated for peaches and for nectarines separately. Peach cultivars had 51 alleles, 15 of them (29%) specific to this group; nectarine cultivars, had 44 alleles, 8 (18%) specific to this group. The mean values of H_o, H_e and F for the nectarine group were lower compared with the peach group, suggesting a different genetic structure between these groups.

Determination of the optimal SSR combination

To determine the optimal SSR combination for cultivar identification, the SSR markers were ranked according to the number of observed genotypes (Table 4). Marker CPPCT-022 produced the largest number of observed genotypes (n = 25) and alleles (n = 9). The lowest values for these 2 parameters were determined for markers

Table 3. Variability parameters of *P. persica* obtained with 14 SSRs makers in 117 cultivars.

SSR/cv group	# Individuals scored	A[a]	Ae[b]	Ho[c]	He[d]	F[e]	# Genotypes
BPPCT-001	117	7	3,4	0,316	0,708	0,553	15
BPPCT-006	117	8	2,3	0,376	0,565	0,335	16
BPPCT-007	117	5	2,0	0,154	0,509	0,698	7
BPPCT-008	117	6	1,4	0,145	0,276	0,473	9
BPPCT-038	117	4	1,6	0,291	0,373	0,221	7
CPPCT-022	117	9	5,4	0,47	0,813	0,422	23
CPPCT-029	117	8	2,1	0,376	0,512	0,266	13
CPPCT-030	117	7	2,5	0,402	0,607	0,339	10
PMS-67	117	5	2,5	0,573	0,607	0,056	9
M		**6,6**	**2,6**	**0,345**	**0,552**	**0,374**	
M peaches		**5,7**	**2,8**	**0,356**	**0,574**	**0,367**	
M nectarines		**4,9**	**2,2**	**0,331**	**0,463**	**0,350**	

A^a: Number of alleles; $A_e{}^b$: Effective number of alleles; $H_o{}^c$: Observed heterozygosity
$H_e{}^d$: Expected heterozygosity; F^e: Wright's Fixation Index; M^f: mean.

BPPCT-038 and BPPCT-007, reaching only 7 observed genotypes and 4 and 5 alleles, respectively.

When using the single marker CPPCT-022, 92 indistinguishable pairs are obtained. As new SSR markers are added to the analysis, the theoretical number of undistinguishable genotypes (X_k) diminishes to finally reach a value of 2.4 (Table 5). When comparing this value with the empirical result, there was a group of 7 pairs of indistinguishable genotypes, a number reached with just the first 7 markers. This means that 10.1% of the cultivars could not be individually resolved. For this reason, the last 2 markers (BPPCT-007 and BPPCT-038) where discarded and an additional set of 5 markers were tested (CPPCT-002, CPPCT-005, UDP98-410, GA-34 and PS9f8). The result was that any of the markers PS9f8, UDP98-410 and GA-34 could differentiate 'Sparkling May' and 'Summer Diamond'. Also, marker CPPCT-002 identified a polymorphism among 'Ross' and 'Ross Peach', and 'Ruby Diamond' was effectively discriminated respect of the pair 'Burnectwo'/'Burnecthree' with markers CPPCT-005 or GA-34. The following pairs of genotypes: 'Burnectwo'/'Burnecthree', 'Ryan's Sun'/'Summer Lady', 'Sun Rise'/'Nectarcrest' and 'Halford'/'Sullivan #4' could not be differentiated by any combination of the whole set of 14 SSR markers tested.

DISCUSSION

Microsatellite polymorphism

A total of 117 peach and nectarine cultivars were studied with a basic set of 9 polymorphic SSR, which amplified a total of 59 alleles. The average number of alleles per locus was 6.6, a value slightly lower than previously reported by Aranzana et al. (2003b) in a population of 212 peach cultivars with 16 SSR markers (7.3), and largely higher than the observed by Sosinski et al. (2000) in a set of 28 peach cultivars with 8 microsatellite markers (2.6), Aranzana et al. (2002) in a set of 24 peach cultivars with 24 polymorphic SSRs (3.2) and Testolin et al. (2000) in a set of 50 peach cultivars with 26 SSR markers (4.5). These differences in information content per marker can be explained by the number of cultivars studied in every case, reflecting the higher chance to find more alleles when the number of genotypes is also higher.

The number of observed alleles per *locus* in peach was lower (4-9 in this work) than those observed in other fruit

Table 4. Primer discrimination power (D) and number of observed genotypes and indistinguishable pairs.

SSR marker	# scored Individuals	Cj	D	# Genotypes	# of indistinguishable pairs (observed value)
CPPCT-022	117	0,180	0,820	25	92
BPPCT-006	117	0,430	0,570	16	101
BPPCT-001	117	0,286	0,714	15	102
CPPCT-029	117	0,483	0,517	13	104
CPPCT-030	117	0,387	0,613	10	107
BPPCT-008	117	0,722	0,278	9	108
PMS-67	117	0,388	0,612	9	108
BPPCT-007	117	0,486	0,514	7	110
BPPCT-038	117	0,624	0,376	7	110

#: Number.

crops such as apples, which ranged from 6-13 with an average of 9.2 (Galli et al. 2005). On the contrary, it was higher than the value determined in a group of 10 sweet cherry cultivars also studied with 9 SSRs, which ranged from 3-6 with an average of 4.1 (Kaçar et al. 2005). However, this kind of comparisons must take into consideration the number of genotypes under study, and the number of sweet cherry cultivars of the cited paper was too small to allow a valid comparison. This result is in agreement with the narrow genetic background known to peach and nectarine modern varieties, which main source of diversity came from a reduced number of genotypes originally bred in North America by the end of XIXth century.

The mean H_o and H_e values found in our work (0.345 and 0.552, respectively) were very close to those observed in a previous work by Aranzana et al. (2003b) (0.350 and 0.500, respectively). In this case, the comparison among both results is possible because of the number of genotypes and markers used.

Allele frequency range was quite diverse, ranging from 0.004 to 0.846 with a mean value of 0.152. A total of 41 *rare alleles* ($p_i \leq 0.1$) were found and, by the opposite, six alleles with $p_i \geq 0.6$ were identified; both diminished severely the heterozygosity of the harboring *loci*. The alleles occurring at higher frequencies at each locus could be considered as set points of sequence repeats and new alleles might sequentially derive from them, by increasing or decreasing the number of repetitions (Xu et al. 2000).

Prunus persica diversity

As mentioned earlier, the dendrogram obtained with our SSR data clearly separate peaches and nectarines into different genetic groups. This is consistent with the breeders practice of not making inter-group crossings. Only a few exceptions to this rule were detected, such as peaches 'Ivory Princess' and 'Crown Princess' that clustered together with nectarines. This "abnormality" could be tentatively explained analyzing the origin of their progenitors; 'Ivory Princess' derives from the cross of 'Crown Princess', a peach, and 'June Pearl', a nectarine. 'Crown Princess' comes from a cross of 'Red Diamond', a nectarine, with an unknown peach cultivar. In any case, both genetic groups are quite alike and to find differences is more difficult than to find genetic similarities.

In spite of that, there were consistent differences between genetic parameters for peaches and nectarines, with nectarines presenting lower diversity indexes expressed in less alleles and in lower values for H_o and H_e. This could mean that the level of heterozygosity is less in nectarines than in peaches, but the different number of individuals in each population (53 nectarines vs. 64 peaches) could alter this conclusion, as discussed before. A larger sample, with

Table 5. Selection of the most efficient minimum set of SSR markers for the identification of the 117 different cultivars.

SSR combination	# of Indistinguishable pairs	
	X_k	Experimentally observed
CPPCT-022	1221,5	92
CPPCT-022 + BPPCT–006	525,2	54
CPPCT-022 + BPPCT–006 + BPPCT-001	150,2	23
CPPCT-022 + BPPCT–006 + BPPCT–001 + CPPCT-029	72,6	17
CPPCT-022 + BPPCT–006 + BPPCT-001 + CPPCT–029 + CPPCT-030	28,1	14
CPPCT-022 + BPPCT–006 + BPPCT–001 + CPPCT–029 + CPPCT–030 + BPPCT-008	20,3	10
CPPCT-022 + BPPCT–006 + BPPCT–001 + CPPCT–029 + CPPCT– 030 + BPPCT-008 + PMS–67	7,9	7
CPPCT-022 + BPPCT–006 + BPPCT–001 + CPPCT–029 + CPPCT–030 + BPPCT-008 + PMS–67 + BPPCT-007	3,8	7
CPPCT-022 + BPPCT–006 + BPPCT–001 + CPPCT–029 + CPPCT–030 + BPPCT-008 + PMS–67 + BPPCT-007 + BPPCT-038	2,4	7

#: Number.

equal number of peaches and nectarines, may be required to confirm this assumption.

Cultivar identification

The use of this particular set of SSR markers allows us to differentiate 88.9% of the analyzed cultivars; 7 of the 9 SSR markers tested showed high discriminating power, confirming the high efficiency of this type of marker for cultivar identification. These results are similar at some extent to those reported by Aranzana et al. (2003b), who were able to differentiate 87% of 212 cultivars of European origin with 16 SSR markers, which includes the markers used in this work. In comparison, 66 apple cultivars (excluding cultivars derived from clonal mutations, such as those of 'Fuji' or 'Gala') were discriminated using just 4 SSR markers (Galli et al. 2005); in sweet cherries, 68 cultivars (89.5% of the studied population) were discriminated using 9 SSR markers (Wünsch and Hormaza, 2002); and 224 grapevine cultivars were differentiated using 8 markers, but in this combining 6 RAPD (dominant) plus 2 SSR (co-dominant) markers (Tessier et al. 1999). These values suggest that peaches and nectarines are not much less diverse in comparison to other fruit crops, but this comparison is difficulted because in each case the criteria for the selection of the markers could have been different or less stringent, or in any way biased. So, the only conclusion possible to be done at this point is that the varieties of this stone fruit are possible to be differentiated with yields similar to other fruit crops, if the SSR markers are carefully selected.

The selection of markers will depend on the nature of the germplasm under study. In our case, the existence of a set of markers applied for the identification of Spanish germplasm was useful but not totally transferable to the Chilean peaches and nectarines varietal collection. A *bis-a-bis* comparison of markers used in both cases revealed that some markers were the most informative in both germplasm collections, notably CPPCT-022, BPPCT-006, BPPCT-001 and some others. However, the hierarchy of "quality" was different among this work and the one based on Spanish cultivars (Aranzana et al. 2003b). For example, marker CPPCT-029 was the fourth most informative in the case of the Chilean varieties, but was only the 10th in the case of varieties from Spain. This result confirms that the validation of a set of SSR markers for the fingerprinting of a particular species requires the evaluation of a set of genotypes that represent the largest possible genetic

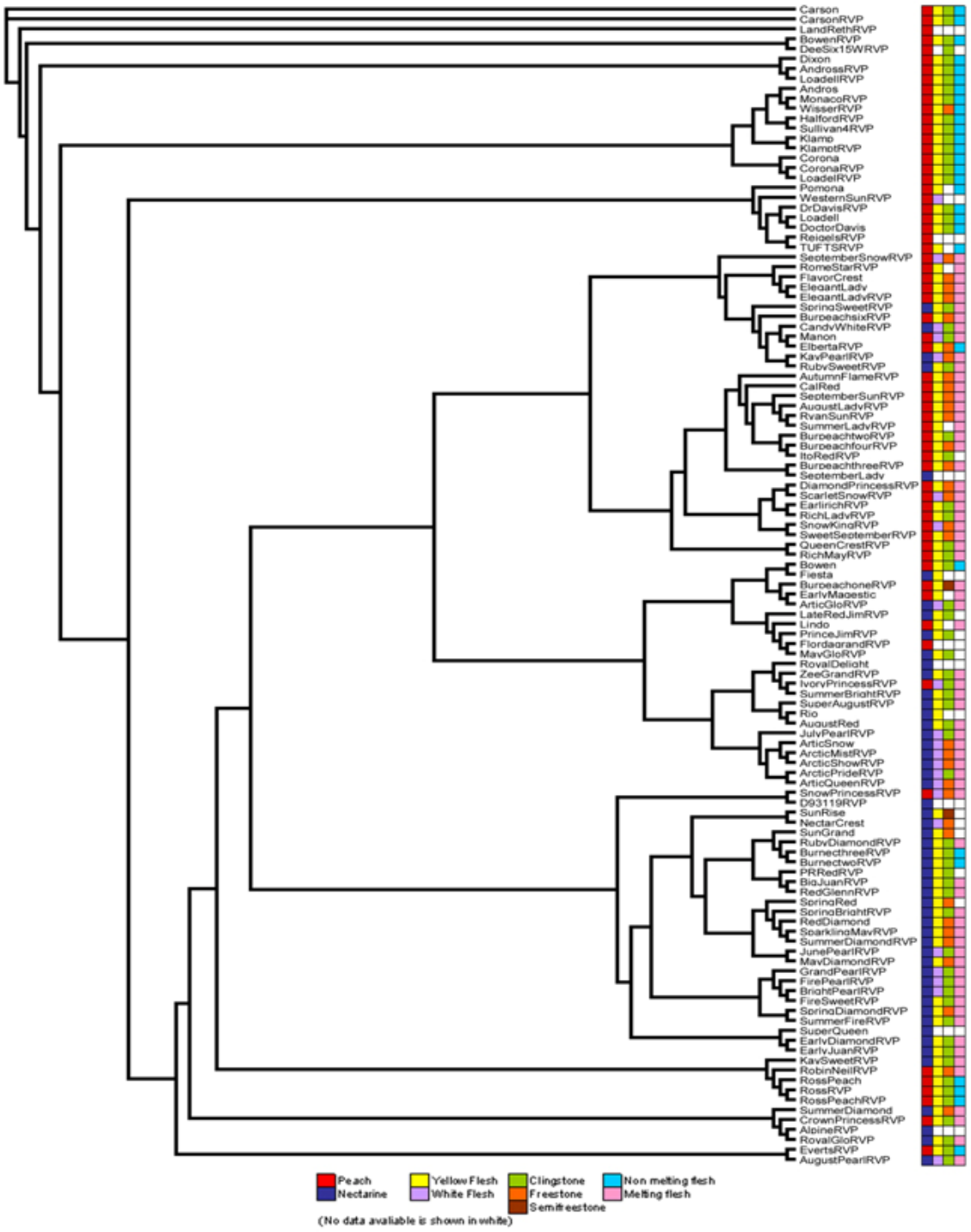

Figure 2. Neighbor joining dendogram of 117 *P. persica* cultivars based on their variation at 9 SSR loci. The color keys refer to peach/nectarine, flesh color, clingstone type and melting/non-melting type, as indicated.

diversity of the species of interest. The most stricking result presented here, in comparison with previous works on peach and nectarine cultivars differentiation, is the identification and validation of this set of 7 SSR markers (Table 4), information that could be very valuable for nurserymen who requires to maintain the "genetic quality" (true-to-typeness and homogeneity) of their genetic stocks.

Some cultivars were not distinguishable using the set of 9 markers. For example, 'Sparkling May' and 'Summer

Diamond', both siblings of 'Red Diamond', could only be differentiated with an additional set of markers. The same was true to diferenciate 'Ross', 'Ross Peach' and 'Ruby Diamond' from 'Burnectwo' and 'Burnecthree'. However, the last two cultivars, that share the same origin (siblings of the nectarines cross 'Grand Diamond' X 'Flameglo'), were not differentiated, even when testing a larger set of markers (results not shown). The same occurred in the following pairs: 'SunRise'/'NectarCrest', 'Halford'/'Sullivan #4' and 'Ryan's Sun'/'Summer Lady' (mutations derived from 'O'Henry'). In order to differentiate these 6 cultivars organized as pairs ('Burnectwo'/'Burnecthree', 'SunRise'/'NectarCrest' y 'Halford'/'Sullivan #4'), a larger number of SSRs may be needed. In the case of 'Ryan's Sun' and 'Summer Lady' that are mutations of the same cultivar, this approach may not work at all, and alternative techniques, such as AFLP or S-SAP may become necessary.

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