Phenotypic and genetic analysis of a peach and a Japanese plum core collection for pre-breeding and distinctness assessment

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ABSTRACT

To know the relationships between phenotypic and genetic variables in a germplasm collection of fruit crops is useful a pre-breeding and cultivar distinctness. A core collection of 23 peaches/nectarines (*Prunus persica* (L.) Batsch), and 16 Japanese plum (*Prunus salicina* Lindl. var. *salicina*) cultivars were evaluated for 33 and 29 characteristics (botanical/productive) respectively during two growing seasons. Also, eight specific simple sequence repeats (SSRs) markers were analyzed in both species. Principal components analyses revealed seven characteristics (related to the size of the fruit and the firmness of the pulp) as the most important for the 23 peach/nectarine cultivars and four characteristics (yield, fruit size, soluble solids, and harvest time) for the 16 Japanese plum cultivars. These analyses revealed three cultivars of peaches (Diamond Princess, Dixon, and Dr. Davis) and three of nectarines (Ruby diamond, Artic sweet, Summer fire) with the highest values for fruit size and pulp firmness. Four Japanese plum cultivars (Angeleno, Flavor Rich, Red Heart, and Pink Delight) showed the highest values of yield, fruit size, soluble solids, and harvest time. Elite germplasms to carry out a breeding program were identified from both the phenotypic and genetic analysis. Additionally, cultivar-specific SSR alleles were identified and are a relevant tool for cultivar distinctness.

Key words: Germplasm collection, *Prunus persica*, *Prunus salicina*, Rosaceae, SSRs.

INTRODUCTION

China is the primary center of origin for peach and Japanese plum; from there, both species were spread throughout the world. Archeological evidence indicates that the human population has been using peaches in China since 8000-7000 BP. However, its cultivation is recorded in Chinese literature from around 4000 years ago. Peach arrived in Rome approximately 2000 years later, and during the Roman empire, it spread throughout modern-day Europe. During the 16th century, peaches were introduced into Florida, Mexico, and South America by Spanish and Portuguese conquers. Between the 18th and 19th centuries, breeding programs in the USA and Europe began.

On the other hand, Japanese plum (*Prunus salicina* Lindl. var. *salicina*) originated from the Yangtze River Basin, and although it is not very important for Chinese culture, it has been cultivated for several thousand years and is still cultivated today (Carrasco et al., 2018). It is not clear when the Japanese plum was introduced into Japan; however, the plum stone has been dating back around 2300 years before the present, and it has been reported that the genetic improvement started in

Japan. Late in the 19th century, Luther Burbank imported 210 Japanese plum seedling germplasms to California, initiating the breeding program for this species in USA. Today most of the cultivars grown around the world were developed by intercrossing those original selections with other plum species and also with apricot.

The USA, France, and Italy developed about 80% of the peach, nectarine, and Japanese plum cultivars currently grown in the world. Whereas South Africa, Australia, China, Japan, Mexico, and Brazil are responsible for developing the remaining cultivars. Initial breeding goals included fruit quality, postharvest life, disease/pest resistance, fruit types, and maturity timing.

In Chile, fruit trees production began approximately 400 years ago. Around the year 1550, Spanish conquers introduced apple trees, stone fruits, and grapes, among others, and during the first decade of the 20th-century, fruits were exported to Europe and USA. From that time, the Chilean industry of fresh fruit production has been based mainly on cultivars developed in California and some European countries. All of these cultivars were introduced to evaluate its productivity and adaptation to local environmental conditions. Currently, the Chilean peach and nectarine exportation is based on 36 cultivars, exporting 45 000 to 51 000 t during the 2016/2017 and 2017/2018 seasons, respectively (ASOEX, 2017). In the case of Japanese plums, 16 cultivars account for 70% of the 85 000 to 90 000 t exported during the 2016/2017 and 2017/2018 seasons (ASOEX, 2017).

The knowledge of the relationships between genetic, morphological, phenological, and agronomic traits of plant collection is vital for agricultural management, breeding practice, and variety testing. In this regards, the phenotypic and genetic evaluation of germplasm collections is a crucial step to identify desirable characteristics and selecting potential parental lines to start the crossing cycle in a breeding program.

Additionally, the increasing number of varieties that every year have been delivered to the agricultural market has made it difficult to determine their distinctness only based on morphological characters, because they are non-stable due to environmental conditions. Moreover, most of the fruit crops are easily misidentified due to the abundance of synonymy and homonymy. This phenomenon leads to high numbers of repetitions in germplasm banks and confusions when they are commercialized. For this reason, it is necessary to use molecular markers to establish the identity of every cultivar.

Additionally, the molecular markers such as simple sequence repeats (SSRs) provide information about the level of genetic diversity and the relationships among cultivars. The main advantages of SSRs are their wide distribution through the genome; usually, they show a high level of variability and are codominant, which allows identifying a homozygous and heterozygous individual for each SSR locus. Fortunately, some specific SSRs are available, and they have been used to analyze the variability pattern of peach and Japanese plums cultivars (Dirlewanger et al., 2002; Mnejja et al., 2004; Carrasco et al., 2012; González et al., 2016).

In this work, we show that the analysis of phenotypic and genetic diversity is a useful pre-breeding tool to evaluate germplasm of peach and Japanese plums. Thereby providing information to select parental lines that show the best phenotype and the maximum genetic differences to each other, maximizing the new genetic combination in the progenies as well as a test for cultivar identification. The goal of this research was to evaluate a core collection of cultivars of Japanese plum and peaches, using phenotypic characteristics and simple sequence repeats (SSR) reported in the literature.

MATERIALS AND METHODS

Plant material and experimental design
During 2014 an orchard with 23 peach/nectarine cultivars (*Prunus persica* (L.) Batsch) and 16 Japanese plum (*Prunus salicina* Lindl. var. *salicina*) was established in the experimental station of Pontificia Universidad Católica de Chile located in Pirque (33°38'5.1'' S; 70°34'24.2'' W), Region Metropolitana, Santiago, Chile. Three plants of each cultivar were planted by the experimental unit (EU). The EUs were distributed following a completely randomized block design with five replicates for each cultivar. The phenotypic analysis was carried out for 15 fruits collected from each replicate associated with each cultivar. The list of each cultivar used in this research is indicated in Table 1. Phenotypic observations of Japanese plum and peach cultivars were carried out during the summer season of 2017 and 2018.

Phenotypic evaluation
A core collection of 16 Japanese plum and 23 peach cultivars were evaluated for 25 botanicals and 13 productive characteristics description (Table 2).

Table 1. *Prunus persica* (23) and *Prunus salicina* (16) cultivars tested in this study.

Cultivar name	Flesh color
Nectarine	
Artic Sweet	White
Artic Snow	White
August Red	Yellow
Candy Ice	White
Candy Pearl	White
Nectar Crest	Yellow
Ruby Diamond	Yellow
Summer Bright	Yellow
Summer Fire	Yellow
Zee Glo	Yellow
Peach	
Andross	Yellow
Carson	Yellow
Diamond Princess	Yellow
Dixon	Yellow
Dr. Davis	Yellow
Fortuna	Yellow
Halford	Yellow
Kurakata	White
Loadell	Yellow
Phillips Cling	Yellow
Ryan sun	Yellow
Rich May	Yellow
Zee Lady	Yellow
Japanese plum	**Skin color**
Angeleno	Dark purple
Aurora	Red
Black Queen	Dark purple
Blue Gusto	Dark purple
Catalina	Dark purple
Flavor Rich	Dark purple
Fortune	Red
Friar	Dark purple
Laroda	Dark purple
Larry Ann	Dark purple
Pink Delight	Yellow
Queen Rosa	Light Red
Red Heart	Red
Santa Rosa	Red
Sapphire	Red
September King	Light Red

Out of 38 characteristics analyzed, only those with complete data set (five replicates and 2 yr of analyses) were considered for each species. Therefore, for Japanese plum and peach cultivars, 29 and 33 characteristics were recorded, respectively. The phenotypic relationships among cultivars were investigated by principal component analysis (PCA) and discriminant analysis (DA; Sarigu et al., 2017). ANOVA was carried out to study the genetic component of phenotypic variability and to calculate the degree of genetic determination (DGD). The Shapiro-Wilk test was applied to verify the normality of the data.

A principal component analysis (PCA) to reduce the dimensionality of the data set into a small number of variables called principal components was carried out to explore a pattern of grouping among cultivars bases on the characteristics used in this study. The three first components with eigenvalue equal or superior to one were chosen. The percentage of variance explained by every component was estimated using 1000 bootstraps. From eigenvector (loadings), the importance of every characteristic was estimated through the coefficient of determination (CD) ($r^2 = ([\sqrt{\text{eigenvalue}}] \times \text{loadings})^2$). Characteristics with r^2 values equal or superior to 0.7 were considered as the most relevant to explain the variability observed among cultivars.

A DA was carried out to verify statistically the groups identified by the PCA and predict the accuracy of every cultivar to a previously defined group. Wilk's Lambda was estimated to determine the significance of the groups previously determined by PCA. The probability values of Wilk's Lambda inferior to 0.05 will indicate that the group is significantly different.

ANOVA was used to estimate the genetic variance of these characteristics, and later broad-sense heritability (H^2) was estimated. The genotypic and phenotypic variance (σ^2_g and σ_p) respectively, were estimated according to Sharma (1988) as follows:

$$\sigma^2_g = (MSG - MSE)/r$$
$$\sigma^2_p = \sigma^2_g + \sigma^2_e \, (MSE)$$

where MSG is the mean square of the genotypes, MSE ($= \sigma^2_e$) is the mean square of error, r is the number of blocks or replicates. The heritability in a broad sense was estimated as $H^2 = \sigma^2_g / \sigma^2_p$ (Sivasubramanian and Menon, 1973). All the statistical analysis was done by PAST3 (Hammer et al., 2001) and NCSS softwares (NCSS, 2020).

Genetic evaluation

DNA extraction was carried out according to the cetyltrimethyl-ammonium bromide (CTAB) method modified by Carrasco et al. (2012). Young leaves (0.5 g) were ground in liquid nitrogen and DNA was extracted with a CTAB hot extraction

Table 2. Description of the botanical and productive characteristics evaluated for 23 peach/nectarine and 16 Japanese plum cultivars.

Botanical characteristics	Species
1. Branchlet diameter (1 yr wood), mm	Pp; Ps
2. Length between nodes, mm	Pp; Ps
3. Number of flower buds	Pp; Ps
4. Length of flower buds, mm	Pp; Ps
5. Width of flower buds, mm	Pp; Ps
6. Number of flower buds through to 10 cm over 1 yr branchlet	Pp; Ps
9. Flower type (Showy [1]/No Showy [0])	Pp
10. Flower weight - five flowers, g	Ps
11. Petal length, mm	Pp; Ps
12. Petal width, mm	Pp; Ps
13. Stamen length, mm	Pp; Ps
14. Pistil length, mm	Pp; Ps
15. Length of the flower pedicel, mm	Ps
16. Stone length, mm	Pp; Ps
17. Stone width, mm	Pp; Ps
18. Stone thickness, mm	Pp; Ps
19. Stone weight, g	Pp; Ps
20. Width of apical zone, mm	Pp; Ps
21. Width of basal zone, mm	Pp; Ps
22. Width of the lateral groove, mm	Pp; Ps
23. Length of lateral groove, mm	Pp; Ps
24. Keel width, mm	Pp; Ps
25. Days from close button to full blossom, d	Pp

Productive characteristics	
1. Fruit type (nectarine = 0; peach = 1), 0/1	Pp
2. Flesh color (white = 0; yellow = 1), 0/1	Pp
3. Fruit weight, g	Pp; Ps
4. Fruit diameter, mm	Pp
5. Fruit length, mm	Pp
6. Cheek firmness, kg[a]	Pp; Ps
7. Shoulder firmness, kg	Pp
8. Suture firmness, kg	Pp
9. Apical firmness, kg	Pp
10. Solid soluble, °Brix[b]	Ps
11. Skin color, 1/2/3/4	Ps
12. Harvest date (days from full blossom to harvest), d	Pp; Ps
13. Yield, t	Ps

Pp: *Prunus persica*; Ps: *Prunus salicina*.
[a]Firmness was measured with a penetrometer (Effegi F357) with an 8 mm plunger.
[b]Solid soluble was measured with a refractometer (BX-50, VEE GEE Scientific).

buffer (50 mM Tris-HCl, pH 8.0; 1.4 M NaCl; 20 mM EDTA; 2% (w/v) CTAB; and 1% (v/v) β-mercaptoethanol). Two extractions were then performed with chloroform/isoamyl alcohol (24:1). Isopropanol was used to precipitate the nucleic acids, and the resulting pellet was dissolved in distilled water. RNA was removed by digestion with deoxyribonuclease-free ribonuclease A. The purified total DNA was quantified by gel electrophoresis, and the quality of the DNA was verified by spectrophotometry (Meisel et al., 2005).

For genetic analysis of Japanese plum and peach cultivars, eight simple sequence repeats (SSRs) markers were selected (Table 3). The PCR was carried out in a total volume of 25 μL under the same conditions described for Carrasco et al. (2012). The final concentrations of the reverse and forward primers were 0.24 and 0.06 μM, respectively. The conditions of DNA amplification were an initial denaturation step of 3 min at 94 ºC; 35 cycles of 40 s at 94 ºC, 40 s at the specific annealing temperature indicated in the Table 3 and 1 min at 72 ºC.

Several PCR amplifications were performed to evaluate the reproducibility of the bands obtained. Furthermore, the SSRs were sequenced with an automatic sequencer (ABI 3100 Avant, Azco Biotech, Oceanside, California, USA). For PCR reactions, one of each primer pair was end-labeled with FAM, HEX, or TAMARA. The SSRs size were analyzed with the Scanner software v. 1.0 (Applied Biosystems, California, USA).

Table 3. SSR primers used for genetic characterization of peach and Japanese plum.

Primer name	Tº	Amplicon (bp)	Sequence 5'→3' SSR
Japanese plum[a]			
CPSCT004	62	122-134	F:GCTCTGAAGCTCTGCATTGA
			R:TTTGAAATGGCTATGGAGTACG
CPSCT0012	62	150-184	F:ACGGGAGACTTTCCCAGAAG
			R:CTTCTCGTTTCCTCCCTCCT
CPSCT0018	62	126-176	F:AGGACATGTGGTCCAACCTC
			R:GGGTTCCCCGTTACTTTCAT
CPSCT0025	56	178-200	F:GCATTGCAAGCATTTGAAGA
			R:GATGCTATCCTTTCCGCATC
CPSCT0029	56	137-161	F:ATGGGCTAGAAGTGGTGGTG
			R:ATTCCGACTCGAAACGAAGA
CPSCT0030	61	181-191	F:CAACAGCGAGTGTCACGTTT
			R:AGGCAACGGACAAAAATCTG
CPSCT0039	62	102-130	F:GCCGCAACTCGTAAGGAATA
			R:TCCACCGTTGATTACCCTTC
CPSCT0044	62	192-218	F:CCAGCACAGAGAAAACGATG
			R:GAGCTCCTACTCTGAGTCTGTAAA
Peach[b]			
CPPCT 029[b]	55	170-194	F:CCAAATTCCAAATCTCCTAACA
			R:TGATCAACTTTGAGATTTGTTGAA
CPPCT 030[b]	50	170-200	F:TGAATATTGTTCCTCAATTC
			R:CTCTAGGCAAGAGATGAGA
BPPCT 001[c]	57	128-159	F:AATTCCCAAAGGATGTGTATGAG
			R:CAGGTGAATGAGCCAAAGC
BPPCT 033[c]	57	164-212	F:GTAGCCGGAGCCGTGTAT
			R:CTAGAACCCTATAAACACATGGC
BPPCT 037[c]	57	146-156	F:CATGGAAGAGGATCAAGTGC
			R:CTTGAAGGTAGTGCCAAAGC
UDP98 408[d]	57	102-109	F:ACAGGCTTGTTGAGCATGTG
			R:CCCTCGTGGGAAAATTTGA
UDP96 015[d]	57	163-185	F:CCTTGACCTATTTGTTCGTCA
			R:ACTAGTCAAACAATCCCCCG
UDP96 003[d]	57	131-149	F:TTGCTCAAAAGTGTCGTTGC
			R:ACACGTAGTGCAACACTGGC

Tº: Annealing temperature (°C); F: forward; R: reverse.
[a]Mnejja et al. (2004).
[b]Aranzana et al. (2002).
[c]Dirlewanger et al. (2002).
[d]Cipriani et al. (1999).

The SSRs data were scored as codominant markers to distinguish homozygotes and heterozygotes for each locus. The parameters determined to analyze the genetic diversity of Japanese plum and peach cultivars were the average number of alleles per locus (A); observed heterozygosity (Ho); expected heterozygosity (He) and fixation index (Fis; Wright, 1978). The genetic differentiation between groups was estimated using AMOVA ϕ_{PT}. The probability of identity (PI) was used to estimate the average probability that two independent cultivars will have the same multilocus genotype (Peakall et al., 2006).

$$PI = 2 \left(\sum p_i^2 \right)^2 \sum p_i^4$$

where p_i is the allele frequency by locus.

Additionally, the discrimination power (D) was estimated according to:

$$D = (1 - PI)^{N(N-1)/2}$$

where PI is the probability of identity, and N is the number of analyzed cultivars, 1 - PI is the exclusion power, and N × (N - 1)/2 is the different pairs of cultivars that can be produced in the current simple. Discrimination power allows calculating the percentage of two cultivars that are genetically different. If D is superior to 95%, it will indicate that cultivars are genetically different (Guzmán et al., 2020). Also, the coefficient relatedness (r) among cultivars was estimated according to Lynch and Ritland (1999), and the assignment of every cultivar to a previously known classification (using PCA and AD) based on morphologic characteristics was carried out. All of the genetic parameters were calculated using the software GenAlex v. 6.4 (Peakall and Smouse, 2006).

RESULTS

Analysis of peach cultivars
The coefficient of variation (CV) in a core collection of 23 peach cultivars for the 33 characteristics studied ranged from 5.9% (fruit length) to 172.5% (keel width). The PCA accounted for 29.9%, 18.4%, and 12.9% of the variance for the first three components, respectively, being the cumulative variance equal to 61.2%. Based on the eigenvector value of the first three components, the CD of 18 variables were estimated. Only seven characteristics were important for describing the variability observed in peach cultivars, these variables were related to productivity and fruit quality. In this regard, fruit weight (CD = 0.80; Min = 144 g; Max = 278 g; CV = 17.1%); fruit diameter (CD = 0.75; Min = 64 mm; Max = 85 mm; CV = 6.8%); fruit length (CD = 0.72; Min = 63.8 mm; Max = 81.6 mm; CV = 6.0%); suture firmness (CD = 0.85; Min = 2.3 kg; Max = 5.7 kg; CV = 22.6%); apical firmness (CD = 0.84; Min = 2.9 kg; Max = 5.7 kg; CV = 16.5%); shoulder firmness (CD = 0.82; Min = 2.1; Max = 5.4; CV = 19.2%) and cheek firmness (CD = 0.81; Min = 2.8; Max = 5.6; CV = 15.0%) showed CD values superior to 0.7; therefore, they were considered responsible for maximum variability observed among the 23 peach cultivars. When a new PCA was applied based on those seven characteristics (Figure 1) the first two principal components explained 87.5% of the total variability observed (PC1 = 63.2%; PC2 = 24.2%) and were able to separate apart peach and nectarine cultivars except for 'Candy Ice' nectarine and 'Rich May' peach.

Discriminant analysis verified that peach and nectarine cultivars are separated into two groups according to those seven characteristics (Wilk's Lambda = 0.20; p < 0.05). Additionally, 87% of the cultivars' classification obtained by DA was in concordance with PCA. Pearson's correlation was significant for every combination of these seven traits (p < 0.001).

The ANOVA of fruit weight, fruit diameter, fruit length, suture firmness, apical firmness, shoulder firmness, and cheek firmness showed a highly significant variation for the 23 analyzed cultivars (p < 0.01). In contrast, the interactions between cultivars and season were nonsignificant (p > 0.05). The results of ANOVA indicated that the mean squares due to genotypes were significant for those seven traits studied (MSG; p < 0.001, Table 4).

The level of SSR variability in peach cultivars was low (Table 5) for the number of alleles (A = 5.1; SE = ± 0.83). In contrast, the average of heterozygosity was high (Ho = 0.56, SE = ± 0.09; He = 0.65, SE = ± 0.04). Also, it was possible to observe a significant deficit of heterozygous (F = 0.11, SE = ± 0.14). These values agree with previous publications (a review about SSRs markers in peach is available in Carrasco et al., 2013) where is reported that peach and nectarine display a low level of SSR variability compared with other stone fruit species (number of alleles per locus = 2.9 and 7.3 and observed heterozygosity = 0.21-0.46) and a deficit of heterozygotes indicating a high level of inbreeding (F = 0.08 to 0.53). The AMOVA analysis and the assignment test showed that there are no differences between nectarine and peach groups (φPT = 0.01; p = 0.35), where they tend to be clustered as only one group with genetically similar cultivars. These results are contrasting with those two clusters previously identified by PCA and AD.

Figure 1. Principal component analysis of 23 peach/nectarines cultivars and seven characteristics.
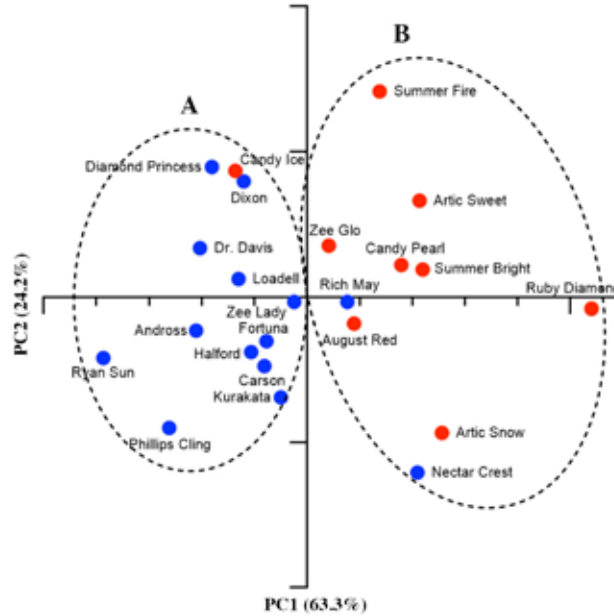
Blue dots: Peach; red dots: nectarine.
A contains the 12 peach and 1 nectarine cultivars which showed bigger fruit size. B contains
8 nectarine and 2 peach cultivars which displayed the higher values for pulp firmness.

Table 4. Genetic parameters of seven characteristics evaluated in peach and four characteristics evaluated in Japanese plum cultivars.

Significant characteristics	Aver	MSG	MSe	r	σ^2g	σ^2p	H^2
Peach (23 cultivars)							
Fruit weight, g	214.0	13 243.2*	5.0	5	2 647.630	2 652.644	99.8
Fruit length, mm	73.6	195.4*	0.7	5	38.931	39.671	98.1
Fruit diameter, mm	5.0	243.4*	4.4	5	47.807	52.172	91.6
Apical firmness, kg	9.6	25.8*	0.001	5	5.169	5.170	99.9
Shoulder firmness, kg	8.7	20.6*	1.8	5	3.752	5.601	66.9
Check firmness, kg	9.0	39.2*	1.2	5	7.603	8.829	86.1
Suture firmness, kg	8.0	34.5*	2.0	5	6.501	8.511	76.4
Japanese plum (16 cultivars)							
Yield, t ha⁻¹	41	355.7*	11.5	5	68.824	80.362	85.7
Fruit weight, g	147	3 977.4*	163.0	5	762.9	925.850	82.4
Solid soluble, °Brix	14	42.4*	1.4	5	8.2	9.633	85.0
Harvest day, d	153	4 762.5*	214.2	5	909.7	1 123.821	80.9

MSG: Mean square of genotypes; MSe: mean square error (environmental variance); r: number of blocks;
σ^2g: genotypic variance; σ^2p: phenotypic variance; H^2: broad-sense heritability ($\sigma^2g/\sigma^2p \times 100$).
*Significant at p > 0.001.

On the other hand, the probability of identity was low (PI = 4.6×10^{-7}), indicating a chance of 1/2173913 to find two individuals with identical genotype. Also, a discriminant power (D = 99.88%) was superior to 95% (Table 5). Only 30 pairwise combinations had some degree of relatedness. From them, 37% showed relatedness coefficient near to the second degree of relatives (half-sibs with a 25% shared loci), and 63% were similar to the third degree of relatives (12.5% shared loci). In this regard, 'Dr. Davis' showed relatedness values between 0.143 to 0.318 with 'Harford', 'Nectar Crest', 'Zee Glo', 'Ryan Sun', 'Loadell', 'Diamond Princess', 'Andross', and 'Candy Ice'. 'Diamond Princess' presented relatedness values between 0.14 to 0.37 with 'Zee Glo', 'Candy Ice', 'Artic Sweet', and 'Nectar Crest'. 'Kurakata' showed relatedness values between 0.157 to 0.31 with 'August Glo', 'Candy Ice', and 'Nectar Crest'. The other 15 pairwise of cultivars had a relatedness coefficient between 0.132 to 0.389.

Table 5. Genetic parameters estimated in the peach and Japanese plum cultivars using SSRs markers.

Species	N	L	P (%)	Na	Ho	He	F	ϕ_{PT}	PI	1 - PI	D (%)
Peaches	23	8	100	5.1	0.56	0.65	0.11	0.01^{ns}	4.6×10^{-7}	0.99	99.88
SE				0.83	0.09	0.04	0.14				
Japanese plum	16	8	100	7.4	0.94	0.77	-0.22	0.0^{ns}	2.3×10^{-9}	0.99	99.99
SE				0.90	0.03	0.02	0.04				

N: Sample size; L: loci number; P: percentage of polymorphic loci; Na: number of alleles by loci; Ho: observed heterozygosity; He: expected heterozygosity; F: inbreeding coefficient; ϕ_{PT}: genetic differentiation among groups; PI: probability of identity; 1 - PI: exclusion power; D: discriminant power; SE: standard error; ns: nonsignificant.

Analysis of Japanese plum cultivars

The coefficient of variation (CV) of 29 characteristics evaluated in a core collection of 16 Japanese plum cultivars ranged from 7.7% (stone length) to 126.8% (stamen length). The CV of productive characteristics (yield, fruit weight, solid soluble, cheek firmness, and harvest days) ranged from 11.9% to 14.8%. The PCA based on 29 characteristics accounted for 18.4%, 14.2%, and 11.9% of the variance for the first three components, with 44.5% being their cumulated variance. The CD was superior to 0.7 only for four characteristics: solid soluble (CD = 0.88; Min = 12.0 °Brix; Max = 19.0 °Brix; CV = 14.8%), fruit weight (CD = 0.81; Min = 122 g; Max = 184 g; CV = 14.8%), yield (CD = 0.80; Min = 30 t; Max = 48 t; CV = 14.4%), and harvest date (CD = 0.70; Min = 121 d; Max = 203 d; CV = 14.4%).

The Pearson's correlation was significantly positive for yield/fruit weight (r = 0.28; p = 0.0003) and solid soluble/harvest date (r = 0.27; p = 0.0005); but was significantly negative for fruit weight/solid soluble (r = -0.18; p = 0.02). A new PCA was built using only these four characteristics, which accounted for 73.3% of the variability (Figure 2; PC1 = 41.3%; PC2 = 32.0%), separating them into two groups. The first cluster (Figure 2) grouped eight cultivars (Aurora, Catalina, Queen Rosa, Fortune, September King, Black Queen, Saphire, and Blue Gusto), with average lower values for yield (37.8 t), fruit size (138.9 g), solid soluble (13.6 °Brix) and harvest date (139.6 d). The second cluster grouped 'Angeleno', 'Larry Ann', 'Friar', 'Laroda', 'Santa Rosa', 'Pink Delight', 'Flavor Rich', and 'Red Heartwhich' with average higher values for yield (43.9 t), fruit size (154.5 g), solid soluble (15.0 °Brix) and harvest date (166.8 d).

Figure 2. Principal component analysis of 16 Japanese plum cultivars and four characteristics.
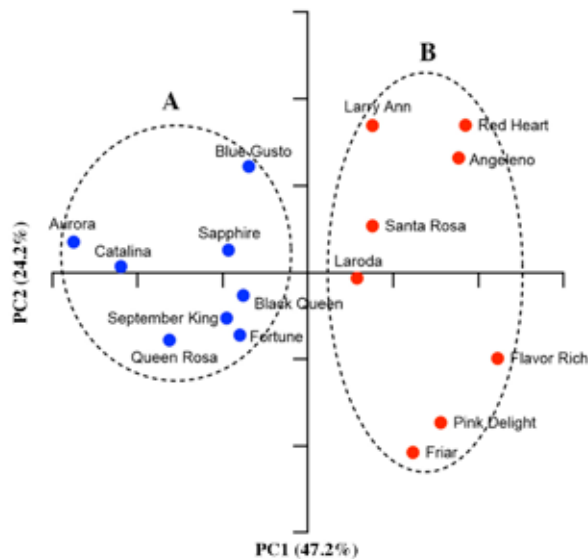
A. Blue dots contain eight cultivars with lower values for yield, fruit size, solid soluble and harvest date.
B. Red dots correspond to eight cultivars that showed higher values for those characteristics.

Discriminant analysis verified these two clusters (Wilk's Lambda = 0.402; p < 0.027) and also confirmed that 88% of the cultivar classification was in agreement with that obtained by PCA. The ANOVA of the solid soluble, fruit weight yield and harvest date, showed a highly significant (p < 0.001) variation among the 16 Japanese plum cultivars. However, the interactions between cultivars and season were nonsignificant (data not shown). For those four traits, the magnitude of genotypic variances ($\sigma^2 g$) was superior to their corresponding environmental deviations (MSe). Moreover, the broad-sense heritability also was high, ranging between 80.9% and 85.6% (Table 4).

The level of genetic diversity in Japanese plum cultivars was high (Table 5) for the number of alleles (A = 7.4; SE = ± 0.90) and heterozygosity (Ho = 0.94; SE = ± 0.03; He = 0.77; SE = ± 0.02). Also, it was possible to observe an excess of heterozygous (F = -0.22; SE = ± 0.04). The relationship among cultivar was analyzed through AMOVA, which found no genetic difference between the two groups previously established by PCA and AD using phenotypic characteristics. On the other hand, AMOVA showed that all the variability of the SSR was distributed between the cultivars.

The PI was very low (PI = 2.3×10^{-9}) for increasing SSR locus combinations (Table 5), and D values reached 99.99%. For most cultivars pairwise, the relatedness coefficient (r) was around zero, even with negative values. Out of 120 pairwise combinations among cultivars, only 7% showed some degree of relatedness. The relatedness pairwise coefficients were 'Angeleno' *vs*. 'Fortune' (r = 0.32); 'Queen Rosa' *vs*. 'Saphire' (r = 0.22); 'Flavor Rich' *vs*. 'September King' (r = 0.20); 'Angeleno' *vs*. 'Black Queen' (r = 0.19); 'Fortune' *vs*. 'Pink Delight' (r = 0.16); 'Friar' *vs*. 'Larry Ann' (r = 0.16); 'Aurora' *vs*. 'Queen Rosa' (r = 0.13) and 'September King' *vs*. 'Friar' (r = 0.13).

DISCUSSION

Multivariate techniques can be useful to analyze large data sets produced by a collection of cultivars. One of them is the principal component analysis (PCA), which allows reducing the number of input phenotypic variables, visualizing a grouping pattern for the studied cultivars and identifying the most relevant variables that explain the observed variability. Several studies have applied PCA to assess phenotypic relationships among important fruit tree crop such as European plum (*Prunus domestica* L. subsp. *domestica*, Sarigu et al., 2017), almond (Khadivi-Khub and Etemadi-Khah, 2015) and peach (Engel et al., 2015).

On the other hand, the discriminate analysis (DA) is another statistic tool that has been used to assess the adequacy of grouping patterns observed with PCA (Sarigu et al., 2017). Discriminate analysis allows maximizing the ratio of variance between-class to the difference within-class to achieve maximal separability of the group previously identified.

The PCA identified two groups of peach cultivars. The CD was estimated to determine the variables that explain the grouping pattern observed among peach cultivars. According to the CD, only characteristics related to productivity were responsible for maximum variability observed among the 23 peach and nectarine cultivars. In this regard, the fruit size (weight, diameter, and length) was significantly superior in peach cultivars than in nectarine cultivars; on the other hand, fruit firmness (cheek, shoulder, suture, and apical) was significantly higher in nectarine cultivars when compared to peach cultivars. Font i Forcada et al. (2014) reported that fruit size and firmness were important to separate peach cultivars. In contrast, it has been found that several botanical characteristics determined the variability pattern observed among peach cultivars. Based on those characteristics, PCA and AD verified that the peach and nectarine cultivars significantly separate into two groups, except for 'Candy Ice' nectarine and 'Rich May' peach. The genetic variance and heritability were estimated using a replicate trial of the available germplasm, and with a broad-sense (H^2) consideration because the nature of peach cultivars corresponds to hybrid material. It is important to note that the H^2 only describes the amount of genetic variation in a population, which implies whether or not a population responds to selection pressure. For the 23 peach cultivars, the H^2 for those seven traits were high, ranging between 66.9% to 99.9% (Table 4). Although fruit size and firmness have been reported to be a polygenic trait with a low to moderate heritability (Font i Forcada et al., 2014), some studies in peach indicate lower values of H^2 for fruit size (H^2 = 0.31 to 0.47; H^2 = 0.35, Biscarini et al., 2017). Similarly, Hansche et al. (1972) reported moderate to high values for narrow heritability for peach (harvest date h^2 = 0.84; fruit length h^2 = 0.31; fruit firmness h^2 = 0.26-0.29). The discrepancy between heritability values estimated in this work and those reported in the literature may be due to many factors such as environmental conditions, agronomical practices experimental design, among others. Those results and our results suggest that the phenotypic variation observed for fruit size and firmness is under genetic control (additive and interaction effects), which are helpful for genetic advances in peach breeding.

The level of SSR variability in peach cultivars was low to moderate, and a significant deficit of heterozygous was shown (Table 5). Although the number of cultivars analyzed is low, these values agree with previous publications where peach and nectarine display a low level of SSR variability compared with other stone fruit species (number of alleles per locus = 2.9 to 7.3 and observed heterozygosity = 0.21 to 0.46) and a deficit of heterozygotes indicating a high level of inbreeding (F = 0.08 to 0.53) (Rojas et al., 2008; Carrasco et al., 2013). This level of inbreeding in peach cultivars can be explained in part by self-pollination that increased the homozygosity in the original parental lines, mainly by narrow number of parental lines that have been used in the modern breeding programs.

The genetic analysis showed that there are no differences between nectarine and peach groups; they tend to cluster as only one group with genetically similar cultivars. Also, the probability of identity and discriminant power indicates that there is no cultivar with an identical multilocus genotype, and the eight SSR loci were able to identify every cultivar (Table 5). Similarly, Rojas et al. (2008) reported that seven SSRs were able to identify 102 peach cultivars, and Wünsch and Hormaza (2002) identified 89.5% of the sweet cherries using nine SSR. The peach cultivars showed values of pairwise relatedness coefficients (r) near to zero and negative, suggesting that most of the pairwise combinations of cultivars (88%) are not genetically related. Unfortunately, the pedigree of the more recently peach cultivars is unknown.

For Japanese plum cultivars, the coefficient of determination was superior only for four characteristics, solid soluble, fruit weight, yield, and harvest date. However, its coefficients of variation were moderate; similar results were found by Cosmulescu et al. (2018) for myrobalan plum cultivars but contrasting with the results obtained for Sundouri et al. (2017) for 14 Japanese plum cultivars. Japanese plums have a more diverse background than other plum species. The PCA and AD were able to identify two groups of cultivars. Similar results were found in apricot cultivars and European plum cultivars (Milošević and Milošević, 2012). They reported that the solid soluble, fruit weight yield, and harvest date, among others, were the most important characteristics to describe the germplasm of these two stone fruit species.

The H^2 for solid soluble, fruit weight, yield and harvest date was superior to 80% (Table 4), indicating that the genetic variance is the most important component of the phenotypic variation. Zaremuk and Alekhina (2013) found a moderate to high H^2 for yield ($H^2 = 0.29\text{-}0.65$) and fruit weight ($H^2 = 0.22\text{-}0.55$) in plums, while Krška et al. (2009) found a high h^2 for fruit size ($h^2 = 0.92$) and fruit firmness ($h^2 = 0.96$) in apricot. Our results indicate that the Japanese cultivars show enough genetic variance to advance in a breeding program for fruit production characteristics.

In contrast with peach and nectarine cultivars, the level of genetic diversity in Japanese plum cultivars was high (number of alleles and heterozygosity, Table 5). Also, it was possible to observe an excess of heterozygous and no genetic difference between the two groups previously established by PCA and AD using phenotypic characteristics. The variability of the SSR was distributed between the cultivars. The level and distribution of the SSR variability are in concordance with those reported for Japanese plums and sweet cherry but they are different from those previously published for peaches and nectarines cultivars, especially for heterozygosity and inbreeding coefficient (F) (Aranzana et al., 2002; Rojas et al., 2008). The reproductive biology can explain in part those differences. In this regard, peach can be self-pollinated; therefore, can maintain an important level of homozygosity while Japanese plums and cherry are mainly self-incompatible, cross-pollinated and displays a high level of heterozygosity. Carrasco et al. (2013) reviewed SSR variability in stone fruit crops.

The low value of PI (PI = 2.3×10^{-9}) for eight SSR locus combinations indicates that there was no cultivar with identical multilocus genotype (Table 5). Moreover, D was superior to the critical value (95%), indicating that the eight loci analyzed allowed these 16 cultivars to be distinguish amongst each other. The genetic analysis of Japanese plum cultivars with SSRs molecular markers has been scarce compared with other stone fruit species. Carrasco et al. (2012) studied 29 cultivars with eight SSRs and 232 ISSRs. Other fruit crop species have received more attention; in this regard, Tessier et al. (1999) described the approach to estimating D where they found that a combination of six RAPDs and two SSR were optimum for the discrimination of 224 grape cultivars. In the same way, Honjo et al. (2011) discriminated 72 strawberry cultivars using four SSRs.

Additionally, the low relatedness coefficient (r) suggests that most cultivars display scarce genetic relationships. Wang (2014) point out that those results are expected where a negative r value will be obtained when the cultivars are less related than the average. The r values of these pairwise combinations were close to the second degree of relatives (for example, half-sibs share 25% of their loci) and third-degree relatives (with 12.5% shared loci). These results are coincident with their pedigree, which indicates that 'Queen Ann' is a common ancestor for 'Flavor Rich', 'Angeleno', 'Fortune', 'Queen Rosa', 'Saphire', and 'Santa Rosa'.

CONCLUSIONS

Size and pulp firmness of fruits are two groups of important characteristics sought in all peach and nectarine genetic improvement programs. Based on the PCA and ANOVA, six superior cultivars could be distinguished for these characteristics. In this regard, three peach cultivars were identified (Diamond Princess, Dixon and Dr. Davis) with the largest fruit size (fruit weight = 252-278 g; fruit diameter = 79.4-85 mm; fruit length = 76-79 mm) and three nectarine cultivars (Ruby diamond, Artic sweet, Summer fire) with the highest fruit firmness (cheek firmness = 4.99-5.44 kg; shoulder firmness = 4.54-5.44 kg, suture firmness = 4.99-5.44 kg and apical firmness = 5.44-5.90 kg). Additionally, genetic analysis with eight SSRs indicated that those six cultivars did not show relatedness except for 'Diamond Princes' and 'Dr. Davis' (r = 0.32).

In the case of Japanese plum, eight cultivars (Larry Ann, Laroda, Angeleno, Flavor Rich, Red Heart, Friar, Santa Rosa, and Pink Delight) showed higher values for yield (33-48 t); fruit weight (123-184 g); solid soluble (12-19 °Brix) and harvest date (142-203 d). The relatedness coefficients (r) were negative, indicating that apparently, the four Japanese plum cultivars do not share any relatedness; however, the pedigree of 'Angeleno' and 'Flavor Rich' indicates that 'Queen Ann' is its common ancestor. These results corroborate the importance of pre-breeding analysis (phenotypic and genetic) to identify and select parental lines that present no relationships between each other and, at the same time, display the best phenotypic behavior. Progeny tests can be applied subsequently to know if the selected parental lines produce the best offspring.

The genetic analysis also allowed us to determine the distinctness of the peach, nectarine, and Japanese plum cultivars using eight SSR. In this regard, the determination of individuality through the use of molecular markers (example SSR) has become increasingly relevant in species of agricultural importance, as an alternative to morphological descriptors because they lack the necessary sensitivity to discriminate between cultivars. Another advantage of the distinctness through SSR, is that they have allowed detecting problems of synonyms and homonymies that affect the identity of an increasing number of cultivars. In summary, our results can contribute to select parental lines for crossings, perform identification of promising segregating lines, and serve as a tool to protect intellectual property for the new cultivars.

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