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GENETIC DIVERSITY OF *Prunus persica* (L.) Batsch AND OTHER SPECIES OF *Prunus* USING MICROSATELLITES

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ABSTRACT

Mexico has three genetic breeding programs for peaches and nectarines (*Prunus persica* (L.) Batsch). The diversity of its germplasm at a DNA level is unknown. Therefore, the aims of this work were: 1) to characterize the diversity of three *P. persica* populations using microsatellites or simple sequence repeats (SSRs); 2) to genetically compare two *P. persica* populations from the *Prunus* breeding programs of the College of Postgraduates and the Center for Genetic Resources and Improvement; and 3) to extrapolate the use of these SSRs to other *Prunus* species. DNA was extracted from leaves of 162 individuals from three locations: Montecillo and Coatepec Harinas, State of Mexico, and Huimilpan, Querétaro. A CTAB-based protocol was used. The variation of six SSRs was examined in polyacrylamide, and the diversity statistics were obtained for loci and populations. On average, 10.8 alleles were found per locus, a polymorphic information content (PIC) of 0.66, genic diversity of 0.69, and heterozygosity of 0.49. The most polymorphic locus was UCDCH15, with a PIC of 0.84 for *P. persica,* and UCDCH14, with 0.9 for the other three *Prunus* species. Regarding *P. persica* populations, only five SSRs were polymorphic. The expected heterozygosity was 0.512, 0.719, and 0.621, and the observed heterozygosity was 0.334, 0.716, and 0.768 for Montecillo, Querétaro, and Coatepec Harinas, respectively, indicating that Querétaro is the most diverse, and Montecillo, the most homogenous. The similarity between Montecillo and Coatepec Harinas was 0.543, followed by Montecillo and Querétaro (0.47) and Coatepec Harinas and Querétaro (0.183). For the other *Prunus* species, the six SSRs were polymorphic, confirming that their use can be extrapolated to related species such as apricot (*Prunus armeniaca* L.), plums (*P. salicina* L.), and cherries (*P. avium* L.). This is the first report on diversity in *P. persica* and some related species by SSRs in Mexico.

Keywords: *Prunus persica (*L.) Batsch, *Prunus armeniaca* L., *Prunus avium* L., *Prunus salicina* L., molecular markers, genetic variability.

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INTRODUCTION

Peach (*Prunus persica (*L.) Batsch) is the third most important deciduous fruit tree in the world after apples and table grapes (FAO, 2023). In Mexico, in 2022, Zacatecas was the main peach-producing state, with almost 11 800 ha planted and a production of over 87 000 Mg out of the country's total of 239 133 Mg of fruit harvested in 33 000 ha (SIAP, 2023). Peach (*P. persica*), apricot (*P. armeniaca*), plum (*P. salicina*), and cherry trees (*P. avium*) are Asian in origin (Li and Wang, 2020) and have a significant impact on human health and the economy.

Hybridization is used to increase the variability in the *Prunus* species in genetic fruit breeding programs. In Mexico, several *Prunus* breeding programs have stood out, led by the National Institute of Forestry, Agricultural, and Livestock Research (INIFAP), Postgraduate College (COLPOS), and the *Prunus* Genetic Resources and Breeding Center (CRGyM), currently active and run by Dr. Salvador Pérez González, which have focused on obtaining new peach and nectarine cultivars (*P. persica*) (Pérez-González, 2007; Calderón-Zavala *et al*., 2019).

Diverse studies have been carried out using molecular markers to know the degree of kinship between individuals, to identify cultivars, and to protect intellectual property (Firas and Abdulkareem, 2015). In genetic diversity studies carried out on *P. persica* and other *Prunus* species, microsatellite or SSR (Simple Sequence Repeat) markers have been used (Sosinski *et al*., 2000; Struss *et al.,* 2003; Trifonova *et al*., 2021). These markers are characterized for being highly polymorphic, codominant, and informative, as well as for the possibility of being extrapolated to other species (García-Gómez *et al*., 2018; Butiuc-Keul *et al*., 2022).

Due to the interest in generating new peach, nectarine, and other *Prunus* species materials that satisfy farmers' demands, it is relevant to characterize the genetic diversity at the molecular level of the germplasm at COLPOS and CRGyM to know the existing degree of divergence and genetic similarity. Therefore, the aims of this work were: 1) to characterize the diversity of three *P. persica* populations using SSRs; 2) to genetically compare two *P. persica* populations from the *Prunus* breeding programs of the COLPOS and the CRGyM; and 3) to extrapolate the use of these SSRs to other *Prunus* species.

MATERIALS AND METHODS

Plant material and location of the experiment

For the genetic diversity study of *P. persica*, a total of 162 individuals were evaluated, out of which 85 were from COLPOS, 58 from CRGyM, and 19 from the Salvador Sánchez Colín Foundation. The individuals from COLPOS Campus Montecillo (19° 30′ N, 98° 53′ W, 2250 m altitude) were from an F_2 population composed of 35 peach trees and 50 nectarine trees. The individuals from the CRGyM established in Huimilpan, Querétaro (20° 22′ N, 100° 16′ W, 2294 m altitude) were 26 peach trees, 12 nectarine trees (derived from an F_2 and advanced selections), and 20 trees from three *Prunus* species that were used to validate the functionality of the SSRs: 11 plum trees (*P. salicina*), seven apricot trees (*P. armeniaca*), and two cherry trees (*P. avium*). Finally, 18 peach trees and one nectarine tree were among the 19 cultivars from the Salvador Sánchez Colín Foundation that came from the COLPOS breeding program.

It was not possible to obtain parental plant material from all individuals, since in some cases they were unknown and in others, the plants had died. Therefore, the sampled plants corresponded to advanced selections, some cultivars, and F_2 segregants. Before sampling, the plants were identified as nectarines or peaches when they were in production. Approximately 15 to 20 leaves were collected per tree at a height of 2 m, distributed in the four cardinal points (the second and third leaves completely extended), which were labeled and transported to the COLPOS Genetics Laboratory. The leaves were cleaned, and a sample was prepared per tree by collecting fragments of the leaves until 0.3 g was obtained, which was then stored at -80 °C until DNA extraction.

DNA extraction

The genomic DNA of peaches and nectarines was extracted following a protocol based on CTAB (cetrimonium bromide) (Doyle, 1991). The samples were homogenized using liquid nitrogen and 8.5 cm-long polypropylene pestles, directly in 2 mL conical-bottom tubes (Axygen). The tissue of each sample was pretreated with 1 mL of saline buffer (0.1 M Tris HCl, 0.001 M EDTA, 2 M NaOH, and 0.05 % bovine albumin). A Vortex Genie 2 shaker (Scientific Industries) was used to homogenize the sample, which was centrifuged at 13 000 rpm at 4 °C for 5 min. The supernatant was discarded, and the tissue was lysed with 1 mL of 2 % CTAB plus 5 μL of β-mercaptoethanol. The mixture was shaken again and incubated for 30 min at 55 °C. After incubation, 400 μL of 25:24:1 phenol:chloroform:isoamyl alcohol were added. The mixture was shaken for 5 min and centrifuged at 13 000 rpm at 4 °C for 10 min. A total of 500 μL of the supernatant was extracted and placed in a new 1.5 mL tube to precipitate the DNA with 50 μL of 7.5 M ammonium acetate and 500 μL of absolute cold isopropanol, leaving the mixture at -20 °C for 2 h. The pellet was sedimented by centrifuging at 13 000 rpm at 4 °C for 10 min. The supernatant was discarded, and the pellet was washed with 1 mL of 70 % cold ethanol. Next, it was centrifuged as in the previous step; the supernatant was discarded, and the pellet was left to dry at room temperature for 2 h. Finally, the DNA pellet was hydrated in 50 μL of TE (Tris-EDTA) pH 8.

The DNA of apricots, cherries, and plums was extracted based on the method described by Struss *et al*. (2003), with some modifications. First, the homogenized tissue was washed two or three times in saline buffer. Then, 800 μL of a lysis buffer, freshly prepared and preheated at 65 °C (2 % CTAB, 100 mM Tris-HCl pH 8, 1.4 M NaCl, 20 mM EDTA, 1 % PVP 40, and 10 mM DTT) were added to each sample, along with 10 μL of proteinase K. The mixture was incubated at 65 °C for 30 min. After incubation, the mixture was purified three times using 500 μL of chloroform:isoamyl alcohol

(24:1), followed by centrifuging at 13 500 rpm at 4 °C for 10 min in each purification, retrieving the supernatant in a new 1.5 mL tube. After the final purification, the DNA in the supernatant was precipitated with 0.6 volumes of cold isopropanol. The mixture was centrifuged at 13 500 rpm at 4 °C for 10 min. The pellet formed in the bottom of the tube was washed with 1 mL of 70 % ethanol, and it was recovered by centrifuging at 13 500 rpm at 4 °C for 5 min. The washed pellet was left to dry at room temperature and resuspended in 50 µL of RNase-free water for 1 h at 4 °C. Then, it was precipitated once again with 5 µL of 3 M sodium acetate and 2 volumes of 100 % ethanol for 1 h at -20 °C. Finally, the DNA was recovered by centrifuging at 13 500 rpm at 4 °C for 15 min, washed twice with 1 mL of 70 % ethanol, air-dried at room temperature, and rehydrated with 50 µL of TE pH 8.

PCR and marker electrophoresis

Six pairs of microsatellites reported in *P. persica* studies (Struss *et al*., 2003; Sosinski *et al*., 2000) were selected based on their ability to detect polymorphism (Table 1).

The annealing temperature of each marker was determined with a 50–60 °C temperature gradient test to eliminate the excess accessory bands using a BioRad C1000 thermocycler (USA). Once the annealing temperatures were determined (Table 1), each marker was amplified in 15 µL reactions with the following components: 10 ng of DNA, 0.25 µM of each primer, 0.2 mM of dNTPs, 1 U of Taq Polymerase (GoTaq Flexi, PROMEGA), and 1.5 mM $MgCl_2$. An Axygen MaxyGen II thermocycler (USA)

Table 1. Flanking sequences, size, and repeat unit of simple sequence repeat (SSR) markers used to determine diversity in *Prunus* species.

SSR/UR	Sequence 5′-3′	RFS	OS	AT
UCDCH14[1] /(CT)18	F-GTACACGGACCCAATCCTG R-TCTAACATCATGTTAAACATCG	155–185	139–185	51 °C
UCDCH15[1] /(CT)15	F-TCACTTTCGTCCATTTTCCC R-TCATTTTGGTCTTTGAGCTCG	78–120	82–140	54 °C
UCDCH19[1] /(CA)12	F-GTACAACCGTGTTAACAGCCTG R-ACCTGCACTACATAAGCATTGG	100–115	125[†] 117–143[¶]	59 °C
UCDCH21[1] /(CA)18	F-TTGTTGACCATCGAATATGAAG R-GAAGGTACATGGCGTGCC	140	86–110	55 °C
PCHGMS1[2] /(AC)12(AT)6	F-GGGTAAATATGCCCATTGTGCAATC R-GGATCATTGAACTACGTCAATCCTC	194	178–208	60 °C
PCHGMS2[2] /(CT)24	F-GTCAATGAGTTCAGTGTCTACACTC R-AATCATAACATCATTCAGCCACTGC	163	147–161	60 °C

[1]Struss *et al*. (2003); [2]Sosinski *et al*. (2000). RFS: reported fragment size; UR: repetition unit; AT: annealing temperature; OS: observed size; F: forward; R: reverse. [†]*P. persica*; [¶]other species.

was used with the following amplification program: one denaturation cycle at 94 °C for 5 min, followed by 35 cycles with denaturation at 94 °C for 1 min, a defined annealing temperature for each marker for 1 min, and extension at 72 °C for 2 min. The protocol ended with an extension cycle at 72 °C for 5 min (Struss *et al*., 2003).

For markers PCHGMS1 and PCHGMS2, the reaction volume was of 10 μL, mixing 4 ng of DNA, 0.12 μM of each primer, 0.2 mM of dNTPs, 0.5 U of Taq Polymerase (GoTaq Flexi, PROMEGA), and 1.5 mM of $MgCl_2$. For these markers, the PCR program consisted of a cycle of denaturation at 94 °C for 5 min, followed by 32 cycles with denaturation at 94 °C for 45 s, the annealing temperatures estimated for each primer for 30 s, and extension at 72 °C for 30 s, followed by a final extension cycle at 72 °C for 5 min (Sosinski *et al*., 2000). At the end of the PCR program, the reactions were chilled at 12 °C and their amplification signal was evaluated in 3 % agarose gels (Sigma, St. Louis, MO, USA), diluting the reactions with loading buffer (Tris 50 mM, EDTA 50 mM pH 8.0, 25 % saccharose, 0.25 % bromophenol blue) in a 2:1 ratio.

The polymorphism of each marker was defined by vertical electrophoresis using 3 μL of reaction mixture in a non-denaturing polyacrylamide gel using the MG33-1063 system (CBS Scientific, USA). Markers UCDCH19, UCDCH21, PCHGMS1, and PCHGMS2 were separated in 8 % 19:1 polyacrylamide gel, while markers UCDCH14 and UCDCH15 were separated in 10 % 29:1 polyacrylamide gel. Electrophoresis was performed for 2–3 hours at 250 V, using a 20 bp ladder (Sigma-Aldrich) as a molecular weight reference. The fragments were then detected by staining with $AgNO_3$ (Sigma, USA) according to the description by CIMMYT (2006) and documented with the MiniBis Pro 16 mm transilluminator (Bio Imaging Systems, Israel). The weight of each band was calculated using the GelAnalyzer software version 19.1 to generate the molecular data matrix. These amplification and electrophoresis conditions were applied to all the *Prunus* species evaluated.

Data analysis

Using the information obtained from the SSR markers, a data matrix was created in Excel. The number of alleles (Na) was calculated, along with the number of genotypes, the observed heterozygosity (*Ho*), the expected heterozygosity (*He*), the polymorphic information content (PIC), and the number of effective alleles (Ne) using the PowerMarker V3.25 (Liu and Muse, 2005) and GenAlEx V6.503 programs (Peakall and Smouse, 2012). The genetic distance was calculated following Nei (1972), and a dendrogram was obtained using the unweighted pair group method average (UPMGA). The analysis of molecular variance (AMOVA) was carried out using GenAlEx V6.503 (Peakall and Smouse, 2012), as well as the calculation of Wright's *F* values for five of the six loci examined, due to one of them being monomorphic.

RESULTS AND DISCUSSION

Diversity of *Prunus persica*

A total of 17 alleles were identified, with a mean of 5.7 per population. The germplasm from each location differed in terms of the allelic variability it carries. For example, 7.8 different alleles were found in the germplasm from Querétaro, while there were 4.8 alleles in the population from Montecillo and 4.4 alleles in Coatepec Harinas (Table 2). The germplasm from Querétaro displayed the highest gene diversity. A study carried out in La Palma, Spain, found that, when comparing peaches from this island with collections from Spain and other countries, the number of alleles per locus ranged between 3.2 and 4.7, with the one from La Palma being the lowest. The authors suggest that this lower diversity in La Palma may be due to its geographic isolation and to the process of hybridization between a limited number of genotypes (Pérez *et al*., 2020). Based on the diversity indicators between populations, 50 % of the alleles were observed to be inherited from one generation to the next (number of alleles (Na) values in regard to the effective number of alleles (Ne)) (Table 2). However, these Ne values are higher than those reported by other authors, whose values fluctuate between 1.19 and 2.84 (Pérez *et al*., 2020).

Table 2. Diversity indicators of the populations studied based on five polymorphic simple sequence repeats (SSRs) in *Prunus persica* (L.) Batsch.

Populations		N	Na	Ne	Ho	He	F
Montecillo	Mean	85	4.8	2.636	0.334	0.512	0.273
	SE	0	1.5	0.700	0.088	0.119	0.132
Querétaro	Mean	38	7.8	4.439	0.716	0.719	-0.016
	SE	0	1.1	0.933	0.094	0.073	0.123
Coatepec Harinas	Mean	19	4.4	2.777	0.768	0.621	-0.248
	SE	0	0.8	0.275	0.076	0.049	0.098

N: total number of samples per population; Na: number of alleles; Ne: effective number of alleles; *Ho*: observed heterozygosity; *He*: expected heterozygosity; *F*: fixation index; SE: standard error.

In genetic diversity studies, two key concepts are found: allele richness and genetic uniformity. The latter is expressed via the expected heterozygosity (*He*), defined as the probability of two randomly chosen alleles in a sample being different. Their values range between 0 and 1 (Hoban *et al*., 2022). In this study, the values of *He* were 0.512 for the Montecillo population, 0.719 for the Querétaro population, and 0.621 for Coatepec Harinas. These results highlight the population from Querétaro as the one with the highest adaptive advantage. However, in this study, all three populations displayed high *He* values, suggesting that together they have a reservoir of alleles capable of producing offspring with greater advantages (Table 2).

These results contrast with those from other investigations, in which the values for *He* were below 0.5 when analyzing three peach tree populations from La Palma (*He* = 0.280), Spain (*He* = 0.398), and other countries (*He* = 0.476) (Pérez *et al.,* 2020). Meanwhile, in three apple tree (*Malus* x *domestica*) populations, a high gene diversity was found, with *He* values greater than 0.8 in populations in Galicia (0.853), Portugal (0.828), and the Canary Islands (0.834) (Pereira-Lorenzo *et al.,* 2018).

Regarding observed heterozygosity (*Ho*), the population from Coatepec Harinas displayed the greatest proportion of heterozygotic individuals, followed by populations from Querétaro and Montecillo (Table 2). The population from Montecillo was the least variable in terms of *Ho*, with a higher proportion of individuals that share the same allele, indicating a tendency towards a particular homozygotic state, since this population is an F_2. This result was corroborated with the fixation index (*F*), which was 0.273 for Montecillo, indicating a high homozygosity in comparison with the populations from Querétaro and Coatepec Harinas, whose *F* values were -0.016 and -0.248, respectively, indicating their tendency towards heterozygosity (Table 2). On average, the *F* value of the three populations was 0.003, indicating an absence of significant genetic differentiation between them.

Differences and similarities between populations

The Nei (1972) genetic distance revealed that the most similar populations were Montecillo and Coatepec Harinas (0.543), followed by Montecillo and Querétaro (0.47) and Coatepec Harinas and Querétaro (0.183). The similarity between Montecillo and Coatepec Harinas is explained because the individuals evaluated come from the same genetic breeding program (COLPOS), which implies the presence of alleles from the same common paternal origin expressed in two different environments. The similarity between the populations from Montecillo and Querétaro suggests that they shared parental origins, a hypothesis that still requires confirmation. On the other hand, the noticeable genetic difference between the populations from Coatepec Harinas and Querétaro is a significant finding, as it indicates favorable genetic diversity due to their belonging to two different breeding programs (COLPOS and CRGyM).

At the subpopulation level (nectarines and peaches), a very similar tendency was found to the one found between populations. The peaches from Montecillo are genetically very similar to those from Coatepec Harinas (0.512), followed by peaches from Montecillo and Querétaro (0.427) and Coatepec Harinas and Querétaro (0.192). In the case of the nectarines, the differences were not noticeable. The genetic distances indicated that the nectarines from Montecillo and Coatepec Harinas share a high level of similarity (0.629), followed by Montecillo and Querétaro (0.524) and Coatepec Harinas and Querétaro (0.384). When comparing peach and nectarine trees, obvious differences were found due to the expression of the pubescence gene. The nectarine and peach trees within Montecillo were highly different (0.076), the same as in Querétaro

(0.073). In the population from Coatepec Harinas (0.24), there were no broad results due to the limited number of nectarines within the population studied (one nectarine and 18 peaches).

The variation distribution by the molecular variance analysis (AMOVA) showed that 63 % of the variation is found within the individuals, 25 % among the three populations, and the lowest part of the variation (12 %), between individuals. These values are similar to those found in a genetic diversity study in five peach tree populations from Asia, the Americas, and Europe, with different degrees of domestication (wild and cultivars), in which the greatest diversity was explained within individuals (68 %), followed by the variation between populations (27 %) (Li *et al*., 2013).

The differences in the AMOVA indicate variability among groups (Laurentin, 2009). In this study, the main difference comes from the individuals that make up each population, highlighting their value within the germplasm population. The value for *Fis*, which represents the inbreeding coefficient and is derived from the *Ho* and *He* values within the populations, indicated a trend towards homozygosity (0.161). This trend could be overestimated, since one of the populations (Montecillo) is an F_2 with a lower *Ho* value than the other two populations (Table 2). This aspect requires greater research in order to understand the history of these populations and why the value of *Fis* pointed out this trend. The analysis without the population from Montecillo (F_2) gave a negative *Fis* (-0.0119), suggesting that the evaluated populations are genetically heterogenous and diverse as a result of different allelic combinations, unlike a positive *Fis* value that indicates that the populations are highly endogamic (Pérez *et al.*, 2020).

Diversity of the six loci

The amplification of the six SSR loci showed that the size of the alleles was within the intervals reported in the literature. These markers, reported by Struss *et al.* (2003) for cherry trees and extrapolated to peach trees, revealed that the marker UCDCH15 was the most polymorphic, while marker UCDCH19 was monomorphic in the three populations with a 125 bp band.

The number of alleles varied between 8 and 16, with an average of 10.8 (Table 3). This average is greater in comparison with other investigations: 4.0 in Chen and Okie (2022), 5.67 in Trifonova *et al*. (2021), and 6.41 in Chavez *et al*. (2014). This variation may be due to the diverse origin of the three populations and the SSR selection in this study, which were chosen for their high informative value, based on a panel of 15 cherry tree cultivars, one plum cultivar ('Santa Rosa'), one apricot cultivar ('Patterson'), and one peach ('Elegant Lady') (Struss *et al.*, 2003).

Per marker, the number of alleles was 14, 16, and 8 for UCDCH14, UCDCH15, and UCDCH21, respectively, which contrasts with reports by Struss *et al*. (2003), who found three alleles for UCDCH14 and UCDCH15 and four alleles for UCDCH21. Regarding the markers PCHGMS1 and PCHGMS2, eight alleles were found for each marker among the 142 materials evaluated, in comparison with the four and two alleles, respectively, reported by Sosinski *et al*. (2000) for a population of 28 peach

Table 3. Diversity parameters calculated for five simple sequence repeats (SSR) markers in 142 *Prunus persica* (L.) Batsch samples.

Marker	Heterozygosity	Number of genotypes	Number of alleles	Gene diversity	PIC
UCDCH21	0.37	12	8	0.64	0.58
UCDCH14	0.70	20	14	0.78	0.76
UCDCH15	0.57	21	16	0.86	0.84
PCHGMS1	0.38	8	8	0.63	0.58
PCHGMS2	0.45	6	8	0.55	0.53
Media	0.49	13	10.8	0.69	0.66

PIC: polymorphic information content.

cultivars. The number of alleles per marker helped identify 13 genotypes out of an average of 64 possible combinations, revealing specific allele combinations in five of the six positions of the peach and nectarine germplasm genome (Table 3).

The variation in the number of alleles is congruent with the discriminant capacity of the selected markers. This parameter is evaluated with the polymorphic information content (PIC), which had an average of 0.66. Marker UCDCH15 stood out as the most informative, with a PIC of 0.84, followed by UCDCH14 with 0.76 (Table 3). PIC values above 0.5 indicate highly informative markers. The five markers used in this work surpassed this criterion, as well as the values reported in other studies. Trifonova *et al*. (2021), who evaluated the genetic diversity of 85 peach materials from the Nikita Botanical Garden in Russia, reported an average PIC of 0.49 for 12 SSRs. Chen and Okie (2022) informed of a PIC of 0.32 in 112 peach materials from genetic breeding programs in the United States using 20 SSRs. Chavez *et al*. (2014) reported a PIC of 0.44 in 164 peach and nectarine materials with 36 SSRs, whereas Demirel *et al.* (2024) obtained a PIC of 0.2 using inter-simple sequence repeat markets (ISSR) to evaluate the genetic diversity in 54 genotypes of *P. persica*. Koohi *et al.* (2020) reported a PIC of 0.8 for 67 EST-SSRs evaluated in 293 *P. persica* individuals.

The heterozygosity (*H*), expected heterozygosity (*He*), and observed heterozygosity (*Ho*) of the markers were 0.49, 0.6, and 0.61, respectively. The value of *H* was greater than that reported by Chen and Okie (2022) (0.33) and similar to the one reported by Sosinski *et al*. (2000) (0.45). The *He* and *Ho* of the evaluated loci were similar to each other (0.61 and 0.6), in comparison to other works in which *Ho* is lower than *He*. For example, Trifonova *et al*. (2021) obtained values of 0.52 for *He* and 0.31 for *Ho*; Chavez *et al*. (2014) reported 0.49 and 0.41 for *He* and *Ho*, respectively; and Koohi *et al.* (2020) obtained 0.83 for *He* and 0.36 for *Ho*.

Heterozygosity reveals both the diversity and the genetic status in which two alleles of the same locus could be in. In this study, approximately 60 % of the individuals were heterozygotic. This can be attributed to both samples belonging to two genetic breeding programs in which one of the goals has been the generation of peaches and nectarines, some of which are F_2 segregating for the trait of pilosity. The gene responsible for the lack of pubescence is recessive (Vendramin *et al.,* 2014). Its expression is visible in 25 % of the second generation, where the heterozygotic expression is double, which led to the values of heterozygosis values found per marker.

Extrapolation of the SSRs used in *P. persica* to other *Prunus* species

The protocol used for DNA extraction in peaches and nectarines displayed limitations when applied to other *Prunus* species (plum, apricot, and cherry trees). Therefore, a new extraction protocol was standardized based on the methodology by Struss *et al*. (2003), with some modifications, which helped reach the purity, concentration, and integrity standards needed to carry out the PCRs and test the standardized microsatellites in peaches and nectarines. In this context, the six SSRs turned out to be polymorphic in the three *Prunus* species different from *P. persica*; that is, there was amplification in cherry, plum, and apricot trees, unlike peach and nectarine trees, where only five were polymorphic (except UCDCH19).

These results are consistent with those reported by Dettori *et al.* (2015), who mentioned the low level of polymorphism of *P. persica* in comparison with other species of the *Prunus* genus. In this study, the averages of the genetic diversity indicators were higher for the other species of *Prunus* than for *P. persica*. At least five markers displayed *H* values greater than 0.5. The three most informative markers were UCDCH14, UCDCH15, and UCDCH19 (Table 4). The latter marker contrasts in its discriminant power between species, since in *P. persica* it was monomorphic and had no informative power, whereas in the other *Prunus* species it did prove to be polymorphic and highly informative.

Table 4. Diversity parameters calculated for six simple sequence repeats (SSRs) in other *Prunus* species.

SSR	Number of genotypes	Number of alleles	Gene diversity	Heterozygosity	PIC
UCDCH21	13.0	12.0	0.71	0.54	0.70
UCDCH14	17.0	16.0	0.91	0.77	0.90
UCDCH15	16.0	13.0	0.90	0.35	0.89
UCDCH19	14.0	10.0	0.83	0.38	0.81
PCHGMS1	7.0	7.0	0.56	0.50	0.53
PCHGMS2	2.0	2.0	0.49	0.85	0.37
Mean	11.5	10.0	0.73	0.56	0.70

The extrapolation of markers developed for a species and applied to another of the same genus or even of different genera has been reported in the literature. Sosinski *et al*. (2000) not only tested *Prunus* but also SSR markers in *Arabidopsis thaliana*, while Bedö *et al.* (2018) managed to extrapolate six SSRs from *P. persica* to *P. armeniaca,* although only five turned out to be polymorphic, unlike our investigation, in which the six SSRs were extrapolated in *P. armeniaca.* This ability to extrapolate markers in species within the same genus helps broaden the use of the markers developed in one species for comparative genomics purposes. Using the six SSRs, the formation of three groups is observed: one including nectarines and peaches, another one with apricots and plums, and the third group containing only cherry trees (Figure 1).

The clustering of species was different when using five markers and omitting PCHGMS2 for being less informative (PIC = 0.37) (Figure 2). This omission helped separate groups by species more clearly, forming four groups: *P. persica, P. armeniaca, P. salicina*, and *P. avium*, with cherries as the furthest species from peaches and nectarines. These results coincide with reports by Dettori *et al.* (2015), who mention that cherry is the most phylogenetically distant species from *P. persica.*

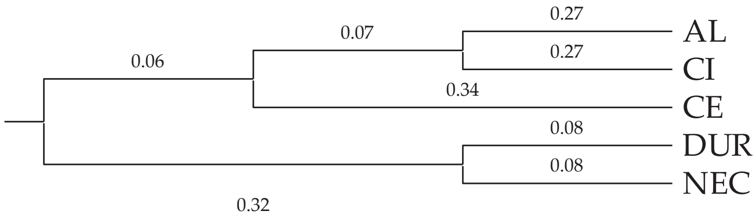

Figure 1. Dendrogram (UPGMA) based on the Nei genetic distances (1972) from six microsatellites and 26 samples of different species. AL: apricot (*Prunus armeniaca* L.); CI: plum (*P. salicina* L.); CE: cherry (*P. avium* L.); DUR: peaches (*P. persica* (L.) Batsch); NEC: nectarines (*P. persica* (L.) Batsch*)*.

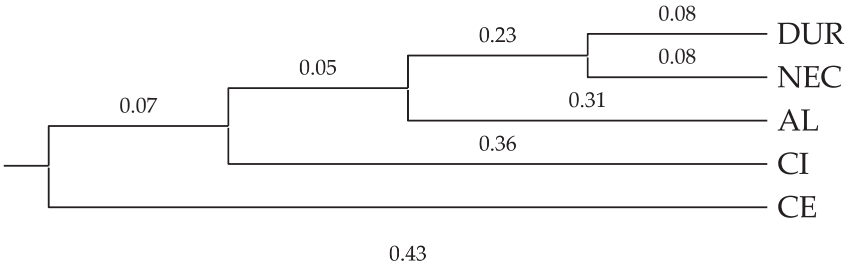

Figure 2. Dendrogram (UPGMA) based on the Nei genetic distances (1972) from five microsatellites and 26 samples of different species. DUR: peaches (*P. persica* (L.) Batsch*)*; NEC: nectarines (*P. persica* (L.) Batsch*)*; AL: apricot (*Prunus armeniaca* L.); CI: plum (*P. salicina* L.); CE: cherry (*P. avium* L.).

CONCLUSIONS

In this work, three collections of peach and nectarine germplasm (*Prunus persica* (L.) Batsch) were characterized using six microsatellites, five of which were polymorphic (UCDCH14, UCDCH15, UCDCH21, PCHGMS1, and PCHGMS1). The populations displayed a high heterozygosity, with the population from Querétaro being the most diverse. At a locus level, a trend towards homozygosity was observed in the population from Montecillo. The genetic distances showed that the populations are more related due to their founder origin. The efficiency of the six markers used in *P. persica* for comparative genomics purposes was shown. This extrapolation revealed that peaches and nectarines are genetically more distant from cherries (*P. avium* L.), followed by plums (*P. salicina* L.) and apricots (*P. armeniaca* L.).

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