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Genetic relationships among *Prunus* rootstocks for sweet cherry (*Prunus avium* L.) cultivars

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Abstract

Sweet cherries can be grafted on a wide range of rootstocks belonging to *Prunus avium*, *Prunus cerasus*, *Prunus mahaleb*, *Prunus angustifolia* or hybrids of different *Prunus* species. Identification of *Prunus* rootstocks using morphological traits is almost impossible particularly during the dormant season. However, molecular analysis carried out on actively growing shoot tips, leaves or dormant buds provides good opportunity to reliably distinguish rootstocks. In this study, DNA was extracted from the leaves of a total of 184 sweet cherry rootstock candidates belonging to *P. avium* L., *P. cerasus* L., *P. mahaleb* L. and *P. angustifolia* L. previously selected from the north-western part of Turkey. The rootstock candidates were tested with ten simple sequence repeat (SSR) primers, developed for the *Prunus* genus. The primers successfully identified all rootstock candidates. The results showed that the number of alleles per locus ranged from 10 (UDAp-401, UCD-CH21 and CPSCT010) to 20 (UCD-CH31) with an average of 13.3 alleles per locus, indicating that the SSRs were highly informative. Unweighted Pair-Group Method with Arithmetic mean analysis demonstrated that *P. avium* accessions are closely related to *P. cerasus*. The reference rootstocks were clustered with their associated botanical species.

Keywords: genetic diversity; genotyping; *Prunus*; SSR

Introduction

The genus *Prunus* includes more than 200 species of flowering shrubs and trees in the *Rosaceae* family and has great economic importance, as it includes the cultivated almond, peach, plum, cherry and apricot (Ercisli, 2004; Yilmaz *et al*., 2009).

All commercially grown sweet cherries grafted or budded on rootstocks belong to different *Prunus* species. Sweet cherry rootstocks can directly influence productivity, precocity, tree size, tree architecture, fruit size and fruit quality of sweet cherry cultivars. The choice of certain rootstocks will also influence many horticultural decisions such as pruning, training, tree support and

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labour management (Jimenez *et al.*, 2007; Cantín *et al.*, 2010; Radunic *et al.*, 2011). Traditionally, sweet cherry rootstocks were identified based on morphological characteristics and those parameters were strongly affected by the environment and the developmental stage of plant as well. This dependence on morphological traits makes rootstock identification nearly impossible particularly during the dormant season of plants. Moreover, botanical classification of species within this genus is sometimes controversial, partly because of the easiness of interspecific hybridization of the *Prunus* genus (Dosba *et al.*, 1994).

For breeding and commercialization of promising rootstock candidates, a precise determination and discrimination of these materials is requested. In the case of rootstocks, it is very difficult to observe their morphological traits after grafting. Therefore, markers independent from the environment are necessary for reliable identification and discrimination of accessions. Superiority of molecular markers over morphological characterization in fruit species is well established and widely accepted (Ercisli *et al.*, 2007; Zamani *et al.*, 2007; Kafkas *et al.*, 2008; Duminil and Di Michele, 2009; Szikriszt *et al.*, 2011).

Microsatellites or simple sequence repeat (SSR) markers have been very useful for studying the extent and distribution of genetic variability in wild and cultivated plants including various *Prunus* species (Cheng and Huang, 2009; Guarino *et al.*, 2009; Nas *et al.*, 2011) and the number of microsatellite loci available, in particular, for the *Prunus* genus has greatly increased. In *Prunus*, microsatellites have been used for germplasm characterization (Lacis *et al.*, 2009), determination of genetic diversity (Bouhadida *et al.*, 2007), germplasm management (Cheng and Huang, 2009), parentage analysis (Yamamoto *et al.*, 2003), cultivar identification (Xuan *et al.*, 2009) and mapping genetic linkage (Lalli *et al.*, 2008). In *Prunus*, microsatellites developed in one species have been used in a different species, demonstrating their transferability and ability to detect polymorphism (Wunsch, 2009).

In cultivated sweet cherries, many reports related to SSR analysis have been published in different countries (Cheng and Huang, 2009; Lacis *et al.*, 2009; Gulen *et al.*, 2010). However, much less has been done to assess genetic diversity of wild cherry species by molecular markers.

The objective of this study was to select a set of microsatellite loci useful to detail polymorphism in different accessions belonging to *Prunus* species used as rootstocks for sweet cherry. It is expected that the information presented here would be useful for selection and more efficient utilization of this germplasm as rootstocks for sweet cherries in future.

Materials and methods

Plant material

For SSR and genetic relationship studies, a total of 184 accessions including representatives of the species *Prunus avium* (110 accessions), *Prunus mahaleb* (40 accessions), *Prunus cerasus* (29 accessions) and *Prunus angustifolia* (5 accessions) were used for SSR analysis. These accessions were previously selected from wild cherry populations in a national-wide sweet cherry rootstock selection study conducted in the north-western part of Turkey (supplementary material available online at http://journals.cambridge.org/). All accessions are maintained in a germplasm collection at the Black Sea Agricultural Research Center in Samsun, Turkey. Three standard *Prunus* rootstocks, SL64 (*P. mahaleb* L.), F12/1 (*P. avium* L.) and Montmorency (*P. cerasus* L.), were also included in the SSR analysis as a reference.

DNA extraction

Genomic DNA was extracted from young leaf tissue using the Wizard® Genomic DNA Purification Kit (Promega, Madison, WI, USA) according to the instructions provided by the manufacturer. Subsequently, an RNase treatment was performed on the eluted DNA samples. Purity and concentration of the DNA were checked both on 1% (w/v) agarose gels and by using a NanoDrop® ND-1000 Spectrophotometer.

SSR analysis

We initially checked 16 SSR primers. These 16 SSR primers are widely used in *Prunus* for molecular characterization (Akpinar *et al.*, 2010; Gulen *et al.*, 2010; Ercisli *et al.*, 2011; Yilmaz *et al.*, 2012). However, ten of them gave better amplification in this study. Therefore, ten SSRs were selected to check polymorphism by capillary electrophoresis in 184 accessions of the four different *Prunus* species (Table 1). Polymerase chain reaction (PCR) was conducted in a volume of 10 μl and contained 15 ng genomic DNA, 5 pmol of each primer, 0.5 mM dNTP, 0.5 unit GoTaq DNA polymerase (Promega), 1.5 mM $MgCl_2$ and 2 μl 5 × buffer. The forward primers were 'labelled' with WellRED fluorescent dyes D2 (black), D3 (green) and D4 (blue) (Pro-ligo, Paris, France). Reactions without DNA were included as negative controls. PCR amplification was performed using the Biometra® PCR System. The amplification conditions consisted of an initial denaturation step of 3 min at 94°C, followed by 35 cycles of 1 min at 94°C, 1 min at 52–56°C

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Table 1. Simple sequence repeat primer pairs used in this study to characterize 184 rootstocks belonging to *Prunus*

SSR locus	Sequence (5′–3′)	Species origin	References
CPSCT010	TTG GGT AAA TAC TTT ATC ATT TCC TCC CTG AAT AAG GGT TGT GC	Plum	Mnejja *et al.* (2005)
Pchgms1	GGG TAA ATA TGC CCA TTG TGC AAT C GGA TCA TTG AAC TAC GTC AAT CCT C	Peach	Sosinski *et al.* (2000)
PS12A02	GCCACCAATGGTTCTTCC AGCACCAGATGCACCTGA	Cherry	Downey and Iezzoni (2000)
UCD-CH17	TGG ACT TCA CTC ATT TCA GAG A ACT GCA GAG AAT TTC CAC AAC CA	Sweet cherry	Struss *et al.* (2003)
UCD-CH21	TTG TTG ACC ATC GAA TAT GAA G GAA GGT ACA TGG CGT GCC	Sweet cherry	Struss *et al.* (2003)
UCD-CH31	TCC GCT TCT CTG TGA GTG TG CGA TAG TTT CCT TCC CAG ACC	Sweet cherry	Struss *et al.* (2003)
UDAp-401	AAA CCC TAG CCG CCA TAA CT GCT AAA GGC CTT CCG ATA CC	Apricot	Messina *et al.* (2004)
UDAp-404	CAT GAA CAG GGT CAA AAG CA TAT ATC CTT ACG CGG CCT CA	Apricot	Messina *et al.* (2004)
UDP96-001	AGT TTG ATT TTC TGA TGC ATC C TGC CAT AAG GAC CGG TAT GT	Peach	Cipriani *et al.* (1999)
UDP96-005	GTA ACG CTC GCT ACC ACA AA CCT GCA TAT CAC CAC CCA G	Peach	Cipriani *et al.* (1999)

and 2 min at 72°C with a final extension at 72°C for 10 min. For the determination of polymorphisms, the PCR products were run on a CEQTM 8800 XL Capillary Genetic Analysis System (Beckman Coulter, Fullerton, CA, USA). The analyses were repeated at least twice to ensure reproducibility of the results. In each run, SL64, F12/1 and Montmorency were included as reference rootstocks.

Genetic analysis

The genetic analysis program 'IDENTITY' 1.0 (Wagner and Sefc, 1999) was used according to Paetkau *et al.* (1995) for the calculation of number of alleles, expected and observed heterozygosity (H_e and H_o), estimated frequency of null alleles, and the probability of genetic identity per locus. Probability of genetic identity corresponds to the probability of two random individuals displaying the same accessions. That means if probability of identity (PI) = 1, it indicates that the primer pair is not able to distinguish between the accessions, and *vice versa* the smaller is the PI value, the more informative the SSR primer pair (locus) is. Null alleles are non-amplified alleles that, when segregating with another allele, result in an apparent homozygote. For microsatellites, such null alleles can arise when mutations occur in the flanking regions, preventing one or both of the primers from binding (Holm *et al.*, 2001). Genetic similarity was determined by the program 'MICROSAT' (version 1.5) (Minch *et al.*, 1995) using the proportion of shared alleles, which was calculated by using 'ps (option 1 − (ps))', as described by Bowcock *et al.* (1994). The results were then converted to a similarity matrix, and a dendrogram was constructed with the unweighted pair-group method with arithmetic mean (UPGMA) method (Sneath and Sokal, 1973) using software NTSYS-pc (Numerical Taxonomy and Multiware Analysis System, version 2.0) (Rohlf, 1988).

Results

In the study, a total of 133 alleles with an average of 13.3 alleles per locus obtained. The number of alleles per primer ranged from 10 (UDAp-401, UCD-CH21 and CPSCTO10 primers) to 20 (UCD-CH31) (Table 2).

We observed an average SSR heterozygosity (H_o) of 0.57, while the H_e was 0.81. The H_o, identified by each primer pair, ranged between 0.34 (UCD-CH21) and 0.81 (UDAp-404) and the H_e was found between 0.70 (UDAp-404) and 0.87 (UCD-CH31 and UDP96-005). UCD-CH31, UDP96-001 and UDP96-005 were the markers with the highest informative value with regard to the lowest PI (0.06) value, whereas the least informative

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Table 2. List of genetic parameters obtained with simple sequence repeat used in this study

Locus	N	H_e	H_o	PI	r
CPSCT010	10	0.77	0.60	0.11	0.09
Pchgms1	15	0.77	0.45	0.13	0.18
PS12A02	15	0.82	0.47	0.10	0.19
UCD-CH17	13	0.86	0.40	0.72	0.25
UCD-CH21	10	0.79	0.34	0.13	0.25
UCD-CH31	20	0.87	0.64	0.06	0.12
UDAp-401	10	0.79	0.67	0.12	0.06
UDAp-404	15	0.70	0.81	0.24	−0.06
UDP96-001	12	0.86	0.56	0.06	0.16
UDP96-005	13	0.87	0.80	0.06	0.04
Total	133				
Average	13.3	0.81	0.57		

N, number of alleles; H_o, observed heterozygosity; H_e, expected heterozygosity; PI, probability of identity; r, null allele frequencies.

locus was UCD-CH17 (PI = 0.72) (Table 2). Null allele frequency was the highest in loci UCD-CH17 and UCD-CH21 (0.25) and was the lowest in UDAp-404 (−0.06) (Table 2).

Genetic similarity measured within the species ranged between 0.05 and 1.00 within *P. avium*, 0.45 and 1.00 within *P. cerasus*, 0.55 and 0.80 within *P. angustifolia* and 0.05 and 1.00 within *P. mahaleb* accessions. The average similarity ratios within the species in a descending order were *P. cerasus* (0.77) > *P. angustifolia* (0.69) > *P. mahaleb* (0.55) > *P. avium* (0.37), respectively.

A tree constructed from the SSR data divided the cultivars into two main clusters according to their taxonomic classification. The first cluster included the *P. avium* and *P. cerasus* accessions, the first subcluster consisted of *P. avium* and the second subcluster included *P. cerasus*. *P. avium* seemed to be more differentiated. *P. cerasus* was divided into five groups, the two biggest groups contained many accessions and these accessions seemed to be identical or very closely related. The second main cluster included the *P. mahaleb* and *P. angustifolia* accessions and was also further divided into two subclusters: its first and second subcluster consisted of the *P. mahaleb* and *P. angustifolia* accessions, respectively. In contrast to *P. cerasus*, *P. mahaleb* and *P. angustifolia* seemed to be more differentiated. The reference rootstocks were also clustered with their associated botanical species (Fig. 1).

Discussion

The results obtained in the present study show that microsatellites could be effectively used for fingerprinting purposes in *Prunus*. In the present study, ten loci in wild *Prunus* accessions were assayed. The number of alleles per locus ranged from 10 to 20 with an average of

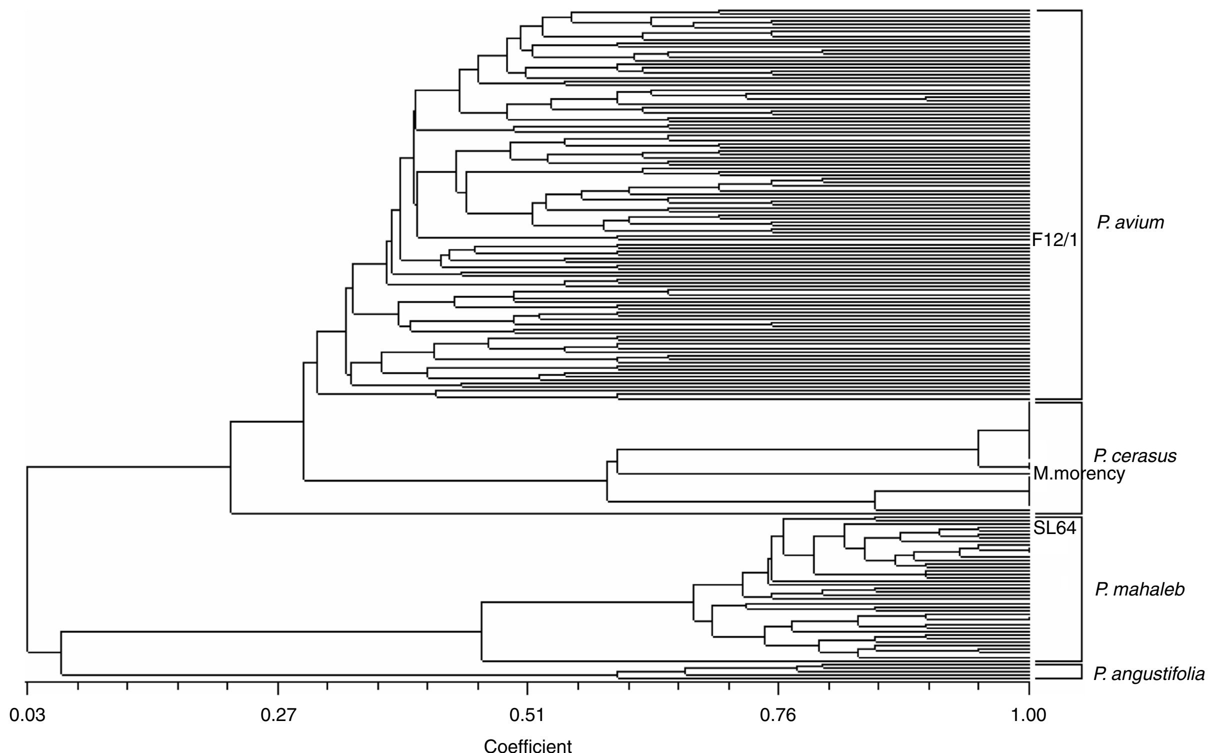

Fig. 1. Dendrogram of 184 *Prunus* accessions based on UPGMA analysis using the genetic similarity matrix generated by the Nei and Li similarity coefficient after amplification with ten pairs of microsatellite primers.

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13.3 putative alleles per locus. Previously, Kacar *et al.* (2005) obtained a total of 37 alleles among ten sweet cherry cultivars by nine SSR primers. Clarke and Tobutt (2003) used 14 sweet cherry cultivars for SSR analysis and determined two to seven alleles per SSR primer. In addition, Vaughan and Russell (2004) used 16 wild cherry accessions for molecular analysis by using ten SSR primers and they detected two to six alleles. In fact, all tested microsatellite primer pairs worked well and produced variable levels of amplifications. The UCD-CH31 locus developed for cherries was the most polymorphic among the ten loci with the highest effective number of alleles (20 alleles) with the lowest PI value (0.06). The CPSCT010, UCD-CH21 and UDAp-401 loci were the less informative with the lowest allele number. Ercisli *et al.* (2011) also found that UDAp-401 and CPSCTO10 were the less informative loci in wild sweet cherries (*P. avium*). The results showed high amplification of cherry groups with plum, apricot and peach indicating a congeneric relationship within *Prunus* species. Ercisli *et al.* (2011) successfully used SSR markers identified in other *Prunus* species to study genetic diversity in wild sweet cherries. Dirlewanger *et al.* (2002), Wunsch and Hormaza (2002) and Bouhadida *et al.* (2009) also showed transportability of SSR markers across *Prunus* species. Our results demonstrated the cross-species transferability of SSR primers developed in cultivated species to wild species in *Prunus* for the discrimination of accessions.

Previously, UCD-CH31 (Struss *et al.*, 2003) and PS12A02 loci were found to be the most informative in other studies (Downey and Iezzoni, 2000; Gulen *et al.*, 2010; Ercisli *et al.*, 2011). According to Sefc *et al.* (2000), the PI value should be over 0.05 and all loci used in this study had PI values higher than 0.05, indicating that the selected loci were highly polymorphic for used materials. As mentioned before, null allele frequency was the highest in loci UCD-CH17 and UCD-CH21 (0.25) and was the lowest in UDAp-404 (−0.06) (Table 2). Null alleles are generally referred to as alleles that fail to amplify during the PCR.

The overall genetic diversity within the tested species was relatively low as evident from the polymorphic ratio of 21% found using SSR primers (Struss *et al.*, 2003), 19% reported by Zhou *et al.* (2002) and 20% reported by Zhou *et al.* (2002) in cherries. We found a high diversity ratio within *P. avium* compared with other species. A higher level of polymorphism was expected in sweet cherry due to its predominant self-incompatibility (Hegedus *et al.*, 2012).

SSR markers have been widely used for molecular characterizations and similarity relationships among *Prunus* accessions and revealed a high level of polymorphism to discriminate among these accessions (Sosinski *et al.*, 2000; Dirlewanger *et al.*, 2002; Pedryc *et al.*, 2009).

The higher levels of within-group variation observed within *P. avium* accessions suggest the development of *P. avium* in the Black Sea and Northeast Anatolia. Introduction and spread of wild and semi-domesticated *Prunus* species, especially from its native Near Eastern range, domestication of indigenous wild *Prunus* species, natural hybridization between indigenous and introduced plants, and human selection may have contributed to this high variation.

The H_o and H_e averaged over the ten SSR loci were, respectively, 0.57 and 0.81, indicating higher mean values than those reported for SSRs in *Prunus* species (Aranzana *et al.*, 2003; Bouhadida *et al.*, 2009). A high allele number and high heterozygosity obtained in the present study reflect the ability of SSR markers to provide a unique genetic profile for individual plant accessions, in particular, for *P. avium*, *P. mahaleb* and *P. angustifolia* accessions.

Such high levels of heterozygosity are commonly observed among clonally propagated, outbreeding, perennial species since they are favoured during selection and are known to confer greater adaptability, vigour and productivity on clonal varieties (Aradhya *et al.*, 1998; Sefc *et al.*, 2000).

Conclusion

In conclusion, the gene pool of the *Prunus* species surveyed in Northeast Anatolia has significant amounts of genetic variation. With regard to germplasm management, our results show that the germplasm collection is highly variable and most variation is common to all genetic groups identified. The *Prunus* germplasm from the region would have economically important adaptive traits that can potentially be incorporated into *Prunus* breeding programmes. Hence, it is expected that the results of this study will assist current *Prunus* rootstock breeding efforts in Turkey as well as maintain the genetic integrity of the genetic resources. The SSR-based phylogeny was also generally consistent with *Prunus* taxonomy based on molecular evidence, suggesting the applicability of SSR analysis for genotyping and phylogenetic studies in the *Prunus* genus.

References

Akpinar AE, Kocal H, Ergul A, Kazan K, Selli ME, Bakir M, Aslantas S, Kaymak S and Saribas R (2010) SSR-based molecular analysis of economically important Turkish apricot cultivars. *Genetics and Molecular Research* 9: 324–332.

https://doi.org/10.1017/S147926211200007X Published online by Cambridge University Press

Aradhya MK, Liana Y, Zee FT and Manshardt RM (1998) Genetic variability in Macadamia. *Genetic Resources and Crop Evolution* 45: 19–32.

Aranzana J, Pineda A, Cosson P, Dirlewanger E, Ascasibar J, Cipriani G, Ryder CD, Testolin R, Abbott A, King GJ, Iezzoni AF and Arús P (2003) A set of simple-sequence repeat (SSR) markers covering the *Prunus* genome. *Theoretical and Applied Genetics* 106: 819–825.

Bouhadida M, Martin JP, Eremin G, Pinochet J, Moreno MA and Gogorcena Y (2007) Chloroplast DNA diversity in *Prunus* and its implication on phylogenetic relationships. *Journal of the American Society for Horticultural Science* 132: 670–679.

Bouhadida M, Casas AM, Gonzalo MJ, Arús P, Moreno MA and Gogorcena Y (2009) Molecular characterization and genetic diversity of *Prunus* rootstocks. *Scientia Horticulturae* 120: 237–245.

Bowcock AM, Ruiz-Linares A, Tomfohrde J, Minch E, Kidd JR and Cavalli-Sforza LL (1994) High resolution of human evolutionary trees with polymorphic microsatellites. *Nature* 368: 455–457.

Cantín CM, Pinochet J, Gogorcena Y and Moreno MA (2010) Fruit quality and yield of 'Van' and 'Stark Hardy Giant' sweet cherry cultivars as influenced by grafting on different rootstocks. *Scientia Horticulturae* 123: 329–335.

Cheng Z and Huang H (2009) SSR fingerprinting Chinese peach cultivars and landraces (*Prunus persica*) and analysis of their genetic relationships. *Scientia Horticulturae* 120: 188–193.

Cipriani G, Lot G, Huang WG, Marrazzo MT, Petelunger E and Testolin R (1999) AC/GT and AG/CT microsatellite repeats in peach (*Prunus persica* (L.) Batsch): isolation, characterisation and cross-species amplification in *Prunus*. *Theoretical and Applied Genetics* 99: 65–72.

Clarke JB and Tobutt KR (2003) Development and characterization of polymorphic microsatellites from *Prunus avium* 'Napoleon'. *Molecular Ecology Notes* 3: 578–580.

Dirlewanger E, Cosson P, Tavaud M, Aranzana MJ, Poizat C, Zanetto A, Arús P and Laigret F (2002) Development of microsatellite markers in peach [*Prunus persica* (L.) Batsch] and their use in genetic diversity analysis in peach and sweet cherry, *Prunus avium* L. *Theoretical and Applied Genetics* 105: 127–138.

Dosba F, Bernhard R and Zanetto A (1994) Importance des ressources génétiques des *Prunus*. *Comptes-rendus des Seances de l'Academie d'Agriculture de France* 80: 45–57.

Downey SL and Iezzoni AF (2000) Polymorphic DNA markers in black cherry (*Prunus serotina*) are identified using sequences from sweet cherry, peach and sour cherry. *Journal of the American Society for Horticultural Science* 125: 76–80.

Duminil J and Di Michele M (2009) 'Plant species delimitation: a comparison of morphological and molecular markers'. *Plant Biosystems* 143: 528–542.

Ercisli S (2004) A short review of the fruit germplasm resources of Turkey. *Genetic Resources and Crop Evolution* 51: 419–435.

Ercisli S, Agar G, Orhan E, Yildirim N and Hizarci Y (2007) Interspecific variability of RAPD and fatty acid composition of some pomegranate cultivars (*Punica granatum* L.) growing in Southern Anatolia Region in Turkey. *Biochemical Systematics and Ecology* 35: 764–769.

Ercisli S, Agar G, Yildirim N, Duralija B, Vokurka A and Karlidag H (2011) Genetic diversity in wild sweet cherries (*Prunus avium* L.) in Turkey revealed by SSR markers. *Genetics and Molecular Research* 10: 1211–1219.

Guarino C, Santoro S, De Simone L and Cipriani G (2009) *Prunus avium*: nuclear DNA study in wild populations and sweet cherry cultivars. *Genome* 52: 320–337.

Gulen H, Ipek A, Ergin S and Akcay MA (2010) Assessment of genetic relationships among 29 introduced and 49 local sweet cherry accessions in Turkey using AFLP and SSR markers. *Journal of Horticultural Science & Biotechnology* 85: 427–431.

Hegedus A, Lenart J and Halasz J (2012) Review of sexual incompatibility in tree fruit species: molecular interactions and evolutionary dynamics. *Biologia Plantarum* 56(2): 201–209.

Holm L-E, Loeschcke V and Bendixen C (2001) Elucidation of the molecular basis of a null allele in a Rainbow trout microsatellite. *Marina Biotechnology* 3: 555–560.

Jimenez S, Pinochet J, Gogorcena Y, Betran JA and Moreno MA (2007) Influence of different vigour cherry rootstocks on leaves and shoots mineral composition. *Scientia Horticulturae* 112: 73–79.

Kacar AY, Iezzoni A and Cetiner S (2005) Sweet cherry cultivar identification by using SSR markers. *Journal of Biological Sciences* 5: 616–619.

Kafkas S, Ozgen M, Dogan Y, Ozcan B, Ercisli S and Serce S (2008) Molecular characterization of mulberry accessions in Turkey by AFLP markers. *Journal of the American Society for Horticultural Science* 133: 593–597.

Lacis G, Rashal I, Ruisa S, Trajkovski V and Iezzoni AF (2009) Assessment of genetic diversity of Latvian and Swedish sweet cherry (*Prunus avium* L.) genetic resources collections by using SSR (microsatellite) markers. *Scientia Horticulturae* 121: 451–457.

Lalli D, Abbott AG, Zhebentyayeva TN, Badenes ML, Damsteegt V, Polak J, Krska B and Salava A (2008) A genetic linkage map for an apricot (*Prunus armeniaca* L.). BC1 population mapping plum pox virus resistance. *Tree Genetics & Genomes* 4: 481–493.

Messina R, Lain O, Marrazzo MT, Cipriani G and Testolin R (2004) New set of microsatellite loci isolated in apricot. *Molecular Ecology Notes* 4: 432–434.

Minch E, Ruiz-Linares A, Goldstein DB, Feldman M and Cavalli-Sforza LL (1995) *Microsat (Version 1.4d): A Computer Program for Calculating Various Statistics on Microsatellite Allele Data*. Stanford: Stanford University Medical Center.

Mnejja M, Garcia-Mas J, Howad W and Arús P (2005) Development and transportability across *Prunus* species of 42 polymorphic almond microsatellites. *Molecular Ecology Notes* 5: 531–535.

Nas MN, Bolek Y and Bardak A (2011) Genetic diversity and phylogenetic relationships of *Prunus microcarpa* C.A. Mey. subsp. *tortusa* analyzed by simple sequence repeats (SSRs). *Scientia Horticulturae* 127: 220–227.

Paetkau D, Calvert W, Stirling I and Strobeck C (1995) Microsatellite analysis of population structure in Canadian polar bears. *Molecular Ecology* 4: 347–354.

Pedryc A, Ruthner SZ, Herman R, Krska B, Hegedus A and Halasz J (2009) Genetic diversity of apricot revealed by a set of SSR markers from linkage group G1. *Scientia Horticulturae* 121: 19–26.

Radunic M, Jazbec A, Pecina M, Cosic T and Pavicic N (2011) Growth and yield of the sweet cherry (*Prunus avium* L.) as affected by training system. *African Journal of Biotechnology* 10: 4901–4906.

https://doi.org/10.1017/S147926211200007X Published online by Cambridge University Press

Rohlf FJ (1988) *NTSYS-PC Numerical Taxonomy and Multivariate Analysis System*. New York: Exeter Publishing.

Sefc K, Lopez MS, Lefort F, Botta R, Roubelakis-Angelakis KA, Ibanez J, Pejic I, Wagner HW, Glossl J and Steinkellner H (2000) Microsatellites variability in grapevine cultivars from different European regions and evaluation of assignment testing to assess the geographic origin of cultivars. *Theoretical and Applied Genetics* 100: 498–505.

Sosinski B, Gannavarapu M, Hager LD, Beck LE, King GJ, Ryder CD, Rajapakse S, Baird WV, Ballard RE and Abbott AG (2000) Characterisation of microsatellite markers in peach [*Prunus persica* (L.) Batsch]. *Theoretical and Applied Genetics* 101: 421–428.

Sneath PH and Sokal RR (1973) *Numerical Taxonomy*. San Francisco, CA: Freeman.

Struss D, Ahmad R, Southwick SM and Boritzki M (2003) Analysis of sweet cherry (*Prunus avium* L.) cultivars using SSR and AFLP markers. *Journal of the American Society for Horticultural Science* 128: 904–909.

Szikriszt B, Hegedus A and Halasz J (2011) Review of genetic diversity studies in almond (*Prunus dulcis*). *Acta Agronomica Hungarica* 59: 379–395.

Vaughan SP and Russell K (2004) Characterization of novel microsatellites and development of multiplex PCR for large-scale population studies in wild cherry, *Prunus avium*. *Molecular Ecology Notes* 4: 429–431.

Wagner HW and Sefc KM (1999) *Identity 1.0*. Vienna: Centre for Applied Genetics, University of Agricultural Science.

Wunsch A (2009) SSR markers for fingerprinting *Prunus* species. *Acta Horticulturae* 814: 689–694.

Wunsch A and Hormaza JI (2002) Molecular characterisation of sweet cherry (*Prunus avium* L.) genotypes using peach [*Prunus persica* (L.) Batsch] SSR sequences. *Heredity* 89: 56–63.

Xuan H, Wang R, Buchele M, Moller O and Hartmann W (2009) Microsatellite markers (SSR) as a tool to assist in identification of sweet (*Prunus avium*) and sour cherry (*Prunus cerasus*). *Acta Horticulturae* 839: 507–514.

Yamamoto T, Mochida K, Imai T, Haji T, Yaegaki H, Yamaguchi M, Matsuta N, Ogiwara I and Hayashi T (2003) Parentage analysis in Japanese peaches using SSR markers. *Breeding Science* 53: 35–40.

Yilmaz KU, Ercisli S, Asma BM, Dogan Y and Kafkas S (2009) Genetic relatedness in *Prunus* genus revealed by inter-simple sequence repeat markers. *HortScience* 44: 293–297.

Yilmaz KU, Paydas-Kargi S, Dogan Y and Kafkas S (2012) Genetic diversity analysis based on ISSR, RAPD and SSR among Turkish apricot germplasms in Iran Caucasian eco-geographical group. *Scientia Horticulturae* doi:10.1016/j.scienta.2012.02.017.

Zamani Z, Sarkhosh A, Fatahi R and Ebadi A (2007) Genetic relationships among pomegranate genotypes studied by fruit characteristics and RAPD markers. *Journal Horticultural Science and Biotechnology* 82: 11–18.

Zhou L, Kapel F, Hampson C, Wiersma PA and Bakkeren G (2002) Genetic analysis and discrimination of sweet cherry cultivars and selections using amplified fragment length polymorphism fingerprints. *Journal of the American Society for Horticultural Science* 127: 786–792.

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