GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Genetic diversity in wild sweet cherries (*Prunus avium*) in Turkey revealed by SSR markers

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Genet. Mol. Res. 10 (2): 1211-1219 (2011)
Received December 6, 2010
Accepted March 24, 2011
Published June 21, 2011
DOI 10.4238/vol10-2gmr1196

ABSTRACT. Wild sweet cherry (*Prunus avium*) trees are abundant in the northern part of Turkey, including the Coruh Valley. We analyzed 18 wild sweet cherry genotypes collected from diverse environments in the upper Coruh Valley in Turkey to determine genetic variation, using 10 SSR primers. These SSR primers generated 46 alleles; the number of alleles per primer ranged from 3 to 7, with a mean of 4.6. The primer PS12A02 gave the highest number of polymorphic bands (N = 7), while CPSCT010, UDAp-401 and UDAp-404 gave the lowest number

(N = 3). Seven groups were separated in the dendrogram, although most of the genotypes did not cluster according to phenological and morphological traits. This level of genetic diversity in these wild sweet cherry genotypes is very high and therefore these trees would be useful as breeders for crosses between cultivated sweet cherry and wild genotypes.

Key words: Genetic diversity; SSR markers; Microsatellites; Wild sweet cherry

INTRODUCTION

Turkey encompasses a high level of plant diversity and is recognized as a center of diversity for globally significant temperate zone fruit crops, like hazelnuts, sweet and sour cherries, apples, chestnuts, walnuts, apricots, pears, quinces, and almonds. Turkey is also described as a country with many microcenters for other crops, such as wheat, flax, barley, melon, cabbage, and bean. The importance of protecting existing plant diversity is highly recognized and various conservation programs exist in the country (Ercisli, 2004; Halilova and Ercisli, 2010).

Located in the northeastern part of the country, the Coruh Valley shows high plant biodiversity in its relatively small area. The valley is classified as one of the 34 hotspots by the World Conservation Union, as the western section of the "Caucasus Ecosystem" (Anonymous, 2009). Its rich biological diversity, in particular for wild edible fruits including wild cherries, is a result of the extreme variations in climate within a very small area and varying altitudes of the valleys where the difference between the lowest and the highest point can reach up to 3000 meters.

Sweet cherry (*Prunus avium* L.) is an important tree crop, which grows both wild and cultivated in many places in Turkey. It is mostly cultivated in temperate regions in the world and is thought to have originated around the Caspian Sea and the Black Sea and now is widely cultivated across Europe and western Asian countries (Webster, 1996). It is believed that the spread of its cultivation through Europe was probably based on domestication of wild individuals that are adaptable to different areas in Europe (Wünsch and Hormaza, 2002).

Currently in Turkey, wild sweet cherries are found in mixed forests (up to 1600 m a.s.l.), mainly in the northeastern part of the country. In particular the Coruh Valley in Northeast Anatolia has notable populations of different wild sweet cherry genotypes that differ from each other in tree vigor and habit, branching habit, fruit skin color and fruit taste, flowering and harvest time (Ercisli, 2004; Turkoglu et al., 2010). Seeds of wild sweet cherries are used as rootstocks for scion cultivars (Turkoglu et al., 2010). The fruit is an important food source in human nutrition, but it has also a significant place in the nutrition chain within the forest ecosystem as food for birds, mammals and insects. Local people in Northeast Anatolia region have traditionally used wild sweet cherry fruits, both as a source of food and as a medicine for hundreds of years. In Turkey, the fruit stalks of wild sweet cherries boiled in water are used as a diuretic and for urinary bladder problems (Baytop, 1984).

The majority of these wild sweet cherry genotypes are potentially useful genetic sources of resistance against diseases and pests, and available for cultivated sweet cherry improvement. Therefore, knowledge of the genetic variation of wild sweet cherry could be

important for their efficient use in further breeding programs and for conservation purposes.

Molecular marker analysis, combined with phenotypic evaluation, is a powerful tool for grouping of genotypes based on genetic similarity data, and for selection of progenitors that might constitute new breeding populations. This tool is also very useful for identification and management of germplasm collections (Benjak et al., 2005; Kafkas et al., 2008).

Among the molecular markers, the microsatellites (or SSR - simple sequence repeats) are polymerase chain reaction (PCR)-based, highly polymorphic, multi-allelic, frequently co-dominant, highly reproducible and randomly and widely distributed in the genome (Powell et al., 1996). In sweet cherries, microsatellites have been used for germplasm characterization (Lacis et al., 2009), determination of genetic diversity (Dirlewanger et al., 2002; Wünsch et al., 2004), germplasm management (Wünsch and Hormaza, 2002), parentage analysis (Schueler et al., 2003), cultivar identification (Xuan et al., 2009; Gulen et al., 2010), and mapping genetic linkage (Olmstead et al., 2008). In *Prunus*, microsatellites developed in one species have been used in different species, demonstrating their transferability and ability to detect polymorphism (Wünsch, 2009).

In cultivated sweet cherries, many reports related to SSR analysis have been published (Wunsch and Hormaza, 2004; Lacis et al., 2009; Cheng and Huang, 2009; Turkoglu et al., 2010). However, much less has been done to assess genetic diversity of wild sweet cherries by molecular markers. A significant report comparing wild sweet cherry populations from Italy, Croatia and Slovenia by SSR markers was published by Guarino et al. (2009).

In the present study, 10 SSRs developed for *Prunus* were used to assess genetic diversity and genetic relationships among wild sweet cherry genotypes, focusing on understanding the variability among wild sweet cherries.

MATERIAL AND METHODS

Plant material

The 18 wild sweet cherry genotypes (*P. avium*) were collected in diverse environments in the upper Coruh Valley and their DNA in leaves were used for microsatellite analysis. Some important tree and fruit characteristics of 18 genotypes are given in Table 1.

DNA extraction

Total genomic DNA was extracted from young leaf from a single tree for each genotype using the CTAB method (Doyle and Doyle, 1987). DNA purity and concentration were checked, both on 1% (w/v) agarose gels by using 1.0X TBA buffer and with a NanoDrop® ND-1000 Spectrophotometer.

SSR and PCR procedure

From the initial screening of 27 SSR primers on agarose gel, a total 10 SSR primers were selected and used in SSR analysis for determining the genetic diversity of 18 genotypes of wild *P. avium* (Table 2). PCR was carried out in a volume of 25 µL including 50 ng template DNA, 1X PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 1.67 pmol of each SSR primer (forward and reverse), and 1.5 U Taq DNA polymerase (Sigma, USA).

Table 1. Some tree and fruit characteristics of wild sweet cherry genotypes.

Genotypes	Tree vigor	Tree habit	Fruit taste	Fruit skin color	Branching habit	Time of beginning of flowering	Time of beginning of fruit ripening
YK01	Medium	Semi-Upright	Bitter	Dark red	Medium	Early	Early
YK02	Strong	Upright	Bitter	Red	Strong	Late	Late
YK03	Medium	Spreading	Bitter	Blackish	Medium	Very Late	Very Late
YK04	Strong	Upright	Sweet	Yellow	Strong	Early	Early
YK05	Medium	Semi-Upright	Bitter	Blackish	Medium	Medium	Medium
YK06	Medium	Semi-Upright	Bitter	Pink	Medium	Medium	Medium
YK07	Medium	Semi-Upright	Sweet	Yellow	Medium	Late	Late
YK08	Strong	Upright	Sweet	Red	Strong	Late	Late
YK09	Medium	Semi-Upright	Sweet	Dark red	Strong	Late	Late
YK10	Strong	Upright	Bitter	Blackish	Strong	Early	Early
YK11	Medium	Upright	Bitter	Dark red	Medium	Medium	Medium
YK12	Medium	Upright	Sweet	Red	Medium	Medium	Medium
YK13	Medium	Semi-Upright	Bitter	Blackish	Medium	Early	Early
YK14	Strong	Upright	Sweet	Blackish	Strong	Late	Late
YK15	Medium	Semi-Upright	Bitter	Red	Medium	Late	Late
YK16	Medium	Semi-Upright	Bitter	Yellow	Medium	Late	Late
YK17	Strong	Semi-Upright	Sweet	Red	Medium	Early	Early
YK18	Medium	Semi-Upright	Bitter	Red	Medium	Late	Late

Table 2. List of microsatellites that produced polymorphic amplification patterns among the genotypes studied.

Locus name	Repeat motif	Specie	Reference	No. of alleles
CPSCT010	$(TA)_9(CT)_{14}$	Plum	Mnejja et al., 2005	3
UDAp-401	$(TC)_{23}$	Apricot	Messina et al., 2004	3
UDAp-404	$(GA)_{21}$	Apricot	Messina et al., 2004	3
Pchgms1	$(AC)_{12}(AT)_6$	Peach	Sosinski et al., 2000	6
UDP96001	CA	Peach	Cipriani et al., 1999	6
UDP96005	AC&CT	Peach	Cipriani et al., 1999	5
PS12A02	$(GA)_{22}$	Sweet cherry	Sosinski et al., 2000	7
UCDCH17	$(CT)_{11}$	Sweet cherry	Struss et al., 2003	4
UCDCH21	$(CA)_{18}$	Sweet cherry	Struss et al., 2003	5
UCDCH31	$(CT)_{26}$	Sweet cherry	Struss et al., 2003	4
Total				46
Average				4.6

PCR amplification program was applied as follows: pre-denaturation at 94°C for 3 min; denaturation at 94°C for 30 s, annealing at 45°C for 30 s, extension at 72°C for 30 s, 30 cycles, and a final extension at 72°C for 5 min. PCR products were separated by electrophoresis on 3% high-resolution agarose gel in 1X TBE buffer at 180 constant volts for 1 h, and visualized by staining with ethidium-bromide and photographed using the gel documentation system 'Bio-Rad Gel Doc 1000' under UV source.

Each polymorphic band was identified and scored as being present (1) or absent (0) on the gels for SSR analyses. A similarity matrix was generated according to simple matching coefficients (Sneath and Sokal, 1973). The data in the similarity matrix were used for cluster analysis using the unweighted pair-group method with arithmetic averages (UPGMA) by using the NTSYS-PC 2.2 software (Numerical Taxonomy and Multiware Analysis System, version 2.0) (Rohlf, 1998).

RESULTS

All 10 loci assayed in wild sweet cherry genotypes possessed a moderate level of polymorphism, with the number of alleles per locus ranging from 3 (CPSCT010, UDAp-401 and UDAp-404) to 7 (PS12A02). The PS12A02 locus was the most polymorphic among the 10 loci, with the highest effective number of alleles and was followed by Pchgms1 and UDP96001 (6 alleles) (Table 2).

The genetic similarity measured within wild sweet cherry genotypes ranged from 0.33 to 0.90 with an average of 0.58. The highest genetic similarity ratio was observed between YK15 and YK17 genotypes (0.90) while the lowest genetic similarity was observed between YK03 and YK18 genotypes (0.33), respectively.

A dendrogram constructed according to SSR data (Figure 1) of 18 wild sweet cherry genotypes divided them into 7 main clusters. The first cluster included YK11, the second cluster included YK18 and the third cluster included YK01 and YK06 genotypes with a 0.40 similarity ratio. The fourth cluster included YK09 and YK14 genotypes, which have a 0.49 similarity ratio (Figure 1). The fifth cluster, which has the majority of genotypes (9 genotypes), was also divided into two subclusters: the first subcluster consisted of YK02, YK05, YK13, YK15, and YK17. These genotypes are found to be genetically very close to each

other. For example, the similarity ratio between YK15 and YK17 is 0.90, and 0.82 for YK02 and YK05 (Figure 1). The second subcluster consisted of genotypes YK07, YK08, YK10, and YK16. Cluster six consisted of YK04 and YK12, and finally the last main cluster consisted only of genotype YK03 (Figure 1).

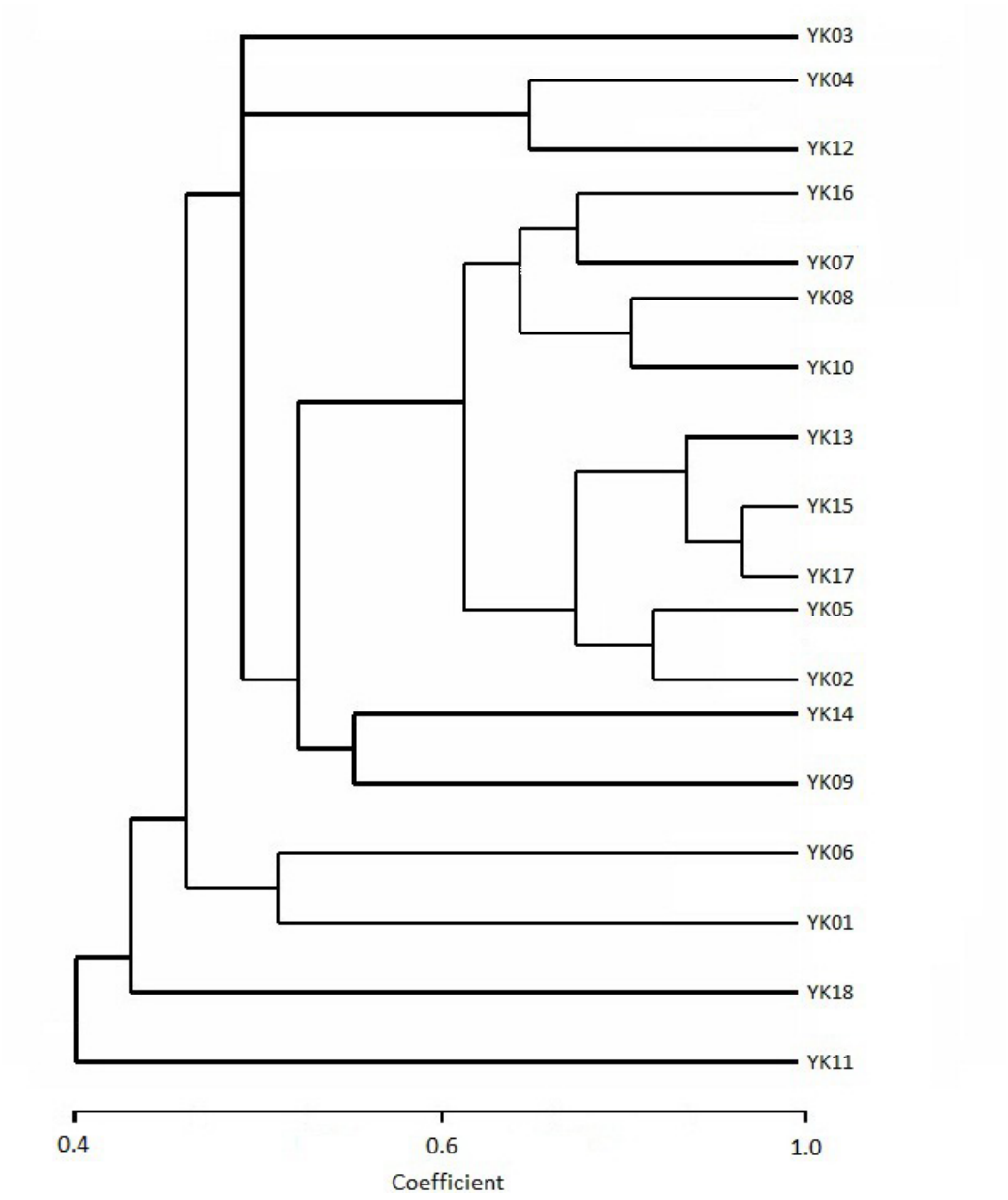

Figure 1. Dendrogram of 18 wild sweet cherry genotypes based on simple matching coefficient and UPGMA analysis of 46 SSR markers.

In the present study, in most cases, wild sweet cherry genotypes within the same cluster did not have similar phenological and morphological characteristics. The majority of genotypes (9 genotypes) placed in cluster five (Figure 1) were found to have a high similarity ratio. However, most of the phenological and morphological characteristics of these 9 genotypes are differed (Table 1). For example, in cluster five, the closest genotypes (with a similarity ratio of 0.90), YK15 and YK17, presented differences in some tree and fruit characteristics. YK15 has medium tree vigor, bitter fruit taste and dark red fruit color. However, YK17 has strong tree vigor, sweet fruit taste and red fruit color (Table 1). The other closest genotypes, YK02 and YK05, with a similarity ratio of 0.82, also have differences in some characteristics. YK02 has strong tree vigor, upright tree habit, red fruit color, strong branching habit, and late flowering and ripening time, while YK05 has medium tree vigor, semi-upright tree habit, blackish fruit color, medium branching habit, and late flowering and ripening characteristics (Table 1).

The other closest genotypes, for example, YK01 and YK06 and YK09 and YK14, also have many different phonological and morphological characteristics (Table 1).

DISCUSSION

Turkey is accepted as an origin and diversity center for sweet cherries. This study is the first report on genetic diversity among a number of wild grown sweet cherries sampled from the Coruh Valley in Turkey.

The primary results show that microsatellites can be effectively used for determining genetic diversity in wild sweet cherries. We obtained high quality, readable and variable levels of amplifications with all microsatellite primer pairs tested. We did not observe any two genotypes that shared identical SSR markers.

In the present study, 10 loci in wild sweet cherry genotypes were assayed. The number of alleles per locus ranged from 3 to 7 with an average of 4.6 putative alleles per locus. Previously, Kacar et al. (2005) obtained a total of 37 alleles among 10 sweet cherry cultivars by 9 SSR primers. Clarke and Tobutt (2003) used 14 sweet cherry cultivars for SSR analysis and determined 2 to 7 alleles per SSR primer. In addition Vaughan and Russell (2004) used 16 wild cherry accessions for molecular analysis by using 10 SSR primers and they obtained 2 to 6 alleles.

The PS12A02 locus developed for sweet cherry was the most polymorphic among the 10 loci, and produced the highest effective number of alleles (7 alleles) and was followed by Pchgms1 and UDP96001 (6 alleles). The results also confirm the high transferability of the SSR markers among different *Prunus* species. This transferability across *Prunus* species has already been confirmed by other authors (Dirlewanger et al., 2002; Wünsch and Hormaza, 2002; Bouhadida et al., 2009). Previously, the PS12A02 locus was found to be the most informative in several studies (Downey and Iezzoni, 2000; Wünsch and Hormaza, 2004; Turkoglu et al., 2010). The second most informative locus, Pchgms1, was also found to be very informative in sweet cherries (Wünsch et al., 2004; Gulen et al., 2010). Previously, SSR markers have been widely used for molecular characterizations and similarity relationships among sweet cherries and have revealed high polymorphism levels that discriminate the accessions (Dirlewanger et al., 2002; Wünsch and Hormaza, 2002; Wünsch et al., 2004; Kacar et al., 2005; Gulen et al., 2010). The relatively high level of intra-group variation observed within *P. avium* genotypes suggests complexities in the development of *P. avium* along the Black Sea

and Northeast Anatolia in Turkey. The continuous seed propagation by birds, natural hybridization between indigenous and introduced plants, and human selection may have contributed to this variation. These heterozygosity are commonly observed among clonally propagated, outbreed, perennial species, since they are favored during selection and are known to confer greater adaptability, vigor and productivity in clonal varieties (Aradhya et al., 1998; Sefc et al., 2000).

In summary, the gene pool of wild sweet cherry in Northeast Anatolia in Turkey has an enough amount of genetic variation. Regarding germplasm management, our results show that the germplasm collection is variable and may be important for future *Prunus avium* breeding programs.

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