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Genetic diversity analysis of Valencia and Navel group sweet orange cultivars by SSR markers

İlknur POLAT*

Batı Akdeniz Agricultural Research Institute, Antalya, Turkey

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Abstract

Sweet orange [*Citrus sinensis* (L.) Osbeck] fruit is one of the main citrus fruits, Navel and Valencia group sweet orange being the most representative and recognizable species of this species. The aims of this study were to determine genetic relationships and diversity of 84 Navel and 36 Valencia groups of sweet orange using SSR (simple sequence repeat) molecular markers. Twenty-six SSR primers were tested on these accessions. Seven SSR primers produced thirteen polymorphic fragments, eight SSR primers produced monomorphic fragments, and eleven SSR primers produced no scorable fragments. Thirteen SSR primers produced a total of 29 fragments and 13 of them were polymorphic. The number of average polymorphic fragments per primer was 1.93. The mean polymorphism information content (PIC) and marker index (MI) are 0.16 and 11.74, respectively. The Dice's similarity coefficient among Navel and Valencia group sweet oranges ranged from 0.42 to 1.00 and matrix correlation (r) was 0.79. In the cluster analysis, Navel group sweet oranges were indicated as a separate group from Valencia group sweet oranges. 'Antalya (40)' was most distinct accessions from the others.

Keywords: *Citrus sinensis* L., Genetic diversity, Genetic resources, SSR

Valencia ve Navel grup portakal çeşitlerinin SSR markörleri yardımıyla genetik çeşitlilik analizi

Özet

Portakal [*Citrus sinensis* (L.) Osbeck] turunçgil yetiştiriciliği içerisinde en önemli türü oluşturur, Navel ve Valencia grubu portakallar ise portakallar içerisinde en fazla yetiştirilenlerdir. Çalışmanın amacı, 84 adet Navel ve 36 adet Valencia grubu

* Sorumlu yazar (Corresponding author): i_polat@hotmail.com

portakalın SSR (simple sequence repeat) moleküler markırı kullanarak genetik akrabalıklarını ve farklılıklarını belirlemektir. Kullanılan 26 SSR primerinden 13 tanesi polimorfik, 8 tanesi monomorfik bant sağlarken, 11 primerden değerlendirilebilecek bant elde edilememiştir. Polimorfizm sağlayan 13 SSR primerinden toplam 29 bant elde edilmiş ve bunların 13 adeti polimorfizm sağlamıştır. Her bir primere düşen ortalama polimorfik bant sayısı 1.93'tür. Ortalama PIC (polymorphism information content) ve MI (marker index) değerleri sırasıyla 0.16 ve 11.74'dür. Navel ve Valencia grubu portakallarında Dice'ın benzerlik indisi (Dice's similarity coefficient) 0.42 ile 1.00 arasında değişim göstermiştir, matriks korelasyon (r=matrix correlation) ise 0.79'dur. Kümeleme analizinde (cluster analysis) ise, Navel grubu portakallar Valencia grubu portakallardan ayrılmıştır. 'Antalya (40)' tüm portakal grupları içerisinde en uzak bireyi oluşturmuştur.

Anahtar kelimeler: *Citrus sinensis* L., Genetik farklılık, Genetik kaynak, SSR

1. Introduction

Sweet orange is an economically important citrus crop in Turkey and worldwide. Total sweet orange production in Turkey is approximately 1 781 258 tons and 1 333 254 tons of this was Washington Navel, 72 419 tons was Yafa, 375 585 tons of this was the other sweet oranges such as Valencia (TUIK, 2013).

The sweet orange originated from Asia and its hybrid characteristic seems to come from a cross between mandarin (*Citrus reticulata* Blanco) and pummelo [*Citrus grandis* (L.) Osbeck] (Davies and Albrigo, 1994; Nicolosi et al., 2000). Citrus varieties show diversity in their morphological, chemical constituents and for convenience. Sweet oranges are classified into four groups: Common (round oranges), low acidity, pigmented (blood) and navel oranges (Hodgson, 1967; Davies and Albrigo, 1994).The round oranges are most important commercially and represent a major portion of sweet oranges. Valencia oranges are included in the round oranges. Navel oranges are the second most widely planted group while blood orange plantings are limited primarily to areas with Mediterranean-type climates (Davies and Albrigo, 1994).

Study of Citrus taxonomy and phylogeny is complicated and quite difficult due to wide cross-compatibility among the species, apomixis, nucellar embryony, high frequency of bud mutation, the long history of cultivation, a long juvenile phase and the paucity of remaining wild citrus stands (Nicolosi et al.,2000).

In the past, systems of citrus genetic classification were based upon mainly morphological characteristics (Nicolosi et al., 2000; Barkley et al., 2006). A number of molecular marker techniques have been used to overcome the limitations of morphological and biochemical markers in citrus genetic classification. Protein, isozymes (Rahman and Nito, 1994), and molecular markers such as restriction fragment length polymorphisms (RFLPs) (Liou et al., 1996), random amplified polymorphic DNA (RAPD) (Machado et al.,1996; Baig et al., 2009; Sun et al.,2012; Malik et al.,2012), sequence-characterized amplified regions (SCARs) (Nicolosi et al., 2000), amplified fragment length polymorphism (AFLP) (Campos et al., 2005), microsatellites simple sequence repeats (SSRs) (Oliveira et al., 2002; Ahmad et al., 2003; Fu et al., 2003; Barkley et al., 2006; Polat, 2009; Jannati et al., 2009; El-Mouei et al., 2011; Uzun et al., 2011; Cristofani-Yaly et al., 2011; Garcia-Lor et al., 2012; Polat et al., 2012; Kacar et al., 2013; Al-Mouei and Choumane, 2014), inter-simple sequence repeats (ISSRs) (De Pasquale et al., 2006), sequence related amplified polymorphism (SRAP) (Uzun et al., 2009; Uzun et al., 2011; Polat et al., 2012; Kacar et al., 2013), sequence-specific amplified polymorphism (S-SAP) and selectively amplified microsatellite polymorphic loci (SAMPL) (Biswas et al., 2011) have been employed to elucidating genetic diversity, determining parentage, and revealing phylogenetic relationships among various *Citrus* species. Compared to morphological data, molecular markers provide abundant information, are highly efficient, and are insensitive to environmental factors (Barkley et al., 2006).

Each molecular marker technique is based on different principles but their application is to bring out the genome-wide variability (Biswas et al., 2011). In general, the choice of molecular marker technique has to be a compromise between reliability and ease of analysis, statistical power and confidence of revealing polymorphisms (Agarwal et al., 2008). SSR markers are codominant, highly polymorphic, easy to use (Barkley et al., 2009) and is, therefore, ideal in the analysis of large genomes (Barkley et al., 2009; Biswas et al., 2011; Amar et al., 2011). In our study, genetic relationships and diversity were determined using SSR molecular marker within 84 Navel and 36 Valencia groups of sweet oranges collected from selections and introductions.

2. Material and Methods

2.1. Plant material

Eighty-four Navel and thirty-six Valencia group genotypes of sweet orange [*Citrus sinensis* (L.) Osbeck] were used. DNA samples of all plant materials from the Tuzcu Citrus Collection (University of Cukurova, Adana, Turkey), Bati Akdeniz Agricultural Research Institute Citrus Collection (Antalya, Turkey) and Alata Horticultural Research Station Citrus Collection (Mersin, Turkey) were obtained from Alata Horticultural Research Station under the project that supported by the Scientific and Technological Research Council of Turkey (TUBITAK) (Table 1).

2.2. SSR analysis

Twenty-six primer pairs (Barkley et al., 2006; Roose, 2009) were used to amplify the DNA. Fifteen primer pairs producing scorable polymorphic bands were used to amplify all of the accessions (Table 2). PCR amplifications were conducted as described by Barkley et al., (2006) with some modifications. Each 10 µl reaction consisted of 1.0 µl primers, 200 mM of each deoxyribonucleotide triphosphate (dNTP), 1.0 µl 10X PCR buffer, 1.0 µl 2.5 mM $MgCl_2$ 4.8 µl double-distilled water, 0.2 µl 0.6 U *Taq* DNA polymerase and 1.0 µl 20 ng DNA. A DNA Thermal Cycler (Bio-Rad, Hercules, CA, USA) was used, and the cycling parameters included 3 min of initial denaturing at 94°C, 35 cycles of 3 steps [30 sec of denaturing at 94°C, 30 sec of annealing at 50°C or 45°C (depending on the primer), and 1 min of elongation at 72°C], and 1 cycle of 10 min at 72°C for extension. PCR products good amplified at annealing temperature of 45°C in TAA52, TAA15 and CAGG9 primers, 50°C in the others primers (Table 2).

PCR products were separated on 2.5% high resolution agarose (Ambresco, Solon, OH USA) gel in 1X TAE buffer at 100 V for 3 h, and photographed (used Kodak Gel Logic 200) under UV light for further analysis. A 100 bp DNA ladder (Vivantis, Oceanside, CA, USA) was used as molecular standard.

Table 1. Plant materials used in this study were identified by Tanaka species name, common or cultivar names and origin or country names

No	Group Name	Origin	Cultivar Name	No	Group Name	Origin	Cultivar Name
1	Navel	USA	Tulegold	61	Navel	Turkey	Washington Navel (Navel 28-M)
2	Navel	USA	Thomson Navel (7365 T)	62	Navel	Turkey	Washington Navel (Navel 29-M)
3	Navel	Nucellar	Washington Navel (A-6) (Antalya (6)	63	Navel	Turkey	Washington Navel (Navel 3-M)
4	Navel	Nucellar	Washington Navel (A-4) (Antalya (4)	64	Navel	Turkey	Washington Navel (Navel 30-M)
5	Navel	Nucellar	Washington Navel Antalya (1)	65	Navel	Turkey	Washington Navel (Navel 31-M)
6	Navel	Nucellar	Tuzcu - 2 N	66	Navel	Turkey	Washington Navel (Navel 32-M)
7	Navel	Nucellar	Washington Navel Antalya (8)	67	Navel	Turkey	Washington Navel (Navel 33-M)
8	Navel	Nucellar	Washington Navel Antalya (2)	68	Navel	Turkey	Washington Navel (Navel 34-M)
9	Navel	Nucellar	Washington Navel Antalya (10)	69	Navel	Turkey	Washington Navel (Navel 35-M)
10	Navel	Nucellar	Washington Navel Antalya (3)	70	Navel	Turkey	Washington Navel (Navel 36-M)
11	Navel	Turkey	Washington Navel Antalya (40)	71	Navel	Turkey	Washington Navel (Navel 37-M)
12	Navel	Turkey	Washington Navel Antalya (39)	72	Navel	Turkey	Washington Navel (Navel 38-M)
13	Navel	TRNC	Washington Navel (Kıbrıs 1)	73	Navel	Turkey	Washington Navel (Navel 39-M)
14	Navel	TRNC	Washington Navel (Kıbrıs 2)	74	Navel	Turkey	Washington Navel (Navel 4-M)
15	Navel	TRNC	Washington Navel (Kıbrıs 3)	75	Navel	Turkey	Washington Navel (Navel 40-M)
16	Navel	Turkey	Washington Navel (Adana)	76	Navel	Turkey	Washington Navel (Navel 41-M)
17	Navel	USA	Skaggs Bonanza Navel	77	Navel	Turkey	Washington Navel (Navel 42-M)
18	Navel	USA	Frost Washington Navel (6615 T)	78	Navel	Turkey	Washington Navel (Navel 5-M)
19	Navel	USA	Newhall Navel (7922 T)	79	Navel	Turkey	Washington Navel (Navel 6-M)
20	Navel	Turkey	Washington Navel Antalya (7)	80	Navel	Turkey	Washington Navel (Navel 7-M)
21	Navel	Turkey	Washington Navel Antalya (37)	81	Navel	Turkey	Washington Navel (Navel 8-M)
22	Navel	France	Navelate SRA 307	82	Navel	Turkey	Washington Navel (Navel 85-M)
23	Navel	France	Navelate SRA 305	83	Navel	Turkey	Washington Navel (Navel 86-M)
24	Navel	Unknown	Cara Cara	84	Navel	Turkey	Washington Navel (Navel 9-M)
25	Navel	Unknown	Spring Navel	85	Valencia	USA	Campbell Valencia (7246 R, N)
26	Navel	Spain	Navelina IVIA 7-5	86	Valencia	USA	Frost Valencia (7246 R, N)
27	Navel	Spain	Navelate IVIA 2-7	87	Valencia	USA	Olinda Valencia (7229 R)
28	Navel	Spain	Morita Navel IVIA	88	Valencia	France	Rhode Red Valencia SRA 360
29	Navel	Spain	Apopka Navel IVIA 201 - 01	89	Valencia	Unknown	Midknight Valencia
30	Navel	Spain	Leng Navel IVIA 216 - 01	90	Valencia	TRNC	KKTC-19 Valencia

Table 1. (Continued)

31	Navel	Spain	Dream Navel IVIA 213 - 01	91	Valencia	TRNC	H.Tokgöz 1/A
32	Navel	Spain	Fisher Navel IVIA 199 - 01	92	Valencia	TRNC	İ.Açıkgöz 1/A
33	Navel	Spain	Cram Navel IVIA 211- 01	93	Valencia	TRNC	İ.Açıkgöz 2/A
34	Navel	Spain	N. Lane Late IVIA 198 - 1	94	Valencia	TRNC	Kasap 1/A
35	Navel	Spain	Navelate IVIA 72 - 12	95	Valencia	TRNC	M.Hoca 1/A
36	Navel	France	Robertson SRA 209 portakalı	96	Valencia	TRNC	M.Hoca 2/A
37	Navel	USA	Navalate 548	97	Valencia	Nucellar	Valencia B 2/3 16-13
38	Navel	Unknown	Fukumoto	98	Valencia	Nucellar	Valencia B 2/3 16-14
39	Navel	TRNC	KKTC-13 Washington Navel	99	Valencia	Nucellar	Valencia B 2/3 16-15
40	Navel	TRNC	KKTC-10 Washington Navel	100	Valencia	Nucellar	Valencia B 2/3 16-16
41	Navel	Unknown	Gillette	101	Valencia	Nucellar	Valencia B 2/3 16-17
42	Navel	Italy	Goldnugget-B	102	Valencia	Nucellar	Valencia B 2/3 17-16
43	Navel	Turkey	Navelli Kan 3-11	103	Valencia	Nucellar	Valencia B 2/3 17-17
44	Navel	Nucellar	Washington Navel (Antalya-9)	104	Valencia	Turkey	91 A Valencia
45	Navel	Turkey	Washington Navel (Navel 10-M)	105	Valencia	Turkey	Valencia (Valencia 22M)
46	Navel	Turkey	Washington Navel (Navel 11-M)	106	Valencia	Turkey	Valencia (Valencia 55A)
47	Navel	Turkey	Washington Navel (Navel 12-M)	107	Valencia	Turkey	Valencia (Valencia 56A)
48	Navel	Turkey	Washington Navel (Navel 14-M)	108	Valencia	Turkey	Valencia (Valencia 57D)
49	Navel	Turkey	Gazipaşa Göçek Köyü Göbekli Portakalı	109	Valencia	Turkey	Valencia (Valencia 58D)
50	Navel	Turkey	Washington Navel (Navel 1-M)	110	Valencia	Turkey	Valencia (Valencia 59A)
51	Navel	Turkey	Washington Navel (Navel 15-M)	111	Valencia	Turkey	Valencia (Valencia 70A)
52	Navel	Turkey	Washington Navel (Navel 17-M)	112	Valencia	Turkey	Valencia (Valencia 72A)
53	Navel	Turkey	Washington Navel (Navel 18-M)	113	Valencia	Turkey	Valencia (Valencia 73A)
54	Navel	Turkey	Washington Navel (Navel 2-M)	114	Valencia	Turkey	Valencia (Valencia 74A)
55	Navel	Turkey	Washington Navel (Navel 20-M)	115	Valencia	Turkey	Valencia (Valencia 75A)
56	Navel	Turkey	Washington Navel (Navel 21-M)	116	Valencia	Turkey	Valencia (Valencia 76A)
57	Navel	Turkey	Washington Navel (Navel 23-M)	117	Valencia	Turkey	Valencia (Valencia 77A)
58	Navel	Turkey	Washington Navel (Navel 24-M)	118	Valencia	Turkey	Valencia (Valencia 88A)
59	Navel	Turkey	Washington Navel (Navel 25-M)	119	Valencia	TRNC	A. Bradi 11/A
60	Navel	Turkey	Washington Navel (Navel 27-M)	120	Valencia	TRNC	A. Bradi 12/A

2.3. Data analysis

A similarity matrix using the similarity coefficient of simple matching was constructed for SSR data based on the presence (1) or absence (0) of fragments for each primer. Statistical analysis was carried out using the software PAST (Paleontological Statistics) (http://folk.uio.no/ohammer/past/). The genetic similarity matrix, neighbor joining (NJ) and principal coordinate analysis (PCO) were constructed based on Dice's coefficient (Dice, 1945).

Polymorphism rates (Pr) were calculated using following formula. Pr= (number of polymorphic bands/total number of bands in that assay unit)x100. Polymorphism information content (PIC) values were determined using following formula as described by Smith et al. (1997). PIC= $1-\Sigma\ fi^2$, where fi^2 is the frequency of the i^{th} allele. Marker index (MI) values were calculated by applying following formula given by Powell et al. (1996) and Smith et al. (1997). MI = Pr x PIC value.

3. Results and Discussion

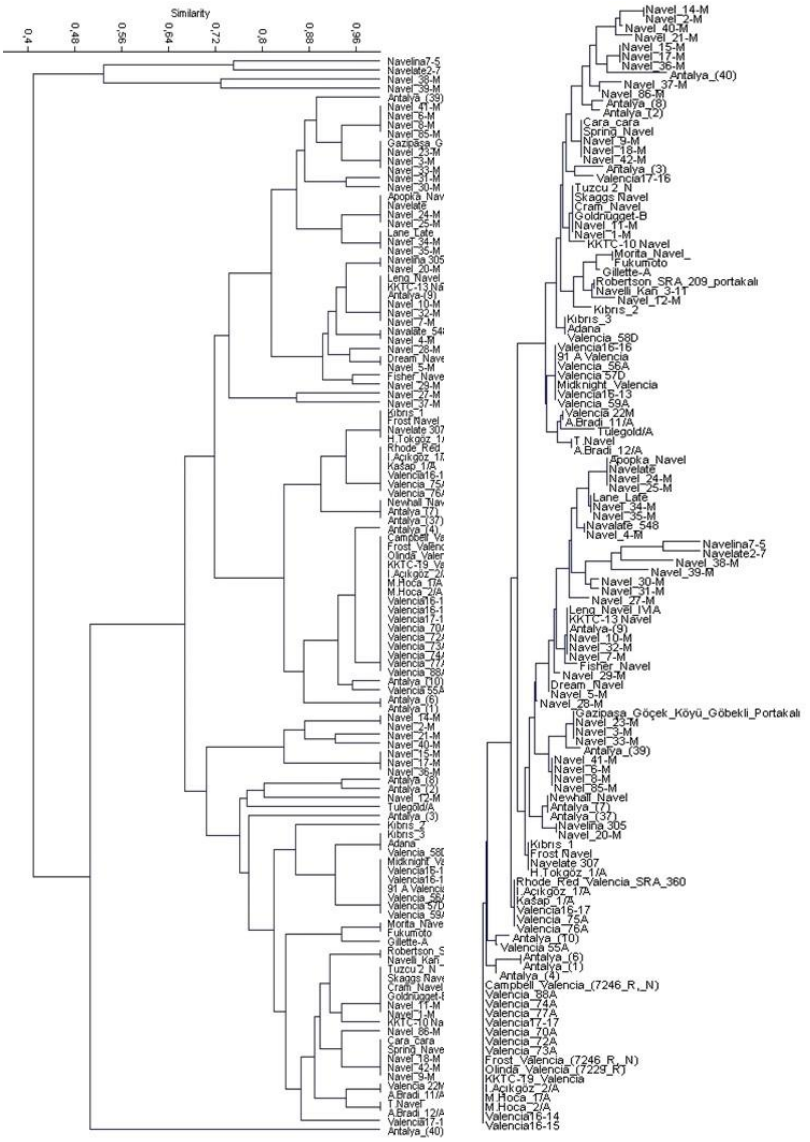
After screening twenty-six SSR primers, fifteen primers produced polymorphic, well-resolved band fragments, eleven primers gave no amplification. When a total of 15 SSR primers were screened, 29 bands were scored. The number of bands scored per primer ranged from 1 (TAA1, CAC23, CT21, AC01 and ATC09) to 3 (CAC33, CAT01, CAG01 and CAC19), with a mean of 1.93. Polymorphism rates ranged from 0% (TAA1, TAA27, CAC23, CAGG9, CT21, AC01, CAC19 and ATC09) to 100% (TAA45, TAA52 and TAA15) (Table 2).

The PIC values for the 15 primers ranged from 0.00 (TAA1, TAA27, CAC23, CAGG9, CT21, AC01, CAC19 and ATC09) to 0.53 (CAG01), with a mean of 0.16 (Table 2). PIC values are generally used in molecular studies as a measure of polymorphism for a marker locus. PIC provides an estimate of the discriminatory power of a locus by taking into account, not only the number of alleles that are expressed but also the relative frequencies of those alleles (Smith et al., 1997). PIC values range from 0 to 1. At a PIC of 0, the marker has only one allele. At a PIC of 1, the marker would have an infinite number of alleles. If a PIC value of greater than 0.7 is considered to

Table 2 Diversity statistics for 15 SSR markers studied in 120 sweet oranges

Locus	Primers sequences 5′-3′	Tm (°C)	Allele sizes (bp)	Na	Pa	Pr (%)	PIC	MI
TAA45	F-GCACCTTTTATACCTGACTCGG R- TTCAGCATTTGAGTTGGTTACG	50	145-200	2	2	100	0.18	18.00
TAA52	F-GATCTTGACTGAACTTAAAG R-ATGTATTGTGTTGATAACG	45	90-110	2	2	100	0.43	43.00
TAA15	F-GAAAGGGTTACTTGACCAGGC R- CTTCCCAGCTGCACAAGC	45	185-195	2	2	100	0.03	3.00
TAA33	F-GGTACTGATAGTACTGCGGCG R-GCTAATCGCTACGTCTTCGC	50	120-180	2	1	50	0.42	21.00
CAC33	F-GGTGATGCTGCTACTGATGC R- CAATTGTGAATTTGTGATTCCG	50	150-200-240	3	2	67	0.46	30.82
CAT01	F-GCTTTCGATCCCTCCACATA R- GATCCCTACAATCCTTGGTCC	50	120-170-180	3	2	67	0.37	24.79
CAG01	F-AACACTCGCACCAAATCCTC R- TAAATGGCAACCCCAGCTTTG	50	150-170-350	3	2	67	0.53	35.51
TAA1	F-GACAACATCAACAACAGCAAGAGC R-AAGAAGAAGAGCCCCCATTAGC	50	195	1	0	0	0.00	0.00
TAA27	F-GGATGAAAAATGCTCAAAATG R- TAGTACCCACAGGGAAGAGAGC	50	190-200	2	0	0	0.00	0.00
CAC23	F-ATCACAATTACTAGCAGCGCC R- TTGCCATTGTAGCATGTTGG	50	260	1	0	0	0.00	0.00
CAGG9	F-AATGCTGAAGATAATCCGCG R- TGCCTTGCTCTCCACTCC	45	110-390	2	0	0	0.00	0.00
CT21	F-CGAACTCATTAAAAGCCGAAAC R- CAACAACCACCACTCTCACG	50	155	1	0	0	0.00	0.00
AC01	F-TTGACATCAACATAAAACAAGAAA R- TTTTAAAATCCCTGACCAGA	50	160	1	0	0	0.00	0.00
CAC19	F-ACAACCTTCAACAAAACCTAGG R- AAGACTTGGTGCGACAGG	50	200-240-260	3	0	0	0.00	0.00
ATC09	F-TTCCTTATGTAATTGCTCTTTG R- TGTGAGTGTTTGTGCGTGTG	50	190	1	0	0	0.00	0.00
Total		-	-	**29**	**13**	**-**	**-**	**-**
Mean		-	-	**1.93**	**0.86**	**36.73**	**0.16**	**11.74**

Na: number of alleles, Pa: Polymorphic of alleles, Pr: Polymorphism rate, PIC: polymorphism information content, MI: marker index



a) Similarity b) Neighbor joining

Figure 1. Dendrogram and neighbor joining of the 120 sweet orange genotypes based on the 15 SSR markers.

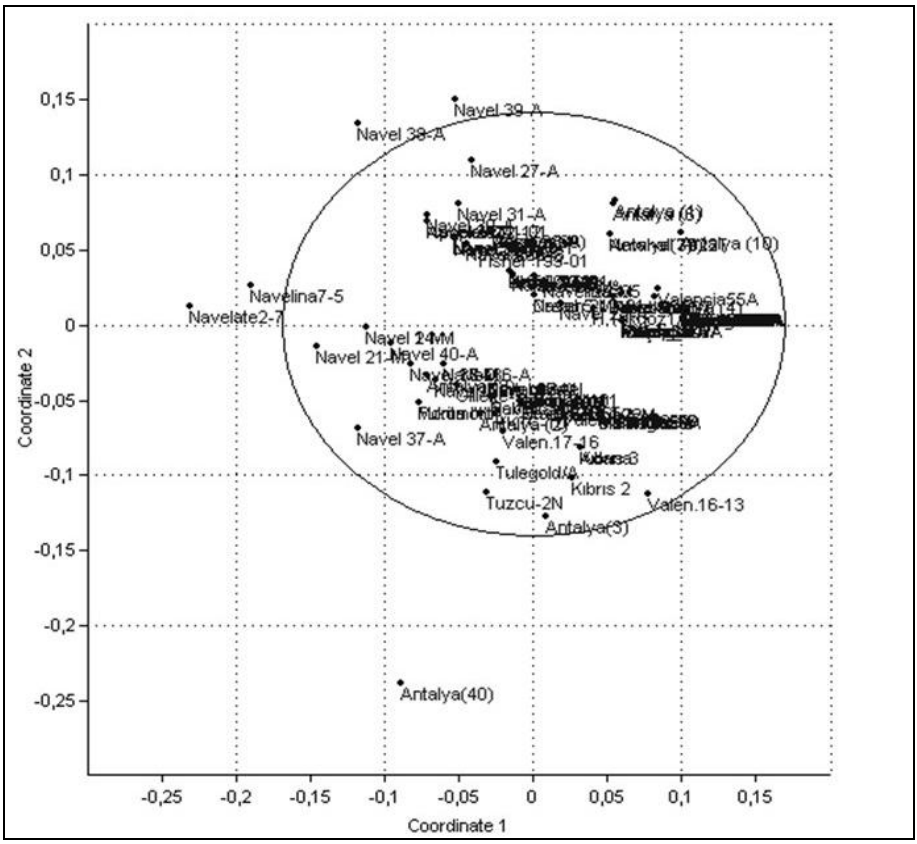


Figure 2. Principal coordinate analysis (PCO) of the 120 sweet orange genotypes based on the 15 SSR markers.

be highly informative. However, a PIC value of 0.44 is considered to be moderately informative. Markers with greater numbers of alleles tend to have higher PIC values and these markers are more informative (Hildebrand et al., 1992). Thus, TAA52, TAA33, CAC33 and CAG01 markers were determined to be moderately informative. The MI value of primers ranged from 0.00 to 43.00 with an average value of 11.74 (Table 2). TAA45, TAA52, TAA15, TAA33, CAC33, CAT01 and CAG01 had polymorphism rate, PIC and MI value. These primers could be considered as informative in revealing the

genetic diversity and determining genetic variation in Valencia and Navel group of sweet orange.

Jannati et al. (2009) used fifteen primer pairs (TAA15, TAA27, TAA41 CAC23, CAC15, CAC33, CAC39, CCT01, CAT01, ATC09, AG14, CTT01, CT21, TC26 and CT19) for genetic diversity analysis of Iranian citrus varieties. All fifteen loci assayed in citrus plant possessed a high level of polymorphism, with the number of alleles per locus ranging from 4 in TAA41 to 12 at CAT01, ATC09, AG14. The most highly polymorphic loci was CAT01 with PIC=0.89. Microsatellite analysis clustered citron and sour orange cvs. cluster but these taxa were quite distant from *Fortunella* sp. A set of informative SSR markers detected considerable levels of genetic variability in the Iranian citrus germplasm. However, sweet oranges (*C. sinensis* L. Osbeck) show low level of genetic diversity. Barkley et al. (2006) were used twenty-four primer pairs to assessing genetic diversity and population structure in a citrus germplasm collection. A total of 275 alleles were detected with a mean number of alleles per locus of 11.5. The PIC values for the 24 markers ranged from 0.247 (CMS8) to 0.916 (TAA41). Although the SSR markers could distinguish between the various *Citrus* species, these SSR markers could not distinguish between accessions which was arisen by apparent spontaneous mutation, such as sweet oranges (*C. sinensis*).

PAST program was originally designed as a follow-up to PALSTAT, a software package for paleontological data analysis written by Ryan et al. (1995). In later years, PAST has grown into a comprehensive statistics package that is used not only by paleontologists, but also in many fields of life science, earth science, and even engineering and economics (Hammer et al., 2001). PAST program was used in DNA fingerprinting (ISSR and RAPD) of *Prosopis cineraria* and *P.juliflora* (Elmeer and Almalki, 2011), molecular (SSR) analysis of old apple cultivars (Király et al., 2012), genetic analysis (RAPD) of *Hibiscus* species (Kadve et al., 2012), molecular (SSR) determination of genetic structure of Brazilian soybean cultivars (Piriolli et al., 2013), SSR-based genetic diversity assessment in tetraploid and hexaploid wheat populations (Abouzied et al., 2013), molecular diversity (SSR) in cultivated groundnut (Goswami et al., 2013).

Dice's similarity was used to the cluster analysis and to generate a dendrogram and neighbor joining showing the relationship among the oranges situated as shown in Figure 1. And also, the result of principal coordinate analysis (PCO) is given in Figure 2. The cophenetic correlation between ultrametric similarities of the tree and the similarity matrix was high

(r = 0.79), suggesting that the cluster analysis strongly represents the similarity matrix. In the cluster analysis (Figure 1), Navel group sweet oranges were indicated as a separate group from Valencia group sweet oranges. 'Antalya (40)' was the most distinct accessions from the others. The same results are seen as a result of PCO (Figure 2) and neighbor joining (Figure 1). Polymorphism was found to be quite low in Valencia group sweet orange. Located within the same cluster 'Navelina 7-5', 'Navelate 2-7', 'Navel 38-M' and 'Navel 39-M' were constituted in a separate the most distant group of Valencia and Navel oranges.

'Washington Navel' orange was imported from Brazil into the United States in 1870. Its origins are uncertain, it is believed to come from a bud sport found in a Selecta orange tree in the early 1800s (Anonymous, 2013). It was the first entry to Turkey in 1945 from California. First cultivation was made in "Antalya Citrus Research Station" and spread from here to all of Turkey (Anonymous, 2012). Today, 'Washington Navel' has been the most cultivated variety of sweet oranges in Turkey (TUIK, 2013). 'Antalya (40)' variety was selected from "Antalya Citrus Research Station" in 1979-1984 (Anonymous, 2012). And also, 'Navel 38-M' and 'Navel 39-M' were selected in Turkey. 'Navelate' sweet orange was occurred from bud mutation on a 'Washington Navel' tree in Vinaros (Castellón), Spain (Zaragoza and Alonso, 1975). 'Navelina' sweet orange was selected as a bud sport selection from the Rubidoux Tract variety block about 1910 (Anonymous, 2013). 'Navelina 7-5' and 'Navelate 2-7' were obtained from Spain via introduction method.

Most of the sweet oranges are diploids with a comparatively small genome size of about 367 Mb (Arumuganathan and Earle, 1991). Sweet oranges usually show low level of genetic diversity (Novelli et al., 2006; Jannati et al. 2009; Polat, 2014). In our results, it was showed that there was a low level of genetic variation among the local and foreign Navel and Valencia group sweet oranges in Turkey. Because, after coming through by introduction, most Turkish sweet orange accessions originated via mutations from domestic and foreign cultivars. Barkley et al. (2006), Jannati et al. (2009), Biswas et al. (2011), Amar et al. (2011), Polat et al. (2012) and El-Mouei et al. (2011) indicated that SSR markers according to other markers were more important tool for cultivar identification, germplasm diversity and phylogenic studying of Citrus. Likewise, our data confirmed that SSR molecular methods are useful tools for the identification of closely accessions. Also, Navel group sweet oranges were indicated as a separate group from Valencia group sweet oranges.

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