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Full Length Research Paper

Molecular diversification and preliminary evaluations of some satsuma selections' performance under mediterranean conditions

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Satsuma (*Citrus unshiu* Marc.) has been cultivated for a long time in Turkey, and therefore variations for agronomical traits are likely among cultivated satsumas due to bud mutations. The objectives of this study were to determine variations for some selected agronomical traits and genetic markers among 21 new satsumas derived from selections. Fruit yield, fruit quality and molecular diversification of these clones were determined. The clones of 62 Adana, 4/2 Izmir and 11/1 Izmir indicated the highest yield. The fruits obtained from all clones were heavier than the control (Owari Satsuma). Molecular analysis, as assessed with 9 random amplification of polymorphic DNA (RAPD) and 14 simple sequence repeats (SSR) primers, indicated that satsuma clones showed a narrow genetic base suggesting that the observed morphological polymorphism within the group must be associated with somatic mutations which were not detected by these molecular markers.

Key words: Mandarins, clonal selection, citrus.

INTRODUCTION

Annual citrus production is approximately 120 million tonnes world-wide (FAO, 2009) and Turkey is one of the important citrus producing countries among the Mediterranean. This region constitutes 17.5% of the world's total citrus production. Total citrus production in Turkey is approximately 3 million tonnes and 25.2% of this was of mandarin (FAO, 2009).

Mandarins exhibit more variation in characters than other citrus species (Reuther et al., 1967), and is the most phenotypically heterogeneous group in *Citrus* (Moore, 2001). Satsuma mandarin is considered to have originated in Japan (Reuther et al., 1967) and also probably as a chance seedling from China (Nishiura, 1964). Chance seedling selections, bud sports and spontaneous mutations are still a major source to find new citrus cultivars. Conventional breeding is slow and difficult due to the complex reproductive biology in citrus (JinPing et al., 2009). Bud mutations arise frequently in citrus, and clonal selection has been used as a traditional breeding method (Uzun et al., 2009).

Although, Turkey is not a natural genetic center of citrus fruits, they have been grown in this country for several years. To find Satsuma varieites which have the features in the direction of the market demands and high yield, the first national citrus budwood selection was carried out between the years 1979-1983 in the Mediterranean and Aegean regions of Turkey. A thorough search of trees in the regions for high yield, trees without pests and diseases and high quality has been repeated every year for 4 years (Özsan et al., 1986). In this project, 21 Satsuma genotypes with high yield and fruit quality were selected from citrus growing areas of Turkey.

RAPD, AFLP, ISSR and SSR markers or isozyme have been used to distinguish between accessions and for investigations of genetic diversity/relatedness and genetic mapping in citrus (Green et al., 1986; Denget al., 1995; Kijas et al., 1995; Machado et al., 1995; Fang et al.,

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Abbreviations: RAPD, Random amplification of polymorphic; **SSR,** simple sequence repeats; **UPGMA,** unweighted pair group method analysis; **ISSR,** ınter-simple sequence repeat.

Table 1. RAPD Primers Used in the study.

RAPD primer	Primer sequence
OPA04	AATCGGGCTG
OPA05	AGGGGTCTTG
OPA09	GGGTAACGCC
OPA13	CAGCACCCAC
OPA14	TCTGTGCTGG
OPA15	TTCCGAACCC
OPA16	AGCCAGCGAA
OPA17	GACCGCTTGT
OPA19	CAAACGTCGG

1997; Fang and Roose, 1997; Masashi et. al., 1998; Federici et al., 1998; Nicolisi et al., 2000; Abkenar et al., 2004; Oliveira et al., 2004; Aka-Kacar et al., 2005; Lin and Chen, 2006; Pang et al., 2007; Wei, 2007; Hvarleva et al., 2008; Rao et al., 2008; Baig et al., 2009; Uzun et al., 2009). Since the introduction of (RAPD) markers in 1990 (Williams et al., 1990), their use in plant genetic analysis has increased in an exponential manner, principally due to its simplicity, quick and easy assay. RAPD analysis has been used for genotype characerization (Khadari et al.,1995; Lu et al., 1996; Nicese et al.,1998), to assess intra-or interpopulation genetic variability (Huff et al., 1993; Alberto et al., 1997; Baig et al., 2009), for genetic mapping (Chaparro et al., 1994; Oliveira et al., 2004) and to identify molecular markers linked to genes of interest (Nair et al., 1996). SSR markers have several advantages over other molecular markers. They are in fact abundant in most genomes and co-dominant, therefore, their information content is very high; SSRs are PCR based, thus requiring little DNA for the amplification; and every SSR locus is defined by a unique pair of primers, so that information exchange between labotaratories is easy (Lacis et al., 2009).

The objective of the study was to determine the variations of some selected agronomical traits of 21 Satsuma mandarins derived from selection breeding programmes and to characterize them by SSR and RAPD genetic markers.

MATERIALS AND METHODS

Plant materials

21 satsuma varieties collected from the Mediterranean and Aegean regions of Turkey between 1979-1983 were used in this study. Thoroughly searching trees in the regions, selections of trees with high yield and quality have been repeated every year for 4 years. The name of Satsuma clones were 61 Adana, 62 Adana, 89 Adana, 3 Izmir, 22 Izmir, 26 Izmir, 30 Izmir, 4/2 Izmir, 8/3 Izmir, 11/1 Izmir, 13/2 Izmir,18/1 Izmir, 19/1 Izmir, 24/2 Izmir, 25/1 Izmir, 27/1 Izmir, 27/2 Izmir,23 Mersin,24 Mersin, 25 Mersin, 27 Mersin and Owari Satsuma which was used as the control for the selections.

Orchard management

The yield and fruit characteristics of 21 (selections and one control) satsuma mandarin types were examined under ecological conditions of Adana province. The study was conducted at the research area of Çukurova University in Adana. Clones were planted on silty-clay-loam textured soil having a pH of 7.3. Plants were spaced with 7x7 m spacing. Clones were grafted on sour orange (*Citrus aurantium* L.) and the experiment was established using completely randomized design. Five replications were established for each genotype. The experimental area was managed according to standard commercial practces.

Characterization for tree yield performance and fruit quality

Selected Satsuma clones were evaluated as follows.

Yield efficinecy

Yield by tree (kg/tree) were recorded during 3 years of production and according to these data, cumulative yield was calculated.

Fruit quality

Commercial harvest of satsumas in Adana is the middle of November. At harvest, 25 fruits were harvested. All fruit samples were assessed for fruit weight (g), fruit height (mm), fruit diameter (mm), index, rind thickness (mm), juice content (%), brix (%), total acids (%) and brix/acid ratio for 3 years.

Molecular analysis

DNA extraction

Ten young leaves were collected from a single tree for each genotype, and immediately frozen in liquid nitrogen and stored at –80°C. Total genomic DNA was extracted from leaf tissue following the protocol for minipreps by using CTAB (Dellaporta et al., 1983). DNA concentrations were assessed by spectrophotometer (Nanodrop ND-1000).

RAPD analysis

Nine RAPD primers (OPA04, OPA05, OPA09, OPA13, OPA14, OPA15, OPA16, OPA17 and OPA19) (Table 1) were used for PCR amplifications to determine genetic characterization of Satsuma mandarins. PCR was performed in 12.5 µl reaction mixture containing the following: 15 ng DNA, 6.25 µl 2X PCR Master Mix, 0.5 µl 25 mM $MgCl_2$, 1.25 µl primer, 0,05 of *Taq* DNA polymerase and distilled water (1.45 µl).

Amplified DNA fragments were loaded in a 1.5% agarose gel in 1X TAE buffer and submitted to electrophoresis for 3 h at 70 volt. DNA fragments were visualized under ultra-violet light and gel image was captured by photo documentation system and the images stored for further analysis. A 1 kb ladder was used as the molecular standard in order to confirm the appropriate RAPD markers.

SSR markers

14 SSR primers characterized for *Citrus* were used in this study (Table 2). PCR amplifications were carried out in 20 µl final reaction

Table 2. The Forward and Reverse SSR Primer Information for This Study.

	SSR Primer
458	F: CCCCCTCTTTTTCTCTTCCA R: TTCTGGGCTGGTAGGTTCAG
571	F: TCGCCCTCCCCCTGAAATTA R: GAAAGCCTGGTGGGAGCAGA
497	F: CGCAATTCAATTCCCTGTCT R: CGTCGAGCAACAAATCAAGA
506	F: AGCGGAGGAAAAAGGAAAAG R: CATATGCCATCACCACCAAA
495	F: GGCCTTAAACCACCTTGACA R: TGAGGCTTTTGCTGTTGTTG
473	F: CTTGGCGTCGAAAAGAAATC R: AGCACGGATGTCAAAATTCC
485	F: CACGACGTTGTAAAACGACAGCTCTCTTCCCTGTGGCTA R: GGTCGAGATTGAGCAGCAGT
697	F: TGCATTTTGTGGGTCTTGCTTG R: GGCCCTGACTGCTGCAAGAT
498	F: CACGACGTTGTAAAACGACGGTAAGGGGCTGGGCAAAAA R: CAGCATCACATATGCAGGCTTGT
MEST 431	F: GAGCTCAAAACAATAGCCGC R: CATACCTCCCCGTCCATCTA
488	F: CACGCTCTTGACTTTCTCCC R: CTTTGCGTGTTTGTGCTGTT
173	F: GCATAGAATAAGAAATGACAGCAAA R: ATGCCTGCACCTTTGGTAAG
54	F: AACACCTTAAGGCTGCAGGA R: CGTTGTTGATGATTCTTGATGA
MEST 121	F: TCCCTATCATCGGCAACTTC R: CAATAATGTTAGGCTGGATGGA

volumes each containing 25 ng of DNA, 2X PCR Master Mix, 5 µl ddH_2O, 0.5 µl of 25 mM $MgCl_2$, 0.05 unit Taq polymerase, 0.025 µM M13 primer, 3'and 5'end primers (F + R).

The DNA amplifications were carried out in a thermocycler (Eppendorf Mastercyler Gradient). The mixture was initially denatured at 94°C for 5 min; followed by 35 cycles at 94°C for 1 min; 55 to 60°C for 30 sec; 72°C for 1 min; and the final extension step at 72°C for 4 min. PCR products were stored at 4°C before analysis.

In SSR analysis, after amplification, an equal volume of formamide loading buffer was added to each reaction tube containing 95% formamide, 10 mM EDTA (pH 8.0), 0.025% of xylene cyanol and 0.025% of bromophenol blue. The samples were heat denatured for 5 min at 95°C and quickly cooled on ice. After loading 1.0 µl of each sample, PCR products were separated in a 25-cm, 8% denaturing polyacrylamide gel (Long Ranger, FMC Biozym, Hessisch Oldendorf, Germany) that had been preheated for 30 min. Electrophoresis was conducted in 1.0 Long Ranger TBE buffer at 1500 V, 50 W, 35 mA and 48°C using a Li-Cor DNA Analyzer 4200 (Licor Biosciences, Bad Homburg, Germany). A 50-350 bp DNA ladder mix (MWG Biotech AG, Ebersberg, Germany)

Table 3. Yield of new Satsuma mandarin clones on sour orange (kg/tree).

Clone	10 year old	11 year old	12 year old	Cumulative yield
61 Adana	37.25 b-e+	81.00 a	94.70 bcd	218.40 efg
62 Adana	24.07 e	116.33 a	147.30 a	289.60 a-d
89 Adana	37.58 b-e	89.12 a	107.70 abc	311.00 ab
3 Izmir	32.37 cde	54.00 a	83.13 cd	197.65 g
22 Izmir	39.63 b-e	102.60 a	108.73 abc	272.90 b-e
26 Izmir	36.23 b-e	93.67 a	122.47 ab	281.30 a-e
30 Izmir	44.37 a-d	122.67 a	115.13 ab	282.16 a-e
4/2 Izmir	53.47 ab	126.28 a	120.80 ab	337.95 a
8/3 Izmir	31.17 cde	62.30 a	93.97 bcd	232.90 d-g
11/1 Izmir	41.65 b-e	119.74 a	130.17 ab	316.13 ab
13/2 Izmir	39.93 b-e	87.60 a	126.73 ab	255.50 b-g
18/1 Izmir	40.63 b-e	108.67 a	89.30 cd	231.56 d-g
19/1 Izmir	40.00 b-e	97.00 a	138.47 ab	301.10 abc
24/2 Izmir	24.63 e	126.00 a	118.63 ab	269.26 b-f
25/1 Izmir	58.80 a	91.78 a	129.35 ab	269.40 b-f
27/1 Izmir	40.63 ab	108.67 a	89.30 cd	273.85 a-e
27/2 Izmir	48.27 abc	105.00 a	94.90 bcd	219.03 efg
23 Mersin	41.93 b-e	87.08 a	111.87 ab	242.95 c-g
24 Mersin	38.70 b-e	91.00 a	107.93 bcd	286.25 a-d
25 Mersin	27.25 de	92.63 a	63.43 de	205.60 fg
27 Mersin	33.07 cde	80.25 a	105.43 abc	279.70 a-e
Owari Satsuma	41.93 a-e	45.00 a	37.00 e	129.86 h
Signifinance	**	*NS*	**	**

**Significant at P<0.01; NS, not significant.
Values followed by a common letter in a column for each clone are not significantly different at P<0.01 by Duncan's Multiple Range test.

was run alongside the amplified PCR products to determine their approx. sizes.

Data analysis

The experiment was carried out during a three year period on 10-11 and 12 year old trees. All data were analyzed using analysis of variance (ANOVA) with the SPSS 17.0 (SPSS, Chicago, USA) program. Mean seperations were determined using Duncan Multiple Range test at a 0.05 significance level.

For molecular analysis, amplified fragments were classified as present (represented with 1) or absent (represented with 0) for both RAPD and SSR analysis. Genetic similarity values (Nei and Li, 1979) were calculated, and Unweighted Pair Group Method analysis (UPGMA) cluster analysis was performed to generate dendrogram with NTSYS-PC version 2.02i programme.

RESULTS AND DISCUSSION

Yield efficiency

Statistically, significant differences were recovered among the clones for most of the traits evaluated. The clones of 62 Adana (101.88 kg/tree), 4/2 Izmir (103.33 kg/tree) and 11/1 Izmir (96.57 kg /tree) gave the highest yield. These means were much higher than Owari Satsuma with 40.49 kg/tree, commonly grown up in the conditions of Çukurova, was used as control (Table 3). Observations of all clones indicated that, 4/2 Izmir clone was the highest (337.95 kg/tree), regarding cumulative yield. In contrast, the lowest cumulative yield (129.86 kg/tree) was determined from Owari Satsuma, as control, among all the selected clones. Also 89 Adana, 11/1 Izmir and 19/1 Izmir were significantly prominent with their high cumulative yields, 311 kg/tree, 316.13 kg/tree and 301,10 kg/tree respectively.

Fruit quality

The heaviest fruits were obtained from the clones of 62 Adana (144.58 g/fruit), 18/1 Izmir (141.28 g/fruit) and 30 Izmir (135.14 g/fruit). The control, Owari Satsuma, had fruit averaging 103.67 g. All clones gave heavier fruits than the control. Köse (2000) analyzed some Satsuma clones in the conditions of Çukurova and determined the fruit weight of Satsuma Sato (145.70 g), Satsuma Suzuki Wase (98.20 g) and Satsuma Owari Sra 12 (75.80 g). Most clones whose performance was tested in this study showed better performance than the cultivars aforementioned.

Table 4. Fruit Quality of New Satsuma Mandarin Clones on Sour Orange Rootstock.

Clone	Fruit weight (g)	Fruit height (mm)	Fruit diameter (mm)	Index	Rind thickness (mm)
61 Adana	126.35 [a]	54.11 [b-d+]	65.87 [abc]	1.224 [abc]	3.10 [a-f]
62 Adana	144.58 [a]	53.52 [cde]	68.14 [ab]	1.282 [ab]	3.15 [a-e]
89 Adana	131.67 [a]	54.69 [ab]	67.09 [ab]	1.234 [abc]	3.12 [a-f]
3 Izmir	134.99 [a]	54.71 [ab]	66.55 [ab]	1.218 [abc]	2.92 [efg]
22 Izmir	121.21 [a]	52.13 [e]	66.75 [ab]	1.288 [a]	2.86 [fg]
26 Izmir	126.09 [a]	52.85 [de]	67.41 [ab]	1.286 [a]	3.34 [a]
30 Izmir	135.14 [a]	53.97 [cd]	68.24 [ab]	1.266 [abc]	3.22 [a-d]
4/2 Izmir	121.26 [a]	52.65 [de]	66.83 [ab]	1.272 [ab]	2.87 [fg]
8/3 Izmir	120.34 [a]	52.20 [e]	66.72 [ab]	1.284 [a]	2.97 [d-g]
11/1 Izmir	131.72 [a]	54.06 [b-d]	66.79 [ab]	1.240 [abc]	3.07 [b-f]
13/2 Izmir	125.74 [a]	56.17 [a]	67.09 [ab]	1.200 [c]	2.82 [g]
18/1 Izmir	141.28 [a]	55.73 [ab]	68.97 [a]	1.242 [abc]	2.96 [d-g]
19/1 Izmir	129.89 [a]	53.82 [cde]	66.76 [ab]	1.248 [abc]	3.27 [ab]
24/2 Izmir	129.07 [a]	54.93 [ab]	66.36 [abc]	1.214 [bc]	3.36 [a]
25/1 Izmir	132.28 [a]	54.30 [b-d]	69.02 [ab]	1.276 [ab]	3.18 [a-e]
27/1 Izmir	119.36 [a]	53.62 [cde]	64.97 [bc]	1.212 [bc]	2.99 [c-g]
27/2 Izmir	122.08 [a]	54.07 [b-d]	65.29 [bc]	1.212 [bc]	2.98 [d-g]
23 Mersin	131.57 [a]	53.77 [cde]	66.70 [ab]	1.244 [abc]	3.05 [b-f]
24 Mersin	131.95 [a]	56.36 [a]	63.12 [c]	1.124 [d]	3.30 [ab]
25 Mersin	127.10 [a]	53.42 [cde]	66.22 [abc]	1.250 [abc]	3.25 [abc]
27 Mersin	130.65 [a]	54.88 [ab]	67.26 [ab]	1.228 [abc]	2.83 [g]
Owari Satsuma	103.67 [a]	48.97 [f]	59.09 [d]	1.218 [abc]	2.83 [g]
Significance	*NS.*	**	**	**	**

**Significant at P<0.01; NS, not significant.
Values followed by a common letter in a column for each clone are not significantly different at P<0.01 by Duncan's Multiple Range test.

All clones gave bigger fruits than the control when they were analyzed in terms of fruit diameter and fruit height. Fruit shape, evaluated by fruit index, was almost similar among the clones tested.

The thinnest peeled ones were from 13/ 2 Izmir (2.82 mm) and the control plants (2,83 mm), while the thickest peeled fruits were being observed in the clones of 24/2 Izmir (3.36 mm), 26 Izmir (3.37 mm) and 24 Mersin (3.30 mm) (Table 4).

The highest amount of fruit juice was obtained from the clone of 19/1 Izmir (46.27 %), 8/3 Izmir (46.20%) and 61A (45.69%) whereas the lowest one was obtained from the clone of the control (39.78%) and 27/1 Izmir (40.37%) (Table 5). Similarly, Urgun (1997) and Köse (2000) notified that the amount of fruit juice in the same Satsuma types (Sugiyama, Silverhill, Ikeda, Hayashi) that they analyzed, showed changes between 40.49 - 45.75%. According to these results, the clones selected show superiorities in terms of fruit juice amount.

22 Izmir (11.38%) clone had the highest total soluble solids, in contrast the lowest was determined from 24 Mersin (10.09%) clone. In terms of total acidity, the highest and the lowest were obtained from 27/1 Izmir (1.47%) and Owari Satsuma (1.16%) respectively (Table 5).

The highest amount of soluble solids/acidity was obtained from the clones of 13/2 Izmir (9.33), control (8.53), 4/2 Izmir (8,78) and 30 Izmir (8,57) but the lowest amount was obtained from the clones of 24 Mersin (7.40) and 27/1 Izmir (7.30) (Table 5).

Molecular analysis

RAPD analysis

The nine 10-mer RAPD primers generated 75 amplification products; the total number of marker ranged from 3 (OPA15) to 12 (OPA16). The sizes of the bands ranged from 250 to 2200 bp (Table 6). In average, each primer gave 8.33 scorable markers per amplification. The similarity value among 22 genotypes ranged from 0.79 to 1. This may imply low genetic variability among the genotypes studied.

Based on RAPD analysis, a dendrogram is illustrated in Figure 1. The results reveal that, 21 genotypes grouped

Table 5. Average fruit quality of new Satsuma mandarin clones on sour orange.

Clone	Juice content (%)	Total soluble solid (TSS) (%)	Total acidity (TA) (%)	TSS/TA
61 Adana	45.69 abc+	11.14 abc	1.39 abc	7.99 bcd
62 Adana	44.30 a-d	10.92 a-e	1.38 bcd	8.05 bcd
89 Adana	43.43 c-f	10.79 be	1.29 d-h	7.77 bcd
3 Izmir	43.70 c-f	10.60 de	1.21 ghi	8.28 a-d
22 Izmir	43.35 c-f	11.38 a	1.36 cde	8.46 abc
26 Izmir	43.10 def	10.54 def	1.30 def	8.13 bcd
30 Izmir	45.59 abc	10.98 a-e	1.22 f-i	8.57 ab
4/2 Izmir	42.37 d-g	11.02 a-d	1.28 e-h	8.78 ab
8/3 Izmir	46.20 ab	10.76 b-e	1.33 cde	8.18 bcd
11/1 Izmir	43.88 b-e	10.91 a-e	1.37 bcd	8.07 bcd
13/2 Izmir	44.22 a-d	10.99 a-d	1.19 i	9.33 a
18/1 Izmir	41.39 e-h	10.74 cde	1.21 hi	8.34 a-d
19/1 Izmir	46.27 a	10.47 ef	1.29 d-g	8.16 bcd
24/2 Izmir	41.70 e-h	10.98 a-d	1.40 abc	7.96 bcd
25/1 Izmir	42.91 d-f	11.35 a	1.46 ab	7.86 bcd
27/1 Izmir	40.37 gh	11.27 ab	1.47 a	7.30 d
27/2 Izmir	42.37 d-g	11.04 a-d	1.38 bcd	8.04 bcd
23 Mersin	42.06 d-h	11.19 abc	1.36 cde	8.26 a-d
24 Mersin	42.48 d-g	10.09 f	1.37 cd	7.40 cd
25 Mersin	44.37 a-d	11.02 a-d	1.34 cde	8.28 a-d
27 Mersin	41.38 fgh	10.80 b-e	1.23 f-i	8.23 bcd
Owari Satsuma	39.78 h	10.55 def	1.16 i	8.53 ab
Significance	**	**	**	*

**Significant at P<0.01; *significant at P<0.05.
Values followed by a common letter in a column for each clone are not significantly different at P<0.05 by Duncan's multiple range test.

Table 6. Band size and band number of primers used for RAPD analysis.

S/N	Primer	Band size	Band number
1	OPA04	250, 350, 500, 510, 750, 760, 1000, 1250, 1500, 1510, 2000	11
2	OPA 05	250, 450, 500, 600, 750, 800,1000,1250,1500, 2000	10
3	OPA09	250, 350, 500, 510, 750, 850, 1000, 1450	8
4	OPA13	300, 480, 600, 730, 800, 1100	6
5	OPA14	730, 850, 1000, 1400,1500	5
6	OPA15	500, 750, 1000	3
7	OPA16	250, 480, 500, 550, 750, 800, 1000, 1450, 1500, 2000, 2100	12
8	OPA 17	250, 400, 500, 250, 350, 480, 498, 500, 600, 750, 1250, 1500, 2000, 2200	11
9	OPA 19	250, 400, 500, 600, 750, 1000, 1100, 1500, 2000	9

in two clusters was based on cutoff value of the average similarity at 0.79. Cluster A included 4/2 Izmir, 25 Mersin, 24/2 Izmir, 62 Adana, 19/1 Izmir genotypes. Cluster B comprised of the other satsumas.

Coletta et al. (1998) used RAPD markers to evaluate genetic similarity among 35 mandarin accessions, including 10 species and 7 hybrids. The genetic similarity within the mandarin group was found to be high, and suggests that cultivated mandarins have a narrow genetic base. Researchers propose that somatic mutations accounts for additional diversity within groups of cultivars, such as 'Satsuma. In their study, satsuma miyagawa wase, Owari, Wase and Unshiu Wase showed close genetic similarity in RAPD analysis.

Machado et al. (1995), random amplified polymorphic DNA (RAPD) analysis was carried out to evaluate polymorphism and genetic similarity between 39 Mediterranean mandarin genotypes. UPGMA cluster analysis

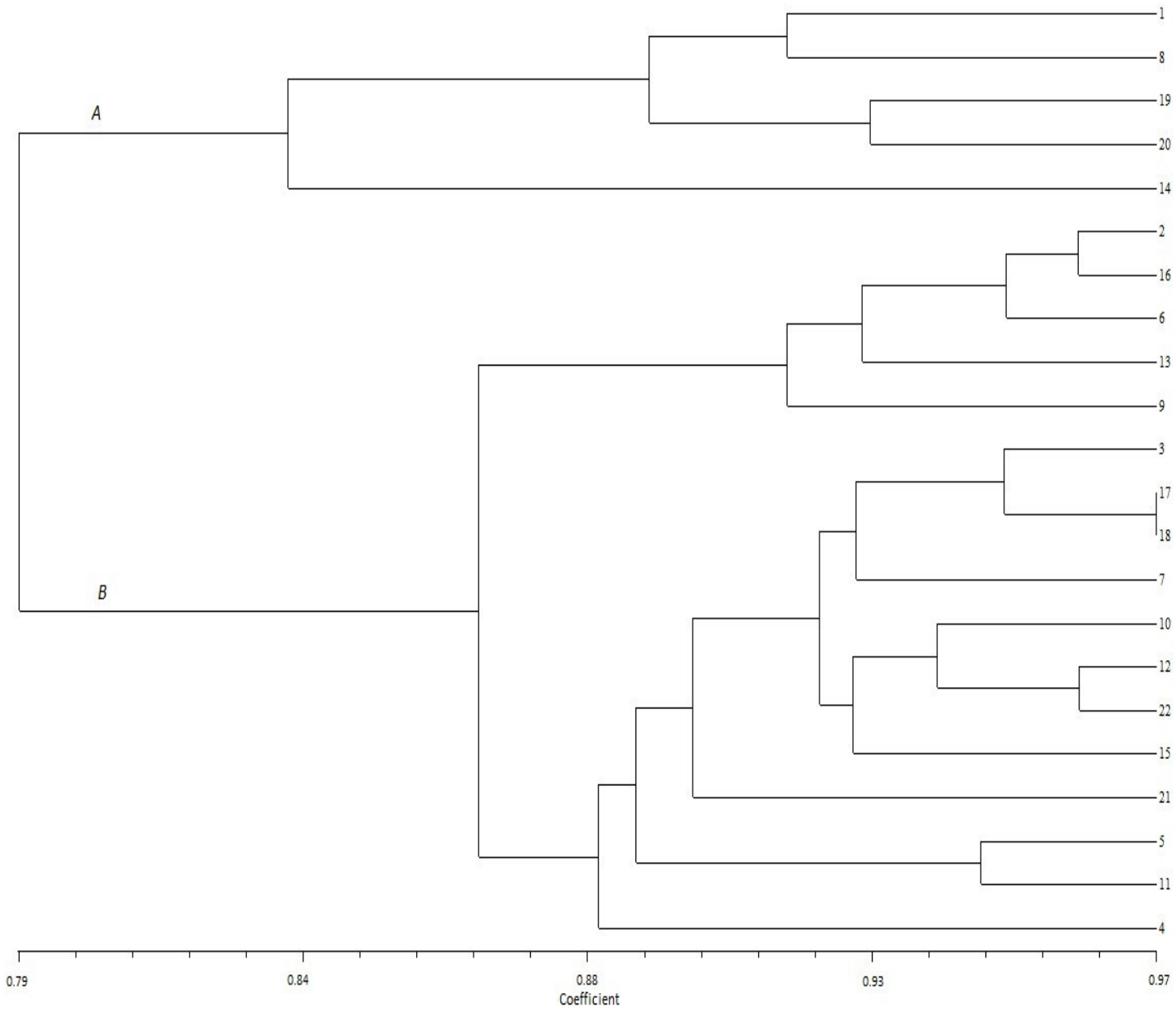

Figure 1. UPGMA dendrogram of 22 satsuma genotypes from RAPD data. Similarity values and matrix corellation are shown at the bottom of the dendrogram. (Matrix correlation: r = 0.82, 1:4/2 Izmir; 2: 26-Izmir; 3: 13/2 Izmir; 4: 27/1 Izmir; 5: 23Mersin; 6: 24 Mersin; 7: 3-Izmir; 8: 25Mersin; 9: 27/2 Izmir; 10: 8/3 Izmir; 11: 22-Izmir; 12: 61Adana; 13: 89 Adana; 14: 19/1 Izmir; 15: 11/1 Izmir; 16: Owari Satsuma; 17: 30-Izmir; 18: 18/1 Izmir; 19: 24/2 Izmir; 20: 62Adana; 21: 27Mersin; 22: 25/1 Izmir).

revealed the low level of genetic variation between accessions of Mediterranean mandarins, whereas their hybrids with other *Citrus* species showed greater genetic dissimilarity. Researchers claimed that twenty accessions yielded very similar patterns, suggesting either that they could be a single clone, or that the technique was not able to detect genomic variation. However, for the other specimens genetic polymorphism can easily be detected by RAPD, although the genetic variation between accessions was quite low.

SSR analysis

The fourteen SSR primer pairs used to characterize the Satsuma clones produced discrete reproducible fragments for genotypes tested. The number of fragments amplified from an individual mandarin clones with each primer pair ranged from one to two. Primer pair 497 was the most informative, producing four different sized fragments (Table 7).

The fourteen SSR primer pairs used on all Satsuma

Table 7. Band size and band number of primers used for SSR analysis.

SSR	Fragment size	Band number
458	233, 238	2
571	280, 285	2
497	355, 357, 358, 362	4
506	165, 167, 170	3
473	220, 234	2
485	216, 219	2
498	362, 370	2
MEST431	346, 357	2
488	160	1
173	204	1
54	173	1
121	198	1
495	272	1
697	393	1

clones produced 25 fragments. Among 25 bands generated by 14 selected primer pairs, 68% were monomorphic that is present in all individuals. The mean number of putative alleles detected per primer pair in this study ranged from 1 to 4 (Table 2) with a mean value of 1.79 alleles per primer pair. This value is very low when compared with other SSR studies on plants (Aka-Kacar et al., 2006).

A dendrogram was constructed from the SSR data (Figure 2). The average similarity value 0.87 was used as a cutoff value for defining the clusters. Two major clusters were formed. Cluster B consisted of genotype “26 Izmir”. Cluster A, the largest group, included the 21 genotypes and this group could be divided into two subgroups. 11/1 Izmir, control , 25/1 Izmir, 18/1 Izmir shared cluster A2 whereas the others take place cluster A1. Genetic similarity within the mandarin genotypes is high (0.87) and suggests that mandarin genotypes used in this study have a narrow genetic base. The dendrogram indicates that the genotypes can be separated into two major groups with a similarity value of 0.87 (A and B).

Based on 25 fragments, a similarity matrix was generated from the 22 Satsuma selections using the Dice coefficient of Nei and Li (1979). The data obtain from RAPD and SSR analyses were combined to perform genetic similarity. The dendogram constructed by UPGMA cluster analysis separated the Satsuma selections into two groups with a similarity value of 0.84 (Figure 3).

The group B occurred as a big group and divided into two groups in itself (Group B1 and Group B2). Group B2 was divided into two groups. The subgroup B.2.2. clustered into two groups. The subgroup B.2.2. contains only genotype 27 Mersin; the other subgroup B2.1. contains genotypes 13/2 Izmir, 30 Izmir, 18/1 Izmir, 3 Izmir, 61 Adana, 25/1 Izmir, 11/1 Izmir, 27/1 Izmir, 23 Mersin, 22 Izmir, 8/3 Izmir. The other subgroup A1 and A2 contains 4/2 Izmir ,25 Mersin, 24/2 Izmir, 62 Adana, 19/1 Izmir which are all similar patterns (Figure 3).

Group A also divided into two group and the A2 is consists of only “genotype 19/1 Izmir” and the other cluster (A1) has 4/2 Izmir, 25 Mersin, 24/2 Izmir, 62 Adana (Figure 3).

SSR markers could not distinguish between accessions within a few groups in which cultivars have arisen by apparent spontaneous mutation, such as sweet oranges (*Camellia sinensis*), Clementines (*Camellia reticulata*), Satsumas (*Camellia reticulata*), and small-fruited acid limes (*Cycloptera aurantifolia*) (Barkley et al., 2006).

Selection of new citrus genotypes collected as natural mutants from citrus orchards was previously reported (Becerra 1979; Robles-González et al., 1993; Uzun et al., 2009). Bud mutations is generally detected in horticultural traits and fruit characteristics in citrus. Contrasting with diversity for agronomic traits, vey low genetic variability has been found in cultivated citrus by use of molecular markers (Uzun et al., 2009).

Previous studies also have found few molecular polymorphisms within groups like these, consisting of cultivars developed by spontaneous mutation (Fang and Roose 1997; Bretó et al., 2001).

Even if mandarins exhibit much variation in characters (Reuther et al., 1967), they have narrow genetic variability. The genetic similarities between mandarins were found close together in other studies. Santos et al. (2003), reported in their molecular characterization studies with 34 mandarin genotypes that the genetic distance between the maximum of 0.32 (0.68 similarity level). Fang and Roose (1997), identified in their study with ISSR markers that five satsuma cultivars were in the same genetic structure. Uzun (2009), studied on the same selected satsuma clones to determine genetic similarity with SRAP markers, reported that polymorphism

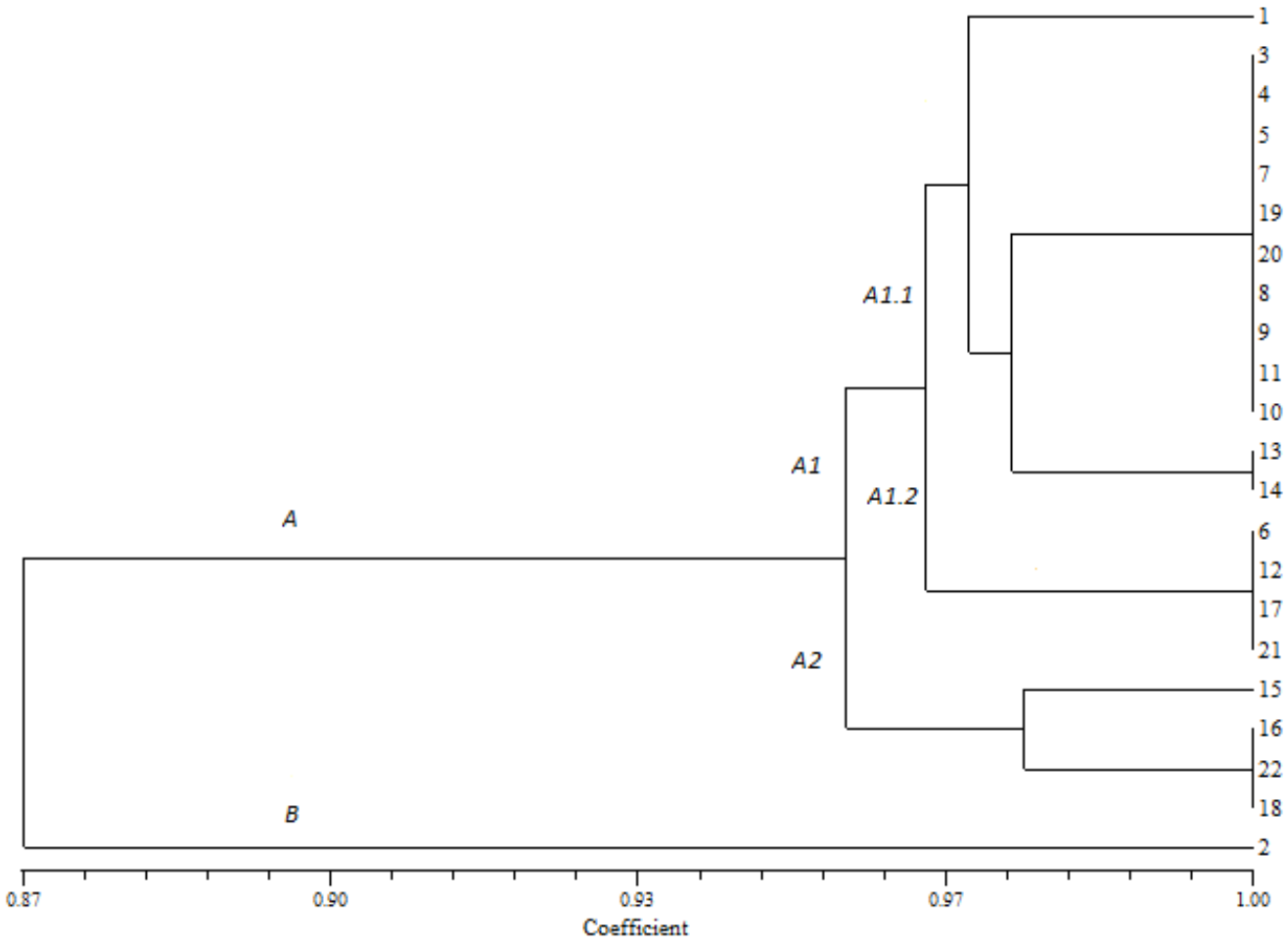

Figure 2. Dendrogram of the genetic relationship of 22 Satsuma clones based on 22 SSR primer pairs. (1:4/2 Izmir; 2: 26-Izmir; 3: 13/2 Izmir; 4: 27/1 Izmir; 5: 23Mersin; 6: 24 Mersin; 7: 3-Izmir; 8: 25Mersin; 9: 27/2 Izmir; 10: 8/3 Izmir; 11: 22-Izmir; 12: 61Adana; 13: 89 Adana; 14: 19/1 Izmir; 15: 11/1 Izmir; 16: Owari Satsuma; 17: 30-Izmir; 18: 18/1 Izmir; 19: 24/2 Izmir; 20: 62Adana; 21: 27Mersin; 22: 25/1 Izmir).

value of the satsuma clones were 0,97-1,00.

RAPD amplification and SSR loci analyses revealed low genetic polymorphism in the grapefruit accessions studied. Varieties of great economic importance and distinct morphological characteristics (for example, pigmented pulp, pale yellow pulp, seeded and seedless fruits) such as 'Marsh Seedless','Duncan', 'Thompson Pink', 'Foster' and 'Red Blush' showed complete genetic similarity. These results suggest that these accessions represent variations of a single clone with different names or that they are in fact different varieties that were derived from somatic mutations that were not detected by the molecular markers used (Corarza-Nunes et al.,2002).

Biotechnological methods are offering several opportunities that can be used in breeding Citrus and relatives, the first step of improving genotypes is to assess the genetic structure of genotypes (Aka-Kacar et al., 2005). Though, morphological and isozyme markers have been employed in assessing the underlying genetic variation of a genotype, the accuracy of the assessment is questionable. The availability of a low number of morphological and biochemical markers, their poor or unknown genetic control, environmental influence on the phenotypic expression, stage specific identification and procedural difficulties are known impediments in using these as genetic markers in genetic diversity analysis. Considering the problems associated with morphological and isozyme markers, researchers searched for alternative tools (Ravi et al., 2003). This study employed SSR and RAPD markers to evaluate genetic polymorphisms between the Satsuma genotypes which were obtained from our selection breeding program.

The fourteen SSR and nine RAPD primer pairs were not able to differentiate all Satsuma selections. These selections have slight morphological differences and may represent sports caused by somatic mutations selected during vegetative propagation from an original cultivar. The genotypes yielded similar patterns, suggesting either

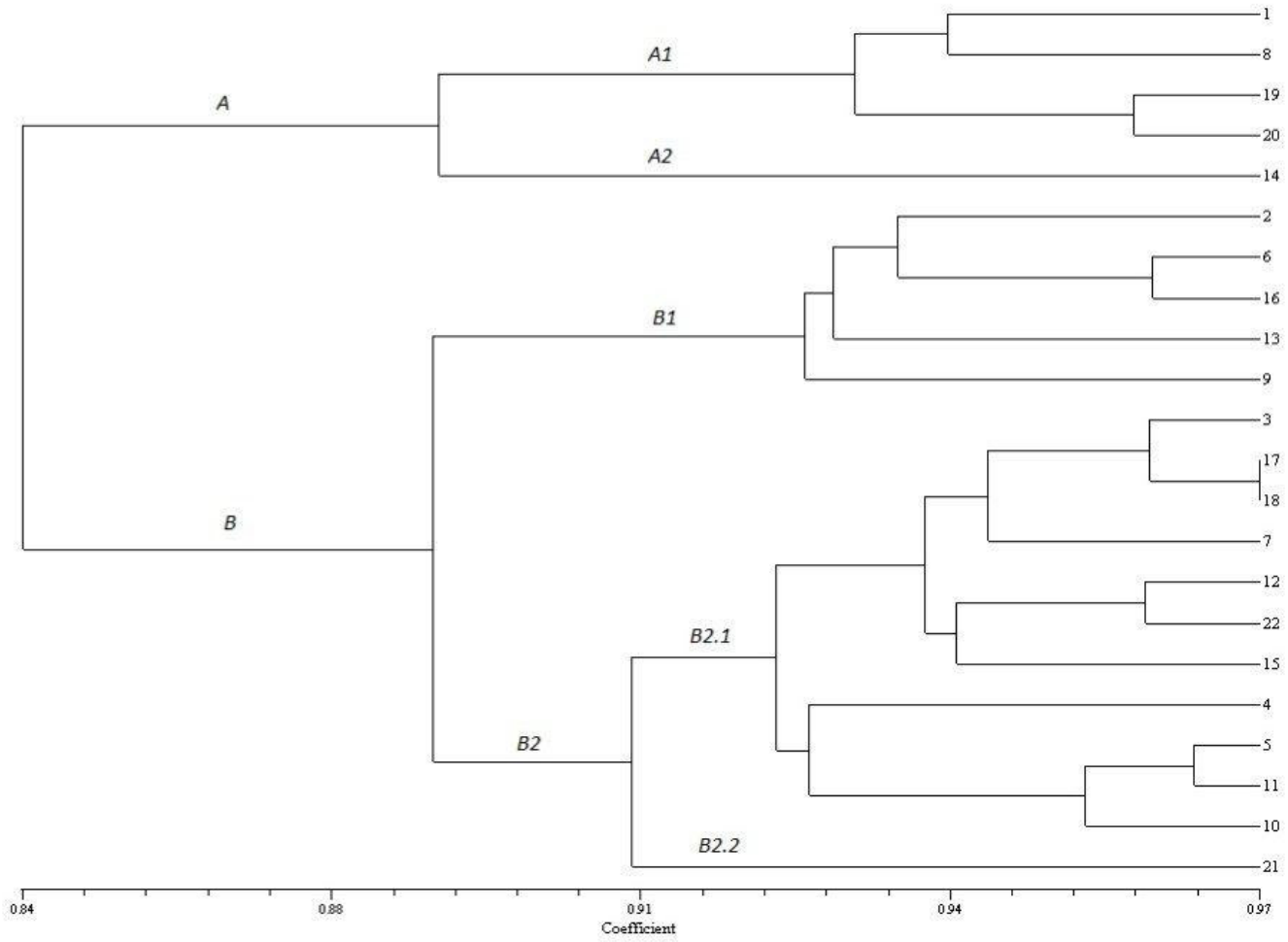

Figure 3. Dendrogram of the genetic relationship of 22 Satsuma clones based on 9 RAPD and 22 SSR primer pairs. (1:4/2 Izmir; 2: 26-Izmir; 3: 13/2 Izmir; 4: 27/1 Izmir; 5: 23Mersin; 6: 24 Mersin; 7: 3-Izmir; 8: 25Mersin; 9: 27/2 Izmir; 10: 8/3 Izmir; 11: 22-Izmir; 12: 61Adana; 13: 89 Adana; 14: 19/1 Izmir; 15: 11/1 Izmir; 16: Owari Satsuma; 17: 30-Izmir; 18: 18/1 Izmir; 19: 24/2 Izmir; 20: 62Adana; 21: 27Mersin; 22: 25/1 Izmir).

they are identical genotypes or we did not have enough markers to detect variation between these genotypes. Our preliminary result indicates that SSR and RAPD markers are useful tools for Satsuma clone varietal identification. But this marker system was not enough to distinguish the genotypes.

In conclusion, these results demonstrated the importance of the evaluation of new natural selections of Satsuma mandarins in order to know their field performance. Among the new 21 satsuma mandarin selections several tree growth, fruit yield, fruit quality, and molecular profile differences were observed under ecological conditions of Adana along three years. According to the results; 30 Izmir, 4/2 Izmir, 62 Adana and 13/2 Izmir clones were found to have better fruit quality and the higher yield than the control. The yield differences among selections began to be more evident since eleven year after planting. Since genotypes showed a real performance in field due to juvenility. As a result, these selections (30 Izmir, 4/2 Izmir, 62 Adana and 13/2 Izmir clones) can be suggested as new satsumas in citrus growing area.

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