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DETERMINATION OF GENOMIC DIVERSITY WITHIN MUTANT LEMON (*CITRUS LIMON* L.) AND MANDARIN (*CITRUS RETICULATA*) USING MOLECULAR MARKERS

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Abstract

Mutation breeding is considered to be successful in order to obtain new cultivars and expanding the genetic source of new varieties of lemon and mandarin in Citrus. In this study, different doses of gamma radiation (^{60}Co) have been used to generate novel mutants of the common variety Yerli Yuvarlak lemon and Yerli mandarin. The differences within the mutants were examined using simple sequence repeats (SSR) and sequence related amplified polymorphism (SRAP) markers. A total of 26 SSR primers and 28 SRAP primer combinations were screened for linkage to the different traits. Six SSR primers were showed polymorphism and closely linked to shoot tip color in lemon. Whereas, all of SRAP primer combinations were not produce any scorable polymorphic bands in both Yerli Yuvarlak lemon and Yerli mandarin. In this study, SSR and SRAP molecular markers were used together for first time to determination the genetic differences in citrus mutation breeding in the world.

Key words: Lemon; Mandarin; Mutation breeding; Shoot tip color; SSR; SRAP.

Introduction

Natural mutations have played an important role in the improvement of cultivars for years (Deng, 2000). There are also many varieties of Citrus obtained in this way (Tanaka, 1925). The first artificial mutation was performed on Citrus seeds using the x-rays by Haskin and Moore in 1935 (Cameron & Frost, 1968). The subsequent applications of artificial mutations were carried out to improve some properties of seedless mandarin (Starrantino *et al.,* 1988; Sutarto *et al*., 2009; Williams & Roose, 2010; Bermejo *et al*., 2011; Bermejo *et al*., 2012), early grapefruit and orange varieties (Donini, 1982; Tang *et al.,* 1994); highly fruitful and compact canopy orange (Donini, 1982), fruit peel color grapefruit (Hensz, 1985; Chapot, 1975), seedless lemon with Mal Secco tolerant (Gulsen *et al.,* 2007) and seedless pummelo (Huang *et al*., 2003; Sutarto *et al*., 2009).

The assessing of the plant variability at molecular level, the examination of genome structures and the establishment of plant gene maps by using molecular marker techniques was of great importance for the continuation of germplasm and plant breeding (Atak *et al.,* 2004). Molecular techniques were used to determine the mutant individuals after mutation breeding in some crops; for instance, on sesame (Uzun *et al.,* 2003), on grape (Stenkamp *et al.,* 2009) and rice (Rashid *et al.,* 2009). Also, studies were carried out on mutation breeding will help to achieve gene identification and/or allele-specific marker development in barley (Kaneko *et al.,* 2000), bread and durum wheat (Monari *et al.,* 2005), rice (Zhao *et al.,* 2008), onion (Kim *et al.,* 2005; Kim *et al.,* 2009), maize (Cassani *et al.,* 2009), grain (Park *et al.,* 2010) and soybean (Lenis *et al.,* 2010).

When we look for studies in citrus, random amplified polymorphic DNA (RAPD) marker was used for the identification of 14 *in vivo* and *in vitro* lemon mutants to compare the zygotic origin of genotype (Deng *et al.,* 1995). But, they are not allowed to formation of commercial accessions and are not the individualls exposed to artificial mutation. Recently, a study was carried out to evaluate and compare the molecular genetics of one particular variety of citrus. Molecular differences between W. Murcott and Tango, molecular regions prone to alteration due to radiation exposure were targeted for analysis (Crowley, 2011).

In our study, SSR markers were used to determine the genetic diversity within stable mutant individualls obtained through ^{60}Co gamma radiations using SSR and SRAP markers. To the best of our knowledge, there is no study related to differentiation on mutant individuals using molecular markers on Citrus. Recently, genotype-fitness correlations have also been reported with DNA markers such as microsatellites, characterized by high mutation rates and specific mutational processes (Tsitrone *et al.,* 2001; Stenkamp *et al.,* 2009).

Simple sequence repeats (SSR) markers have been the most commonly utilized markers in molecular biology for mapping, genetic diversity, phylogenetic construction, and fingerprinting because they are co-dominant, highly polymorphic, and easy to use (Zane *et al.,* 2002; Barkley *et al.,* 2009). SSRs have been used for several purposes, such the study of genetic diversity, relationships of *Citrus* species, fingerprint accessions, evaluate phylogenetic relationships among accessions, mapping and examine the level of genetic diversity in Citrus (Novelli *et al.,* 2006; Barkley *et al.,* 2009; Polat & Turgutoğlu, 2012; Hamza, 2013). Sequence-related amplified polymorphism (SRAP) marker is a PCR-based marker system as described by Li and Quiros (2001). SRAP markers have been used determination of genetic diversity, evaluate and phylogenetic relationships among accessions in Citrus (Uzun *et al*., 2009a; Polat *et al*., 2012; Kacar *et al*., 2013).

The purpose of this study is to use SSR and SRAP molecular markers to identify mutant and non-mutant individual's genetic diversity in Yerli Yuvarlak lemon and Yerli mandarin.

Material and Methods

Plant materials: Nine stable mutant and 1 non-mutant Yerli Yuvarlak lemons, and thirty-four stable mutant and 1 non-mutant Yerli mandarins were used for this study (Table 1). Yerli Yuvarlak lemon (*Citrus limon* (L.) Burm. F.) is yielding regular, abundant water and Mal secco [*Phomatracheiphila* (Petri) Kantschaveliand Gikashvili] tolerant common lemon variety, however, is quite thorny. The leaves are reddish/purple when young. By the time the leaves becom pale green on the underside and darker green on top. Yerli mandarin (*Citrus reticulata* Blanco) is common mandarin variety a yielding variety characterized by plenty of juicy fruits with typical mandarin aroma, but, the fruit is quite seeded.

Table 1. Plant materials, ^{60}Co doses and plant numbers.

Varieties	^{60}Co doses (Gray)	Plant numbers
Yerli mandarin	20	7
	40	6
	60	9
	100	10
	120	2
	0 (control)	1
Yerli Yuvarlak lemon	60	1
	80	4
	90	4
	0 (control)	1

The genomic DNA isolation: The total genomic DNA was extracted from young leaves by the CTAB method as described by Doyle and Doyle (1990).

SSR analysis.

Twenty six SSR primers (Table 1) described by Barkley *et al.* (2006) and Roose, (2009) were used in the study. PCR amplifications were conducted as described by Polat, (2009). Each 10-µl reaction consisted of 1.0 µl of primers, 200 mM of each dNTP, 1.0 µl of 10X PCR Buffer (Biorun, Nantes, France), 1.0 µl of MgCl2 (2.5 mM $MgCl_2$ 4.8 µl ddH2O, 0.2 µl Taq (0.6 U Taq DNA polymerase), and 1.0 µl DNA(20 ng DNA). DNA Thermal Cycler (Biorad DNA-Engine Gradient Cycler, Hercules, CA, USA) was used and cycling parameters included 3 min of denaturing at 94ºC, thirty-five cycles of three steps: 30 second of denaturing at 94ºC, 30 second of annealing at 50ºC, 55ºC or 40ºC (annealing temperatures were specific for each primer (Table 2) and 1 min of elongation at 72ºC, and for extension, one cycle 10 min at 72ºC.

SRAP analysis: Twenty-eight primer combinations were used (Table 3). PCR reaction components and PCR cycling parameters were performed as described by Uzun *et al.* (2009a) with some modifications. Each 15-µl reaction consisted of 1.0 µl of primers, 200 mM of each dNTP, 1.5 µl of 10X PCR Buffer (Biorun, Nantes, France), 1.5 mM of $MgCl_2$, 5.3 µl ddH_2O, 0.2 µl Taq (0.6 U Taq DNA polymerase, Biorun) and 1.0 µl template DNA (20 ng DNA). DNA Thermal Cycler (Biorad DNA-Engine Gradient Cycler, Hercules, CA, USA) was used and cycling parameters included 5 min of denaturing at 94ºC, five cycles of three steps: 1 min of denaturing at 94ºC, 1 min of annealing at 35ºC and 2 min of elongation at 72ºC. In the following 35 cycles the annealing temperature was increased to 50ºC, and for extension, one cycle 5 min at 72ºC.

All of PCR products were separated on 2, 5% high resolution agarose gel in 1X TAE buffer at 100 V for 3.0 h, and photographed (used Kodak Gel Logic 200) under UV light for further analysis. A 100 bp DNA ladder was used as molecular standard in order to confirm the appropriate SSR and SRAP markers.

Results

Twenty-eight SRAP primer combinations (Table 3) were screened, and total 149 bands scored in Yerli mandarin, 204 bands scored in Yerli Yuvarlak lemon. But, all fragments scored for each primer combination were monomorphic (Table 4). So, all of SRAP primer combinations were not produced any scorable polymorphic bands in Yerli Yuvarlak Lemon and Yerli Mandarin. So, used SRAP primer combinations were not successful to determination of genetic diversity. There is no polymorphism, which is also not convenient to mutation breeding.

Twenty-six SSR primers were screened (Table 2). A total of 66 PCR fragments were amplified with 26 SSR primers and 7 of them were polymorphic in Yerli Yuvarlak lemon. However, total 58 PCR fragments were amplified with 26 SSR primers and none of them were polymorphic in Yerli mandarin (Table 5). Six of the twenty-six SSR primers were produced scorable polymorphic bands for two mutants in Yerli Yuvarlak lemon. These primers are CAT01, TAA1, CT21, CT02, CAC39 and CAGG09. Polymorphic SSR primers and band sizes in Yerli Yuvarlak Lemon are presented in Table 6. As shown in Table 6, CAT01 (160 bp), CAGG09 (130 bp), CT21 (900 bp), CT02 (850 bp) and CAC39 (190 bp) primers do not produce fragment on mutant 1. However; TAA1 (180 bp) ve CT21 (750 bp) primers produce no band both on mutant 1and mutant 2. Polymorphism by CAT01 primer is given in Fig. 1.

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Table 2. Forward and reverse primer sequences and annealing temperature for 26 SSR markers.

Locus	F-Primer	R-Primer	Repeat motif	Annealing temperature (ºC)
TAA1	GACAACATCAACAACAGCAAGAGC	AAGAAGAAGAGCCCCCATTAGC	TAA	50
TAA45	GCACCTTTTATACCTGACTCGG	TTCAGCATTTGAGTTGGTTACG	TAA	50
TAA52	GATCTTGACTGAACTTAAAG	ATGTATTGTGTTGATAACG	TAA	40
CAC19	ACAACCTTCAACAAAACCTAGG	AAGACTTGGTGCGACAGG	CAC	50
TAA15	GAAAGGGTTACTTGACCAGGC	CTTCCCAGCTGCACAAGC	TAA	40
TAA27	GGATGAAAAATGCTCAAAATG	TAGTACCCACAGGGAAGAGAGC	TAA	50
TAA41	AGGTCTACATTGGCATTGTC	ACATGCAGTGCTATAATGAATG	TAA	50
CAC23	ATCACAATTACTAGCAGCGCC	TTGCCATTGTAGCATGTTGG	CAC	50
CAGG9	AATGCTGAAGATAATCCGCG	TGCCTTGCTCTCCACTCC	AGG	40
TAA3	AGAGAAGAAACATTTGCGGAGC	GAGATGGGACTTGGTTCATCACG	TAA	50
CAC15	TAAATCTCCACTCTGCAAAAGC	GATAGGAAGCGTCGTAGACCC	CAC	55
CAC33	GGTGATGCTGCTACTGATGC	CAATTGTGAATTTGTGATTCCG	CAC	50
CAC39	AGAAGCCATCTCTCTGCTGC	AATTCAGTCCCATTCCATTCC	CAC	50
TAA33	GGTACTGATAGTACTGCGGCG	GCTAATCGCTACGTCTTCGC	TAA	50
CCT01	TCAACACCTCGAACAGAAGG	CCCACATGCTAGCACAAAGA	CCT	50
GT03	GCCTTCTTGATTTACCGGAC	TGCTCCGAACTTCATCATTG	GT	50
CT02	ACGGTGCGTTTTGAGGTAAG	TGACTGTTGGATTTGGGATG	CT	50
AC01	TTTGACATCAACATAAAACAAGAAA	TTTTAAAATCCCTGACCAGA	AC	50
CAG01	AACACTCGCACCAAATCCTC	TAAATGGCAACCCCAGCTTTG	CAG	50
CAT01	GCTTTCGATCCCTCCACATA	GATCCCTACAATCCTTGGTCC	CAT	50
ATC09	TTCCTTATGTAATTGCTCTTTG	TGTGAGTGTTTGTGCGTGTG	ATC	50
AG14	AAAGGGAAAGCCCTAATCTCA	CTTCCTCTTGCGGAGTGTTC	AG	50
CTT01	TCAGACATTGAGTTGCTCG	TAACCACTTAGGCTTCGGCA	CTT	50
CT21	CGAACTCATTAAAAGCCGAAAC	CAACAACCACCACTCTCACG	CT	50
TC26	CTTCCTCTTGCGGAGTGTTC	GAGGGAAAGCCCTAATCTCA	TC	50
CT19	CGCCAAGCTTACCACTCACTAC	GCCACGATTTGTAGGGGATAG	CT	50

Table 3. The forward and reverse SRAP primer sequences information.

Marker names	F-Primers	R-Primers
Me11-Em16	TGAGTCCAAACCGGAAC	GACTGCGTACGAATTGTC
Me10-Em13	TGAGTCCAAACCGGAAA	GACTGCGTACGAATTCTG
Me8-Em2	TGAGTCCAAACCGGACT	GACTGCGTACGAATTTGC
Me8-Em15	TGAGTCCAAACCGGACT	GACTGCGTACGAATTGAT
Me4-Em11	TGAGTCCAAACCGGACC	GACTGCGTACGAATTCTA
Me12-Em6	TGAGTCCAAACCGGAGA	GACTGCGTACGAATTGCA
Me2-Em5	TGAGTCCAAACCGGAGC	GACTGCGTACGAATTAAC
Me11-Em7	TGAGTCCAAACCGGAAC	GACTGCGTACGAATTCAA
Me8-Em10	TGAGTCCAAACCGGACT	GACTGCGTACGAATTCAT
Me9-Em4	TGAGTCCAAACCGGAGG	GACTGCGTACGAATTTGA
Me10-Em7	TGAGTCCAAACCGGAAA	GACTGCGTACGAATTCAA
Me5-Em16	TGAGTCCAAACCGGAAG	GACTGCGTACGAATTGTC
Me5-Em13	TGAGTCCAAACCGGAAG	GACTGCGTACGAATTCTG
Me1-Em2	TGAGTCCAAACCGGATA	GACTGCGTACGAATTTGC
Me9-Em15	TGAGTCCAAACCGGAGG	GACTGCGTACGAATTGAT
Me14-Em11	TGAGTCCAAACCGGCTA	GACTGCGTACGAATTCTA
Me3-Em6	TGAGTCCAAACCGGAAT	GACTGCGTACGAATTGCA
Me6-Em15	TGAGTCCAAACCGGACA	GACTGCGTACGAATTGAT
Me6-Em7	TGAGTCCAAACCGGACA	GACTGCGTACGAATTCAA
Me3-Em10	TGAGTCCAAACCGGAAT	GACTGCGTACGAATTCAT
Me14-Em4	TGAGTCCAAACCGGCTA	GACTGCGTACGAATTTGA
Me4-Em9	TGAGTCCAAACCGGACC	GACTGCGTACGAATTCAG
Me13-Em11	TGAGTCCAAACCGGAAG	GACTGCGTACGAATTCTA
Me4-Em12	TGAGTCCAAACCGGACC	GACTGCGTACGAATTCTC
Me7-Em11	TGAGTCCAAACCGGACG	GACTGCGTACGAATTCTA
Me10-Em9	TGAGTCCAAACCGGAAA	GACTGCGTACGAATTCAG
Me6-Em5	TGAGTCCAAACCGGACA	GACTGCGTACGAATTAAC
Me3-Em16	TGAGTCCAAACCGGAAT	GACTGCGTACGAATTGTC

Table 4. Number of total, polymorphic and monomorphic bands, fragment size using SRAP primers.

Primer code	Total bands		Polymorphicbands		Monomorphicbands		Fragment size (bp)	
	Yerli mandarin	Yerli Yuvarlak lemon	Yerli mandarin	Yerli Yuvarlak lemon	Yerli mandarin	Yerli Yuvarlak lemon	Yerli mandarin	Yerli Yuvarlak lemon
Me11-Em16	4	5	0	0	4	5	300-600	200-650
Me10-Em13	6	6	0	0	6	6	300-1200	300-1200
Me8-Em2	5	7	0	0	5	7	300-600	250-1000
Me8-Em15	3	5	0	0	3	5	500-1200	300-1200
Me4-Em11	6	7	0	0	6	7	300-1200	250-1300
Me12-Em6	5	7	0	0	5	7	250-800	200-1300
Me2-Em5	4	7	0	0	4	7	450-800	400-1400
Me11-Em7	7	7	0	0	7	7	300-1200	250-1300
Me8-Em10	5	6	0	0	5	6	400-1200	350-1200
Me9-Em4	7	8	0	0	7	8	150-900	150-1400
Me10-Em7	5	7	0	0	5	7	350-1100	300-1300
Me5-Em16	5	7	0	0	5	7	250-800	200-1300
Me5-Em13	6	9	0	0	6	9	300-1100	250-1400
Me1-Em2	5	7	0	0	5	7	230-850	300-1300
Me9-Em15	7	8	0	0	7	8	250-800	200-1300
Me14-Em11	5	7	0	0	5	7	150-800	250-1200
Me3-Em6	4	9	0	0	4	9	180-750	150-1100
Me6-Em15	6	9	0	0	6	9	120-900	180-1200
Me6-Em7	7	7	0	0	7	7	150-1250	200-1100
Me3-Em10	4	7	0	0	4	7	100-600	150-1300
Me14-Em4	5	7	0	0	5	7	150-1000	200-1200
Me4-Em9	7	9	0	0	7	9	200-1100	250-1400
Me13-Em11	4	7	0	0	4	7	120-400	150-400
Me4-Em12	5	8	0	0	5	8	180-1150	120-1300
Me7-Em11	4	7	0	0	4	7	150-550	220-1200
Me10-Em9	6	7	0	0	6	7	150-500	200-1100
Me6-Em5	6	8	0	0	6	8	300-1200	120-1300
Me3-Em16	6	9	0	0	6	9	180-1150	150-1200
Total	**149**	**204**	**0**	**0**	**149**	**204**	**-**	**-**

Table 5. Number of total, polymorphic and monomorphic bands, fragment size using SSR primers.

Primer code	Total bands		Polymorphicbands		Monomorphicbands		Fragment size range (bp)	
	Yerli mandarin	Yerli Yuvarlak lemon	Yerli mandarin	Yerli Yuvarlak lemon	Yerli mandarin	Yerli Yuvarlak lemon	Yerli mandarin	Yerli Yuvarlak lemon
TAA1	1	2	0	1	0	1	180	180-200
TAA45	4	4	0	0	4	4	90-200	90-200
TAA52	1	1	0	0	1	1	120	120
CAC19	6	4	0	0	6	4	150-320	150-3201
TAA15	5	5	0	0	0	5	200-700	180-700
TAA27	1	2	0	0	1	2	200	200-210
TAA41	1	1	0	0	1	1	150	150
CAC23	3	3	0	0	3	3	90-250	90-250
CAGG9	1	2	0	1	1	1	130	110-130
TAA3	1	3	0	0	1	3	150	150-400
CAC15	1	1	0	0	1	1	600	650
CAC33	3	2	0	0	3	2	180-220	180-200
CAC39	1	2	0	1	1	1	180	180-190
TAA33	2	2	0	0	2	2	80-120	80-120
CCT01	2	3	0	0	2	3	180-210	180-450
GT03	1	1	0	0	1	1	800	1200
CT02	4	5	0	1	4	4	180-500	150-850
AC01	3	2	0	0	3	2	150-350	550-700
CAG01	1	1	0	0	1	1	130	130
CAT01	4	5	0	1	4	4	120-280	130-330
ATC09	3	2	0	0	3	2	120-220	100-180
AG14	1	2	0	0	1	2	150	150-800
CTT01	4	5	0	0	4	5	290-1000	250-1200
CT21	1	3	0	2	1	1	150	550-900
TC26	1	1	0	0	1	1	150	150
CT19	2	2	0	0	2	2	150-450	450-700
Total	**58**	**66**	**0**	**7**	**52**	**59**	**-**	**-**

Table 6. Polymorphic SSR primers and band sizes in Yerli Yuvarlak lemon.

	CAT01	TAA1	CAGG9	CT21		CT02	CAC39
	160 bp	180 bp	130 bp	900 bp	750 bp	850 bp	190 bp
Mutant 1	-	-	-	-	-	-	-
Mutant 2	+	-	+	+	-	+	+
Mutant 3	+	+	+	+	+	+	+
Mutant 4	+	+	+	+	+	+	+
Mutant 5	+	+	+	+	+	+	+
Mutant 6	+	+	+	+	+	+	+
Mutant 7	+	+	+	+	+	+	+
Mutant 8	+	+	+	+	+	+	+
Mutant 9	+	+	+	+	+	+	+

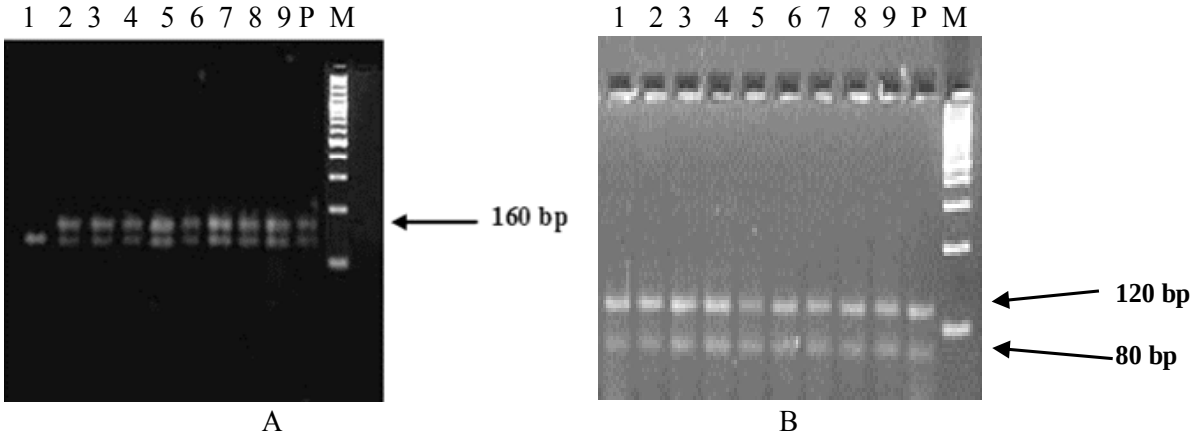

Fig. 1. Image of amplification profile using CAT01 (A) and TAA33 (B) SSR loci. M: ladder marker, P: non mutant parent, 1-9: mutant individuals

Discussion

SRAP markers have been used for determining genetic diversity of *Citrus* and found very informative and useful system (Uzun *et al.,* 2009a; Uzun *et al.,* 2009b; Uzun *et al.,* 2011; Polat *et al.,* 2012). However, SRAP markers did not provide genetic diversity of mutant plants by using ^{60}Co gamma radiations in our study. Therefore, SRAP markers are not suitable for use in Citrus mutation breeding. It would be better to expand the number of SRAP combinations that were tested.

SSR markers have been found very useful to determination of mutant accessions. In our study, CAGC09, CT21, CT02, CAC39 and TAA1 primers produced polymorphic bands in Yerli Yuvarlak lemon. In a previous study with citrus germplasm collections, CAT01, CT21, CT02 primers were reported to be highly polymorphic (Barkley *et al.,* 2006).

Mutant 1 and mutant 2 were obtained from 60 and 80 Gray doses of ^{60}Co gamma radiations, respectively. The applications with the doses of 5 and 7 krad (50 and 70 gray) have provided seedless and *mal secco* disease resistance on stable mutant Kütdiken lemons (Gulsen *et al.,* 2007).

Polymorphic six SSR primers closely linked to shoot tip color of two mutants in Yerli Yuvarlak lemon was identified. Lemon leaves are reddish/purple when the shoots were young, and become pale green on the underside and darker green on top as they grow up (Morton, 1987). But, mutant 1 and mutant 2 lemons have green leaves both young and mature.

However, none of SSR primer was produced scorable polymorphic bands in Yerli mandarin in our study. Citrus has quite complex genetic structure. Mandarins are thought to be ancestral species, whereas lemons are classified as hybrid origin. The lemons have higher frequency of heterozygotes than the mandarins (Barkley *et al.,* 2006). Therefore to determine genetic diversity would be difficult, compared to lemon.

Mutation breeding is an established method for crop improvement, and has played a major role in the development of many new good traits in *Citrus* species. Mutations breeding was reported in lemon (Starrantino *et al.,* 1988; Spiegel-Roy *et al.,* 1990; Gulsen *et al.,* 2007), mandarin (Starrantino *et al*., 1988; Distefano *et al.,* 2009; Sutarto *et al*., 2009; Williams & Roose, 2010; Bermejo *et al*., 2011; Bermejo *et al*., 2012), grapefruit (Donini, 1982; Tang *et al.,* 1994; Hensz, 1985), pummelo (Huang *et al*., 2003; Sutarto *et al*., 2009) and sweet orange (Donini, 1982). Gamma irradiation causes chromosomal re-arrangements that may be related to multiple trait alterations (Fehr, 1993). This may provide additional opportunity for further lemon and mandarin improvement.

Mutations in either structural or regulatory genes can produce mutants with missing or reduced pigmentation (Holton & Cornish, 1995). Sometimes, color change is formed in citrus as a result of natural mutation. Tetraploid plants obtained from one type of sector mutant (termed 'gigas') and albino plants obtained from another type of sector mutant confirmed that some genetic mutations observed in fruit rind can be recovered in nucellar seedlings in Citrus. Propagation of plants from mutant sectors may yield cultivars with improved fruit color, altered maturation date, and reduced disease or mite susceptibility and may eventually lead to breeding of seedless triploid hybrids (Bowman *et al.,* 1991). A spontaneous mutant of *Citrus sinensis* L. Osbeck, navel negra, produces fruit with an abnormal brown-colored flavedo during ripening (Alos *et al.,* 2008). Peel color is one of the main features affecting citrus quality. Clementine is a widespread citrus species with several mutants showing a delay in pigmentation and harvesting (Distefano *et al.,* 2009). Mutation breeding were used in order to provide a color change in some important plants such as chrysanthemum (Matsumura *et al.,* 2010) and rice (Wu *et al.,* 2003). But, we could not have any literature about color change of shoot tip in mutant lemons.

Determination of the plant variability at molecular level, the examination of genome structures by using molecular marker techniques were of great importance for the continuation of germplasm and plant breeding (Atak *et al.,* 2004). Molecular techniques are used to determine the diversity of individuals obtained by mutation breeding. Molecular and biochemical aspects of Tibetan β-amylase-less mutant barley were investigated. Following Southern analysis and polymerase chain reaction, it is suggested that these mutants result from the same insert mutation in the latter part of the β-amylase structural gene (Kaneko *et al.,* 2000). AFLP marker linked to the closed capsule trait was determineted in mutant sesames (Uzun *et al.,* 2003). RAPD molecular marker techniques to variability obtained from mutation breeding (gamma rays) were used in sugarcane. A total of 85 loci were amplified, out of which 76.47% were polymorphic and 23.53% were monomorphic. Mutants P4 (40 Gy) and P4 (10 Gy) were genetically distinct from other mutants (Khan *et al.,* 2010).

Molecular characterization of mutated waxy loci in four bread wheat cultivars and in four 'durum' wheat cultivars was conducted by means of PCR, Southern and DNA sequence analyses (Monari *et al.,* 2005). A fourth primer set, specific for this mutation, was derived. Zhao *et al.* (2008) searched the candidate gene for these two allelic LPA mutations using T-DNA insertion mutants, mutation detection by CEL I facilitated mismatch cleavage, and gene sequencing. A CAPS marker (LPA1-CAPS) was developed for distinguishing the lpa 1-1 allele from lpa 1-2.

RAPD markers were used to genetic diversity within commercial individuals with zygotic origin of lemons under *In vitro* and *In vivo* conditions (Deng *et al.,* 1995). However, genetic diversity was determined using SRAP and SSR markers on mutant lemon and mandarin. Furthermore, a marker was detected, showing linkage to color of shoot tip. However, there is not any other literature about molecular characterization of obtained mutant individuals by using mutation breeding in Citrus. So, this work is of importance in Citrus. SSR markers seem a good molecular tool for determining variability in mutation breeding (gamma rays) programs of Yerli Yuvarlak lemon.

Conclusion

Molecular markers were used for the first time in this study to determine the genetic diversity of individuals obtained from mutation breeding in *Citrus*. The SSR and SRAP molecular markers were used to identify of mutant and non-mutant individual's genetic diversity in Yerli Yuvarlak lemon and Yerli mandarin. SRAP markers were not suitable for assessing genetic diversity for both Yerli Yuvarlak lemon and Yerli mandarin. However, SSR markers have identified for individual's genetic differences in the two mutants Yerli Yuvarlak lemon. Six SSR primers closely linked to shoot tip color in mutant 1 and mutant 2 Yerli Yuvarlak lemon were determined.

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