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Development of BAC-End based simple sequence repeat (SSR) markers in apple

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ABSTRACT

A genome-wide bacterial artificial chromosome (BAC) physical map of the apple, *Malus domestica* Borkh., has been recently developed. This study addresses development of SSR markers from the BAC-end sequences in apple. Previously designed 187 BAC-SSR primer pairs were subjected firstly to gradient-PCR for amplification and to determine their optimum annealing temperatures. Then, the amplified primer pairs were tested in two F1 segregating populations: 'Kasel-37' x 'Delbarestivale', and 'Kasel-41' X 'Williams Pride'. The PCR reactions were performed in 12 F1 progenies and in the parents to determine segregation types of the primer pairs. There was no amplification in 20 SSR primer pairs, and 87 of them were monomorphic in the two populations. As a result, 80 of the primer pairs showed segregation in each of the two F1 populations.

Keywords: SSR, apple, polymorphism, microsatellite

Introduction

Apples have been grown for thousands of years in Asia and Europe, and have been cultivated for more than 4000 years ago (Özçağıran et al. 2004). Apple, *Malus domestica,* is one of the most popular tree fruit species in the rose family (*Rosaceae*) which belongs to the Maloideae subfamily of the Rosaceae. It is one of the most widely cultivated tree fruits; and the most widely known of genus *Malus*. Although it is functionally diploid (2n=2x=34); it has been suggested that the Maloideae are of allopolyploid origin.

According to FAO data, Turkey is among the top ten apple producers in the world. Apples are grown in many regions in Turkey, and approximately 50 percent of all commercial apple production comes from three provinces; Isparta, Karaman and Nigde. These provinces are located in the southern part of Central Anatolia and the Northern Mediterranean Regions. In addition to them; commercial apples are also grown in Antalya, Eregli, Denizli, Yalova and Amasya.

Amasya apple is Turkey's premier apple cultivars that its taste and aroma are more attractive among all other commercial varieties. In addition to aroma compounds; sugars and organic acids along with other pleasant fruit characteristics are noteworthy. Trade value of this cultivar in international market

is very low; because of low yield, alternate bearing and fruit size. Therefore, it is necessary to select the best ones with high quality and yield.

Bacterial artificial chromosome (BAC) libraries have been used in genomics research due to their large DNA inserts, high cloning efficiency, and stable maintenance of foreign DNA. In plants, BAC libraries have been constructed for a variety of species such as *Arabidopsis* (Choi et al. 1995), rice (Wang et al. 1995), maize (Yim et al. 2002), sorghum (Woo et al. 1994), soybean (Shoemaker et al. 1996; Salimath and Bhattacharyya 1999; Tomkins et al. 1999; Meksem et al. 2000), papaya (Ming et al. 2001), and apple (Vinatzer et al. 1998; Xu et al 2001). These libraries have made invaluable contributions to plant genomic studies including map-based or positional cloning of genes, genome-wide physical map construction (Mozo et al. 1999; Klein et al. 2000; Chen et al. 2002; Xuand Korban 2002; Shultz et al. 2006; Han et al. 2007).

SSR markers have several advantages over other molecular markers. They are infact neutral, co-dominant, highly polymorphic markers, widely used in genetic mapping, fingerprinting and diversity studies.Furthermore, the high information content of microsatellites can be fully applied to QTL mapping and in general to apple breeding, enormously.

BAC-SSR primer pairs designed by Han et al. (2009) were used in this study.Here, we report segregation types of these BAC-end sequence derived SSR primers in two segregating F_1 populations in apple.

Materials and methods

Plant material and DNA extraction

Twelve progenies of two F_1 populations were used for this study. The first population (A) was derived from 'Kaşel-41' x 'Williams Pride' cross, and the second population (B) was derived from a cross between 'Kaşel-37' and 'Delbarestivale'. All crosses were made in Eğirdir Fruiticulture Research Station in Isparta province of Turkey.

DNAs were isolated according to the CTAB-based protocol (Doyle and Doyle, 1990) with minor modifications (Kafkas et al. 2006). After calculating concentration of DNA with QubitFluorometre (Invitrogen) and diluted to a concentration of 10 ng/µl for SSR-PCR reactions. 187 previously designed SSR primers by Han et al. (2009) were used to test in two F_1 populations in this study.

PCR conditions

Firstly, gradient PCR was performed in a 1.5% agarose gel to determine optimum annealing temperatures of the primer pairs. M13 universal primer 5'-TGTAAAACGACGGCCAGT-3' is attached to the forward primer at the 5' end, were synthesised by labelling with 6-FAM, VIC, NED and PET fluorescent dyes (Schuelke 2000).

PCRs were carried out in 12.5- µL volumes containing 10 ng of DNA, 75 mMTris-HCl (pH: 8.8, 20 mM $(NH_4)_2SO_4$), 2.0 mMMgCl_2, %0.01 Tween 20, 200mM dNTP, 10nM forward primer that added M13 Universal (5 `-TGTAAAACGACGGCCAGT-3 ') primer and 200 nM FAM, VIC, NED and PET, 200 nMreverse primer and 0.6 U of *Taq* polymerase (Fermentas).

The PCR conditions comprised an initial denaturation step at 94°C for 5 min, followed by 30 cycles at 94°C for 30s, 52-60°C for 45s and 72°C for 60s, plus 10 cycles at 94°C for 30s, annealing temperature 52°C for 45 s and 72°C for 60s, plus and a final extension at 72°C for 10 min. (Schuelke, 2000).

The products of gradient PCR reactions were stained with ethidium bromide in 1.5% agarose gel, and were photographed under UV light. Segregation types of the primer pairs were determined by testing them using 12 F_1 progenies in two populations.

In order to identify the allele sizes of SSR primer pairs, the electrophoresis of PCR reactions were done on automatic sequencing device, the model of ABI 3130xl. In the consequence of capillary electrophoresis, the allele sizes produced by SSR primer pairs were defined on the Genemapper 4.0 software.

In this study, a total of 187 SSR primer pairs from Han et al. (2009), were used to determine segregation types of the primer pairs in 'Kaşel-41' x 'Williams Pride' and 'Kaşel-37' x 'Delbarestivale' F_1 segregating populations.

The possible segregation types in F_1 populations using SSR markers are given in Table 1. The segregation types considered 'abxcd', 'efxeg' and 'hkxhk' as heterozygous in both parents, are called as common markers. 'nnxnp' and 'lmxll' as heterozygous in the male and female, respectively (Van Ooijen and Voorrips, 2001).

SSR alleles (band) can also be scored as presence/absence, but some genetic information will be lost. In this case, three segregation types ('lmxll', 'nnxnp', 'hkxhk') will be available. The single allele is present in one of the parents and available in 50% of individuals was scored as 'lmxll' 'nnxnp'. If one allele is present in both parents and also available in 75% of individual was scored as 'hkxhk' (Table 2).

Results

All 187 SSR primer pairs used in this study were firstly tested by gradient PCR and 20 of them did not have amplification in both populations. After gradient PCR, all the primer pairs were screened using 12 F_1 individuals in each of two segregated populations. After capillary electrophoresis of the PCR reactions, 87 SSR primer pairs were monomorphic, and 80 of them were polymorphic in two populations (Table 3).

In 'Kaşel-41' x 'Williams Pride' F_1 population; 25 SSR primer pairs scored as dominant whereas 55 primer pairs had co-dominant segregating patterns: fourteen of them had 'abxcd', 11 of them had 'efxeg', 4 of them had 'hkxhk', 16 of them had 'lmxll', and 11 of them had 'nnxnp' segregation (Table 2).

In 'Kaşel -37' x 'Delbarestivale' F_1 population; 24 SSR primer pairs scored as dominant whereas 56 co-dominant markers were produced: 10 markers had 'abxcd' segregation, 13 markers had 'efxeg', 3 markers had 'hkxhk', 20 marker had 'lmxll', and 13 markers had 'nnxnp' segregation (Table 2).

The list of primers which showed polymorphism in two populations is given in Table 3. In both of the populations, 91 primers showed polymorphism. Some of primer pairs were monomorphic in population 'A' and was polymorphicin population 'B' or *vice versa*. According to the results, segregation patterns of polymorphic primer pairs were different in each population. Segregating patterns were determined for each primer as co-dominant (abxcd-efxeg- hkxhk-lmxll- nnxnp) or dominant (hkxhk- lmxll- nnxnp). In addition, some of primer pairs had amplification in more than one locus. Some primer pairs showed two co-dominant segregating loci such as 'BACSSR187' primer in both 'A' and 'B' populations, some of them showed co-dominant and dominant loci such as 'BACSSR40' and 'BACSSR155' loci in 'B' population (Table 4).

Discussion

Molecular markers can be used to study the relationship between an inherited trait and its genetic cause. It is known that pieces of DNA that lie near each other on a chromosome tend to be inherited together. This property enables the use of a marker, which can be used to determine the precise inheritance pattern of the gene that has not yet been exactly localized.

Genetic markers are employed in genealogical DNA testing for genetic genealogy to determine genetic distance between individuals or populations. Genetic markers have to be easily identifiable, associated with a specific locus, and highly polymorphic, because homozygotes do not provide any information. Some of the methods used to study for these purposes are RFLP, Amplified fragment length polymorphism (AFLP), RAPD, and SSR markers. SSR markers are rapid and relatively simple to use, and their banding pattern is almost always easy to interpret. (Smeets et al. 1989). Finding a set of highly polymorphic BAC-SSR markers may help future studies in Amasya apple genome.

A total of 187 BAC-SSR primers were tested in two apple F_1 populations in this study. The screening of BAC-SSR over in two populations allowed determining level of polymorphism in two F_1 segregating populations. The role of polymorphic assay procedures in plant breeding was quickly realized for cultivar and parental identification, gene identification and selection (Ainsworth and Sharp 1989; Soller and Beckmann, 1983).

For assessing the size of the amplified amplicons of cultivars, M13 universal labeled primers which have been already discussed by Schuelke (2000) were used. The absolute fragment size could be determined in the model of ABI 3130xl. The differences of the allele sizes in some primer pairs between the parents in this study were ±1 or ±2 bases. This range of allele sizes can't determine in other platforms such as polyacrylamide gel electrophoresis (This et al. 2004). The results of SSR electrophoresis are reproducible and exchangeable between laboratories (Jones et al. 1997).

All the tested SSR primer pairs in two populations in this study showed different segregating patterns. The primer pairs with different segregating patterns or having different allele sizes can be used in the characterization of apple germplasm or genetic mapping studies in different populations in apple. Moreover, these markers correspond to physical location of DNA on their chromosomes and marker loci in genetic mapping studies and allow the detection of difference between the individuals.

As a result of this study, polymorphism levels and segregation types of 91(51.1%) newly developed BAC-SSR primer pairs were determined they showed a high degree of polymorphism in two F_1 populations.

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Table 1. Co-dominant Segregation types in F_1 populations.

Segregation types	Female parent		Male Parent	
	Allele size(1)	Allele size(2)	Allele size(1)	Allele size(2)
abxcd (1:1:1:1)	a	b	c	d
efxeg (1:1:1:1)	e	f	e	g
hkxhk (1:2:1)	h	k	h	k
lmxll (1:1)	l	m	l	l
nnxnp (1:1)	n	n	n	p

Table 2. Dominant segregation types in F_1 populations and information about scoring system .

Segregation types	Female parent	Male Parent	Present allele size In individuals	Absent allele size In individuals
	Present allele size	Absent allele size		
hkxhk (1:3)	h-	h-	h-	kk
lmxll (1:1)	lm	ll	lm	ll
nnxnp (1:1)	nn	np	nn	np

Table 3. The results of screened SSR primer pairs in two population: (Pop A) 'Kaşel-41' x 'Williams Pride', (Pop B) 'Kaşel-37' x 'Delbarestivale'

Explanation of testing primers	K-41 x WP (Pop A)	K-37 x Delbarestivale (Pop B)
Number of tested primer pairs	187	187
Number of primer pairs not amplified in gradient PCR	20	20
Number of primer pairs amplified monomorphic	87	87
Number of primer pairs amplified polymorphic	80	80
Number of primer pairs amplified co-dominant	55	56
Number of primer pairs amplified dominant	25	24
Number of primer pairs had abxcd segregation	14	10
Number of primer pairs had efxeg segregation	11	13
Number of primer pairs had hkxhk segregation	4	3
Number of primer pairs had lmxll segregation	16*	20*
Number of primer pairs had nnxnp segregation	11*	13*

*one primer had two loci (lmxll and nnxnp)

Table 4. The information of SSR primer pairs which amplified polymorphisms in two population: (Pop A) 'Kaşel-41' x 'Williams Pride', (Pop B) 'Kaşel-37' x 'Delbarestivale'.

No	Primer	'Kaşel-41' x 'Williams Pride'	'Kaşel-37' x 'Delbarestivale'
		(Pop A)	(Pop B)
		Segregation patterns of markers	Segregation patterns of markers
1	BACSSR2	hkxhk	efxeg
2	BACSSR6	lmxll*/nnxnp*	lmxll*/nnxnp*/nnxnp*
3	BACSSR9	Monomorphic	nnxnp
4	BACSSR10	lmxll	efxeg
5	BACSSR11	lmxll	hkxhk
6	BACSSR12	lmxll	lmxll
7	BACSSR14	lmxll*/nnxnp*/nnxnp*	Monomorphic
8	BACSSR16	lmxll**/nnxnp**	lmxll*/nnxnp*/nnxnp*
9	BACSSR18	lmxll	lmxll
10	BACSSR19	hkxhk*/nnxnp*/nnxnp*	hkxhk*/lmxll*/nnxnp*
11	BACSSR20	abxcd	efxeg
12	BACSSR22	nnxnp	Monomorphic
13	BACSSR24	lmxll*	lmxll*/nnxnp*
14	BACSSR29	nnxnp*	nnxnp
15	BACSSR30	lmxll*/nnxnp*	lmxll*/nnxnp*
16	BACSSR32	lmxll*/lmxll*/nnxnp*	lmxll*/hkxhk*
17	BACSSR34	abxcd	efxeg
18	BACSSR35	efxeg	lmxll
19	BACSSR37	efxeg	efxeg
20	BACSSR39	lmxll*/nnxnp*/nnxnp*	lmxll
21	BACSSR40	Monomorphic	lmxll//nnxnp*
22	BACSSR42	abxcd	abxcd
23	BACSSR43	lmxll*/nnxnp*	lmxll*/nnxnp*
24	BACSSR45	Monomorphic	lmxll*/nnxnp*
25	BACSSR46	abxcd	lmxll
26	BACSSR47	efxeg	lmxll
27	BACSSR48	lmxll*/lmxll*/nnxnp*	Monomorphic
28	BACSSR51	efxeg	lmxll
29	BACSSR53	lmxll*/nnxnp*	lmxll*/nnxnp*

Continuing table 4

No	Primer	'Kaşel-41' x 'Williams Pride'	'Kaşel-37' x 'Delbarestivale'
		(Pop A)	(Pop B)
		Segregation patterns of markers	Segregation patterns of markers
30	BACSSR57	nnxnp	Monomorphic
31	BACSSR59	abxcd	efxeg
32	BACSSR61	Monomorphic	nnxnp
33	BACSSR62	lmxll*/lmxll*/nnxnp*	lmxll*/nnxnp*
34	BACSSR63	lmxll*/nnxnp*	lmxll*/nnxnp*/nnxnp*
35	BACSSR64	lmxll	abxcd
36	BACSSR65	lmxll	lmxll
37	BACSSR67	abxcd	lmxll
38	BACSSR68	abxcd	abxcd
39	BACSSR69	nnxnp	nnxnp
40	BACSSR70	lmxll*/lmxll*/nnxnp*	efxeg
41	BACSSR71	efxeg	abxcd
42	BACSSR72	abxcd	abxcd
43	BACSSR75	abxcd	abxcd
44	BACSSR78	Monomorphic	lmxll*/lmxll*
45	BACSSR79	lmxll	abxcd
46	BACSSR82	abxcd	efxeg
47	BACSSR83	abxcd	abxcd
48	BACSSR84	abxcd	lmxll*/nnxnp*
49	BACSSR87	Monomorphic	efxeg
50	BACSSR88	nnxnp	Monomorphic
51	BACSSR90	lmxll*/lmxll*/nnxnp*/nnxnp*	lmxll*/nnxnp*/nnxnp*
52	BACSSR91	nnxnp	nnxnp
53	BACSSR92	lmxll	lmxll
54	BACSSR93	Monomorphic	lmxll*/nnxnp*
55	BACSSR94	lmxll	lmxll*/nnxnp*/nnxnp*
56	BACSSR96	lmxll*/nnxnp*	lmxll*/nnxnp*/nnxnp*
57	BACSSR98	lmxll	abxcd
58	BACSSR99	efxeg	efxeg
59	BACSSR101	lmxll*/lmxll*/nnxnp*	efxeg
60	BACSSR105	Monomorphic	lmxll

Continuing table 4

No	Primer	'Kaşel-41' x 'Williams Pride'	'Kaşel-37' x 'Delbarestivale'
		(Pop A)	(Pop B)
		Segregation patterns of markers	Segregation patterns of markers
61	BACSSR108	lmxll*/nnxnp*	nnxnp*
62	BACSSR112	efxeg	lmxll*/nnxnp*/nnxnp*
63	BACSSR116	lmxll*/nnxnp*/nnxnp*	Monomorphic
64	BACSSR117	nnxnp	Monomorphic
65	BACSSR118	nnxnp	nnxnp*
66	BACSSR119	efxeg	Monomorphic
67	BACSSR120	lmxll*/nnxnp*/nnxnp*	Monomorphic
68	BACSSR122	abxcd	efxeg
69	BACSSR123	lmxll*/hkxhk*	Monomorphic
70	BACSSR128	efxeg	llxlm
71	BACSSR132	hkxhk	lmxll*/hkxhk*
72	BACSSR133	abxcd	abxcd
73	BACSSR136	nnxnp	nnxnp
74	BACSSR137	Monomorphic	nnxnp
75	BACSSR139	efxeg	lmxll
76	BACSSR143	Monomorphic	nnxnp
77	BACSSR145	efxeg	lmxll
78	BACSSR149	hkxhk*	lmxll*
79	BACSSR153	hkxhk*	nnxnp
80	BACSSR155	lmxll	lmxll /hkxhk*/nnxnp*
81	BACSSR156	lmxll	lmxll
82	BACSSR162	lmxll	lmxll
83	BACSSR164	lmxll	efxeg
84	BACSSR169	lmxll*/nnxnp*/nnxnp*	Monomorphic
85	BACSSR173	nnxnp	nnxnp
86	BACSSR174	Monomorphic	nnxnp
87	BACSSR178	nnxnp	nnxnp
88	BACSSR180	lmxll	lmxll
89	BACSSR181	hkxhk	hkxhk
90	BACSSR182	hkxhk	hkxhk
91	BACSSR187	nnxnp/lmxll	nnxnp/lmxll

* Dominantly scored markers

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