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Development of a simple sequence repeat (SSR) marker set to fingerprint local and modern potato varieties grown in central Anatolian Plateau in Turkey

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Most of the potato cultivars grown in Turkey are of foreign origin and there are some local landrace varieties. Tuber seeds of most of these varieties are not produced and distributed in an organized way and many disputes appear as to the identity of the cultivar. An efficient variety identification system is required to solve these problems. In the present study, 16 selected good quality simple sequence repeat (SSR) markers were evaluated in a total of 15 varieties, six of which were local landraces and nine were modern genotypes. Landraces were Başçiftlik Beyazi, Aleddiyan Sarisi, Aleddiyan Beyazi, Aybasti Beyazi, Aybasti Sarisi and Gölköy. Modern varieties were Marfona, Agria, Hermes, Cosmos, Burren, Milva, Agata, Latona and Slaney. Fifteen of the used 16 markers were polymorphic on used cultivars. Markers had one to six band configurations on each genotype (average 3.69). Diversity index values of the markers varied from 0 to 0.720 (average 0.528). Marker data showed that three of the six potato landrace varieties with different local names were essentially the same. Using a set of five SSR markers, all cultivars tested could be easily identified, proving the power of SSR markers as fast and reliable tools for genetic fingerprinting purposes.

Key Words: Diversity, microsatellite markers, polymorphism, *Solanum tuberosum*.

INTRODUCTION

Potato is an economically significant crop worldwide including Turkey. However, since Turkey does not have a major potato breeding program, most of the varieties grown are of foreign origin. There are about 80 modern potato varieties used and only two are of Turkish origin. There are also some landraces grown in Turkey. Most of the landraces have not been characterized based on molecular biology techniques so far, although they are grown in considerable amounts. Some of these landraces are preferred by consumers because of their alleged superior taste. Farmers name landraces based on their growing area and sell accordingly. With so many cultivars and landraces grown, there appear to be some problems

relating to the identification of tuber seeds sold to farmers as well as tubers sold to consumers.

Simple sequence repeats (SSRs), also called micro-satellites, are effective marker systems used in potato as well as in many other crops. SSR marker systems have many advantages over other markers such as ease of analysis, high polymorphism rate, high reliability, co-dominance and transferability among related species (Rafaleski et al., 1996; Yildirim et al., 2009). Milbourne et al. (1998) developed 112 SSR markers and used them in a panel of six potato genotypes, two diploids and two tetraploids. They reported that 98 markers had high level of polymorphism. Using 65 of these markers, investi-gators mapped 89 loci in two potato mapping populations. Ghislain et al. (2004) developed 48 SSR markers and used them to identify 931 potato germplasms. In addition, they mapped 31 of these markers using mapping populations. Feingold et al. (2005) developed 94

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SSR markers and used 61 of them for mapping and 30 for genetic characterization of 30 potato cultivars. These studies indicate that SSR markers are useful genomic tools in potato as in other crop species. SSR markers are used to differentiate potato cultivars. Reid and Kerr (2007) used 28 SSR markers in 121 potato cultivars and obtained 5 - 57 configurations per marker. The investigators determined diversity indices varying from 0.64 to 0.97. Screening 30 South and North American and European cultivars with 61 SSR markers, Feingold et al. (2005) determined 1 - 24 (average 11.6) configurations per marker. The diversity index values of the cultivars varied from 0 to 0.95 (average 0.81). Milbourne et al. (1997) studied 16 potato cultivars with 17 SSR markers and obtained an average of 0.73 diversity index per marker. Moisan-Thiery et al. (2005) screened 286 French potato cultivars with four SSR markers. They obtained 12 - 15 configurations for three markers and 55 configurations for one marker. The diversity indices of these four markers were between 0.79 and 0.91 (average 0.84). Ruiz de Galerrata et al. (2007) studied 19 potato landraces from Canary Islands using 19 SSR markers. The investigators found a total of 62 configurations. Thus, SSRs are useful tools for cultivar identification in potato.

There are many landrace and modern potato varieties grown in Turkey. On occasions, farmers complain that the tuber seeds they buy are not the ones they intend to buy. In addition, due to the lack of genetic characterization, tubers of the same landrace are sold on markets with different names coming from the area in which they are grown. On these occasions, it may be necessary to differentiate potato varieties rapidly, reliably and efficiently to help farmers and consumers make their decisions.

The aim of the current study was to determine a set of SSR markers that can be used rapidly and reliably to characterize major potato landraces and modern cultivars used in the Central Anatolia Plateau, which will be useful to guide the farmers in buying the correct tuber seeds as well as in forming expert opinion in court cases.

MATERIALS AND METHODS

In this investigation, six potato landraces and nine modern varieties grown in Tokat province located in the Central Anatolian Plateau in Turkey were fingerprinted using 16 SSR markers. Landraces were Başçiftlik Beyazi, Aleddiyan Sarisi, Aleddiyan Beyazi, Aybasti Beyazi, Aybasti Sarisi and Gölköy, while modern varieties were Marfona, Agria, Hermes, Cosmos, Burren, Milva, Agata, Latona and Slaney. Genomic DNA was isolated using Fermentas Genomic DNA Isolation Kit (Catalog No: K0512). A young leaf about 2 cm in diameter was ground in an Eppendorf tube with liquid nitrogen using pipette tips. DNA quality was checked and the amount was adjusted using a spectrophotometer and a 1% agarose gel. DNA concentrations of the samples were adjusted to 50 ng/μl.

In this study, SSR markers were used, in which specifically designed primers around microsatellite repeats were employed in polymerase chain reaction (PCR) reactions. Eight STM (19, 30, 31, 37, 1052, 1106, 2013 and 3012) and eight STI (24, 30, 32, 33, 42, 53, 57 and 58) SSR markers were used (Table 1). STMs were developed by Milbourne et al. (1998) and STIs by Feingold et al.

(2005). All SSR markers were selected based on their good quality (low copy number and absence of stutter bands) and high diversity index values (Ghislain et al., 2004; Milbourne et al., 1998; and Feingold et al., 2005). Total PCR reaction volume was 40 μl. A PCR reaction composition was as follows: 250 nM of each primer, 0.2 mM of each nucleotide (dNTPs), 1.5 mM $MgCl_2$, 0.5 units of *Taq*-DNA polymerase (Promega) and 50 ng genomic DNA as template. Typical PCR conditions were: following 5 min at 94°C, 32 cycles of 1 min at 94°C, 1 min at 50 - 60°C (depending upon primer annealing temperature), 1 min at 72°C and final extension for 5 min at 72°C. PCR products were run on a 3% metaphore agarose gel system (Cambrex Corporation, USA) with 1% tris/borate/ethylene-diaminetetraacetic acid (EDTA) (TBE) buffer. DNA was visualized by the addition of ethidium bromide to the gel and its photograph was taken using a gel imaging system (Vilber Lourmat CN–08, France). DNA bands were scored by Biocapt software (Version: 11.02). Most of the loci in potato, a clonally propagated plant, are expected to be heterozygous. Therefore, an SSR marker amplifying a single locus can yield up to four bands in potato, a tetraploid species. Therefore, some bands could appear as more than one copy and consequently as denser than others. This fact, also known as dosage effect, makes the bands difficult to analyze and decrease the reliability of the DNA analysis (Moisan-Thiery, 2005). In order to overcome this problem, potato researchers prefer scoring the configuration of all bands as a dominant marker rather than scoring each band separately (Milbourne et al., 1997; Feingold et al., 2005). In the present study, the varieties with the same band configurations were considered diploid genotypes having the same dominant allele of the marker. Assuming that each SSR marker amplifies a single locus, the diversity index values that show the differentiating power of a marker was calculated according to Milbourne et al. (1997):

$$DI = 1 - \Sigma\, fg^2$$

Where, DI, Diversity index; fg, frequency of each genotype.

Relationships among the varieties were calculated according to Nei (1978). Based on these relationship values, dendograms were prepared using UPGMA algorithm by POPGENE software (Version 1.31; Yeh et al., 1997).

RESULTS AND DISCUSSION

Results from 16 SSR markers used on 15 potato varieties studied are given in Table 2. A sample gel picture of an SSR marker is given in Figure 1. Number of different bands produced by each SSR markers varied from two to six bands (average 3.75) (Table 3). The highest number of different bands was produced by STM30 and STM 1106. Polymorphism was observed with all markers used except for STI30. An average of 3.69 configurations was obtained from 16 SSR markers (Table 3). The highest number of configurations was observed in STM1052 and STM31 as six configurations, followed by five configurations in STM30, STM19 and STM1106; four in STI33, STI42, STI24 and STM37; three in STM3012, STI32 and STI57 and two in STI58, STI53 and STM2013, respectively. Number of configurations per variety (3.69/ 15) in the present study is 0.246. This value is somewhat low compared to the ones from Feingold et al. (2005) and Milbourne et al. (1997) where the number of configurations per marker is 0.387 in 30 varieties (11.6 configurations/30

Table 1. Some properties of SSR primers studied (Milbourne et al., 1998; Feingold et al., 2005).

Primer	Primer sequence	Annealing temp. (ºC)	Expected size (bp)	Chromosome
STM0030-F	AGAGATCGATGTAAAACACGT	53	147	XII
STM0030-R	GTGGCATTTTGATGGATT			
STM0019-F	AATAGGTGTACTGACTCTCAATG	47	155	VI
STM0019-R	TTGAAGTAAAAGTCCTAGTATGTG			
STM2013-F	TTCGGAATTACCCTCTGCC	55	160	VII
STM2013-R	AAAAAAAGAACGCGCACG			
STM1106-F	TCCAGCTGATTGGTTAGGTTG	55	156	X
STM1106-R	ATGCGAATCTACTCGTCATGG			
STI57-F	CCTTGTAGAACAGCAGTGGTC	60	196	IX
STI57-R	TCCGCCAAGACTGATGCA			
STI33-F	TGAGGGTTTTCAGAAAGGGA	64	134	VII
STI33-R	CATCCTTGCAACAACCTCCT			
STI30-F	TTGACCCTCCAACTATAGATTCTTC	60	107	XII
STI30-R	TGACAACTTTAAAGCATATGTCAGC			
STI42-F	TCACGAGGTGCCCAACTG	60	133	VII
STI42-R	TCCATTCGTCAACAAGGT			
STI24-F	CGCCATTCTCTCAGATCACTC	60	168	II
STI24-R	GCTGCAGCAGTTGTTGTTGT			
STM3012-F	CAACTCAAACCAGAAGGCAAA	57	193	IX
STM3012-R	GAGAAATGGGCACAAAAAACA			
STM1052-F	CAATTTCGTTTTTTCATGTGACAC	50	248	VII
STM1052-R	ATGGCGTAATTTGATTTAATACGTAA			
STM0031-F	CATACGCACGCACGTACAC	57	172	VII
STM0031-R	TTCAACCTATCATTTTGTGAGTCG			
STI58-F	CAAGCACGTTACAACAAGCAA	60	121	V
STI58-R	TTGAAGCATCACATACACAAACA			
STI53-F	TCAGACCGGGTTCGATGG	60	160	II
STI53-R	CGGCTTGAATCATTGCCCA			
STM0037-F	AATTTAACTTAGAAGATTAGTCTC	53	90	XI
STM0037-R	ATTTGGTTGGGTATGATA			
STI32-F	TGGGAAGAATCCTGAAATGG	64	121	V
STI32-R	TGCTCTACCAATTAACGGCA			

Table 2. SSR marker sizes and configurations of local and modern potato varieties grown in Tokat Province.

Marker	Feature	BB	MR	AG	HR	CS	BR	ML	AT	LT	SL	GL	AS	AB	AL	AY
STM30	Bands	150 163	150 163	140 143	143 163	150 163	163 166	137 143 150	143 163	150 163	143 163	163	163 166	150 163	150 163	150 163
	Config.	A	A	E	C	A	B	D	C	A	C	B	B	A	A	A
STM19	Bands	158	150 185	158	158 185	150	158 192	150 185	158 185	150 185	158 185	158	158	158	158	150 185
	Config.	A	B	A	C	D	E	B	C	B	C	A	A	A	A	B

Table 2. Cont'd

STM2013	Bands	143 155	143 155	143 155	140 150	140 150	140 150	143 155	140 150	140 150	143 155	143 155	143 155	143 155	143 155	140 150
	Config.	A	A	A	B	B	B	A	B	B	A	A	A	A	A	B
STM1106	Bands	185 240	160 200 240	160 200 240	160 200	160 200	220 240	160 200 240	170 185	160 200	160 200	160 200	160 200	185 240	185 240	160 200
	Config.	A	D	D	E	E	C	D	B	E	E	E	E	A	A	E
STI57	Bands	185 205	185	193	185	185	185 205	185	193	193	185	193	193	185 205	185 205	193
	Config.	A	C	B	C	C	A	C	B	B	C	B	B	A	A	B
STI33	Bands	111 129	111 129	111 129	108 129	129	111 129	111 129	108 129	111 129	111 129	129	129	111 129	111 129	129 132
	Config.	A	A	A	C	B	A	A	C	A	A	B	B	A	A	D
STI30	Bands	107 110	107 110	107 110	107 110	107 110	107 110	107 110	107 110	107 110	107 110	107 110	107 110	107 110	107 110	107 110
	Config.	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
STI42	Bands	126 137	122 133 141	122 133	122 133	133 141	118 122	133 141	118 122	122 133	133 141	122 133	122 133	126 137	126 137	122 133
	Config.	A	C	D	D	C	B	C	B	D	C	D	D	A	A	D
STI24	Bands	168 177	168	168	143 163 177	168 177	168	168	168 177	143 163 177	168 177	163 177	163 177	168 177	168 177	143 163 177
	Config.	A	C	C	B	A	C	C	A	B	A	D	D	A	A	B
STM3012	Bands	166 198	166 198	166 198	166	198 205	166 198	166 198	198 205	198 205	198 205	198 205	198 205	166 198	166 198	198 205
	Config.	A	A	A	C	B	A	A	B	B	B	B	B	A	A	B
STM1052	Bands	200 206 222 240	206 240	206 240	206	206 240	206 222	206 240	200	222 240	206 240	206	206	206 240	206 240	240
	Config.	A	A	A	B	A	C	A	D	E	A	B	B	A	A	F

Table 2. Contd.

STM31	Bands	129	129	113 193	129 172	113	172 193	172 193	172 193	172 193	172 193	129	129	129	129	129 172 193
	Config.	A	A	B	C	D	F	F	F	F	F	A	A	A	A	E
STI58	Bands	96	96	96	96	96	96	96	96	102	96	96	96	96	96	102
	Config.	A	A	A	A	A	A	A	A	B	A	A	A	A	A	B
STI53	Bands	130 162	130	130	130	130	162	130	130	130	130	130	130	130	130	130
	Config.	A	A	A	A	A	B	A	A	A	A	A	A	A	A	A
STM37	Bands	80	80	80	87	80	77 94	80	80	77	77 94	77	77	80	80	77
	Config.	A	A	A	C	A	D	A	A	B	D	B	B	A	A	B
STI32	Bands	114 121	114 121	114 121	114 121	114 121	126	114 121	114 121	114 121	126	114 121	114 121 126	114 121	114 121	114 121
	Config.	A	A	A	A	A	B	A	A	A	B	A	C	A	A	A

BB, Başçiftlik Beyazi; MR, Marfona; AG, Agria; HR, Hermes; CS, Cosmos; BR, Burren; ML, Milva; AT, Agata, LT, Latona, SL, Slaney, GL, Gölköy; AS, Aleddiyan Sarisi; AB, Aybasti Beyazi; AL, Aleddiyan Beyazi; AY, Aybasti Sarisi.
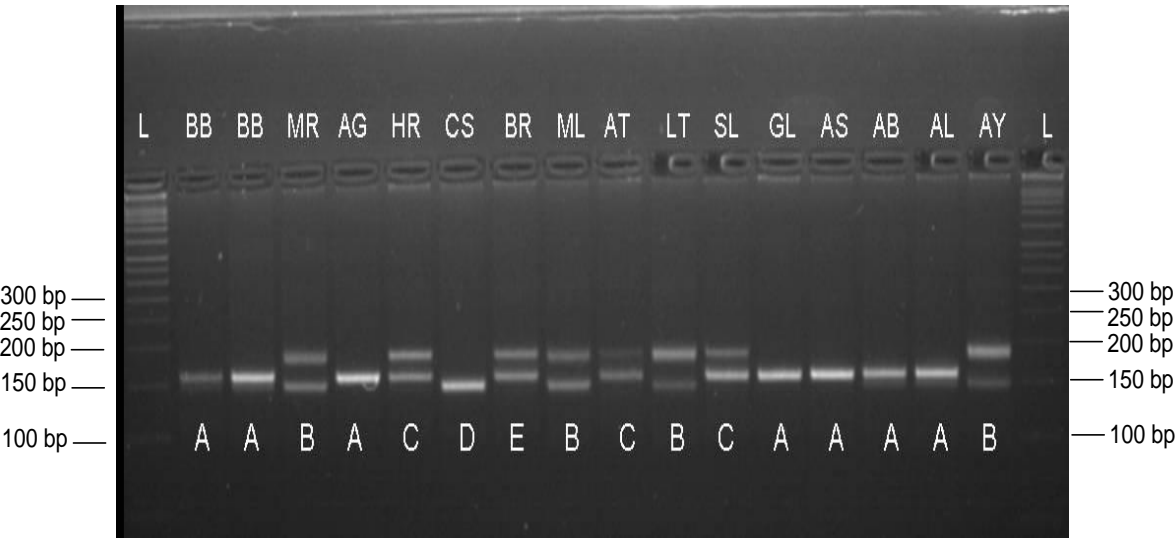

Figure 1. STM 19 SSR marker configurations of some local and modern potato varieties. BB, Başçiftlik Beyazi; MR, Marfona; AG, Agria; HR, Hermes; CS, Cosmos; BR, Burren; ML, Milva; AT, Agata, LT, Latona, SL, Slaney, GL, Gölköy; AS, Aleddiyan Sarisi; AB, Aybasti Beyazi; AL, Aleddiyan Beyazi; AY, Aybasti Sarisi. L: 50 bp DNA ladder (SIGMA S7025). Letters below the bands show the marker band configurations considering all bands produced in a variety.

varieties) and 0.360 in 16 varieties (5.76 configurations/ 16 varieties), respectively. However, the average configuration number in the present study is higher than that of Ruiz de Galerrata et al. (2007) who found an average of 0.171 configurations in 19 Spanish landraces. Relatively, low number of configurations in the present study can be

Table 3. Number of bands and configurations, and diversity index values of SSR markers.

SSR marker	Total number of different bands	Number of configurations	Diversity index
STM30	6	5	0.693
STM19	4	5	0.720
STM2013	4	2	0.480
STM1106	6	5	0.693
STI57	3	3	0.658
STI33	4	4	0.531
STI30	2	1	0
STI42	5	4	0.711
STI24	4	4	0.711
STM3012	3	3	0.560
STM1052	4	6	0.658
STM31	4	6	0.710
STI58	2	2	0.231
STI53	2	2	0.124
STM37	4	4	0.622
STI32	3	3	0.338
Average	3.75	3.69	0.528

ascribed to the use of metaphore agarose gel, where only the major bands could be detected, while weak bands were not clearly observed. Data from these weak bands are mostly unreliable (Palombi and Damiano, 2002). Thus, metaphore agarose gel system takes into account only reliable information and losses only a few. Another possible reason for the low number of band configurations demonstrated in the present study was that six of the 15 varieties used were closely related local varieties. Actually, three of the 15 varieties used were exactly the same. This fact lowered the diversity indices as demonstrated in the low number of band configurations recorded in the whole varieties tested. Nevertheless, the SSR marker system worked perfectly to differentiate potato varieties studied.

Diversity index (DI) values, which shows the informativeness of a marker, ranged from 0 to 0.720 (Table 3). STM19 had the highest value and was followed by STI42, STI24, STM31 and STM1106, respectively, with diversity indices of about or over 0.7. Eleven of the 16 studied SSR mar-kers had DI values over 0.5. These values are relatively low compared to the ones reported in literature. Milbourne et al. (1997) found that average diversity index value of 17 SSR markers used in 16 varieties was 0.73. Studying 286 French cultivars with five SSR markers, Moisan-Thiery et al. (2005) found diversity index values ranging from 0.79 to 0.91. In another study, Reid and Kerr (2007) evaluated 28 SSR markers in 121 potato varieties, where they found diversity index values between 0.64 - 0.97 in six best markers.

A dendogram based on the relationship values among the varieties was prepared using marker information (Figure 2). Three local varieties with white flesh tubers (Başçiftlik Beyazi, Aybasti Beyazi and Aleddiyan Beyazi) had completely the same marker configurations. Thus, although named differently according to the growing area, these local varieties are the same. This could be another reason why the number of configurations per variety was low in the present study. Three local varieties with yellow flesh tubers appeared to be closely related to each other. SSR markers could differentiate all modern varieties studied. Only Marfona and Milva cultivars were close to each other and all other cultivars were easily distinguished. As few as five SSR markers (STM19, STM31, STM3012, STI32 and STI42) were able to differentiate all varieties studied in this investigation.

In conclusion, evaluation of 16 selected good quality SSR markers in six landraces and nine modern potato varieties turned out to be very efficient in distinguishing among potato varieties. Five SSR markers (STM19, STM31, STM3012, STI32 and STI42) were enough to differentiate all cultivars studied in the present study. Marker data revealed that three landraces with different local names were in fact the same genotypes. All these findings showed the great potential of SSR markers as fast and efficient tools for genetic fingerprinting purposes.
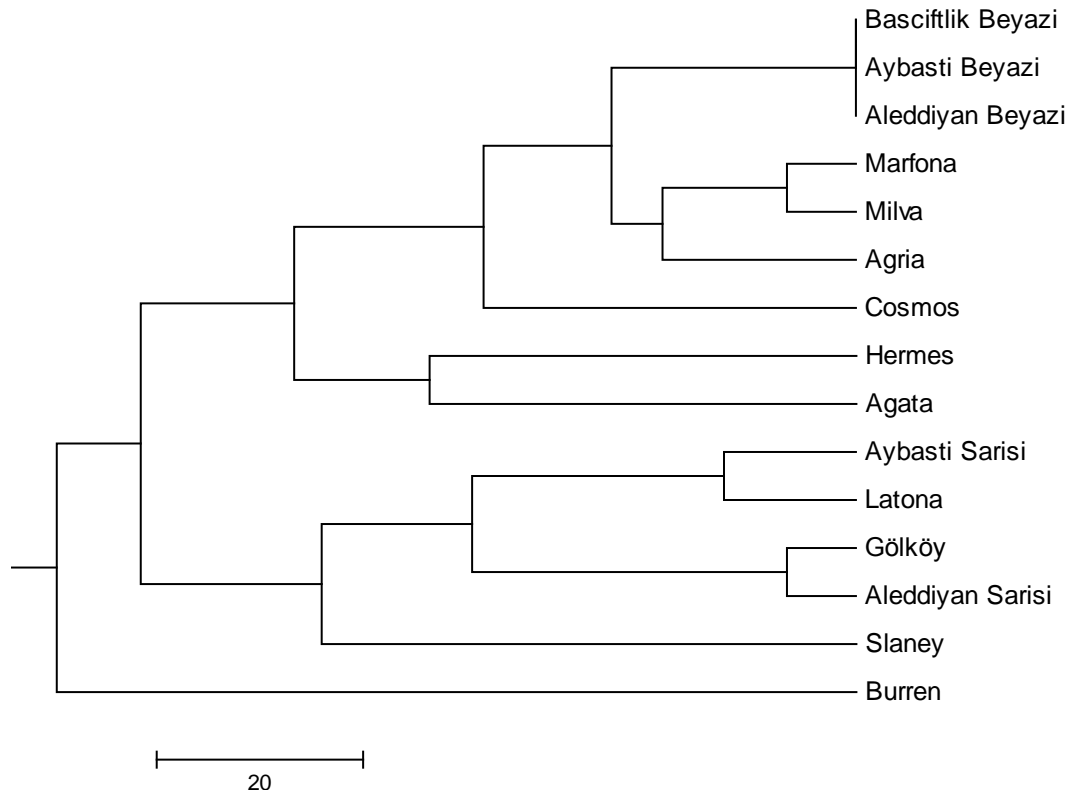
Figure 2. Tree dendogram prepared based on SSR marker data from 15 potato varieties grown in Central Anatolian Plateau of Turkey.

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