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ISOLATION OF DIFFERENT GENOTYPES IN 'BAŞÇIFTLIK BEYAZI' POTATO LANDRACE USING SSR MARKERS

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ABSTRACT

Potato is an important crop in Turkey. Along with modern cultivars, there are some potato landrace cultivars grown mainly for their superior taste. 'Başçiftlik Beyazı' is such a landrace cultivar grown in the Mid-Black Sea region of Turkey and used as baked potato. Since this cultivar is not registered, certified seed is not available. Therefore, tuber production suffers from serious yield and quality losses mainly due to viral diseases. The aim of the present study was to determine a main genotype and different genotypes within the cultivar using 16 SSR markers. SSR analyses were conducted over 191 'Başçiftlik Beyazi' plants. Nine of the 16 SSR markers were polymorphic. The data showed that landrace cultivar 'Başçiftlik Beyazi' consisted of 23 different genotypes. The same marker profile was present in 150 of the 191 plants. SSR analyses conducted on some modern and landrace cultivars grown in the region showed that the different genotypes were not the remnants of the previous years' potato crop. This variation could be directly used in potato breeding. SSR marker data also indicated that three local cultivars with different names were actually the same.

Key words: Potato, SSR markers, landrace cultivar.

INTRODUCTION

Potato is an important cash crop in Turkey. Most of the potato cultivars grown in Turkey are of foreign origin. There are some landrace cultivars grown by farmers mostly for their superior taste for direct consumption. 'Başçiftlik Beyazı' (in English meaning: Headfarm White) is one of such cultivars grown in the mid-Blacksea Region of Turkey in Tokat and Ordu provinces. Tubers of this landrace have white-colored flesh and are mostly used for baked potato. However, the landrace has not been genetically identified yet and, therefore, has not been registered. There is not any authority to maintain its clean seed and poor seed quality hampers production of good quality tubers and also decreases the tuber yields.

Landrace cultivars are significant sources of genetic variation. Ispizua *et al*. (2007) identified 72 different genotypes in 155 potato accessions that belong to 59 landraces. Ruiz de Galareta *et al*. (2007) determined 62 different potato genotypes in 19 landraces from two sub-species. Zimmerer and Douches (1991) found 30 different genotypes among 139 plants from six potato landraces. The investigators commented that the main source of genetic variation in landraces of potato, a clonally propagated crop, is probably sexual recombination rather than mutations.

DNA markers are effectively used to differentiate genotypes of related origin. SSRs (microsatellites) are one of such marker systems used in potato as well as many other crops. SSRs have advantages over other markers. They are easy to analyze, highly polymorphic, highly reliable, codominant and transferable among related species (Rafaleski *et al*., 1996; Yildirim *et al*. 2009). Milbourne *et al*., (1998) developed 112 SSR markers and used them in six potato genotypes. The investigators mapped 89 loci using 65 of these markers in two potato mapping populations. Ghislain *et al*. (2004) developed 48 SSR markers and used them to fingerprint 931 potato germplasms. They mapped 31 of these markers. Feingold *et al*. (2005) developed 94 SSR markers and used 61 of them for mapping and 30 of them for genetic characterization of 30 potato cultivars. All these studies show the usefulness of SSR markers as genomic tools in potato.

Diversity indices and number of alleles show the usefulness of markers. Reid and Kerr (2007) obtained 5-57 configurations per SSR marker as the average of 28 SSR markers in 121 cultivars. They determined that the diversity indices of these markers varied from 0.64 to 0.97. Feingold *et al*. (2005) found 1-24 (average 11.6) configurations per marker when they screened 30 South and North American and European cultivars with 61 SSR markers. 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. The diversity indices of these four markers were between 0.79 and 0.91 (average 0.84). Thus, SSRs are useful tools for cultivar identification in potato.

Although Turkey is not an origin center of potato, the growing of this crop has long been conducted in Turkey. There are some landrace cultivars still used in certain regions of Turkey. These cultivars are not genetically identified. The objective of the present study was to determine a main genotype and other different genotypes in 'Başçiftlik Beyazi' potato landrace using SSR markers.

Table 1. 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	4	3	0.296
STM19	3	3	0.061
STM2013	4	4	0.081
STM1106	4	4	0.208
STI57	3	3	0.181
STI33	3	3	0.137
STI30	2	2	0.179
STI42	4	5	0.121
STI24	3	3	0.189
STM3012	2	1	0.000
STM1052	3	1	0.000
STM31	1	1	0.000
STI58	2	1	0.000
STI53	1	1	0.000
STM37	2	1	0.000
STI32	2	1	0.000
Average	2.69	2.32	0.097

MATERIALS AND METHODS

In order to identify genetic constitution of 'Başçiftlik Beyazi' landrace cultivar, farmers' fields were sampled in the Başçiftlik district of Tokat province of Turkey. A total of 191 plants were labeled during vegetative growth and their tubers were harvested. Since the most common type and different genotypes were targeted within 'Başçiftlik Beyazi' landrace, about the same numbers of both typical and different looking plants were sampled.

After the first indications about the presence of different genotypes within the landrace based on SSR tests, the question of whether these genotypes were the remnants of some modern cultivars grown in the fields in the previous years needed to be answered. For this purpose, nine modern cultivars and five landrace cultivars were also subjected to SSR analyses.

Genomic DNA was isolated using a genomic DNA isolation kit (Fermentas Catalog No: K0512). A young leaf about 2 cm long was ground in an Eppendorf tube with liquid nitrogen. DNA quality was checked and amount was adjusted using a spectrophotometer and on a 1% agarose gel. DNA concentrations of the samples were adjusted to 50 ng/µl.

SSR markers used were eight STMs (19, 30, 31, 37, 1052, 1106, 2013 and 3012) and eight STIs (24, 30, 32, 33, 42, 53, 57 and 58). 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*., 2008 for STMs and Feingold *et al*., 2005 for STIs).

Total PCR reaction volume was 40 µl. The PCR reaction composition was as follows: 250 nM of each primers, 0.2 mM each nucleotide, 1.5 mM $MgCl_2$, 0.5 units of *Taq*-DNA polymerase (Promega) and 50 ng genomic DNA as template.

Table 2. Genotype groups of plants within 'Başçiftlik Beyazı' potato landrace based on SSR marker data

Genotype group No:	Number of plants	Plant number
1	150	Remaining plants
2	1	168
3	1	114
4	1	38
5	1	150
6	1	178
7	1	49
8	1	189
9	12	11, 21, 73, 90, 91, 97, 124, 138, 140, 147, 148, 167
10	1	66
11	1	53
12	1	26
13	1	60
14	5	3, 40, 55, 85, 113
15	1	95
16	2	135, 137
17	1	46
18	1	65
19	1	41
20	2	8, 16
21	2	24, 133
22	1	106
23	2	31, 34

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) and 1 min. at 72 °C, and 5 min. at 72 °C.

PCR products were run on a 3% Metaphore Agarose gel system with 1% TBE buffer. DNA was visualized by ethidium bromide added to the gel and photographed using a gel imaging system (Vilber Lourmat CN–08). 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, although an SSR marker amplifies a single locus, it 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 of 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 genotypes with the same band configurations were considered diploid having the same dominant allele of the marker. Assuming that each SSR marker amplified a single locus, Diversity Index values that showed the differentiating power of a marker was calculated as follows (Milbourne *et al*. 1997):

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

fg: Frequency of each genotype.

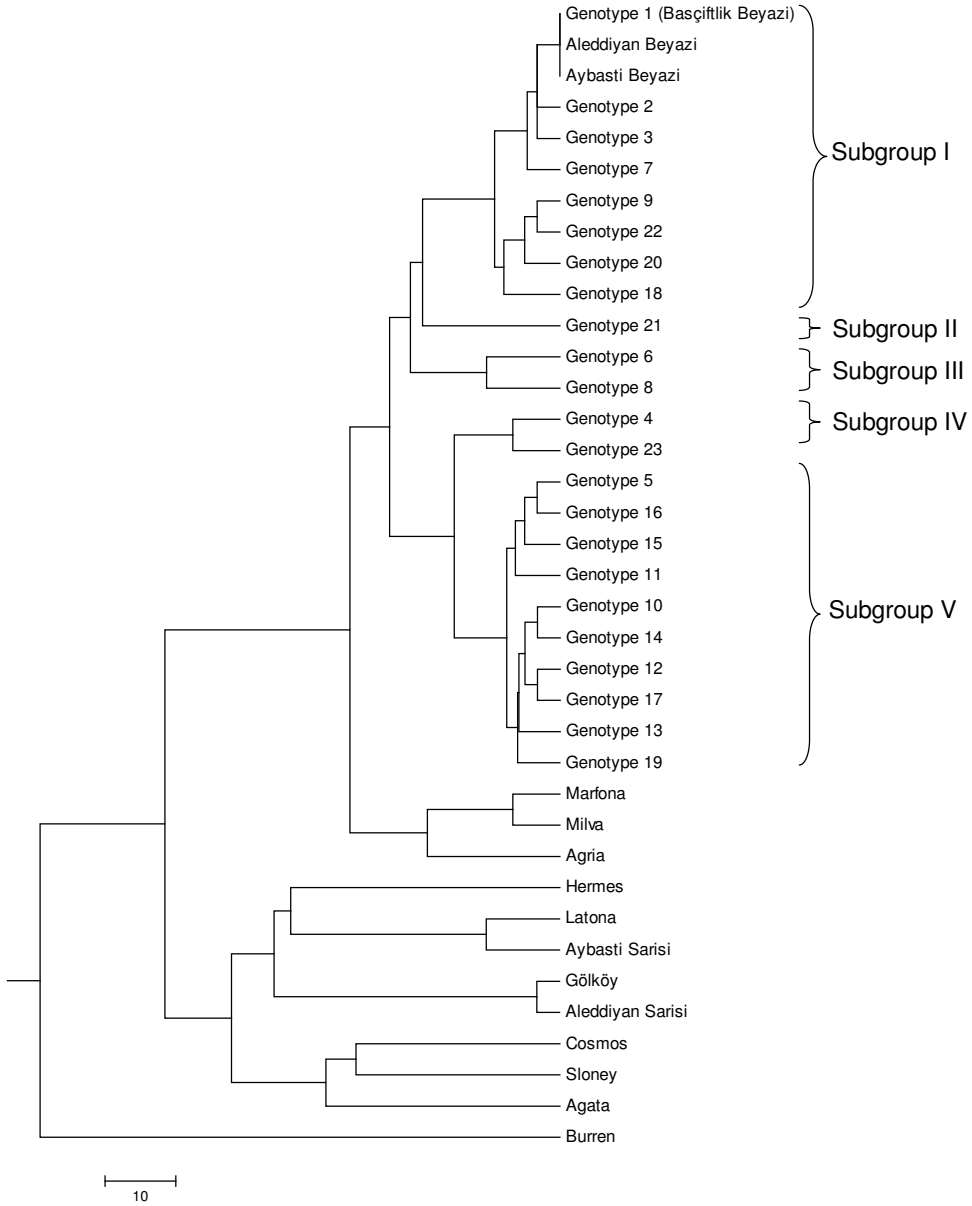

Figure 1. Dendogram analysis of genotypes within 'Başçiftlik Beyazı' potato landrace and some modern and landrace cultivars grown in the region based on SSR marker profiles.

Relationships among the genotypes 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

Nine of the 16 SSR markers used (STM30, STM19, STM2013, STM1106, STI57, STI33, STI30, STI42 and STI24) showed polymorphism in 191 sampled 'Başçiftlik Beyazi' plants (Table 1). PCR reactions produced 1 to 4 bands (average 2.69). Average number of marker configurations, which are the different combinations of bands, was 2.52. The highest number of marker configurations was obtained from STI42 (five configurations) followed by STM2013 and STM1106 (four configurations), STM30, STM19, STI57, STI33 and STI24 (three configurations) and STI30 (two configurations). Number of bands and configurations produced among 'Başçiftlik Beyazi' plants were quite low as compared to the numbers obtained in other studies (Feingold *et al*. 2005; Moisan-Thiery *et al*., 2005; Reid and Kerr, 2007). This was anticipated since the plants from a clonally propagated landrace are expected to be genetically related and they do not produce many different bands and marker configurations.

For diversity indices, which show polymorphism levels of markers, STM30 had the highest value (0.296) followed by STM1106, STI24, STI57 and STI30. These values are relatively low as compared to the values given in literature (Milbourne *et al*., 1997; Moisan-Thiery *et al*., 2005; Reid and Kerr, 2007). The low level of diversity indices is again the result of close relationship among the plants of 'Başçiftlik Beyazi' landrace.

SSR marker data showed that 150 of the 191 sampled plants had the same marker profile, and the other 41 had different profiles (Table 2). There were 23 different genotype groups in 'Başçiftlik Beyazi' landrace. These were assigned group numbers and plants within each genotype group entered the dendogram analysis as one genotype. The most common genotype group had 150 plants and was accepted to be 'Başçiftlik Beyazi' main genotype. The second most common genotype group (Genotype 9) had twelve plants and the third (Genotype 14) had five. Genotype groups (16, 20, 21 and 2) had two plants. Other genotype groups had only one plant.

According to the dendogram analysis, genotypes in 'Başçiftlik Beyazi' could be separated into five subgroups (Figure 1). Common genotype (Genotype 1) and Genotypes 2, 3, 7, 9, 22, 20 and 18 constituted subgroup I. Genotype 21 alone constituted subgroup II. Subgroup III consisted of Genotypes 6 and 8, and subgroup IV consisted of Genotypes 4 and 23. The other genotypes were in subgroup V.

In order to determine whether the different genotypes in 'Başçiftlik Beyazi' landrace were the remnants of other cultivars grown in the fields previous years, 16 SSR markers were also used to compare the different genotypes with nine modern and five landrace cultivars grown in the region. A gel picture of an SSR marker using such cultivars is given in Figure 2. Marker data from other cultivars were also included in the dendogram analysis (Figure 1). None of the genotypes in 'Başçiftlik Beyazi' had the same marker profile as modern cultivars grown in the region. Besides, all 23 genotypes appeared to be close in dendogram analysis. These results show that genotypes in 'Başçiftlik Beyazi' are not the remnants of other cultivars grown in the region, and they are different clones in 'Başçiftlik Beyazi' landrace.

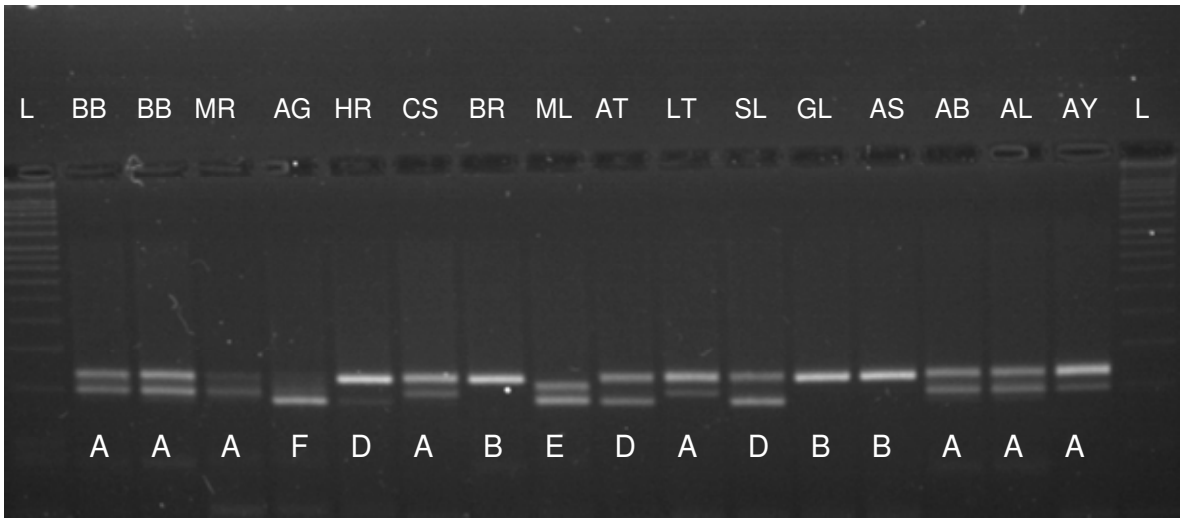

Figure 2. STM30 SSR marker configurations of some local and modern potato cultivars. MR, Marfona; AG, Agria; HR, Hermes; CS, Cosmos; BR, Burren; ML, Milva; AT, Agata, LT, Latona, SL, Slaney, GL, 'Gölköy'; AS, 'Aleddiyan Sarisi'; AB, 'Aybasti Beyazi'; AL, 'Aleddiyan Beyazi'; AY, 'Aybasti Sarisi'. L: DNA ladder. Letters below the bands show the marker band configurations considering all bands produced in a cultivar.

Twenty-three different genotypes in 191 sampled plants of 'Başçiftlik Beyazi' landrace cultivars indicate a quite high genetic variation for a clonally propagated crop. About half of the sampled plants were taken based on their morphological appearance, such as plant structure and leaf shape. This fact definitely increased the variation in sampled plants and helped to isolate as many different genotypes as possible, which will be subjected to agronomical, chemical and quality analyses later. This level of variation is quite high for a clonally propagated crop such as potato in a region which is not one of the origin centers for potato. High levels of variations within potato landraces were also reported by other investigators. Ispizua *et al*., (2007) determined 72 different genotypes in 155 accessions from 59 landraces. Ruiz de Galareta *et al*., (2007) found 62 different genotypes in 19 local potato landraces. Zimmerer and Douches (1991) found 30 different genotypes in 139 plants from six potato landraces. Therefore, it seems that, despite the clonally propagating nature of the crop, a good level of variation is present in potato landraces.

Dendogram analysis showed that marker profiles of two landraces with some acreage in the region, 'Aleddiyan Beyazi' and 'Aybastı Beyazı', are the same as that of 'Başçiftlik Beyazi' main genotype. Apparently, these are the same landrace but given different names in places where they are produced. All three of them have white fleshed tubers. This is another example of the power of SSR data in the identification of genotypes.

In conclusion, this study revealed the distinguishing power of SSR markers among related genotypes. It also showed that three local cultivars with different names are actually the same landrace. In addition, 23 different genotypes were identified within 'Başçiftlik Beyazi' local landrace cultivar. This variation could be directly used for plant breeding purposes.

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