TÜBİTAK

Turkish Journal of Agriculture and Forestry

http://journals.tubitak.gov.tr/agriculture/

Research Article

Turk J Agric For
(2021) 45: 784-796

doi:10.3906/tar-2102-12

The first report about genetic diversity analysis among endemic wild rhubarb (*Rheum ribes* L.) populations through iPBS markers

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Received: 03.02.2021 • **Accepted/Published Online:** 29.09.2021 • **Final Version:** 16.12.2021

Abstract: Approximately 30% of plant species of Turkey, which is among the richest countries in terms of biodiversity, has been endemic. Wild rhubarb (*Rheum ribes* L.) is a wild vegetable grows especially in the eastern region of Turkey and is an endemic species. In this study, genetic relationships among 80 wild rhubarb genotypes collected from some regions of Lake Van Basin, which are in the distribution area, were tried to be determined by iPBS marker system. At the same time, a commercial variety of *R. rhabarbarum*, which is a cultivated species, was used as control. PCR studies were conducted with 23 iPBS primers to determine genetic relationships, and a total of 340 scorable bands were obtained. 100% polymorphism rate was obtained from all primers studied. While the average PIC value was found to be 0.90, the highest value was found to be 0.97 from the primer # 2220. It was determined that the genotypes were divided into 3 basic groups in the dendogram created with UPGMA based on Jaccard similarity coefficient.

Key words: iPBS, genetic variation, population structure, *Rheum ribes* L., wild rhubarb

1. Introduction

The genus *Rheum* L., known as rhubarb, belongs to the Polygonaceae family and has 60 species that spread around the world (Tabin et al., 2018). Only *Rheum ribes* L. is naturally occurring in Turkey (Tosun and Kizilay, 2003), and it has also found in Iran, Pakistan, Afghanistan, Iraq, Armenia, and Lebanon (Bazzaz et al, 2005; Ekincialp et al., 2019). It is perennial plant and consumed as vegetables (Naemi et al., 2014); it could be used as a medicine for diabetes, (Raafat et al., 2014; Adham and Naqishbandi, 2015; Raafat and El-Lakany, 2018), diarrhea, cancer, and Alzheimer's (Zahedi et al., 2015; Khiveh et al., 2017; Aygün et al., 2020).

The diversity in plant genetic resources enables the development of new varieties with preferred characteristics such as resistance to diseases and pests, yield potential and large seeds, etc. (Govindaraj et al., 2014). Determining the nature and level of genetic diversity within and among populations plays an important role in developing plants and making effective use of them. Different agronomic and morphological criteria are used to detect genetic diversity among plant species (Erdinc et al., 2013a; Erdinc et al., 2017; Nadeem et al.2018).

During the last 30 years, rapid developments in the field of molecular genetics have increased the effectiveness of molecular genetic studies in plant breeding (Nadeem et al. 2018). Molecular markers are widely used to track locus and genome regions during the plant breeding process (Erdinc et al., 2013b; Varshney et al., 2007). Molecular markers are gene or DNA sequences located in a known region on a chromosome and associated with a particular trait (Al-Samarai and Al-Kazaz, 2015), and there are different molecular marker systems.

Kalendar et al. (2010) reported the iPBS (inter Primer Binding Site) marker system, which is qualified as universal. Due to the presence of a universal tRNA complement as the primary binding site of reverse transcriptase in long terminal repeat retrotransposons, the iPBS marker system can be used in all plant species without sequence information (Yıldız et al., 2020). This method has been applied successfully in several plant species such as wild chickpea (Andeden et al., 2013), grape (Guo et al.,

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2014), peas (Baloch et al., 2015), beans (Nemli et al., 2015; Öztürk et al., 2020), okra (Yıldız et al., 2015), *Leonurus cardiaca* (Borna et al., 2017), *Fagaceae* (Coutinho et al., 2018), *Ranunculaceae* (Hossein-Pour et al., 2019), oregano (Karagoz et al., 2020), pepper (Yıldız et al., 2020).

R. ribes is grown naturally in Turkey. Determination of the genetic diversity and population structure of this species will be a guide in the breeding process, in the culture studies and in the protection of this species. To date, AFLP (Kuhl and DeBoer, 2008), SSR (Tanhuanpaa et al., 2019; Ekincialp et al., 2019), ISSR (Hu et al., 2011, Hu et al., 2014; Ekincialp et al., 2019) are the marker systems have been used to determine genetic diversity in the genus *Rheum*. In the present study, it was aimed to determine genetic diversity and population structure in 80 wild rhubarb genotypes collected from Van Lake Basin using iPBS-Retrotransposon marker system. Determination of genetic differences in wild rhubarb species with iPBS marker system will be revealed for the first time in the present study.

2. Materials and methods

2.1. Plant materials and DNA isolation

In the study, 80 *R. ribes* L. genotypes and 1 *R. rhabarbarum* L. genotype were used as plant materials. *R. ribes* L genotypes were collected from 4 different locations in the Lake Van Basin (Turkey) where *R. ribes* widely spread out (Table 1). Sampling was accompanied with a GPS device in May and June 2015. Fresh leaf samples of each genotype were brought to the laboratory in the cold chain and stored at –80 °C until the DNA isolation process was performed. The modified CTAB protocol of Doyle and Doyle (1990) was performed for DNA isolation (Baloch et al., 2016).

2.2. iPBS-retrotransposon amplification

A total of 50 iPBS-retrotransposon primers were screened in 8 randomly selected wild rhubarb genotypes, and the 23 most polymorphic primers were selected for studying all genotypes. Sequence and annealing temperatures of these 23 primers are given in Table 2. PCR reaction content and conditions were carried out according to the protocol reported by Kalendar et al. (2010). According to this protocol, the PCR reaction was carried out in a total volume of 25 µl, containing 1X Dream Taq Green PCR buffer, 0.2 mM dNTPs, 10 µM primer, 1 unit Dream Taq DNA polymerase and 10 ng DNA. PCR condition was initiated with 4 min of denaturation at 95 °C; 35 cycles of denaturation at 95 °C for 15 s, annealing for 1 min at 50–65 °C (depending on the primer), 1 min at 68 °C, and the final extension phase by holding at 72 °C for 5 min. The PCR products obtained were electrophoresed in 1.7% (w/v) agarose gel prepared using 1xTBE buffer solution and stained with ethidium bromide and photographed under UV viewer Gel Doc XR + system (Bio-Rad, USA) (Figure 1).

2.3. Analysis of data

Only clear and clean bands were considered in the gel images for data analysis. Scoring was made according to the binary data system and recorded as "0" in the absence of "1" in the presence of a band. The analysis of the data was carried out in the PAST3 computer program. Genetic similarity between genotypes was determined by the Jaccard similarity coefficient (Jaccard, 1908). The dendogram, which shows the genetic relationship between wild rhubarb genotypes, was created by UPGMA method using similarity matrices. The PIC (polymorphic information content) was calculated according to Powell et al., 1996 and Smith et al., 1997. Effective number of alleles (*ne*), gene diversity (*h*), Shannon information index (*I*) (Yeh et al., 2000) were calculated in the POP-GENE version 1.32 computer program. Population structure was analyzed with the model-based approach of the Bayesian method in the computer program STRUCTURE ver. 2.3.2 (Pritchard, 2000). To predict the most expected K value, values of ΔK and optimal K were computed using STRUCTURE Harvester (Earl, 2012).

3. Results

In the present study, a total of 340 scorable bands were obtained from 23 iPBS primers to determine genetic variation in a population consisting of eighty *R. ribes* L. and one *R. rhabarbarum* L. genotype. All bands obtained were polymorphic (Table 3).

While the lowest band production per primer was obtained from primer # 2388 with 5 bands, the highest band production was obtained from the primers # 2232 and 2253 with 23 bands. Average band production per primer was determined as 14.78. All primers showed 100% polymorphism. The average polymorphism information content (PIC) value was calculated as 0.90 for all studied genotypes. The minimum PIC value was obtained from the primer # 2239 with 0.66, while the highest PIC value was obtained from the primer # 2220 with 0.97 (Table 3).

The *ne* value for the twenty-three iPBS primers ranged from 1.33 (the primer # 2085) to 1.73 (the primer # 2230). The average *ne* value was calculated as 1.53. Average *h* value was calculated as 0.33. The lowest *h* value was obtained from the primer # 2085 with 0.24 and the highest *h* value from the primer # 2230 with 0.41. The average *I* value was calculated as 0.5; the maximum value was determined as 0.60 (the primer # 2230) and the minimum value was 0.39 (the primer # 2085) (Table 3).

Paired genetic similarity coefficients were calculated according to Jaccard to estimate the variation among eighty-one genotypes. According to the obtained genetic similarity genetic similarity (GS) coefficients, the most similar genotypes were YYUBAH39 - YYUMUR60 (GS=0.954) and the other similar genotypes were

Table 1. Geographical data of 80 wild rhubarb genotypes.

#	Genotype name	Collection site	Coordinates		
			Altitude (m)	Latitude (N)	Longitude (E)
1	YYUERC-01	ERÇEK- Karakoç Village Irgat Mountain	1983	38 36’ 23,41”	43 44’ 12, 28”
2	YYUERC-02	ERÇEK- Karakoç Village Irgat Mountain	2019	38 36’ 22, 52”	43 44’ 10,2”
3	YYUERC-03	ERÇEK- Karakoç Village Irgat Mountain	2015	38 36’ 23,14”	43 44’ 10,2”
4	YYUERC-04	ERÇEK- Karakoç Village Irgat Mountain	2016	38 36’ 23, 23”	43 44’ 7,83”
5	YYUERC-05	ERÇEK- Karakoç Village Irgat Mountain	2018	38 36’ 23,26”	43 44’ 6,37”
6	YYUERC-06	ERÇEK- Karakoç Village Irgat Mountain	2064	38 36’ 23,21”	43 44’ 2,62”
7	YYUERC-07	ERÇEK- Karakoç Village Irgat Mountain	2066	38 36’ 23,46”	43 44’ 1,27”
8	YYUERC-08	ERÇEK- Karakoç Village Irgat Mountain	2081	38 36’ 22,62”	43 44’ 0,01”
9	YYUERC-09	ERÇEK- Karakoç Village Irgat Mountain	2076	38 36’ 22,02”	43 43’ 58,22”
10	YYUERC-10	ERÇEK- Karakoç Village Irgat Mountain	2083	38 36’ 21,76”	43 43’ 57,77”
11	YYUERC-11	ERÇEK- Karakoç Village Irgat Mountain	2083	38 36’ 21,54”	43 43’ 57,77”
12	YYUERC-12	ERÇEK- Karakoç Village Irgat Mountain	2082	38 36’ 21,53”	43 43’ 55,39”
13	YYUERC-13	ERÇEK- Karakoç Village Irgat Mountain	2126	38 36’ 18,25”	43 43’ 55,39”
14	YYUERC-14	ERÇEK- Karakoç Village Irgat Mountain	2128	38 36’ 18,12”	43 43’ 54,3”
15	YYUERC-15	ERÇEK- Karakoç Village Irgat Mountain	2147	38 36’ 12,69”	43 43’ 50,44”
16	YYUERC-16	ERÇEK- Karakoç Village Irgat Mountain	2138	38 36’ 12,24”	43 43’ 50,98”
17	YYUERC-17	ERÇEK- Karakoç Village Irgat Mountain	2122	38 36’ 10,01”	43 43’ 50,53”
18	YYUERC-18	ERÇEK- Karakoç Village Irgat Mountain	2117	38 36’ 11,01”	43 43’ 50,7”
19	YYUERC-19	ERÇEK- Karakoç Village Irgat Mountain	2128	38 36’ 11,19”	43 43’ 50,73”
20	YYUERC-20	ERÇEK- Karakoç Village Irgat Mountain	2119	38 36’ 11,05”	43 43’ 507,3”
21	YYUBAH-21	BAHÇESARAY	1925	38 0’ 29,67”	42 44’ 45, 74”
22	YYUBAH-22	BAHÇESARAY	1960	38 0’ 31, 26”	42 44’ 31,17”
23	YYUBAH-23	BAHÇESARAY	1960	38 0’ 31,21”	42 44’ 31,18”
24	YYUBAH-24	BAHÇESARAY	1960	38 0’ 31,32”	42 44’ 30,95”
25	YYUBAH-25	BAHÇESARAY	1960	38 0’ 30,92”	42 44’ 31,68”
26	YYUBAH-26	BAHÇESARAY	1970	38 0’ 30,52”	42 44’ 31,45”
27	YYUBAH-27	BAHÇESARAY	1980	38 0’ 30,08”	42 44’ 31,47”
28	YYUBAH-28	BAHÇESARAY	1980	38 0’ 30,08”	42 44’ 31,47”
29	YYUBAH-29	BAHÇESARAY	1985	38 0’ 29,71”	42 44’ 32,48”
30	YYUBAH-30	BAHÇESARAY	1985	38 0’ 29,48”	42 44’ 32,39”
31	YYUBAH-31	BAHÇESARAY	1990	38 0’ 29,33”	42 44’ 32,54”
32	YYUBAH-32	BAHÇESARAY	1985	38 0’ 29,62”	42 44’ 32,57”
33	YYUBAH-33	BAHÇESARAY	1985	38 0’ 29,6”	42 44’ 32,85”
34	YYUBAH-34	BAHÇESARAY	1980	38 0’ 29,64”	42 44’ 33,07”
35	YYUBAH-35	BAHÇESARAY	1975	38 0’ 29,85”	42 44’ 33,26”
36	YYUBAH-36	BAHÇESARAY	1970	38 0’ 30,17”	42 44’ 33,57”
37	YYUBAH-37	BAHÇESARAY	1965	38 0’ 30,01”	42 44’ 33,72”
38	YYUBAH-38	BAHÇESARAY	1960	38 0’ 30”	42 44’ 33,91”

Table 1. (Continued).

39	YYUBAH-39	BAHÇESARAY	1960	38 0’ 30”	42 44’ 33,91”
40	YYUBAH-40	BAHÇESARAY	1960	38 0’ 30,31”	42 44’ 34,03”
41	YYUMUR-41	MURADİYE-Doğangün Village	2245	38 45’ 28,41”	43 45’ 1,25”
42	YYUMUR-42	MURADİYE-Doğangün Village	2250	38 45’ 27,94”	43 45’ 1,18”
43	YYUMUR-43	MURADİYE-Doğangün Village	2255	38 45’ 27,56”	43 45’ 2,15”
44	YYUMUR-44	MURADİYE-Doğangün Village	2265	38 45’ 25,46”	43 44 59,14”
45	YYUMUR-45	MURADİYE-Doğangün Village	2280	38 45’ 22,66”	43 44’ 54,96”
46	YYUMUR-46	MURADİYE-Doğangün Village	2290	38 45’ 20,92”	43 44’ 54,67”
47	YYUMUR-47	MURADİYE-Doğangün Village	2335	38 45’ 18,58”	43 44’ 54,46”
48	YYUMUR-48	MURADİYE-Doğangün Village	2340	38 45’ 16,69”	43 44’ 53,73”
49	YYUMUR-49	MURADİYE-Doğangün Village	2350	38 45’ 15,83”	43 44’ 53,92”
50	YYUMUR-50	MURADİYE-Doğangün Village	2360	38 45’ 15,66”	43 44’ 53,24”
51	YYUMUR-51	MURADİYE-Doğangün Village	2360	38 45’ 15,69”	43 44 53,23”
52	YYUMUR-52	MURADİYE-Doğangün Village	2370	38 45’ 14,38”	43 44’ 53,11”
53	YYUMUR-53	MURADİYE-Doğangün Village	2370	38 45’ 13,74”	43 44’ 53,34”
54	YYUMUR-54	MURADİYE-Doğangün Village	2395	38 45’ 13,01”	43 44 51,63”
55	YYUMUR-55	MURADİYE-Doğangün Village	2395	38 45’ 12,53”	43 44’ 52,42”
56	YYUMUR-56	MURADİYE-Doğangün Village	2395	38 45 12,71”	43 44’ 52,32”
57	YYUMUR-57	MURADİYE-Doğangün Village	2395	38 45’ 12,93”	43 44’ 52,64”
58	YYUMUR-58	MURADİYE-Doğangün Village	2395	38 45’ 12,46”	43 44’ 53,11”
59	YYUMUR-59	MURADİYE-Doğangün Village	2395	38 45’ 12,32”	43 44’ 53,83”
60	YYUMUR-60	MURADİYE-Doğangün Village	2420	38 45 10,82”	43 44’ 53,12”
61	YYUMER-61	Mount Erek (Merkez=Centrum)	2110	38 29’ 50,76”	43 29’ 0,76”
62	YYUMER-62	Mount Erek (Merkez=Centrum)	2110	38 29’ 50,39”	43 29’ 0,76”
63	YYUMER-63	Mount Erek (Merkez=Centrum)	2095	38 29’ 49,45”	43 29’ 0,45”
64	YYUMER-64	Mount Erek (Merkez=Centrum)	2145	38 29’ 46,58”	43 28’ 55,7”
65	YYUMER-65	Mount Erek (Merkez=Centrum)	2145	38 29’ 44,17”	43 28’ 54,53”
66	YYUMER-66	Mount Erek (Merkez=Centrum)	2145	38 29’ 44,9”	43 28 53,78”
67	YYUMER-67	Mount Erek (Merkez=Centrum)	2150	38 29’ 45,54”	43 28’ 54,42”
68	YYUMER-68	Mount Erek (Merkez=Centrum)	2165	38 29’ 44,31”	43 28 54,365”
69	YYUMER-69	Mount Erek (Merkez=Centrum)	2135	38 29’ 39,82”	43 28’ 54,46”
70	YYUMER-70	Mount Erek (Merkez=Centrum)	2135	38 29’ 39,82”	43 28’ 54,46”
71	YYUMER-71	Mount Erek (Merkez=Centrum)	2135	38 29’39,82”	43 28’ 54,46”
72	YYUMER-72	Mount Erek (Merkez=Centrum)	2135	38 29’ 40,62”	43 28’ 54,07”
73	YYUMER-73	Mount Erek (Merkez=Centrum)	2145	38 29’ 40,16”	43 28’ 54,56”
74	YYUMER-74	Mount Erek (Merkez=Centrum)	2145	38 29’ 39,25”	43 28’ 54,36”
75	YYUMER-75	Mount Erek (Merkez=Centrum)	2145	38 39’ 39,45”	43 28’ 54,33”
76	YYUMER-76	Mount Erek (Merkez=Centrum)	2155	38 29’ 39,09”	43 28’ 54,56”
77	YYUMER-77	Mount Erek (Merkez=Centrum)	2155	38 29’ 39,09”	43 28’ 54,56”
78	YYUMER-78	Mount Erek (Merkez=Centrum)	2165	38 29’ 38,13”	43 28’ 54,41”
79	YYUMER-79	Mount Erek (Merkez=Centrum)	2165	38 29’ 38,43”	43 28’ 54,23”
80	YYUMER-80	Mount Erek (Merkez=Centrum)	2165	38 29’ 37,98”	43 28’ 54,33”

Table 2. Sequence and annealing temperature data of the studied 23 iPBS primers.

Primer	Sequence	Ann. Temp. (°C)	Primer	Sequence	Ann. Temp. (°C)
2074	GCTCTGATACCA	50	2253	TCGAGGCTCTAGATACCA	51
2085	ATGCCGATACCA	53	2272	GGCTCAGATGCCA	55
2095	GCTCGGATACCA	53	2277	GGCGATGATACCA	50
2220	ACCTGGCTCATGATGCCA	57	2295	AGAACGGCTCTGATACCA	60
2222	ACTTGGATGCCGATACCA	53	2374	CCCAGCAAACCA	53
2228	CATTGGCTCTTGATACCA	53	2375	TCGCATCAACCA	50
2229	CGACCTGTTCTGATACCA	52	2388	TTGGAAGACCCA	50
2230	TCTAGGCGTCTGATACCA	53	2390	GCAACAACCCCA	55
2232	AGAGAGGCTCGGATACCA	55	2394	GAGCCTAGGCCA	55
2239	ACCTAGGCTCGGATGCCA	55	2401	AGTTAAGCTTTGATACCA	53
2249	AACCGACCTCTGATACCA	51	2415	CATCGTAGGTGGGCGCCA	60
2251	GAACAGGCGATGATACCA	53			

YYUBAH22 - YYUBAH23 (GB = 0.947) and YYUMUR42 - YYUMER70 ((GS=0.903). The most distant genotypes were determined as YYUBAH39 - YYUERC04 GS=0.029, followed by YYUMUR53 - YYUERC03 (GS=0.032)and YYUMER78 - YYUMER80 (GS=0.034). The mean genetic similarity value for all genotypes was calculated as 0.159.

A dendogram was constructed to determine the genetic relatedness among the studied genotypes using binary genetic similarity values. The dendogram obtained by UPGMA-based analysis divided all genotypes into 3 groups as A, B, and C. Group A is the smallest group with 3 genotypes. Group B is represented by 15 genotypes. Group C, which has the most crowded genotype, contains 63 genotypes. All groups branched out into smaller subgroups. The genotype belonging to the *R. rhabarbarum* L. species was included in group C and was genetically similar to the YYUMER78 genotype (Figure 2).

In order to better understand the genetic variation between genotypes, a principal coordinate analysis (PCoA) was also performed according to the assembly regions of the genotypes. All genotypes are divided into 3 groups as A, B, and C. Groups A and B consisted of Muradiye (YYUMUR) and Mount Erek (Merkez=Centrum, YYUMER) genotypes, while group C was a mixed group containing wild rhubarb genotypes of all locations and the genotype *R. rhabarbarum* L. (Figure 3).

The population structure was analyzed by STRUCTURE, a computer program based on the Bayesian clustering method. In STRUCTURE analysis, the highest K value was found to be 4. With this K value, the studied population consisting of 80 *R. ribes* genotypes and one *R. rhabarbarum* L. genotype was divided into 4 subpopulations (Subpopulations I, II, III, and IV). The subpopulations I., II., III. and IV. consisted of 55, 14, 6 and 6 genotypes, respectively (Table 4). The genotype of *R. rhabarbarum* L. was included in the subpopulation I having the most genotypes (Figure 4).

Analysis made to determine the genetic relationship between populations formed by genotypes belonging to different locations distinguished YYUERC population from other populations. In the dendogram obtained, YYUBAH population and *R. rhabarbarum* L. genotype were in the first branch, while YYUMUR and YYUMER populations were in the second branch (Figure 5). Genetic similarity coefficient between populations ranged from 0.1185 to 0.1698 (Table 5). According to the results of the analysis, while the closest populations were YYUMUR with YYUMER, the most distant populations were YYUERC and *R. rhabarbarum* L.

4. Discussion and conclusion

In the present study, 340 bands were produced in total and the average number of polymorphic bands per primer was calculated as 14.78. Guo et al. (2014) reported the average number of bands per primary iPBS markers in grape varieties as 5.7. Baloch et al. (2015) reported the value for the same parameter in their study with iPBS markers in peas was 6.75. The average number of polymorphic bands reported in the mentioned studies was smaller than the value we obtained. However, in another study conducted with iPBS markers, Hossein-Pour et al. (2019) determined the number of polymorphic bands as 20.3 in *Adonis* L. (*Ranunculaceae*) population collected from different regions of Turkey. Obtaining such different values is not

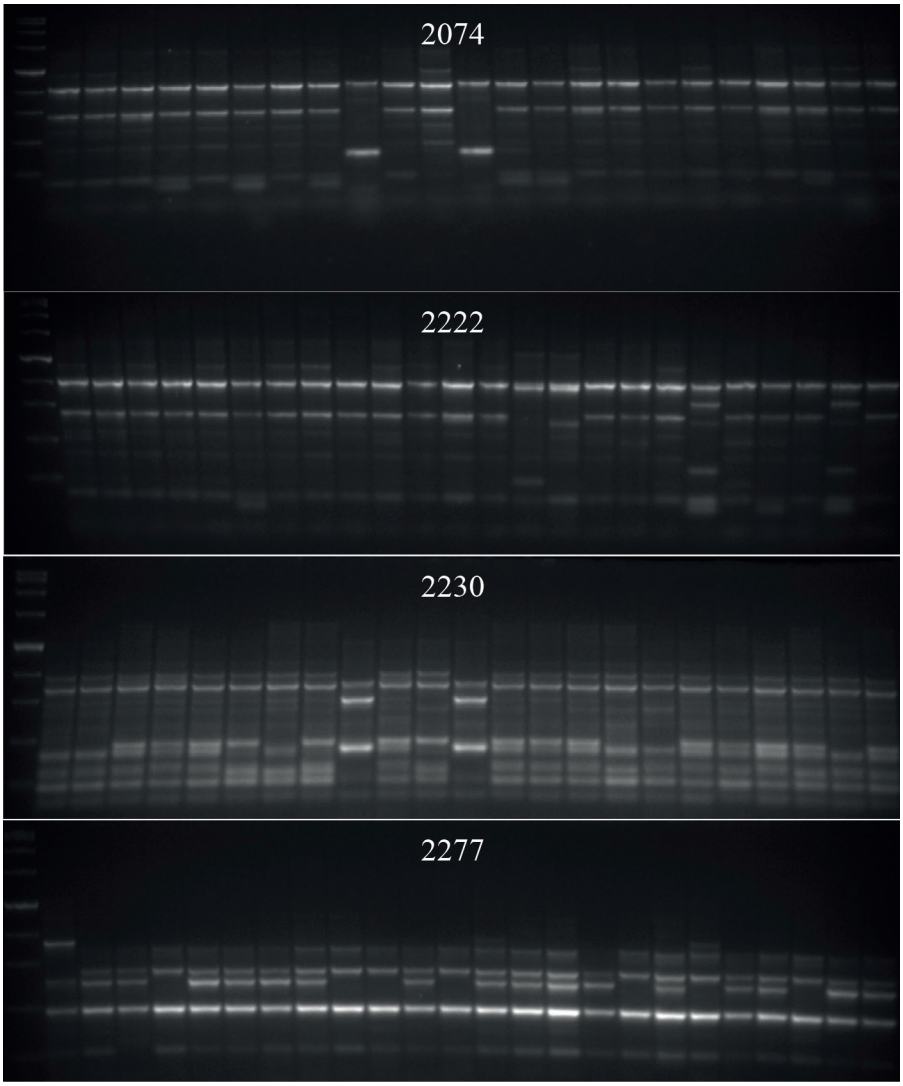

Figure 1. Agarose gel image of some iPBS primers.

entirely related to the marker technique but is due to the results obtained from different plant species. All bands (100%) produced by iPBS markers in the present study showed polymorphism. Hu et al. (2014) detected genetic variation with ISSR markers in 5 different populations of *R. tanguticum* species. The rate of polymorphism obtained from the populations varied between 42.81% and 51.81%, and the average polymorphism rate was reported to be 48.61%. This polymorphism value is a very low value compared to the value of the present study because there are different Rheum species were used in the mentioned studies. Therefore, discrepancy between the results of the study and of the previous studies was probably caused by species differences. Hu et al. (2011) obtained 99.42% polymorphism by using ISSR primers in *R. tanguticum* Maxim. ex Balf., which is similar to the results we obtained. Another parameter used to evaluate polymorphism is the PIC value. PIC is a commonly used value to indicate the polymorphism level of a marker locus used in linkage analysis in genetic studies (Shete et al., 2000). In the present study, a high PIC value (0.90) was obtained. A similar result (PIC = 0.91) was obtained from a study on wild chickpea with iPBS primers (Andeden et al., 2013). However, there are also some other studies in which lower PIC values were obtained using iPBS primers, such as the study of Nemli et al. (2015) on beans, Yıldız et al. (2020) on pepper, Koçak et al., (2020) on *Fritillaria imperialis* L., Öztürk et al. (2020) on bean and Barut et al. (2020) on quinoa with 0.71, 0.66, 0.33, and 0.41 PIC values, respectively.

According to Jaccard similarity coefficient, the most similar genotypes were determined to be YYUMUR59-YYUMUR60 and YYUBAH22-YYUBAH23. When the most similar genotypes are considered based on the location, it is understood that they are taken from the same

Table 3. iPBS primers and parameters of genetic diversity of 80 wild rhubarb genotypes and *R. rhabarbarum* L. genotype.

Primer	Amplified bands		% Polymorphism	PIC	*ne*	*h*	*I*
	Total	Polymorphic					
2074	15	15	100	0.94	1.59	0.36	0.55
2085	20	20	100	0.87	1.33	0.24	0.39
2095	12	12	100	0.96	1.58	0.36	0.55
2220	16	16	100	0.97	1.47	0.29	0.45
2222	14	14	100	0.94	1.53	0.34	0.52
2228	20	20	100	0.92	1.49	0.31	0.48
2229	16	16	100	0.91	1.51	0.31	0.47
2230	13	13	100	0.94	1.73	0.41	0.60
2232	23	23	100	0.93	1.41	0.27	0.44
2239	16	16	100	0.66	1.45	0.30	0.47
2249	14	14	100	0.91	1.59	0.36	0.54
2251	13	13	100	0.90	1.57	0.34	0.52
2253	23	23	100	0.93	1.44	0.29	0.46
2272	19	19	100	0.85	1.58	0.35	0.53
2277	10	10	100	0.85	1.64	0.38	0.56
2295	15	15	100	0.94	1.50	0.32	0.50
2374	11	11	100	0.96	1.65	0.38	0.56
2375	11	11	100	0.95	1.54	0.33	0.49
2388	5	5	100	0.93	1.70	0.40	0.59
2390	11	11	100	0.89	1.64	0.38	0.56
2394	15	15	100	0.96	1.43	0.29	0.46
2401	11	11	100	0.75	1.38	0.26	0.42
2415	17	17	100	0.84	1.48	0.31	0.48
Total	340	340					
Average	14.78	14.78	100	0.90	1.53	0.33	0.50

Effective number of alleles (*ne*), gene diversity (*h*), Shannon information index (*I*), and polymorphism information content (PIC).

altitude and very close regions. Since these genotypes are very similar, gene flow among them could be possible by pollination and, therefore, they are likely to be genetically similar. Genetically similar genotypes of the genotypes found in close regions with the analysis results show that the iPBS marker system is successful in revealing the genetic variation in wild rhubarb genotypes. Genotypes most distant from each other in terms of genetic similarity are YYUBAH39-YYUERC04 and YYUMUR53-YYUERC03 genotypes collected from different locations and altitudes. Since these genotypes differ genetically, they can be used as parents in future breeding studies. Although *R. rhabarbarum* L. belongs to a different species than other genotypes, it did not have the highest distance genetically with any genotype. The pairwise similarity coefficient is 0.20 with the closest genotype (YYUMER79), while it is 0.04 with the farthest genotype (YYUERC05). The average pairwise similarity coefficient with all other genotypes is 0.13. It appears that with this value, the genetic relationship among wild rhubarb genotypes is quite low. Average *ne* value was calculated to be 1.53. Yıldız et al. (2020) reported that the *ne* value with iPBS markers in pepper was 1.21. Average *h* and *I* values in the present study are 0.33 and 0.50, respectively. Different mean *h* and *I* values using iPBS primers were obtained by different plant species: 0.31 and 0.86, respectively in wild chickpeas (Andeden et al. 2013); 0.07 and 0.12, respectively in okra, (Yıldız et al. 2015), 0.26 and 0.21, respectively in peas, (Baloch et al. 2015), and 0.15 and 0.25, respectively in pepper (Yıldız et al. 2020). All genotypes were divided into 3 groups according to the dendogram created by UPGMA-

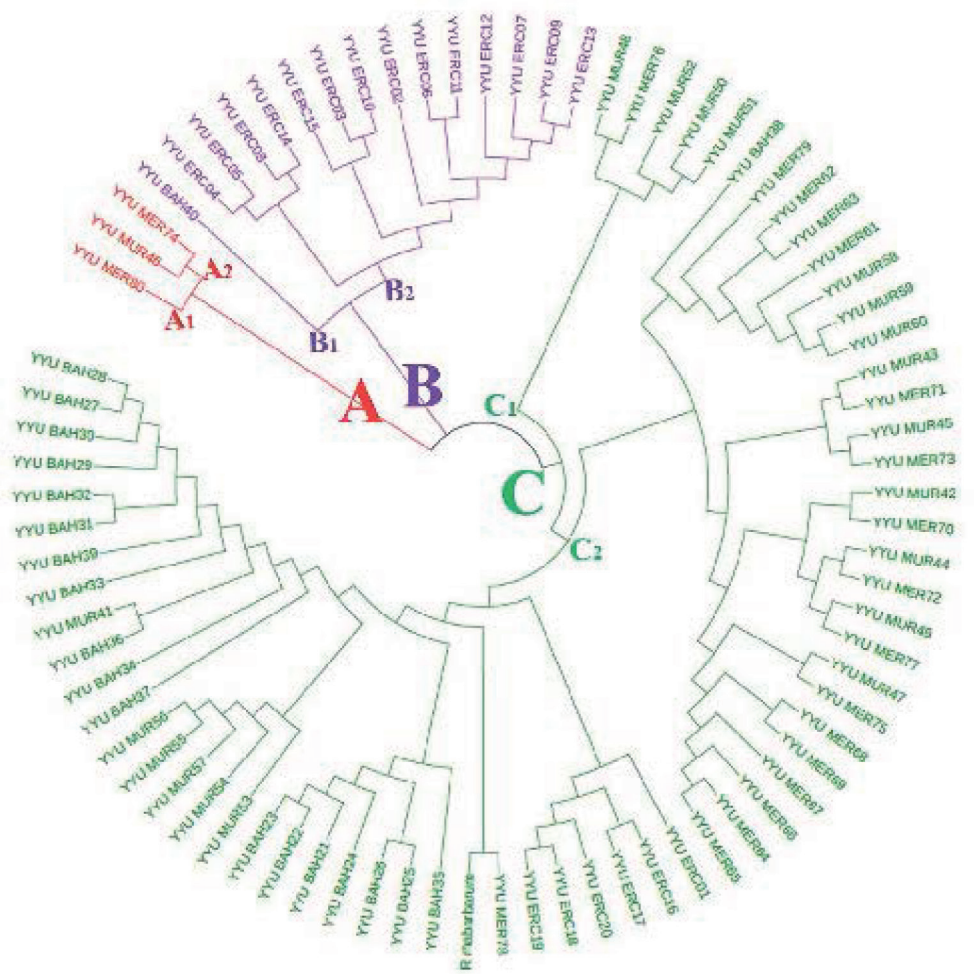

Figure 2. UPGMA based genetic clustering of 80 wild rhubarb genotypes and *R. rhabarbarum* L. cultivar.

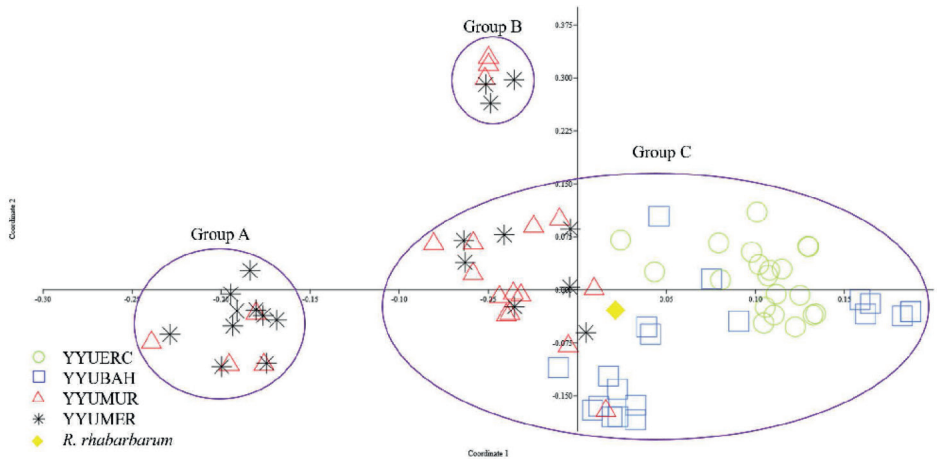

Figure 3. Genetic clustering of 80 wild rhubarb genotypes and one *R. rhabarbarum* L. genotype based on principal coordinate analysis (PCoA).

based cluster analysis. When examined according to the collection locations, Group A consists of 2 YYUMER and 1 YYUMUR genotypes. Group C consists of a completely mixed population with genotypes collected from all locations and the genotype belonging to *R. rhabarbarum* L. Group B consists entirely of YYUERC genotypes, except

Table 4. Distribution of wild rhubarb genotypes to subpopulations according to membership coefficient.

Genotype name	*Subpopulation*				Genotype name	*Subpopulation*			
	I	II	III	IV		I	II	III	IV
YYUERC-01	0.959	0.008	0.001	0.031	YYUMUR-42	0.002	0.994	0.001	0.004
YYUERC-02	0.990	0.003	0.001	0.006	YYUMUR-43	0.784	0.199	0.015	0.002
YYUERC-03	0.948	0.002	0.043	0.006	YYUMUR-44	0.001	0.997	0.001	0.001
YYUERC-04	0.991	0.006	0.001	0.002	YYUMUR-45	0.870	0.115	0.011	0.004
YYUERC-05	0.990	0.003	0.003	0.005	YYUMUR-46	0.915	0.042	0.041	0.001
YYUERC-06	0.991	0.003	0.002	0.004	YYUMUR-47	0.006	0.991	0.002	0.002
YYUERC-07	0.986	0.006	0.001	0.006	YYUMUR-48	0.971	0.023	0.005	0.002
YYUERC-08	0.979	0.014	0.006	0.001	YYUMUR-49	0.008	0.990	0.001	0.001
YYUERC-09	0.992	0.003	0.002	0.003	YYUMUR-50	0.812	0.008	0.176	0.003
YYUERC-10	0.979	0.008	0.011	0.002	YYUMUR-51	0.989	0.009	0.001	0.001
YYUERC-11	0.990	0.003	0.004	0.003	YYUMUR-52	0.857	0.014	0.125	0.005
YYUERC-12	0.992	0.003	0.002	0.004	YYUMUR-53	0.982	0.010	0.006	0.002
YYUERC-13	0.982	0.002	0.009	0.007	YYUMUR-54	0.993	0.004	0.001	0.002
YYUERC-14	0.934	0.034	0.030	0.001	YYUMUR-55	0.984	0.010	0.004	0.002
YYUERC-15	0.965	0.003	0.006	0.025	YYUMUR-56	0.969	0.004	0.026	0.002
YYUERC-16	0.992	0.003	0.003	0.002	YYUMUR-57	0.984	0.007	0.008	0.001
YYUERC-17	0.992	0.005	0.001	0.001	YYUMUR-58	0.039	0.028	0.931	0.002
YYUERC-18	0.992	0.004	0.002	0.003	YYUMUR-59	0.000	0.000	0.999	0.000
YYUERC-19	0.977	0.005	0.001	0.017	YYUMUR-60	0.001	0.001	0.997	0.001
YYUERC-20	0.984	0.008	0.001	0.007	YYUMER-61	0.088	0.012	0.898	0.003
YYUBAH-21	0.004	0.004	0.001	0.991	YYUMER-62	0.003	0.027	0.969	0.001
YYUBAH-22	0.001	0.000	0.000	0.999	YYUMER-63	0.025	0.077	0.898	0.001
YYUBAH-23	0.001	0.001	0.001	0.998	YYUMER-64	0.002	0.959	0.039	0.001
YYUBAH-24	0.074	0.040	0.003	0.884	YYUMER-65	0.023	0.974	0.002	0.001
YYUBAH-25	0.078	0.004	0.012	0.905	YYUMER-66	0.005	0.966	0.011	0.018
YYUBAH-26	0.148	0.006	0.006	0.840	YYUMER-67	0.003	0.989	0.003	0.005
YYUBAH-27	0.984	0.009	0.002	0.004	YYUMER-68	0.002	0.996	0.001	0.001
YYUBAH-28	0.989	0.008	0.002	0.001	YYUMER-69	0.005	0.891	0.103	0.001
YYUBAH-29	0.989	0.006	0.001	0.003	YYUMER-70	0.002	0.993	0.001	0.005
YYUBAH-30	0.993	0.003	0.001	0.002	YYUMER-71	0.785	0.197	0.014	0.004
YYUBAH-31	0.989	0.008	0.001	0.002	YYUMER-72	0.002	0.996	0.001	0.001
YYUBAH-32	0.989	0.005	0.002	0.004	YYUMER-73	0.900	0.074	0.024	0.003
YYUBAH-33	0.969	0.004	0.023	0.004	YYUMER-74	0.916	0.036	0.047	0.002
YYUBAH-34	0.945	0.050	0.003	0.001	YYUMER-75	0.004	0.991	0.003	0.002
YYUBAH-35	0.956	0.011	0.005	0.028	YYUMER-76	0.973	0.021	0.003	0.002
YYUBAH-36	0.992	0.005	0.002	0.002	YYUMER-77	0.002	0.996	0.001	0.001
YYUBAH-37	0.981	0.011	0.002	0.006	YYUMER-78	0.699	0.244	0.012	0.044
YYUBAH-38	0.756	0.015	0.214	0.015	YYUMER-79	0.667	0.123	0.201	0.009
YYUBAH-39	0.990	0.004	0.004	0.002	YYUMER-80	0.968	0.024	0.001	0.006
YYUBAH-40	0.991	0.002	0.003	0.003	*R. rhabarbarum*	0.972	0.012	0.006	0.010
YYUMUR-41	0.989	0.008	0.001	0.002					

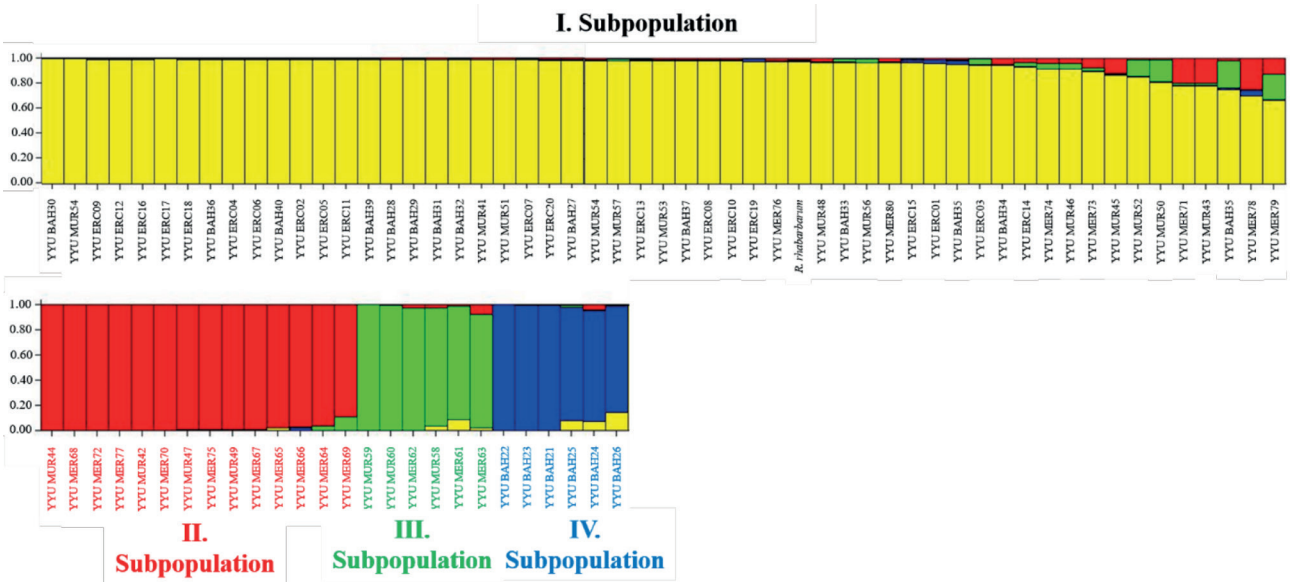

Figure 4. Population structure analysis of wild rhubarb genotypes and one *R. rhabarbarum* genotype using iPBS markers.

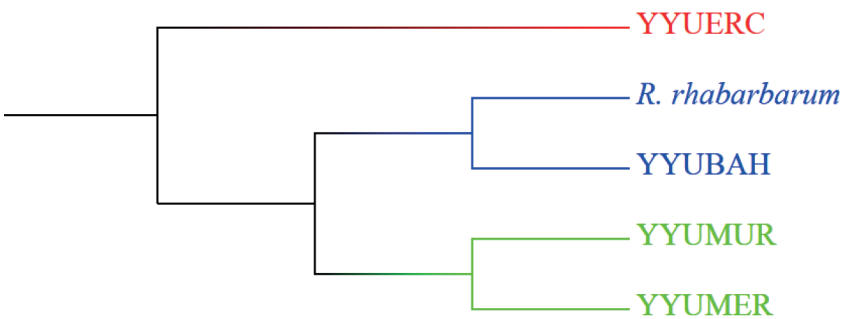

Figure 5. UPGMA based genetic clustering of wild rhubarb populations from different locations in Lake Van Basin and *R. rhabarbarum* L. genotype.

Table 5. Genetic similarity index among wild rhubarb populations from different locations in Lake Van Basin and *R. rhabarbarum* L. genotype.

	YYUERC	YYUBAH	YYUMUR	YYUMER
YYUBAH	0.1366			
YYUMUR	0.1238	0.1422		
YYUMER	0.1211	0.1395	0.1698	
R. rhabarbarum	0.1185	0.1487	0.1400	0.1349

for the 1 YYUBAH genotype. YYUBAH genotype in Group B branched separately from all YYUERC genotypes within the group. Ekincialp et al. (2019) detected genetic variation with SSR and ISSR markers using the same genotypes. The dendograms they obtained with both SSR and ISSR data divided all genotypes into 3 groups. However, the number of individuals of the groups formed by each dendogram and the clustering positions of the genotypes differed. The dendogram we obtained showed differences from the study mentioned. The different results can be explained by the different marker systems used. It is seen that the locations where the genotypes are collected are effective in the formation of genetic distinction, but it does not provide distinction clearly.

Genotypes were also divided into 3 groups by PCoA analysis. Two of these groups include individuals (YYUMER and YYUMUR) located separately from each other but of the same geographic location. The other group has the

largest number of individuals and includes examined within itself, it is seen that YYUMER and YYUMUR genotypes are located closely, similar to the other two small groups. While YYUERC genotypes are located closely among themselves, YYUBAH genotypes are gathered in a relatively large area. Bayesian-based population structure analysis divided the genotypes into 4 subpopulations. Ekincialp et al. (2019) used the same genotypes and reported 2 subpopulations (K=2) with ISSR and SSR. In different species of *Rheum*, Wang et al. (2012a) and Tabin et al. (2016) found 3 subpopulations (K=3) and Wang et al. (2012b) declared 2 subpopulations (K=2) with ISSR markers. In population structure analysis, individuals with a membership coefficient of 0.8 or higher are considered pure, while individuals with a lower membership coefficient are considered to be a mixture of at least two different subpopulations (Fukunaga et al. 2005). Five individuals belonging to the subpopulation and membership coefficient lower than 0.8 and therefore these genotypes are probably not pure. All other genotypes are possible pure individuals due to their membership coefficient greater than 0.8.

It has been observed that the genetic diversity of wild rhubarb genotypes used in the study can be comprehensively determined with the iPBS marker system. Especially, the high polymorphism ratio of iPBS primers and the high number of bands obtained from these primers showed that this marker system can give enough information about the genetic diversity of the studied population. Inter-primer binding site (iPBS) might be an all-inclusive strategy for DNA fingerprinting and retrotransposon isolation; it is an amplification technique and do not require sequence data, and the iPBS procedure has effectively been utilized for assessment of genetic reletadness in plants (Öztürk et al. 2020). According to the cluster analysis of the genotypes collected from four different locations, it was observed that there was no grouping according to the regions they were collected, but the closest populations were YYUMER and YYUMUR, while the YYUERC population was the most different. This study demonstrated that the iPBS marker system could be used for prebreeding selection of wild rhubarb parent candidates, which could reveal variation.

Acknowledgment

This study was supported by Van Yüzüncü Yıl University Scientific Research Projects Unit with project number FHD-2017-6080.

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