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ANALYSIS OF *ONOBRYCHIS* GENETIC DIVERSITY USING SSR MARKERS FROM RELATED LEGUME SPECIES

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ABSTRACT

Availability of legume microsatellite markers for *Onobrychis* taxa was limited. However, cross genera utilization of such markers has been of great interest due to the high cost and labor. In the present study, we attempted to transfer microsatellite markers from *Phaseolus vulgaris* L. and *Medicago truncatula* Gaertn. to *Onobrychis* genus. Additionally, transferred markers were used to identify genetic diversity among *Onobrychis* taxa collected from different regions of Turkey. Of the 95 SSR primer pairs previously used for *P. vulgaris* and *M. truncatula*, 18 primers were successfully amplified and showed polymorphism among 58 *Onobrychis* taxa. Eighteen SSR primers observed 79 loci resulting in 725 alleles. The highest number of loci was obtained from BM175 and MTIC84 primers. Gene diversity and polymorphism information content values showed that *P. vulgaris* primers produced the most informative loci on *Onobrychis* genomes. The highest genetic diversity values were obtained for *Onobrychis argyrea* Boiss. subsp *argyrea* Boiss. (53) while the lowest from *Onobrychis cornuta* (L.) Desv.(1). The average diversity values were the highest on *Hymenobrychis* section which was followed by *Heliobrychis*, *Onobrychis*, *Laphobrychis* and *Dendobrychis* sections. Magnitude of genetic variation was the highest within *Onobrychis* section in which genetic similarity values ranged from 0.013 to 0.399. The SSR and phylogenetic analysis results showed that sections were separated similar to their morphological characteristics. However, *Hymenobrychis* and *Heliobrychis* clearly separated from other sections. Our study showed that *Onobrychis* genomes could be successfully studied using other legume SSR markers. Therefore, they can be used for conservation of *Onobrychis* species as well as improving new varieties for feed use.

Key words: *Onobrychis*, SSR transferability, *Medicago truncatula*, *Phaseolus vulgaris*

INTRODUCTION

The genus of *Onobrychis* Adans includes about 170 species around the world. It is cross-pollinated, diploid (2n = 14, 16) or tetraploid (2n = 28), perennial or annual herbs or shrubs (Akçelik *et al*., 2012; Hejazi *et al*., 2010). Only recently has haploid chromosome size of *Onobrychis viciifolia* Scop. been found to be 1223 Mbp (http://data.kew.org/cvalues). It extends from the Mediterranean region to Caucasia, the Zagros Mountains, and central Asia. The genus is concentrated in Turkey (55 species), Iran (58 species) and Caucasia (39 species). Thus, Iran and Turkey appear to be the main centers of genetic diversity (Hedge, 1970; Davis *et al*., 1988; Aktoklu, 2001; Yıldız *et al*., 1999). Agronomically, they have positive characteristics such as a deep tap root, that allows the plant to be very resistant to drought and high palatability with valuable protein and non-bloating properties (Ahuja *et al*., 1983; Koivisto and Lane, 2001). Especially, cultivated *Onobrychis* species (*O. viciifolia*) is known as bloat-safe legume because of foliar condensed tannins (Li *et al*., 1993; Çelikta *et al*., 2006).

Today, sainfoin (*O. viciifolia*) is especially popular in Turkey, where about 153,000 ha were grown in 2011. However, in recent years, cultivation of sainfoin has been adversely affected by two different rootworm pests (*Bembecia scopigera* (Scopoli.) and *Sphenoptera carceli* Cast. and Groy.). These pests, which stop development of plant by opening tunnels to the central part of the inside root, cause the plant to die in second and third years. Although it is long-lived plant (5 or 6 years), it can be lost as of second years in pests infested fields.

Breeding resistant varieties is the most effective method against these pests and the other biotic and abiotic stress factors. Wild *Onobrychis* species have a very rich genetic diversity for adaptation to high temperature, rainfall, drought, salinity, diseases and pests, and they can be used in breeding programs for improving resistant varieties (Hart, 2001). Therefore collection and characterization of these species are of great importance for using them in breeding programs. While morphological characterization is a necessary step in plant breeding, several molecular tools have been used for plant variety characterization and identification. Especially, microsatellites or simple sequence repeats

(SSRs) have become one of the most useful molecular marker systems in plant breeding (Demdoum *et al*., 2012).

In most public domains, thousands of legume SSR sequence information is available (http://phaseolusgenes.bioinformatics.ucdavis.edu for *Phaseolus*, http://www.medicago.org/ for *Medicago*, http://marker.kazusa.or.jp/ for *Lotus* and *Glycine*). SSR markers were developed and used for characterization of many legume genus like *Medicago* (He *et al*., 2009), different legumes (Chandra, 2010), *Lens* (Reddy *et al*., 2010), *Trifolium* (Dias *et al*., 2008; Kölliker *et al*., 2001), *Lespedeza* (Wang *et al*., 2009), *Vigna* (Gupta and Gopalakrishna, 2010) *Onobrychis* (Demdoum *et al*., 2012). However, there is limited data about the microsatellites and genetic diversity of *Onobrychis* taxa except Demdoum *et al*. (2012). In this study, EST-SSRs from *M. truncatula* and *Glycine max* (L.) Merr. were tested for transferability in various species of *Onobrychis* (*Onobrychis pyrenaica* Sennen, *Onobrychis argentea* Boiss. and *O. viciifolia*). In our study, different EST-SSRs and SSRs from *M. truncatula* and *P. vulgaris* were tested for transferability in different Turkish *Onobrychis* taxa.

M. truncatula genome is an important reference species for legumes and non-legumes alike (Barker *et al*., 1990; Cook, 1999). This species has been preferred as a model organism for legume biology because it has a small diploid (2n = 16) genome (500-550 Mbp total and 200-250 Mbp to be sequenced in gene-rich euchromatic regions), is self-fertile, has a rapid generation time and prolific seed production, and is amenable to genetic transformation (Erayman *et al*., 2014; Young *et al*., 2005; Barker *et al*., 1990; Chabaud *et al*., 2003; Araújo *et al*., 2004; Crane *et al*., 2006).

However, there are not adequate number of microsatellites available for *Onobrychis*. Simple genetic similarities among legumes and interspecies availability of SSR markers (Choudhary *et al*., 2008; Reddy *et al*., 2010) suggested that some SSR markers could be used to dissect *Onobrychis* genomes. Therefore, this study was conducted to identify additional SSR markers from *Medicago* and *Phaseolus* genomes to understand the genetic relationship and variation among *Onobrychis* genus collected from different regions of Turkey. This study is likely to assist exploiting genetic variation and improve *Onobrychis* and its cultivation as an important alternative feed crop.

MATERIALS AND METHODS

Plant material and genomic DNA extraction: Fifty eight *Onobrychis* taxa were used in this study (Table 1). Seeds were collected from natural flora of Turkey between years 2006 and 2008 and they were planted to experimental field of Department of Field Crops, Ankara University. Additionally, *M. truncatula* which was kindly provided from U.S. National Plant Germplasm System (NPGS) and *P. vulgaris* species was used as a positive control when testing SSR transferability (Table 1). One plant per population was randomly selected and young healthy leaves from each *Onobrychis* taxa were collected for DNA extraction. DNA was extracted using Fermentas (K512) plant DNA isolation kit. Extracted DNA was quantified nanodrop spectrophotometer (ACTGene UVS-99, NJ, USA) and diluted to 10 ng/µl before PCR amplification.

SSR analysis: Ninety five microsatellites from *M. truncatula* (68 primers), *P. vulgaris* (27 primers) were tested for transferability in *O. viciifolia* and selected fourteen SSR primer pairs (Table 2) were used in reactions. PCR amplification reactions were performed in a final volume of 25 µl containing 0.2 µl of each deoxynucleotide, 0.2 µl of TaqDNA polymerase (5 U/µl), 2.0 µl DNA template (10 ng), 0.2 µl each of primer, 2.5 µl of 10X PCR buffer, 1.5 µl of $MgCl_2$ (25 mM) and 14.8 µl of sterilized distilled water. PCR was carried out using a Multigene thermocycler (Labnet International, NJ, USA). The PCR conditions were optimized with a 5 min initial denaturation at 95°C followed by 35 cycles of 94 °C for 30 s, with the annealing temperature ranging from 50 °C to 61 °C (annealing temperature was optimized for each SSR locus) for 30 s and an extension at 72 °C for 1 min and final extension step at 72 °C for 10 min before cooling to 4 °C. PCR products were separated by electrophoresis using 3% 'Nu Micropor agarose (Prona, EU) agarose gels in 1x TBE buffer at 5 V/cm, stained with ethidium bromide (0.5 µg/ml) and visualised under UV light using the Gel-Documentation Unit (DNR MiniLumi, Israel) and photographed. Band scoring was carried out using DNA size standards (100 bp or 1 kb, Fermentas Cat No: SM0241) depending on the SSR amplification range. The presence and absence of each band of a particular molecular weight in SSR profiles of all the taxa was scored manually.

Data analysis: To characterize genetic variation, observed number of alleles (Na), effective number of alleles (Ne), Nei's gene diversity (He) and Shannon's information index (I) were calculated. All of the above calculations were performed using POPGENE program ver. 2.02 g (Yeh *et al*., 1999). Coefficient of similarity among species was calculated according to Nei (1972). The UPGMA tree and Principal Component Analysis (PCA) were constructed using NTSYS v2.02 (Rohlf, 1998). Mantel's test was also performed to compare genetic similarity with geographical similarity such as altitude and coordinates using NTSYS v2.02. The polymorphism information content (PIC) of each of the analyzed SSRs was calculated using Power Marker ver. 3.0 (Lui, 2003).

RESULTS

A total of 95 SSR primer pairs, derived either from *M. truncatula* (68) or *P. vulgaris* (27) genomes, were used to detect loci on *Onobrychis* taxa. From *M. truncatula* and *P. vulgaris*, 19% of the primer pairs produced bands and were successfully transferred to *Onobrychis* genome, respectively. In the present study, we also used the 18 SSR markers to investigate genetic diversity on *Onobrychis* taxa collected from different regions of Turkey between 2006-2008 (Table 1). Of the 18 SSRs, all were polymorphic and resulted in 79 loci among 58 *Onobrychis taxa*. A total of 725 alleles were obtained by the 79 loci rendering average of 9.18 alleles per locus. The highest numbers of loci (seven loci) were obtained from the BM175 and MTIC84 primers while there were only two loci for each of the BM137, BM141 and MTIC326 primer pairs.

Gene diversity (h*) and polymorphism information content (PIC) values indicated *P. vulgaris* primers produced the most informative loci on *Onobrychis* genomes (Table 3). The highest rate of genetic diversity information was obtained from BM141 and Pv-att001 while the lowest was from MTIC58, MTIC89 and MTIC 93 (Table 3).

Although effective alleles, gene diversity and Shannon's information index values were higher in *P. vulgaris* and *M. truncatula*, the highest values in *Onobrychis* taxa were obtained for *O. argyrea* subsp *argyrea* (53) while lowest were from *O. cornuta* (1) (Table 2). Additionally the average values were the highest on *Hymenobrychis* section which was followed by *Heliobrychis*, *Onobrychis*, *Laphobrychis* and *Dendobrychis* sections.

Ecological distance or altitude does not seem to be the factor for the genetic diversity among and within sections since Mantel's test showed no significant correlation between genetic distance and geographical distance (r = -0.020; p = 0.291; 1000 permutation). Similarity index obtained from SSR patterns shows the relative genetic similarities within *Onobrychis* taxa ranging from 0.013 and 0.399 (Table 4). When *P. vulgaris* and *M. truncatula* were involved in the matrix, genetic difference was as high as 0.608 between *M. truncatula* and *Onobrychis lasistanica* Širj. (37); (Table 4). Within the section, the highest similarity (0.013) was between *O. argyrea* subsp *argyrea* (53) and *Onobrychis huetiana* Boiss. (56), *Onobrychis occulta* Hedge & Hub.-Mor. (11) and *Onobrychis araxina* Schischkin (25); *Onobrychis armena* Boiss.(20) and *O. araxina* (25) while *Onobrychis ornata* Desv. (57) had the largest genetic difference (0.399) from *Onobrychis kotschyana* Fenzl (32), *Onobrychis altissima* Grossh. (36) and *O. lasistanica* (37). Since number of the species was the largest, magnitude of variation was the highest within the *Onobrychis* section as expected (Table 4).

According to the SSR results and the resulting dendogram, common bean seemed to have slightly more similar to *Onobrychis* genomes than did *M. truncatula* (Figure 1). The dendogram comprised two main groups in which Group I involved *Hymenobrychis* and *Heliobrychis*; Group II involved *Onobrychis*, *Lapobrychis* and *Dendorychis* (Figure 1). In group I, *Hymenobrychis* and *Heliobrychis* formed two subgroups and each section was clearly separated from each other. However, Group II was also comprised of two subgroups. One of the two subgroups contained *Laphobrychis* and *Dendorychis* with some of the *Onobrychis* species. Although *Lapobrychis* was clearly distinct, *Dendobrychis* was placed with *Onobrychis stenostachya* Freyn subsp. *sosnowskyi* (Grossh.) Hedge (21), *Onobrychis stenostachya* subsp. *sosnowskyi* (23), *Onobrychis stenostachya* subsp. s*osnowskyi* (22), *O. araxina* (25) and *O. armena* (20) (Figure 1).

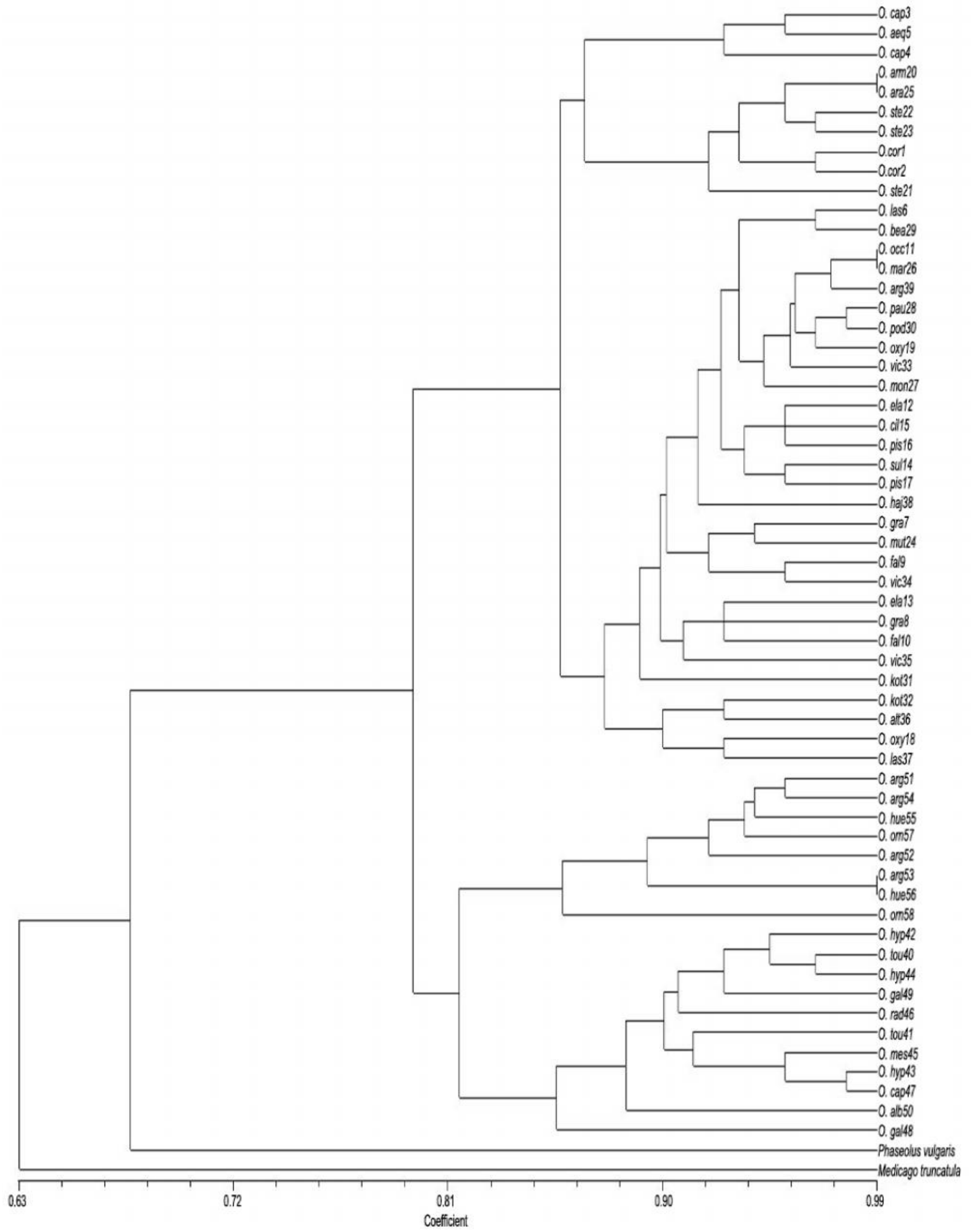

Figure 1. Dendogram of *Onobrychis* taxa.

Table 1. List and origin of the fifty-eight *Onobrychis* taxa according to sections

No	S. No	Name of taxa and sections	Abbreviations	Lati. (N)	Long. (E)	Alti. (m)
		***Dendobrychis* section**				
1	27	*O. cornuta*	Between Erzincan to Bayburt road	39°53'09"	39°21'18"	2084
2	78	*O. cornuta*	Konya, between Beyreli to Ta kent road	36°49'56"	32°27'57"	1891
		***Laphobrychis* section**				
3	1	*Onobrychis caput-galli* Lam.	Çanakkale, between Eceabat to Yalova road	40°14'14"	26°27'04"	17
4	37	*O. caput-galli*	Konya, Bucakkı la	36°56' 52"	33°02'37"	427
5	40	*Onobrychis aequidentata* d'Urv.	K. Mara , entrance of Süleymanlı	37°52'20"	36°50'07"	2607
		Onobrychis section				
6	4	*Onobrychis lasiostachya* Boiss.	Denizli, Pamukkale	37°54'59"	29°09'43"	330
7	5	*Onobrychis gracilis* Besser	Adana, Pozantı	37°24'31"	31°52'44"	780
8	51	*O. gracilis*	Mara , Süleymanlı	37°51'16"	36°49'42"	825
9	6	*Onobrychis fallax* Freyn & Sint. ex Freyn var. *longifolia* Aktoklu	Malatya, Arguvan	39°00'02"	38°12'27"	1410
10	54	*Onobrychis fallax* Freyn & Sint. ex Freyn	Malatya, Pötürge	38°12'31"	38°53'05"	1156
11	8	*O. occulta*	Sivas, Kangal	39°12'32"	37°21'14"	1599
12	12	*Onobrychis elata* Boiss. & Balansa	Nev ehir, Zelve 1	38°40'43"	34°51'09"	1046
13	44	*O. elata*	Kayseri, between Hisarcık to Hacılara road	38°38'17"	35°29'36"	1443
14	14	*Onobrychis sulphurea* Boiss. Et Bal. var. *sulphurea* (C. Koch) Tvzel.	Kayseri, Hisarcık	38°37'38"	35°31'39"	1514
15	15	*Onobrychis cilicica* Kit Tan & Sorger	Mersin, between Mut to Kırobası	36°41'38"	33°37'27"	1095
16	16	*Onobrychis pisidica* Boiss.	Isparta, arkikaraa aç, Örenköy village	38°06'04"	31°13'27"	1341
17	88	*O. pisidica*	Isparta, arkikara aç	38°09'25"	31°16'42"	1211
18	20	*Onobrychis oxyodonta* Boiss.	Ankara, Çubuk II Dam	40°17'27"	33°00'57"	1161
19	63	*O. oxyodonta*	Karabük, Araç	41°12'42"	32°57'21"	370
20	18	*O. armena*	Between Erzincan to Kelkit, Yeniyol village	39°53'14"	39°21'01"	2102
21	21	*Onobrychis stenostachya* subsp. *Sosnowskyi*	Kars, Akyaka	40°45'10"	43°38'00"	1536
22	35	*Onobrychis stenostachya* subsp. *Sosnowskyi*	Erzurum, Tortum	40°13'42"	41°30'23"	1987
23	92	*Onobrychis stenostachya* subsp. *Sosnowskyi*	Erzurum, Hınıs	39°33'43"	41°44'32"	878
24	23	*Onobrychis mutensis* Kit Tan & Sorger	Mersin, Mut, A a ı Köselerli village	36°32'18"	33°26'32"	125
25	28	*O. araxina*	Kars, Susuz, Kiziro lu village	40°55'38"	43°03'30"	2796
26	29	*Onobrychis marashensis* H.Duman & Vural	K. Mara , Ahır mountain	37°38'33"	37°02'08"	1850
27	30	*Onobrychis montana* DC.	Between Bayburt to A kale, Kop mountain	40°01'52"	40°30'57"	2408
28	36	*Onobrychis paucijuga* Bornm.	Konya, Cihanbeyli	38°42'49"	33°00'38"	940
29	43	*Onobrychis beata* Širj.	Adana, Karaisalı, Kızılda plateau	37°24'23"	35°02'55"	1435
30	47	*Onobrychis podperae* Širj.	Kütahya, between Gediz to Emet road	39°02'22"	29°25'42"	820
31	56	*O. kotschyana*	Gaziantep, campus of G. Antep Üniversity	37°01'09"	37°18'57"	897
32	68	*O. kotschyana*	. Urfa, Hilvan, parting of the Bozova road	37°31'59"	38°53'08"	629
33	48	*O. viciifolia*	Kütahya, Gediz, Çavdarisar	39°05'53"	19°28'51"	887
34	60	*O. viciifolia*	Bolu, Mengen	40°54'07"	32°04'09"	642
35	87	*O. viciifolia*	Erzurum, Hınıs	39°33'43"	41°44'32"	1878
36	70	*O. altissima*	Kars, Susuz, Mezra Village	40°41'54"	43°09'55"	1819
37	94	*O. lasistanica*	Trabzon, Köprüba ı, Kemer gateway	40°38'00"	40°01'00"	2426
38	96	*Onobrychis hajastana* Grossh.	Erzurum, Ilıca, E erti village	40°06'27"	40°58'39"	2000
39	97	*Onobrychis argaea* Boiss. & Balansa	Kayseri, Erciyes mountain, Tekir plateau	38°32'28"	35°31'26"	2227
		***Hymenobrychis* section**				
40	13	*Onobrychis tournefortii* Desv.	Nev ehir, Zelve 1	38°40'09"	35°51'49"	1060
41	26	*O. tournefortii*	Sivas, Ta lıdere	39°37'03"	37°01'04"	1312
42	17	*Onobrychis hypargyrea* Boiss.	Ankara, Çubuk II Dam	40°17'27"	33°00'57"	1161
43	46	*O. hypargyrea*	Kütahya, Gediz, U ak road	38°55'52"	29°18'33"	687
44	62	*O. hypargyrea*	Between Karabük to Araç road	41°12'35"	32°48'50"	365
45	22	*Onobrychis meschetica* Grossh.	Kars, Akyaka	40°45'10"	43°38'00"	1536

46	33	*Onobrychis radiata* M.Bieb.	Kars, between Kötek to Paslı road	40°45'25"	42°58'00"	1609
47	52	*Onobrychis cappadocica* Boiss.	Malatya to Elazı road	38°21'09"	38°35'23"	771
48	55	*Onobrychis galegifolia* Boiss.	Adıyaman, Gölba ı	37°50'44"	37°18'57"	897
49	57	*O. galegifolia*	Gaziantep, campus of G. Antep Üniversity	37°02'01"	37°18'06"	894
50	73	*Onobrychis albiflora* Hub.-Mor.	Sivas, between Sincan to Karaman road	39°27'34"	37°49'14"	1246
		***Heliobrychis* section**				
51	7	*O. argyrea* subsp *argyrea*	Between Sivas to Gürün road	38°43'33"	37°16'39"	1377
52	58	*O. argyrea* subsp *argyrea*	Adana, Pozantı, Ulukı la	37°31'00"	34°37'44"	1225
53	66	*O. argyrea* subsp *argyrea*	Çankırı, between Ilgaz to Tosya road	40°54'07"	33°47'24"	818
54	39	*Onobrychis argyrea* Boiss. subsp *isaurica* Hedge Et Hub.-Mor.	Konya, Hadim, Bademli village	37°02'47"	32°43'31"	941
55	9	*O. huetiana*	Sivas, Koyulhisar	40°17'26"	37°49'00"	799
56	67	*O. huetiana*	Çorum, skilip	40°43'50"	34°27'49"	903
57	10	*O. ornata*	Ankara, Bala, Beynam village	39°40'56"	32°49'25"	1077
58	75	*O. ornata*	Entrance of Çankırı	40°44'48"	33°37'32"	696
59		*P. vulgaris*				
60		*M. truncatula*	Australia			

S. No: section number.

Table 2. Primers and related information.

Markers	Sample Size	Primers (5'-3')	Allel No	Gene Diversity	PIC	No. of Allels	Polymorphic Bands	Bands per locus	Reference
AJ248338	60	F: GCAAAGGTGAACGACTT R: CTACCTACGCCACCTCTT	2	0.124	0.103	33	6	5.500	Nagar 2011
AW698894	60	F: ATTCAGCAGGAGGAGCAT R:TGCAACCCAGACACTTTCA	2	0.190	0.159	29	4	7.250	Nagar 2011
MTIC21	60	F: GGTGATTGACTGTGGTGTCG R: TCCGGTCTCCCAGGTTCTA	2	0.151	0.125	82	6	13.667	Julier *et al*. 2003
MTIC230	60	F: GTAAGCGCCTGCTTGGACT R: GAGATTCTGCCAAAATGCAA	2	0.215	0.181	78	4	19.500	Julier *et al*. 2003
MTIC272	60	F: AGGTGGATGGAGAGAGTCA R: TCATGAATAGTGGCACTCAA	2	0.289	0.229	47	3	15.667	Julier *et al*. 2003
MTIC289	60	F: GCTGGTGTCAAAGAGGTCTA R: AGATTTGAAACTGCCCTACA	2	0.077	0.070	13	5	2.600	Julier *et al*. 2003
MTIC326	60	F: GATCACCCTTTATGGAGTTTGAA R: CGACTTCAATTGACCCCCTA	2	0.253	0.197	38	2	19.000	Julier *et al*. 2003
MTIC35	60	F: GAAGAAGAAAAAGAGATAGATCT GTGGR:GGCAGGAACAGATCCTTGAA	2	0.166	0.135	21	3	7.000	Julier *et al*. 2003
MTIC58	60	F: CATCATTAACAACAACGGCAAT R: TGCAAACACAGAACCGAAGA	2	0.074	0.069	12	5	2.400	Julier *et al*. 2003
MTIC79	60	F: AAAATCCAAAGCCCTATCACA R: AGCGTGAGATTTTTCCATCG	2	0.124	0.100	30	5	6.000	Julier *et al*. 2003
MTIC82	60	F: CACTTTCCACACTCAAACCA R: GAGAGGATTTCGGTGATGT	2	0.147	0.125	54	5	10.800	Julier *et al*. 2003
MTIC84	60	F: TCTGAGAGAGAGACAAACAAAAC AAR: GGGAAAAGGTGTAGCCATTG	2	0.063	0.061	14	7	2.000	Julier *et al*. 2003
MTIC94	60	F: GCTACAACAGCGCTACATCG R: CAGGGTCAGAGCAACAATCA	2	0.084	0.078	11	4	2.750	Julier *et al*. 2003
BM175	60	F: CAACAGTTAAAGGTCGTCAAATT R: CCACTCTTAGCATCAACTGGA	2	0.205	0.167	73	7	10.429	
PV-at001	60	F: GGGAGGGTAGGGAAGCAGTG R: GCGAACCACGTTCATGAATGA	2	0.428	0.336	54	2	27.000	Yu *et al*. 2000
BM137	60	F: CCGTATCCGAGCACCGTAAC R: CGCTTACTCACTGTACGCACG	2	0.176	0.151	13	2	6.500	Gaitan-Solis *et al*. 2002
BM141	60	F: TGAGGAGGAACAATGGTGGC R: CTCACAAACCACAACGCACC	2	0.459	0.352	97	4	24.250	Gaitan-Solis *et al*. 2003
BM152	60	F:AAGAGGAGGTCGAAACCTTAAATCG R: CCGGGACTTGCCAGAAGAAC	2	0.151	0.135	26	5	5.200	Gaitan-Solis *et al*. 2004

Table 3. Observed number of alleles, Effective number of alleles, Nei's (1973) gene diversity, Shannon's Information index.

Locus	na*	ne*	h*	I*
O. cap3	2	1.1633	0.1404	0.2688
O. cap4	2	1.1633	0.1404	0.2688
O. aeq5	2	1.2225	0.1820	0.3279
O. las6	2	1.2225	0.1820	0.3279
O. gra7	2	1.2225	0.1820	0.3279
O. fal9	2	1.1926	0.1615	0.2993
O. occ11	2	1.2839	0.2211	0.3798
O. ela12	2	1.2839	0.2211	0.3798
O. sul14	2	1.3792	0.2750	0.4471
O. cil15	2	1.4117	0.2916	0.4671
O. pis16	2	1.3471	0.2577	0.4260
O.arm20	2	1.1345	0.1186	0.2359
O. oxy18	2	1.5429	0.3519	0.5367
O. ste21	2	1.2530	0.2019	0.3546
O. mut24	2	1.2530	0.2019	0.3546
O. ara25	2	1.1633	0.1404	0.2688
O. mar26	2	1.3153	0.2397	0.4036
O. mon27	2	1.1926	0.1615	0.2993
O. ste22	2	1.1633	0.1404	0.2688
O. pau28	2	1.2530	0.2019	0.3546
O. bea29	2	1.3153	0.2397	0.4036
O. ela13	2	1.4117	0.2916	0.4671
O. pod30	2	1.2530	0.2019	0.3546
O. vic33	2	1.3153	0.2397	0.4036
O. gra8	2	1.4117	0.2916	0.4671
O. fal10	2	1.4772	0.3230	0.5039
O. kot31	2	1.4117	0.2916	0.4671
O. vic34	2	1.3153	0.2397	0.4036
O. oxy19	2	1.2530	0.2019	0.3546
O. kot32	2	1.4772	0.3230	0.5039
O. alt36	2	1.5429	0.3519	0.5367
O. vic35	2	1.4117	0.2916	0.4671
O. pis17	2	1.4443	0.3076	0.4860
O. ste23	2	1.2530	0.2019	0.3546
O. las37	2	1.5429	0.3519	0.5367
O. haj38	2	1.3792	0.2750	0.4471
O. arg39	2	1.3153	0.2397	0.4036
O. arg51	2	1.4443	0.3076	0.4860
O. hue55	2	1.3471	0.2577	0.4260
O. orn57	2	1.3471	0.2577	0.4260
O. arg54	2	1.4443	0.3076	0.4860
O. arg52	2	1.2839	0.2211	0.3798
O. arg53	2	1.5429	0.3519	0.5367
O. hue56	2	1.5100	0.3378	0.5208
O. orn58	2	1.4772	0.3230	0.5039
O. cor1	2	1.1064	0.0961	0.2004
O. cor2	2	1.1345	0.1186	0.2359
O. tou40	2	1.4443	0.3076	0.4860
O. hyp42	2	1.5100	0.3378	0.5208
O. mes45	2	1.4772	0.3230	0.5039
O. tou41	2	1.3792	0.2750	0.4471
O. rad46	2	1.5100	0.3378	0.5208
O. hyp43	2	1.3792	0.2750	0.4471
O. cap47	2	1.3153	0.2397	0.4036
O. gal48	2	1.4117	0.2916	0.4671
O. gal49	2	1.4117	0.2916	0.4671
O. hyp44	2	1.4117	0.2916	0.4671
O. alb50	2	1.4443	0.3076	0.4860

na = Observed number of alleles ne = Effective number of alleles [Kimura and Crow (1964)] h = Nei's (1973) gene diversity
I = Shannon's Information index [Lewontin (1972)]

Table 4. Genetic similarity values within *Onobrychis* taxa

	O. cap3	O. cap4	O. aeq5	O. las6	O. gra7	O. fal9	O. occ11	O. ela12	O. sul14	O. cil15	O. pis16	O. arm20	O. oxy18	O. ste21	O. mut24	O. ara25	O. mar26	O. mon27	O. ste22	O. pau28	O. bea29	O. ela13	O. pod30	O. vic33	O. gra8	O. fal10	O. kot31	O. vic34	O. oxy19	O. kot32	O. alt36	O. vic35	O. pis17	O. ste23	O. las37	O. haj38	O. arg39	O. arg51	O. hue55	O. orn57	O. arg54	O. arg52	O. arg53	O. hue56	O. orn58	O. cor1	O. cor2	O. tou40	O. hyp42	O. mes45	O. tou41	O. rad46	O. hyp43	O. cap47	O. gal48	O. gal49	O. hyp44	O. alb50	P. vulgaris	M. truncatula
O. cap3	1.00																																																										
O. cap4	0.33	1.00																																																									
O. aeq5	0.56	0.40	1.00																																																								
O. las6	0.17	0.17	0.23	1.00																																																							
O. gra7	0.17	0.17	0.33	0.46	1.00																																																						
O. fal9	0.30	0.08	0.25	0.50	0.36	1.00																																																					
O. occ11	0.33	0.23	0.20	0.50	0.39	0.42	1.00																																																				
O. ela12	0.46	0.33	0.39	0.64	0.39	0.42	0.67	1.00																																																			
O. sul14	0.36	0.27	0.31	0.40	0.31	0.25	0.53	0.64	1.00																																																		
O. cil15	0.33	0.25	0.29	0.47	0.38	0.31	0.60	0.71	0.69	1.00																																																	
O. pis16	0.29	0.20	0.25	0.43	0.43	0.36	0.57	0.69	0.67	0.73	1.00																																																
O. arm20	0.10	0.00	0.18	0.44	0.30	0.50	0.15	0.25	0.20	0.27	0.31	1.00																																															
O. oxy18	0.26	0.20	0.24	0.37	0.30	0.25	0.47	0.56	0.63	0.68	0.58	0.21	1.00																																														
O. ste21	0.07	0.07	0.13	0.55	0.31	0.33	0.27	0.36	0.22	0.28	0.31	0.40	0.23	1.00																																													
O. mut24	0.25	0.15	0.21	0.42	0.55	0.46	0.58	0.58	0.47	0.64	0.62	0.27	0.42	0.29	1.00																																												
O. ara25	0.20	0.09	0.27	0.40	0.27	0.44	0.14	0.33	0.27	0.33	0.39	0.83	0.26	0.36	0.25	1.00																																											
O. mar26	0.31	0.21	0.19	0.46	0.36	0.39	0.91	0.62	0.60	0.67	0.64	0.23	0.53	0.25	0.54	0.21	1.00																																										
O. mon27	0.08	0.18	0.07	0.50	0.25	0.40	0.55	0.55	0.43	0.50	0.58	0.33	0.39	0.33	0.46	0.30	0.64	1.00																																									
O. ste22	0.09	0.09	0.17	0.40	0.17	0.30	0.23	0.23	0.19	0.25	0.20	0.57	0.20	0.36	0.15	0.50	0.31	0.30	1.00																																								
O. pau28	0.15	0.25	0.21	0.70	0.42	0.46	0.73	0.58	0.47	0.53	0.50	0.27	0.42	0.39	0.50	0.25	0.67	0.60	0.36	1.00																																							
O. bea29	0.21	0.13	0.27	0.73	0.58	0.39	0.62	0.62	0.60	0.67	0.64	0.46	0.53	0.43	0.54	0.42	0.69	0.50	0.42	0.67	1.00																																						
O. ela13	0.25	0.25	0.29	0.47	0.47	0.31	0.60	0.60	0.50	0.65	0.53	0.19	0.52	0.28	0.64	0.18	0.56	0.40	0.18	0.53	0.56	1.00																																					
O. pod30	0.15	0.25	0.13	0.55	0.31	0.33	0.73	0.58	0.47	0.64	0.50	0.17	0.42	0.29	0.64	0.15	0.67	0.60	0.25	0.80	0.54	0.64	1.00																																				
O. vic33	0.21	0.21	0.19	0.46	0.36	0.29	0.75	0.62	0.60	0.67	0.53	0.14	0.61	0.25	0.54	0.13	0.69	0.50	0.21	0.67	0.57	0.56	0.67	1.00																																			
O. gra8	0.33	0.33	0.47	0.47	0.57	0.40	0.50	0.60	0.59	0.65	0.63	0.36	0.60	0.28	0.53	0.43	0.56	0.40	0.33	0.53	0.67	0.65	0.44	0.47	1.00																																		
O. fal10	0.29	0.22	0.33	0.50	0.41	0.35	0.53	0.63	0.53	0.67	0.65	0.24	0.55	0.32	0.56	0.29	0.50	0.35	0.22	0.56	0.59	0.67	0.56	0.50	0.67	1.00																																	
O. kot31	0.25	0.18	0.22	0.38	0.38	0.40	0.60	0.41	0.42	0.47	0.53	0.27	0.46	0.21	0.44	0.25	0.67	0.40	0.33	0.53	0.56	0.40	0.44	0.47	0.56	0.50	1.00																																
O. vic34	0.21	0.13	0.19	0.46	0.46	0.64	0.50	0.40	0.41	0.47	0.53	0.46	0.45	0.33	0.54	0.42	0.57	0.50	0.31	0.54	0.57	0.39	0.43	0.38	0.56	0.42	0.56	1.00																															
O. oxy19	0.25	0.25	0.21	0.42	0.31	0.23	0.73	0.58	0.57	0.64	0.50	0.08	0.42	0.20	0.64	0.07	0.67	0.46	0.15	0.64	0.54	0.64	0.80	0.67	0.44	0.56	0.44	0.33	1.00																														
O. kot32	0.29	0.22	0.26	0.33	0.33	0.28	0.44	0.53	0.53	0.58	0.56	0.17	0.55	0.19	0.56	0.22	0.42	0.28	0.16	0.39	0.42	0.50	0.47	0.50	0.50	0.60	0.50	0.35	0.47	1.00																													
O. alt36	0.20	0.14	0.18	0.37	0.30	0.25	0.47	0.47	0.48	0.52	0.50	0.15	0.57	0.23	0.50	0.14	0.45	0.32	0.14	0.42	0.45	0.60	0.50	0.53	0.46	0.70	0.46	0.32	0.50	0.70	1.00																												
O. vic35	0.18	0.11	0.22	0.47	0.47	0.31	0.41	0.41	0.42	0.56	0.44	0.27	0.52	0.28	0.53	0.25	0.39	0.24	0.25	0.44	0.56	0.56	0.44	0.56	0.56	0.67	0.47	0.39	0.44	0.58	0.60	1.00																											
O. pis17	0.24	0.17	0.21	0.44	0.35	0.29	0.56	0.56	0.75	0.71	0.69	0.25	0.57	0.26	0.60	0.24	0.63	0.47	0.24	0.50	0.63	0.61	0.60	0.63	0.53	0.63	0.53	0.44	0.60	0.63	0.65	0.61	1.00																										
O. ste23	0.15	0.15	0.21	0.55	0.31	0.33	0.27	0.36	0.29	0.35	0.31	0.56	0.29	0.50	0.29	0.50	0.33	0.33	0.67	0.39	0.54	0.28	0.29	0.25	0.44	0.32	0.35	0.43	0.20	0.25	0.23	0.35	0.33	1.00																									
O. las37	0.26	0.20	0.24	0.37	0.30	0.25	0.47	0.56	0.63	0.68	0.58	0.21	0.71	0.23	0.50	0.26	0.53	0.39	0.20	0.42	0.53	0.68	0.50	0.53	0.60	0.62	0.46	0.38	0.50	0.70	0.71	0.52	0.65	0.29	1.00																								
O. haj38	0.12	0.19	0.11	0.40	0.24	0.25	0.53	0.44	0.53	0.59	0.47	0.20	0.55	0.22	0.47	0.19	0.60	0.54	0.27	0.57	0.50	0.59	0.69	0.60	0.50	0.53	0.42	0.41	0.57	0.45	0.55	0.50	0.65	0.29	0.63	1.00																							
O. arg39	0.21	0.21	0.19	0.46	0.36	0.29	0.75	0.62	0.60	0.67	0.64	0.23	0.61	0.25	0.54	0.21	0.83	0.64	0.31	0.67	0.69	0.56	0.67	0.69	0.56	0.50	0.67	0.47	0.67	0.42	0.45	0.39	0.63	0.33	0.53	0.60	1.00																						
O. arg51	0.11	0.00	0.10	0.21	0.15	0.29	0.14	0.14	0.08	0.12	0.13	0.25	0.22	0.26	0.14	0.24	0.13	0.10	0.24	0.14	0.18	0.16	0.09	0.08	0.21	0.19	0.21	0.24	0.04	0.15	0.18	0.21	0.11	0.26	0.18	0.12	0.13	1.00																					
O. hue55	0.13	0.00	0.11	0.25	0.18	0.36	0.16	0.16	0.09	0.13	0.14	0.31	0.15	0.31	0.17	0.29	0.15	0.12	0.29	0.17	0.21	0.18	0.11	0.10	0.24	0.22	0.18	0.28	0.05	0.12	0.15	0.24	0.13	0.31	0.15	0.14	0.10	0.69	1.00																				
O. orn57	0.13	0.00	0.11	0.11	0.11	0.19	0.05	0.05	0.04	0.04	0.04	0.13	0.11	0.17	0.05	0.13	0.05	0.00	0.13	0.05	0.10	0.08	0.00	0.00	0.13	0.12	0.13	0.15	0.00	0.04	0.07	0.13	0.04	0.17	0.07	0.04	0.05	0.69	0.60	1.00																			
O. arg54	0.17	0.00	0.15	0.21	0.21	0.29	0.19	0.19	0.17	0.21	0.23	0.33	0.27	0.26	0.20	0.31	0.24	0.16	0.31	0.14	0.30	0.21	0.09	0.13	0.32	0.24	0.32	0.30	0.09	0.15	0.18	0.26	0.20	0.33	0.22	0.17	0.24	0.77	0.69	0.69	1.00																		
O. arg52	0.14	0.00	0.13	0.29	0.20	0.42	0.18	0.18	0.15	0.20	0.22	0.50	0.17	0.27	0.19	0.46	0.24	0.21	0.33	0.19	0.31	0.14	0.12	0.11	0.26	0.18	0.26	0.40	0.06	0.13	0.12	0.20	0.19	0.36	0.17	0.15	0.17	0.56	0.57	0.47	0.67	1.00																	
O. arg53	0.14	0.04	0.13	0.30	0.18	0.32	0.22	0.22	0.19	0.23	0.20	0.28	0.33	0.23	0.17	0.26	0.26	0.19	0.33	0.23	0.32	0.23	0.17	0.16	0.33	0.26	0.33	0.32	0.13	0.21	0.24	0.28	0.22	0.35	0.29	0.24	0.26	0.74	0.50	0.50	0.65	0.47	1.00																
O. hue56	0.15	0.05	0.14	0.32	0.19	0.33	0.23	0.23	0.20	0.24	0.21	0.29	0.30	0.24	0.18	0.28	0.27	0.20	0.35	0.24	0.33	0.24	0.18	0.17	0.35	0.27	0.29	0.33	0.13	0.22	0.25	0.29	0.23	0.37	0.30	0.25	0.22	0.68	0.53	0.45	0.60	0.50	0.94	1.00															
O. orn58	0.16	0.05	0.09	0.26	0.20	0.28	0.30	0.24	0.21	0.25	0.22	0.17	0.26	0.14	0.25	0.16	0.29	0.15	0.16	0.25	0.29	0.30	0.25	0.23	0.30	0.28	0.25	0.29	0.19	0.23	0.26	0.30	0.24	0.19	0.26	0.26	0.23	0.48	0.47	0.33	0.41	0.37	0.55	0.57	1.00														
O. cor1	0.00	0.00	0.00	0.20	0.10	0.20	0.20	0.20	0.10	0.10	0.10	0.30	0.10	0.30	0.20	0.30	0.20	0.20	0.40	0.20	0.20	0.10	0.20	0.20	0.10	0.10	0.10	0.20	0.10	0.10	0.10	0.10	0.10	0.30	0.10	0.10	0.20	0.20	0.20	0.10	0.20	0.20	0.20	0.20	0.20	1.00													
O. cor2	0.00	0.10	0.00	0.30	0.20	0.20	0.30	0.30	0.10	0.20	0.20	0.30	0.20	0.40	0.30	0.20	0.20	0.30	0.40	0.30	0.20	0.20	0.30	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.60	0.20	0.20	0.20	0.20	0.20	0.10	0.20	0.20	0.20	0.20	0.10	0.50	1.00												
O. tou40	0.20	0.30	0.30	0.30	0.20	0.20	0.30	0.40	0.30	0.30	0.30	0.20	0.30	0.30	0.30	0.20	0.20	0.20	0.20	0.30	0.20	0.40	0.30	0.20	0.50	0.30	0.20	0.20	0.20	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.20	0.40	0.40	0.30	0.40	0.30	0.40	0.50	0.30	0.20	0.30	1.00											
O. hyp42	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.30	0.30	0.30	0.30	0.20	0.30	0.20	0.20	0.30	0.20	0.30	0.20	0.20	0.20	0.30	0.20	0.20	0.40	0.30	0.20	0.30	0.10	0.20	0.20	0.20	0.20	0.40	0.30	0.30	0.20	0.30	0.30	0.30	0.40	0.40	0.40	0.40	0.30	0.20	0.30	0.80	1.00										
O. mes45	0.20	0.20	0.20	0.10	0.20	0.30	0.20	0.20	0.20	0.30	0.30	0.20	0.30	0.20	0.30	0.20	0.20	0.20	0.20	0.20	0.20	0.30	0.20	0.20	0.40	0.30	0.30	0.30	0.10	0.30	0.20	0.20	0.20	0.20	0.30	0.20	0.20	0.40	0.30	0.30	0.40	0.30	0.40	0.40	0.40	0.30	0.20	0.60	0.50	1.00									
O. tou41	0.10	0.10	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.30	0.20	0.30	0.20	0.20	0.30	0.10	0.10	0.20	0.20	0.20	0.30	0.20	0.10	0.40	0.30	0.10	0.30	0.10	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.10	0.30	0.40	0.30	0.30	0.30	0.30	0.30	0.40	0.30	0.20	0.60	0.50	0.50	1.00								
O. rad46	0.30	0.20	0.30	0.10	0.20	0.20	0.20	0.20	0.30	0.30	0.30	0.20	0.30	0.10	0.20	0.20	0.30	0.10	0.20	0.20	0.20	0.30	0.20	0.20	0.40	0.30	0.30	0.30	0.10	0.20	0.20	0.20	0.20	0.20	0.30	0.30	0.30	0.40	0.30	0.40	0.50	0.40	0.50	0.50	0.40	0.10	0.10	0.70	0.60	0.70	0.50	1.00							
O. hyp43	0.20	0.20	0.20	0.20	0.20	0.30	0.20	0.30	0.20	0.30	0.30	0.20	0.30	0.20	0.30	0.30	0.20	0.20	0.20	0.20	0.20	0.40	0.20	0.20	0.40	0.30	0.20	0.30	0.20	0.30	0.20	0.20	0.20	0.20	0.30	0.20	0.20	0.30	0.30	0.30	0.30	0.30	0.30	0.40	0.40	0.30	0.20	0.60	0.60	0.80	0.60	0.60	1.00						
O. cap47	0.10	0.10	0.20	0.20	0.30	0.20	0.20	0.20	0.20	0.30	0.30	0.20	0.30	0.20	0.30	0.30	0.20	0.10	0.10	0.20	0.20	0.30	0.20	0.20	0.40	0.30	0.10	0.20	0.10	0.20	0.20	0.30	0.20	0.20	0.30	0.20	0.20	0.30	0.30	0.20	0.30	0.30	0.30	0.30	0.40	0.30	0.10	0.50	0.50	0.70	0.60	0.50	0.80	1.00					
O. gal48	0.30	0.20	0.20	0.20	0.10	0.20	0.20	0.30	0.30	0.30	0.20	0.10	0.40	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.30	0.20	0.20	0.30	0.30	0.20	0.20	0.20	0.30	0.30	0.20	0.20	0.20	0.30	0.20	0.30	0.40	0.20	0.30	0.30	0.20	0.50	0.50	0.30	0.10	0.10	0.50	0.40	0.50	0.30	0.50	0.50	0.40	1.00				
O. gal49	0.20	0.30	0.20	0.20	0.30	0.20	0.30	0.30	0.40	0.40	0.40	0.30	0.40	0.20	0.40	0.30	0.30	0.30	0.20	0.30	0.30	0.40	0.30	0.30	0.60	0.40	0.30	0.40	0.20	0.30	0.30	0.30	0.30	0.30	0.40	0.40	0.30	0.20	0.20	0.10	0.30	0.30	0.30	0.30	0.30	0.10	0.20	0.60	0.60	0.50	0.50	0.60	0.60	0.60	0.30	1.00			
O. hyp44	0.20	0.30	0.20	0.20	0.30	0.20	0.30	0.30	0.30	0.30	0.40	0.20	0.30	0.30	0.40	0.30	0.30	0.20	0.20	0.30	0.30	0.40	0.30	0.30	0.50	0.40	0.20	0.30	0.20	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.20	0.30	0.30	0.30	0.30	0.30	0.20	0.30	0.80	0.70	0.70	0.60	0.60	0.80	0.70	0.50	0.80	1.00		
O. alb50	0.10	0.20	0.20	0.10	0.20	0.10	0.10	0.20	0.20	0.20	0.20	0.10	0.30	0.10	0.10	0.20	0.20	0.20	0.20	0.10	0.10	0.20	0.10	0.10	0.30	0.20	0.20	0.20	0.10	0.20	0.20	0.10	0.20	0.20	0.30	0.20	0.20	0.30	0.20	0.20	0.30	0.20	0.30	0.30	0.20	0.20	0.20	0.60	0.60	0.50	0.40	0.50	0.60	0.40	0.40	0.50	0.60	1.00	
P. vulgaris	0.00	0.10	0.10	0.20	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.20	0.20	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.20	0.10	0.10	0.10	0.10	0.10	0.00	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.00	0.10	0.10	0.10	1.00	
M. truncatula	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00

DISCUSSION

Many attempts have been made to transfer markers among Legume species such as from *G. max* to *Arachis hypogaea* L. (He *et al*., 2006), from *M. truncatula*, *Pisum sativum* L. and *Trifolium pretense* L. to *Lens culinaris* Medik. (Reddy *et al*., 2010). However, there has been limited attempt to transfer SSR markers to *Onobrychis* sprecies. In this study, we attempted to transfer SSR markers derived from *M. truncatula* and *P. vulgaris* to wide range of *Onobrychis* genomes collected in Turkey. Additionally, the amplified primers were used to detect genetic diversity within *Onobrychis* genus.

Transferability rate of both *P. vulgaris* and *M. truncatula* markers to *Onobrychis* genomes was the similar. Although rate of transfer was lower than the previous study (Dendoum *et al*., 2012), number of polymorphic markers was adequate to genetically differentiate the *Onobrychis* taxa collected in Turkey. This implies that related legume genomes could be useful SSR marker sources to dissect *Onobrychis* genomes which scarcely have had the mentioned marker system so far. Amplified markers were usually weaker than the donor species. Weaker bands amplified in related genomes were typical in transferability studies (Kuleung *et al*., 2004). Amplified markers from donor genomes resulted in similar banding patterns in *Onobrychis* genome, suggesting that they were derived from the same loci and that these allelic regions of the primer binding sites are conserved. We expected that number of the markers amplified in the *Onobyrchis* genome was to be higher since all three genomes were related. This degree of site loss is likely reflective of the genomic divergence in *Onobrychis* genome.

Although *M. truncatula* and *P. vulgaris* primers yielded similar amplification rates, the latter renders more information about *Onobrychis* genome. This may be due to the fact that compact genome of *M. truncatula* remained more conserved during diversification process while extension of the genomes likely modified the SSR regions as in *P. vulgaris*. Extension and large variation of the legume genomes due to the retroelements are also reported (Pearce *et al*., 1996; Pearce *et al*., 2000). Although the number of samples was higher in *Onobrychis*, the genetic diversity values seemed to be lower than *Hymenobrychis* and *Heliobrychis*. This may imply that *Hymenobrychis* and *Heliobrychis* recently diverged from *Onobrychis* section.

Onobrychis taxa are represented by two sub-genuses (Onobrychis and Sisyrosema) in Turkey. While flowers are usually pink, rarely white or cream and fruit is almost sessile in sub-genus Onobrychis, flowers are yellow, rarely cream or white and fruit is apparent stalk in sub-genus Sisyrosema. However, sub-genus Onobrychis has three sections (*Dendobrychis*, *Laphobrychis* and *Onobrychis*) and sub-genus Sisyrosema has two sections (*Hymenobrychis* and *Heliobrychis*). Additionally, distinction of sections was performed according to annual or perennial herbs or shrubs and standard and wing length ratio of taxa (Hedge, 1970; Davis *et al*., 1988). The dendogram obtained in this study supports such morphological distinction. This suggested that SSR regions were more conserved between *Onobrychis* and *Dendobrychis* and to some extend *Lapobrychis* than between *Onobrychis* and other two sections. More recently, using DNA sequences of intergenic spacer regions of chloroplast genomes, Carbonero (2012) constructed a dendogram among for *Onobrychis* species and found also that *Lapobrychis* and *Dendobrychis* were more similar to *Onobrychis* than were others.

About 10% difference between *P. vulgaris* and *M. truncatula* in similarity to *Onobrychis* genomes did not appear to yield more transferable markers. It is probably due to the less number of primers used in our study. Genetic diversity results were generally in corroboration with morphological distinction of sections. The number of polymorphic SSR markers to dissect *Onobrychis* genomes is limited. Transferability of SSR markers from related genera seems to be a good source for conservation and breeding of *Onobrychis* varieties.

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