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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Evaluation of the Genetic Structure of Some Accessions Belonging to *Onobrychis* spp. Using Microsatellite DNA Markers*

Onobrychis Türlerine Ait Bazı Aksesyonların Mikrosatellit DNA Markırı Kullanılarak Genetik Yapısının Değerlendirilmesi

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Abstract

Turkey is in a very convenient position for animal husbandry in terms of both natural resources and ecological conditions. Forage crops, which has a very important place in agricultural activities, is the insurance of plant and animal production. Sainfoin is a perennial forage legume species that grown in the northern temperate regions of the world from the Mediterranean region and the Caucasus, and to Central Asia. In this study the genetic diversity of 100 genotypes representing 44 accessions from 18 different *Onobrychis* species (*O. arenaria* subsp. *arenaria*, *O. inermis*, *O. petraea*, *O. cyri*, *O. iberica*, *O. altissima*, *O. vassilczenkoi*, *O. conferta* subsp. *argentea*, *O. alba* subsp. *laconica*, *O. biebersteinii*, *O. grandis*, *O. kachetica*, *O. kemulariae*, *O. oxyodonta*, *O. megataphros*, *O. pallasii*, *Onobrychis* spp., and *O. viciifolia*) were evaluated using 8 simple sequence repeat (microsatellite) markers. Based on the results, OVK036, OVK094, OVK125, OVM033, OVK161, OVK046, OVM061, and OVK174 loci were polymorphic. The observed number of alleles per SSR locus ranged from 6 to 21 alleles (mean of 11.625). Maximum allele frequency ranged from 0.51 to 0.93 with a mean value of 0.73. The PIC value ranged from 0.124 to 0.244. The mean polymorphism information content of loci was 0.188. Genetic diversity coefficients according to the UPGMA ranged from 0.000 to 0.9375. Cluster analysis divided the 100 sainfoin genotypes into two main groups (Cluster-I and Cluster-II). All diploid genotypes (except for 1 diploid genotype) used in the study formed a separate group within Cluster-I. The results revealed that SSR markers used in this study are useful for molecular characterization and assessing genetic diversity of sainfoin accessions. The obtained SSR alleles and genetic variability in a studied certain loci provided significant information about the genetic structure of sainfoin accessions that could be used as parental lines in sainfoin breeding programs.

Keywords: Breeding, Molecular characterization, Sainfoin, SSR, UPGMA.

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Öz

Türkiye hem doğal kaynaklar hem de ekolojik koşullar bakımından hayvancılığa oldukça elverişli bir konuma sahiptir. Tarımsal faaliyetler içerisinde çok önemli bir yere sahip olan yem bitkileri tarımı, bitkisel ve hayvansal üretimin sigortası konumundadır. Korunga, dünyanın kuzey ılıman bölgelerinde özellikle Akdeniz bölgesi ve Kafkaslardan Orta Asya'ya kadar yetişen çok yıllık bir baklagil yem bitkisi türüdür. Bu çalışmada 18 farklı *Onobrychis* türüne ait 44 aksesyonu temsil eden 100 genotipin (*O. arenaria* subsp. *arenaria, O. inermis, O. petraea, O. cyri, O. iberica, O. altissima, O. vassilczenkoi, O. conferta* subsp. *argentea, O. alba* subsp. *laconica, O. biebersteinii, O. grandis, O. kachetica, O. kemulariae, O. oxyodonta, O. megataphros, O. pallasii, Onobrychis* spp. ve *O. viciifolia*) genetik çeşitliliği 8 adet basit dizi tekrarları (mikrosatellit) belirteci kullanılarak incelenmiştir. Mikrosatellit analizi sonuçlarına göre, OVK036, OVK094, OVK125, OVM033, OVK161, OVK046, OVM061 ve OVK174 lokusları polimorfik olarak saptanmıştır. SSR lokusu başına gözlenen allel sayısı 6 ile 21 arasında gözlenmiştir (ortalama 11.625). Maksimum allel frekansı 0.51 ile 0.93 arasında değişmekte olup, ortalama 0.73 değerindedir. PIC değeri 0.124 ile 0.244 arasında hesaplanmış, ortalama polimorfik bilgi içeriği 0.188 olarak belirlenmiştir. UPGMA'ya göre genetik çeşitlilik katsayıları 0.000 ile 0.9375 arasında değişmiştir. Dendrogram analizine göre, 100 korunga genotipi iki ana gruba (Grup-I ve Grup-II) ayrılmıştır. Çalışmada kullanılan tüm diploid genotipler (1 diploid genotip hariç) Grup-I içerisinde ayrı bir grup oluşturmuştur. Bu çalışmada kullanılan SSR belirteçleri, korunga aksesyonlarının moleküler karakterizasyonu ve genetik çeşitliliğinin değerlendirilmesi için yararlı olduğu sonucuna varılmıştır. Spesifik lokuslardan elde edilen SSR alelleri ve genetik varyasyon, korunga ıslah programlarında ebeveyn hatları olarak kullanılabilecek korunga aksesyonlarının genetik yapısı hakkında önemli bilgiler sağlamıştır.

Anahtar Kelimeler: Islah, Moleküler karakterizasyon, Korunga, SSR, UPGMA.

1. Introduction

Turkey is one of the countries with high biodiversity in terms of plant flora. The reasons such as being at the intersection of 3 different phytogeographical regions (the Mediterranean, the Irano-Turanian and Euro-Siberian), climate, topography, geological and geomorphological structure, existence of different habitats, and altitude differences contribute to its high biodiversity (Sekercioglu et al., 2011; Noroozi et al., 2019). Turkey hosts approximately 12000 plant species and 32% of these species are endemic (Sekercioglu et al., 2011). In the world, the plant families that take the first 3 ranks based on the number of species are Orchidaceae, Asteraceae and Fabaceae, respectively (Lewis et al., 2005; Christenhusz and Byng, 2016). Fabaceae is the second largest family in Turkey with 1145 species, 69 genera (TUBIVES, 2022). There are 60 taxa of *Onobrychis* genus, which ranks 4th in terms of the number of species in the legume family (TUBIVES, 2022).

Although *O. viciifolia* is the most widely cultivated species of the genus *Onobrychis*, *O. arenaria subsp. arenaria*, and *O. transcaucasica* are also cultivated in some countries. Sainfoin is a common name given to those cultivated species of *Onobrychis.* Sainfoin is a perennial forage legume species that can be easily grown in arid and calcareous soils, especially in the northern temperate regions of the world, ranging from the Mediterranean region and the Caucasus, and to Central Asia (Aktoklu, 1995; Bhattarai et al., 2016). Turkey is the one of the micro gene centers for sainfoin (Yildiz et al., 1999; Carbonero et al., 2011). Sainfoin is an important forage species in terms of many features such as high palatability, high nutritional value, anthelmintic activity, being a good pasture for bees, and usage in soil improvement (Delgado et al., 2008; Ozbek, 2011; Ozalp and Temel, 2016). Ozerbey-03 is Turkey's first registered sainfoin variety in 2003 as a result of the sainfoin breeding program started in 1975. Since 2003, the number of registered varieties in Turkey has increased to seven with the varieties developed by public institutions or the private sectors (Anonymous, 2021).

Microsatellites [Simple Sequence Repeats (SSR)], which are widely distributed throughout eukaryotic genomes, are codominant DNA markers (Vieira et al., 2016; Bagshaw et al., 2017). In plant genetic diversity or genetic characterization studies, microsatellites are widely preferred due to their advantageous characteristics such as being highly informative, multi-allelic nature, high polymorphism level, reproducibility and being transferable between closely related species due to conserved flanking regions (Vieira et al., 2016; Sevim et al., 2017). There were previous reports evaluating genetic structure and diversity in sainfoin populations/accessions. Some of them have used RAPD analysis (Nosrati et al., 2012; Rasouli et al., 2013; Hejrankesh et al., 2014; Ertus, 2021), ISSR analysis (Toluei et al., 2012; Zarrabian et al., 2013; Zarrabian and Majidi, 2015; Nosrati et al., 2016; Ertus, 2021), AFLP analysis (Bhattarai, 2017), and SSR analysis (Demdoum et al., 2012; Kempf et al., 2016; Ozkan and Bilgen, 2019; Shen et al., 2019; Sutcu, 2020). When the studies were examined, it was observed that either dominant marker systems or SSR analysis developed for closely related species were used for determination of the genetic structure and diversity of sainfoin species. There are few studies using SSR markers developed specifically for *Onobrychis* species by Kempf et al. (2016). Therefore, *Onobrychis* species-specific SSR markers were preferred in this study to evaluate the genetic structures of 100 genotypes representing 44 accessions from 18 different *Onobrychis* species using SSR markers. This study also provides the determination of the genetic diversity parameters, and the contribution to the breeding program by molecular phylogenetic analysis.

2. Materials and Methods

2.1. Plant Material

100 genotypes representing 44 accessions from 18 different *Onobrychis* species (*O. arenaria* subsp. *arenaria*, *O. inermis*, *O. petraea*, *O. cyri*, *O. iberica*, *O. altissima*, *O. vassilczenkoi*, *O. conferta* subsp. *argentea*, *O. alba* subsp. *laconica*, *O. biebersteinii*, *O. grandis*, *O. kachetica*, *O. kemulariae*, *O. oxyodonta*, *O. megataphros*, *O. pallasii*, *Onobrychis* spp., *O. viciifolia*) were used as a plant material (*Table 1*). All of the *Onobrychis* accessions used in the study were obtained from USDA gene bank located in Pulman, WA.

Table 1. List of studied Onobrychis accessions number, their origin, sample code, and ploidy levels

Accession No	Species name	Origin	Sample code	Ploidy
PI 312954	*Onobrychis arenaria* subsp. *arenaria*	Stavropol, Russia	1-1, 1-2, 1-3, 1-4	Tetraploid
PI 312943	*Onobrychis inermis*	Stavropol, Russia	6-1, 6-2, 6-3	Tetraploid
PI 312935	*Onobrychis inermis*	Former Soviet Union	8-1, 8-2, 8-3, 8-4	Tetraploid
PI 312942	*Onobrychis inermis*	Former Soviet Union	10-1	Tetraploid
PI 312946	*Onobrychis petraea*	Stavropol, Russia	11-1, 11-2, 11-3, 11-4	Tetraploid
PI 316295	*Onobrychis petraea*	Germany	12-1, 12-2, 12-3	Tetraploid
W6 17878	*Onobrychis cyri*	Russia	13-1, 13-2, 13-3	Tetraploid
PI 315085	*Onobrychis iberica*	Former Soviet Union	17-1, 17-2, 17-3	Tetraploid
PI 312909	*Onobrychis altissima*	Former Soviet Union	23-1, 23-2, 23-3, 23-4	Tetraploid
PI 300580	*Onobrychis vassilczenkoi*	Former Soviet Union	26-1, 26-2, 26-3	Diploid
PI 280259	*Onobrychis conferta* subsp. *argentea*	Spain	30-1	Tetraploid
PI 325448	*Onobrychis altissima*	Stavropol, Russia	42-1	Tetraploid
PI 642147	*Onobrychis alba* subsp. *laconica*	Bulgaria	43-1, 43-2, 43-3	Diploid
PI 227377	*Onobrychis biebersteinii*	Iran	44-1, 44-2, 44-3, 44-4	Tetraploid
PI 297923	*Onobrychis grandis*	Australia	47-1, 47-2, 47-3	Tetraploid
PI 325438	*Onobrychis inermis*	Stavropol, Russia	48-1	Tetraploid
PI 314931	*Onobrychis iberica*	Former Soviet Union	49-1	Tetraploid
PI 314469	*Onobrychis kachetica*	Former Soviet Union	50-1, 50-2	Diploid
PI 312464	*Onobrychis kemulariae*	Former Soviet Union	51-1	Tetraploid
PI 301107	*Onobrychis megataphros*	Turkey	52-1, 52-2, 52-3	Diploid
PI 312945	*Onobrychis oxyodonta*	Former Soviet Union	54-1, 54-2, 54-3	Tetraploid
W6 21877	*Onobrychis pallasii*	Ukraine	55-1	Diploid
PI 312947	*Onobrychis petraea*	Stavropol, Russia	57-1, 57-2, 57-3	Diploid
PI 440574	*Onobrychis vassilczenkoi*	Stavropol, Russia	60-1	Diploid
PI 314468	*Onobrychis cyri*	Former Soviet Union	63-1, 63-2, 63-3, 63-4	Tetraploid
PI 372809	*Onobrychis inermis*	Czech Republic	69-1	Tetraploid
PI 440572	*Onobrychis inermis*	Russia	70-1	Tetraploid
PI 464819	*Onobrychis* spp.	Turkey	78-1, 78-2	Diploid
PI 312940	*Onobrychis inermis*	Stavropol, Russia	81-1, 81-2, 81-3, 81-4	Tetraploid
PI 312936	*Onobrychis inermis*	Former Soviet Union	82-1, 82-2, 82-3, 82-4	Tetraploid
PI 312937	*Onobrychis inermis*	Stavropol, Russia	83-1, 83-2, 83-3	Tetraploid
PI 312938	*Onobrychis inermis*	Former Soviet Union	84-1, 84-2, 84-3	Tetraploid
PI 312939	*Onobrychis inermis*	Stavropol, Russia	85-1, 85-2, 85-3	Tetraploid

Table 1(continuance).

Accession No	Species name	Origin	Sample code	Ploidy
PI 312941	*Onobrychis inermis*	Stavropol, Russia	86-1, 86-2, 86-3	Tetraploid
PI 225729	*Onobrychis* spp.	Turkey	90-1, 90-2, 90-3	Tetraploid
PI 325445	*Onobrychis* spp.	Former Soviet Union	93-1	Tetraploid
PI 567875	*Onobrychis* spp.	Diyarbakır, Turkey	94-1	Diploid
PI 225730	*Onobrychis* spp.	Turkey	97-1	Tetraploid
PI 205200-2	*Onobrychis. viciifolia*	Turkey	95	Tetraploid
PI 236486-1	*Onobrychis viciifolia*	Turkey	96	Tetraploid
PI 250024	*Onobrychis viciifolia*	Iran	97	Tetraploid
PI 273784	*Onobrychis viciifolia*	Lithuania	98	Tetraploid
PI 192994	*Onobrychis viciifolia*	-	99	Tetraploid
PI 639688	*Onobrychis viciifolia*	Wyoming, USA	100	Tetraploid

2.2. DNA Extraction

Fresh leaves of 100 sainfoin genotypes were used for CTAB (Cetyltrimethylammonium bromide)-based DNA extraction protocol with slight modifications (Doyle and Doyle, 1990). Each fresh leaf sample was ground using a ball mill (Retsch MM400) just before starting DNA isolation. The Nanodrop®1000 and Nanodrop® LITE spectrophotometer was used to quantify and qualify the isolated DNAs. The DNA samples were diluted as 50ng/µL and preserved at -20 °C for downstream SSR analysis.

2.3. SSR and Data Analysis

Eight SSRs (namely OVK036, OVK046, OVK094, OVK125, OVK161, OVK174, OVM033, and OVM061) were chosen from species specific SSR primers designed by Kempf et al. (2016). Three-primer strategy of Schuelke (2000) (M13 tailed forward primer, reverse primer and 6-FAM™, NED™, PET™ or VIC™ fluorescent labeled M13 tail primer) was used during PCR amplification. Both the PCR mixture preparation and the PCR amplifications were performed using the protocol described by Kempf et al. (2016). The presence of amplified PCR products for each SSR primer was performed by agarose gel electrophoresis [1.5% agarose, RedSafe Nucleic Acid Staining Solution (3 ul/100 ml), 1X TBE (Tris-Borate-EDTA) buffer, 110 V, 1 h]. The amplified PCR bands were visualized Gel Imaging System Vilber Lourmat Quantum. 3500 Genetic Analyzer (Applied Biosystems, Life Technologies, UK) capillary electrophoresis was used to DNA fragment analysis, and GeneMapper Software 5.0 (Applied Biosystems) was used to determine the SSR alleles.

The observed allele number (NoA), the frequency of each allele (AF), Shannon's information index (I), Nei's 1987 unbiased genetic diversity (uh) were calculated with GenAlEx version 6.5 program (Peakall and Smouse, 2012) to evaluate genetic structure and diversity of studied sainfoin genotypes. The polymorphism detection ability of studied SSR markers [Polymorphic information contents (PIC)] was calculated with the formula given in Roldan-Ruiz et al. (2000) for each locus. The UPGMA (the unweighted pair group method) dendrogram based on Nei's unbiased genetic distance value was constructed with POPULATIONS 1.2.32 (Population Genetic Software) (Langella, 1999) and TreeView 1.6.6 (Page, 1996) programs.

3. Results and Discussion

The eight SSR markers generated 93 polymorphic alleles for the studied *Onobrychis* accessions (*Table 2*). The genetic diversity parameters, the observed allele number (NoA), the frequency of each allele (AF), Polymorphic information contents (PIC), Shannon's information index (I), Nei's (1987) unbiased genetic diversity (uh) were consequently used (*Table 2*). The number of alleles varied from 6 to 21 alleles per locus with a mean value of 11.625. The lowest number of allele was obtained from OVK174 whereas the highest number of

allele was obtained from the OVK094 marker. When the studies using the same SSR markers were examined, it was observed that the number of alleles was similar to each other (Kempf et al., 2016; Ozkan and Bilgen, 2019; Sutcu, 2020; Sutcu et al., 2022). It can be said that the small differences determined as a result of the comparison are due to the variety in the number and type of *Onobrychis* species used in these studies (*Table 3*). Successful fingerprinting of polyploids with a high level of precision is significant for plant breeding studies. Therefore, having a higher number of alleles per locus is especially important in polyploid species for genetic diversity studies due to its high discriminatory power. The frequency of 18 out of 93 alleles evaluated in the study was observed to be less than 0.01 and these alleles were named as rare allele. Polymorphism information content (PIC) of the loci ranged from 0.124 to 0.244 for 8 SSR loci. It was observed that the results were compatible with each other in the comparison of PIC values using the same SSR markers with our study (*Table 3*) (Kempf et al., 2016; Ozkan and Bilgen, 2019; Sutcu, 2020; Sutcu et al., 2022). Similar PIC values were calculated in studies conducted by different researchers.

Table 2. Genetic diversity parameters in Onobrychis accessions using SSR markers

SSR Locus	NoA*	Band Range (bp)	Min. AF*	Max. AF*	PIC*	I*	uh*
OVK036	9	148-171	0.02	0.87	0.234	0.370	0.237
OVK046	13	150-184	0.01	0.55	0.244	0.380	0.246
OVK094	21	229-288	0.01	0.60	0.162	0.270	0.163
OVK125	9	194-221	0.02	0.77	0.226	0.362	0.228
OVK161	15	215-282	0.01	0.65	0.131	0.223	0.132
OVK174	6	247-265	0.01	0.93	0.124	0.212	0.125
OVM033	10	306-340	0.01	0.51	0.241	0.364	0.243
OVM061	10	142-195	0.01	0.93	0.143	0.253	0.144
Mean	11.625	-	0.013	0.73	0.188	0.301	0.187

*NoA=Observed allele number, AF= Allele frequency, PIC=Polymorphic information contents, Min.=Minimum, Max.=Maximum, I=Shannon's information index, uh= Nei's (1987) unbiased genetic diversity

Shannon's information index (I) was calculated as 0.212 for OVK174 and 0.380 for OVK046 marker. In a previous study conducted with different *Onobrychis* genotypes by same SSR loci, the mean I value of 0.375 was reported (Sutcu et al., 2022). Ozkan and Bilgen (2019) studied the molecular characterization of two Turkish cultivars and 3 populations of *O. viciifolia* using same SSRs, and I value was calculated as 0.322. In Nosrati et al. (2012) 5 wild sainfoin populations collected from different regions were evaluated with 5 RAPD primers, and the I value was calculated between 0.364 and 0.461. Nosrati et al. (2016) used ISSR primers on sainfoin, the I value was calculated between 0.181 and 0.277. Zarrabian et al. (2013) conducted a study on a total of 80 sainfoin accessions, 46 Iranian and 34 exotics, and calculated I value ranged from 0.33 to 0.57. The Nei's 1987 unbiased genetic diversity (uh) changed from 0.125 to 0.246 with a mean of 0.187 (*Table 2*). Ozkan and Bilgen (2019) reported the unbiased genetic diversity value as 0.222. Sutcu et al. (2022) found the mean uh value as 0.243. When the h value obtained in different studies using various types of markers on sainfoin was examined, similar results were observed. It was reported that the h value was between 0.118 and 0.179 (Nosrati et al., 2016), 0.246 and 0.318 (Nosrati et al., 2012). Ertus (2021) reported the mean genetic diversity level as 0.3365, 0.2656, and 0.3018 for 23 cultivated landraces and one registered Turkish variety for RAPD, ISSR primers and RAPD/ISSR combination, respectively. According to the findings obtained in molecular characterization studies on sainfoin species, it can be concluded that the genetic diversity parameters obtained from our study were in concordance with the data presented from other researchers.

Table 3. Observed allele number and polymorphism information contents of studied Onobrychis-specific SSR primers evaluated by different researchers

SSR locus	Our study	Kempf et al. (2016)	Ozkan and Bilgen (2019)	Sutcu (2020)
	NoA	NoA	NoA	NoA
OVK036	9	7	8	6
OVK046	13	12	11	10
OVK094	21	14	8	15
OVK125	9	9	5	14
OVK161	15	12	9	12
OVK174	6	5	3	5
OVM033	10	8	7	7
OVM061	10	10	7	9
	Mean PIC	**Mean PIC**	**Mean PIC**	**Mean PIC**
OVK036	0.234	0.350	0.193	0.363
OVK046	0.244	0.310	0.215	0.286
OVK094	0.162	0.240	0.194	0.189
OVK125	0.226	0.290	0.296	0.285
OVK161	0.131	0.250	0.147	0.200
OVK174	0.124	0.230	0.245	0.120
OVM033	0.241	0.290	0.241	0.297
OVM061	0.143	0.190	0.208	0.241

The constructed dendrogram from the UPGMA analysis was shown in *Figure 1*. Genetic diversity coefficients ranged from 0.000 to 0.9375. The genetic diversity coefficient values in this study were similar to those recorded in the literature for sainfoin populations/accessions (Nosrati et al., 2012; Ozkan and Bilgen, 2019; Sutcu, 2020; Ertus, 2021; Sutcu et al., 2022). UPGMA clustering method provided two main clusters for all *Onobrychis* accessions. Cluster-I included 98 genotypes; 31 *O. inermis*, 9 *O. petraea*, 8 *Onobrychis* spp., 7 *O. cyri*, 6 *O. viciifolia*, 5 *O. altissima*, 4 *O. biebersteinii*, 4 *O. iberica*, 4 *O. vassilczenkoi*, 4 *O. arenaria* subsp. *arenaria*, 3 *O. grandis*, 3 *O. megataphros*, 3 *O. alba* subsp. *laconica*, 2 *O. oxyodonta*, 2 *O. kachetica*, 1 *O. kemulariae*, 1 *O. pallasii*, 1 *O. conferta* subsp *argentea* accession. Cluster-II included only 2 (*O. petraea* and *O. oxyodonta*) genotypes. When the Cluster-I is examined in detail, it is seen that it is divided into 5 subgroups. While each of the 95, 82-1, 47-3 and 11-3 genotypes in Cluster-I constitutes a separate subgroup, the remaining 94 genotypes form the second subgroup. This second group is divided into two subgroups, one consisting of 58 genotypes and the other consisting of 36 genotypes. These groups are also divided into subgroups within themselves. Except for one genotype, all diploid genotypes (#18) used in the study formed a separate group within this large group containing 58 genotypes. Within this group formed by diploid genotypes, only 2 *O. inermis*, 1 *O. altissima* and 2 genotypes of *Onobrychis* species were included among tetraploids. The only diploid genotype (*O. petrae*) did not included in this group, while a few tetraploid genotypes such as *O. biebersteini* and *O. inermis* together formed a subgroup within the other large group consisting of 36 genotypes. The most genetically similar genotype pairs in Cluster-I were 26-2 to 26-3 (*O. vassilczenkoi* genotypes), 63-1 to 63-2 (*O. cyri* genotypes) and 1-1 to 1-2 (*Onobrychis arenaria* subsp. *arenaria* genotypes). In the study, some sainfoin species are represented with higher genotype numbers (four plants/accessions) than others. These genotypes were scattered in the dendogram. For example, there were 4 genotypes of *O. viciifolia*, while two of them were together; the other two were distributed in different groups in the dendogram. It can be explained that the reasons for being in different subgroups were (1) being very similar to each other morphogically, (2) the confusion during collection, misdiagnosis, controversial taxonomy of the genus, (3) abundant exchange of genetic material due to their coexistence in nature, and (4) even the low number of loci used in the study.

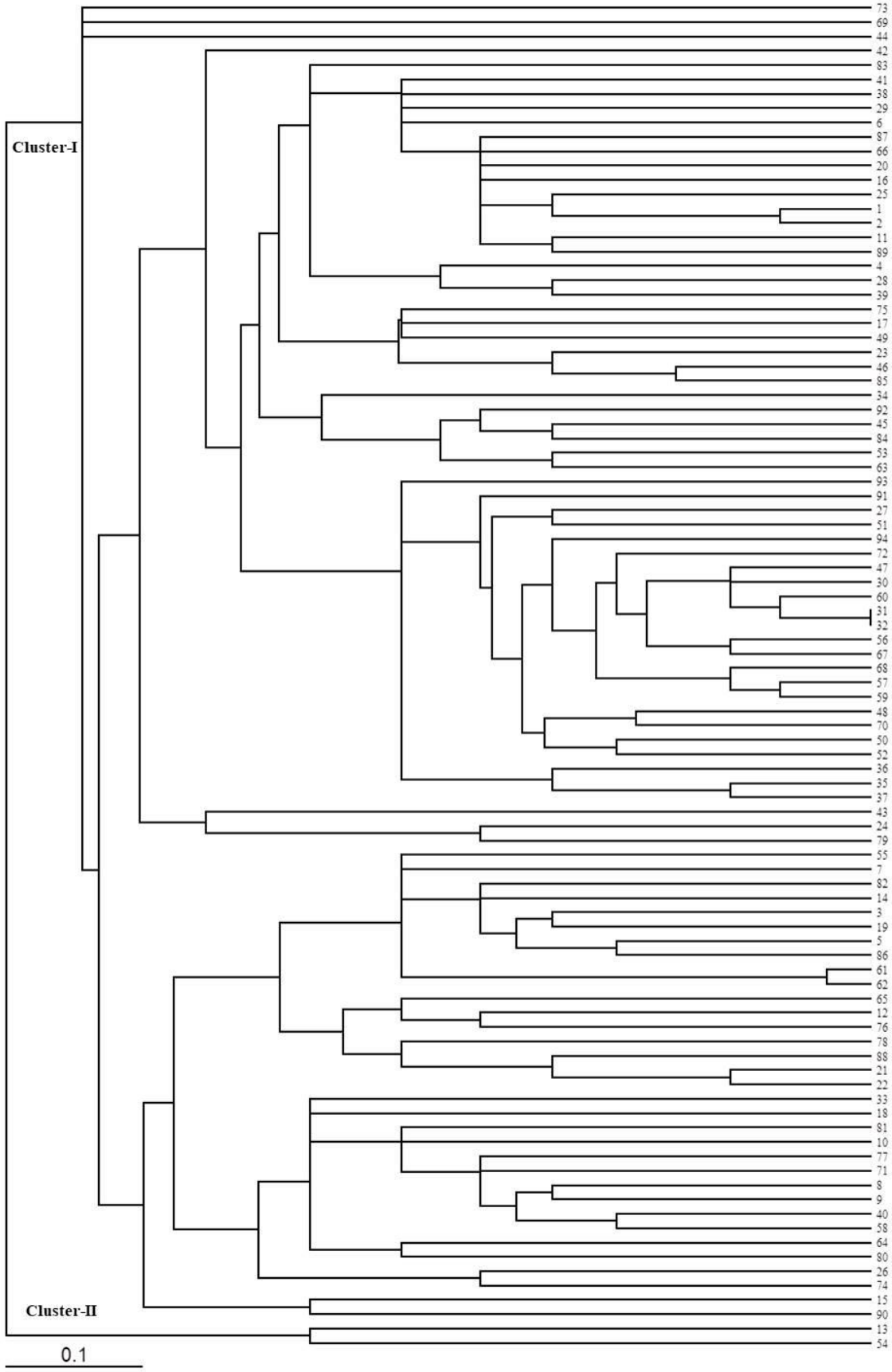

Figure 1 UPGMA dendrogram for Onobrychis accessions based on Nei's genetic distance values.

4. Conclusions

Sainfoin is a legume plant that has an important place as a fodder plant, and fast and effective breeding studies are needed to increase the number of new local varieties. In order to achieve more effective and successful results, it is necessary to include molecular breeding approaches into the classical breeding processes. Molecular markers provide deeper insights for the understanding of the genetic structure of crops and they are important for successful plant breeding programs (Nadeem et al., 2018). This study was conducted to evaluate the genetic diversity of *Onobrychis* species by using eight species specific SSR markers for 100 different genotypes belonging to 18 different *Onobrychis* species. The studied genotypes have high possibility to be selected for breeding program of sainfoin due to their genetic potential. It has been concluded that obtained SSR data provided more reliable results to distinguish genotypes from each other, and can be, therefore, used ongoing/future breeding programs of sainfoin to develop new varieties.

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