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Karyological Characteristics of Some Endemic *Onobrychis* Taxa Belonging to *Onobrychis* Section Naturally Grown in Turkey

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ABSTRACT

Karyotype properties of of six endemic *Onobrychis* taxa (*O. beata*, *O. cilicica*, *O. fallax*, *O. podperae*, *O. sulphurea* and *O. lasistanica*) naturally grown in Turkey were determined using squash preparation method and similarity of these endemics with cultivated taxon (*O. viciifolia*) were revealed. Ploidy levels of *Onobrychis* taxa were diploid (2n=14) except *O. lasistanica* and *O. viciifolia* (2n=28). Basic chromosome number is x=7 and chromosomes ranged from median to sub median with regard to centromere position. While the longest total chromosome length was measured in *O. cilicica* (28.21 µm), the shortest total chromosome length was in *O. beata* (21.47 µm). *O. cilicica* and *O. sulphurea* have satellite on chromosome 1 and chromosome 2, respectively. Hierarchical cluster analysis was performed to determine the relationships among the *Onobrychis* taxa and they were separated into three groups. *O. fallax, and O. podperae* were in the first group while *O. sulphurea* and *O. cilicica* were in the second group. *O. beata, O. lasistanica*, and *O. viciifolia* were assigned to the third group.

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Introduction

Sainfoin (*Onobrychis viciifolia* Scop.) is grown successfully in arid and semi-arid areas. It is cultivated in 196.180 hectares within forage sown in Turkey (TUIK, 2017). Forage quality of sainfoin is very high in terms of crude fat and protein and minerals (Acikgoz, 2001). It is used as a soil improvement plant due to the strong root system and nitrogen fixation characteristics (Acikgoz, 2001; Altin et al., 2005; Elci, 2005).

Wild *Onobrychis* taxa have an important role in forage breeding program because of high tolerance to biotic and abiotic stress conditions. *Onobrychis* genus encompasses about 170 taxa around the world and they are spread from the Mediterranean Region to the Zagros Mountains. Turkey is known as an important diversity center includes about 55 taxa and 28 of them are endemic (Hedge, 1970; Aktoklu, 2001; Avci et al., 2014). The studies on wild *Onobrychis* taxa for morphological, palynological, molecular and cytological contribure the *Onobrychis* breeding process.

Cytological and cytotaxonomic information of wild *Onobrychis* taxa are quite useful in determining the phylogenetic relationships of the taxa (Elci and Sancak, 2009). Hejazi and Mahdi (2010) performed a karyological study of 20 taxa (45 populations) of the genus *Onobrychis* Adans. from different geographic origins and they found the two usual basic chromosome numbers in the genus, x=7 abd x=8. Sepet et al. (2011) determined chromosome numbers and morphology of eight species of *Onobrychis* in Turkey and reported the chromosome numbers as 2n=14, 16 and 28. Ghanavati et al. (2012) stated that counting ploidy levels in somatic cells in the metaphase of *Onobrychis* species was difficult. Akcelik Somay et al. (2012) reported that karyotype analysis of the species of *Onobrychis* was undertaken using squash method and the chromosome numbers of *Onobrychis* species were determined as 2n=14 and 2n=16.

In this study, karyologic properties were determined in six endemic taxa (*Onobrychis fallax* Freyn & Sint. ex Freyn var. *longifolia* Aktoklu var. Nov., *Onobrychis sulphurea* Boiss. & Bal. var. *sulphurea* C. Koch Tvzel, *Onobrychis podporea* Sirj., *Onobrychis cilicica* Kit Tan & Sorger, *Onobrychis beata* Sirj., *Onobrychis lasistanica* Sirj.) for the first time and one cultivated form (*Onobrychis viciifolia* Scop.) belong to *Onobrychis* section and phylogenetic relationship of these taxa were revealed.

Material and Methods

Seeds of *Onobrychis* taxa were collected from natural habitat in Turkey between 2006 - 2009 years within a project (Project no: 106O040) which was supported by Scientific and Technological Research Council of Turkey (TÜBİTAK) and these taxa were identified by Prof. Dr. Ahmet Duran (Table 1) and these original seeds were used to observe chromosome characteristics. The images of sepal and petal of these taxa were given in Figure 1. The fruit pods were removed and seeds were kept at -20°C until used. These seeds were applied to mechanical scarification with sandpaper for dormancy breaking because of hard or impermeable seed coats before study as described by Avci and Kaya (2013).

Table 1 Names, locations and coordinate informations of *Onobrychis* taxa

N	Taxa name	Location	Latitude	Longitude	Height (m)
1*	*O. fallax var. longifolia*	Malatya, Arguvan, Çobandere Village, Şotik Stream Valley	39°00′02″	38°12′27″	1410
2*	*O. sulphurea var. sulphurea*	Kayseri, Hisarcık, Kıranardı oak groove	38°37′38″	35°31′39″	1514
3*	*O. cilicica*	Mersin, between Mut to Kırobası	36°41′38″	33°37′27″	1095
4*	*O. beata*	Adana, Karaisali, Koca Çukur Plateau	37°24′23″	35°02′35″	1435
5*	*O. podperae*	Kütahya, Gediz Range	39°02′22″	29°25′42″	820
6*	*O. lasistanica*	Trabzon, Köprübaşı, Kemer Passage	40°38′00″	40°01′00″	2426
7	*O. viciifolia*	Kütahya, Gediz, Çavdarhisar	39º05'53"	19º28'51"	887

N: Number, *: shows endemic taxa

Figure 1 Flower images of investigated Onobrychis taxa indicate sepal, banner petal, wing, keel, stamen and pistil

The root tips were obtained from germinated seeds at 20°C and pre-treatment was performed with α-monobromo naphthalene (0.5%) for 4 hours at 4°C. Root tips were subsequently treated with 10% formaldehyde and 1% chromic acid (1:1) for 16 hours for fixation and were rinsed with distilled water for 3 hours after fixation (Hejazi and Mahdi, 2010). 1N NaOH solution was used for hydrolysing in 60°C between 8 and 12 minutes depending on taxa. Staining was performed with the hematoxylin-iron method and root tips were kept in the dark for 4 hours (Ghanavati, et al., 2012). After staining process, softening treatment was applied with cellulase enzyme (Cellulase from *Trichoderma* sp., Sigma catalogue no: CO615) for 3 minutes at room temperature due to hardening. Squashing method was used for the preparation of root tips between 1 to 2 mm lengths by using one drop 45% acetic acid – lactic acid (10:1) solution. Images that were used in the karyological analysis were captured from 5 different somatic cells using Canon EOS 2000 camera integrated to Zeiss Axio Scope A1 microscope. Chromosome length (CL), short arm length (SA), long arm length (LA), satellite length [(SAT), (if there is satellite)] were measured in Zeiss Axio Vision software. Chromosome length was determined with the sum of short arm length, long arm length and satellite length (CL=LA+SA+SAT). Positions of centromere and chromosome classification were designated with dividing long arm length to short arm length (Levan et al., 1964). Centromere indexes (CI) were derived from dividing short arm length to total chromosome length (TCL) [(CI=SA/TCL×100)] as described by Hejazi and Mahdi (2010). Relative length (RL) and arm ratio (AR) values were generated due to the formula given by Akçelik Somay et al. (2012). Ideograms were created by using Microsoft Excel software, Five chromosome characteristics (LA, SA, CI, RL and TCL) were used in generating of dendrogram for each taxon and statistical analysis was performed in SPSS 16 software.

Results and Discussion

The karyological characteristics and their similarities were revealed in seven *Onobrychis* taxa, of whose cell images, karyotpe and ideograms were given in Figure 2, Figure 3 and Figure 4 respectively. Cellulase enzyme was used to overcome hard cell walls of *Onobrychis* taxa during preparation and photography. Ghanavati et al. (2012) confirmed that enzyme treatments on root tips for hard cell walls facilitated the preparation of *Onobrychis* species. The hydrolysis time to have optimized to obtain good cell staining showed variation among different *Onobrychis* taxa. The best hydrolysis time was 12 minutes in *O. cilicica* and *O. lasistanica*, 10 minutes in *O. fallax, O. beata, and O. podperae*, and 7 minutes in *O. sulphurea*. Yildirim (2007) reported that the hydrolysis time changed according to the species. Sepet et al. (2011) and Akcelik Somay et al. (2012) stated that the hydrolysis time of *Onobrychis* taxa ranged from 10 to 18 and 7 to 12 minutes, respectively.

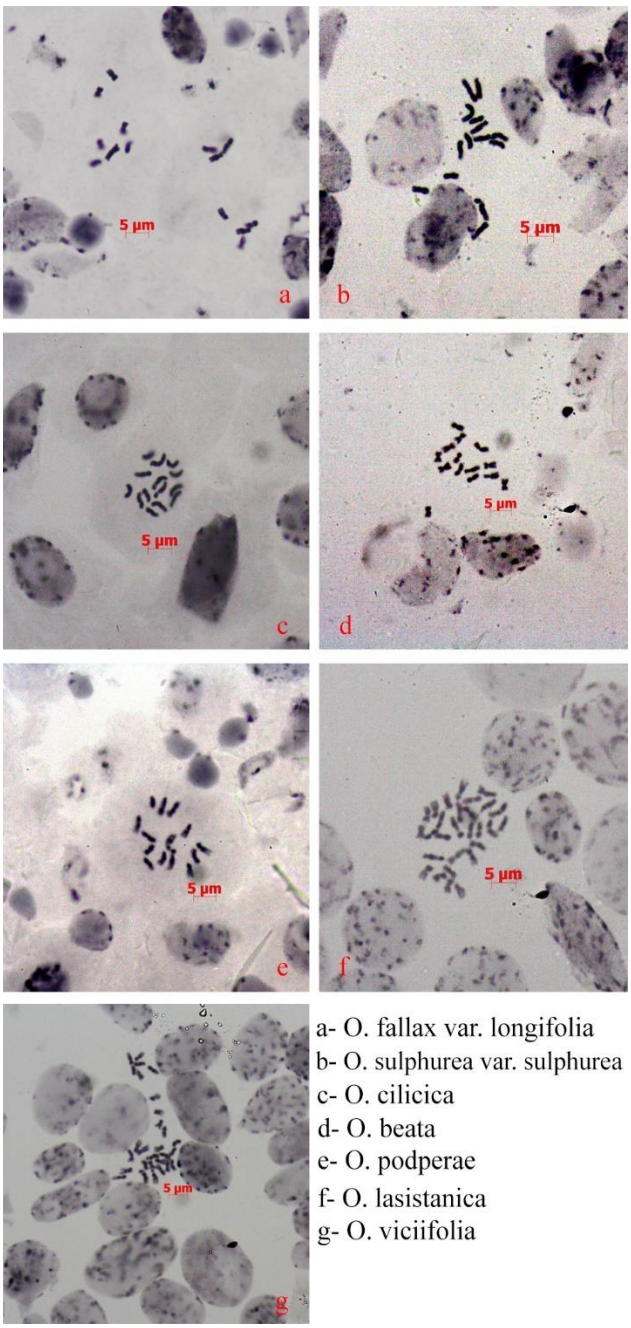

Figure 2. Cell images of investigated Onobrychis taxa in mitotic metaphase stage

Karyological characteristics of all the endemic taxa were determined for the first time, except for *O. fallax*. Basic chromosome number of *Onobrychis* taxa was x=7 and five of them were diploid (2n=14), whereas two of them were tetraploid (2n=28), as illustrated in Table 2 and Figure 2, Figure 3 and Figure 4. In some studies, basic chromosome number of *Onobrychis* genus was observed to be both x=7 and x=8 (Abou-El-Enain 2002; Hejazi and Mahdi, 2010; Sepet et al., 2011; Akcelik Somay et al., 2012). Ranjbar et al. (2009) and Ghanavati et al. (2012) indicated that *Onobrychis* genus has different ploidy levels such as 2n=2x=14, 2n=2x=16, 2n=4x=28 and 2n=4x=32.

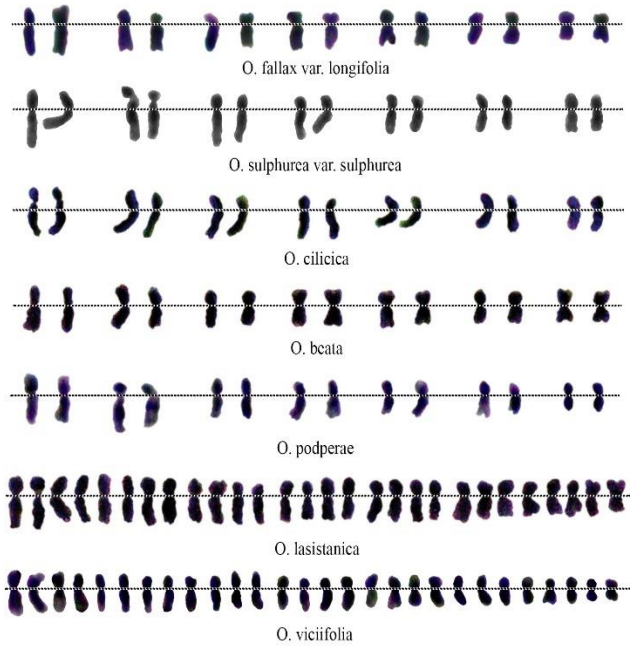

Figure 3 Karyotype arrangement of the investigated Onobrychis taxa

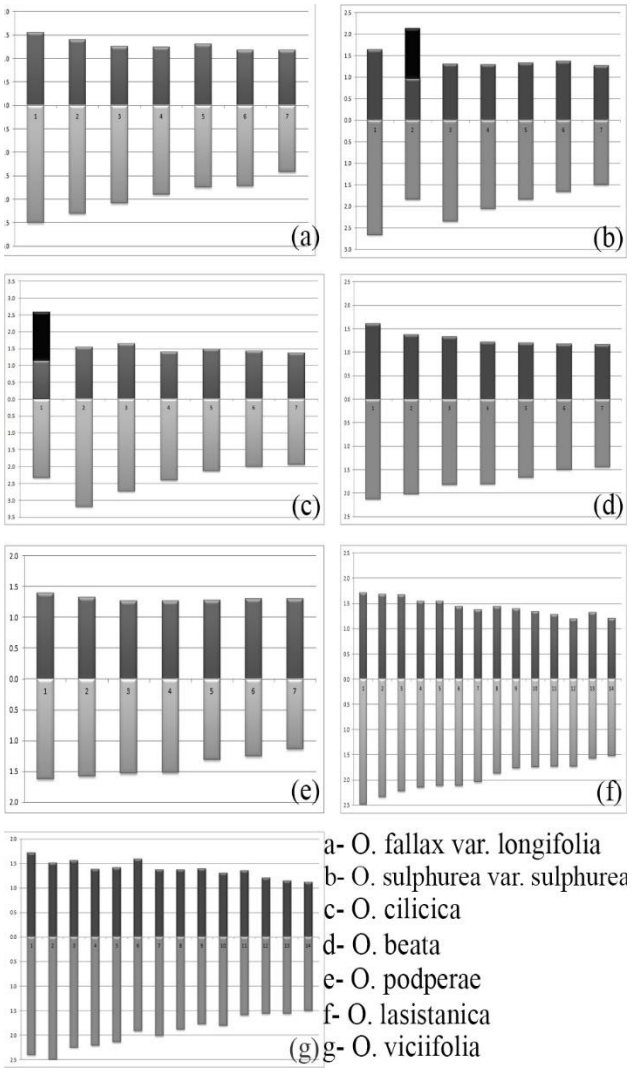

Figure 4 Ideograms of investigated *Onobrychis* taxa

The total chromosome length varied between 21.47 µm and 28.21 µm in *O. beata* and *O.cilicica*, respectively (Table 2). The chromosome type according to centromeric position ranged from median to sub median as indicated by Hejazi and Mahdi (2010), Sepet et al. (2011) and Akcelik Somay et al. (2012) in certain *Onobrychis* taxa (Table 3).

O. fallax including two varieties such as fallax and longifolia is an endemic taxon in Turkey. Sepet et al. (2011) reported that *O. fallax* var. *fallax* showed diploid ploidy level (2n=2x=14) and median-centromeric chromosomes (m). Our findings of *O. fallax* var. *longifolia* revealed considerable similarities with the other variety. Karyological properties of *O. fallax* show that these varieties are not conspicuously different from each other.

The *O. sulphurea* taxon is localized to the middle, south, and east of Turkey. There are three different varieties, namely *O. sulphurea* var. *vanensis*, *O. sulphurea* var. s*ulphurea*, and *O. sulphurea* var. *pallida*. The total chromosome length and karyotype formula of *O. sulphurea* var. *sulphurea* were 24.27 µm, and 5m+2sm, respectively and this taxon included satellite on chromosome 2 with the length of 1.16 µm. (Table 2 and Table 3).

The longest total chromosome among the *Onobrychis* taxa belonged to *O. cilicica* (28.21 µm) and centromere position of chromosomes varied from median to submedian, 4 m+3 sm. Chromosome 1 included a satellite with the length of 1.44 µm and the centromeric index ranged from 23.37 to 41.69 (Table 2 and Table 3).

O. beata, which spreads in a very local area in the southern region of Turkey, had the shortest total chromosome (21.47 µm) among the *Onobrychis* taxa, including only the median chromosome (Table 2). The centromeric index of *O. beata* varied from 40.26 to 44.66 (Table 3).

The total chromosome length of *O. podperae* was 22.64 µm, and its karyotype formula was 5m+2sm (Table 2). Centromeric index ranged from 32.73 to 44.90. Additionally, the arm ratio of *O. podperae* varied from 1.23 to 2.06 (Table 3).

O. lasistanica and *O. viciifolia* which were similar to each other in terms of chromosome characteristics like ploidy level (2n=28) and centromere position (median) except other investigated taxa. Also, the total chromosome lengths of *O. lasistanica* and *O. viciifolia* were very close to each other like 23.75 and 23.33 µm, respectively (Table 2). While the centromeric index of *O. lasistanica* varied from 41.18 to 44.82, this value ranged from 39.59 to 44.64 in *O. viciifolia* (Table 3). Hejazi and Mahdi (2010) reported that *O. viciifolia* had tetraploid ploidy level (2n=28) and chromosomes varied from median to submedian in different populations. Also, we found that centromeric index ranged from 36.00 to 43.00 in this study. However, the presence of satellite showed variability. While some *O. viciifolia* populations included satellite, the others were not as similar to our findings. Our findings showed similarity with the Sepet et al. (2011) who determined the ploidy level of *O. viciifolia* was tetraploid (2n=28) and its chromosomes were median.

Table 2 Ploidy level, basic chromosome number, total chromosome length (TCL) and karyotype formula (KF) of the *Onobrychis* taxa

Taxa name	2n	x	TCL (µm)	KF
O. fallax var. longifolia	14	7	22.78	7 m
O. sulphurea var. sulphurea	14	7	24.27	5 m+2 sm
O.cilicica	14	7	28.21	4 m+3 sm
O.beata	14	7	21.47	7 m
O.podperae	14	7	22.64	5 m+2 sm
O.lasistanica	28	7	23.75	7m
O.viciifolia	28	7	23.33	7m

Table 3 Chromosome characteristics of the investigated *Onobrychis* taxa

Taxa Name	CNTN	Chromosome arms (µm)		CL (µm)	SAT	AR	CI	CT
		LA	SA					
O. fallax var. longifolia	I	2.50±0.39	1.50±0.14	4.06±0.50	-	1.60±0.17	38.42±2.65	m
	II	2.30±0.20	1.40±0.28	3.70±0.40	-	1.64±0.33	37.84±4.37	m
	III	2.09±0.32	1.26±0.13	3.35±0.44	-	1.66±0.11	37.61±1.64	m
	IV	1.90±0.20	1.24±0.25	3.14±0.27	-	1.53±0.39	39.49±6.02	m
	V	1.74±0.12	1.30±0.19	3.04±0.26	-	1.34±0.17	42.76±3.26	m
	VI	1.72±0.12	1.17±0.23	2.89±0.24	-	1.47±0.28	40.48±4.76	m
	VII	1.42±0.18	1.18±0.12	2.60±0.28	-	1.20±0.11	45.38±2.21	m
O. sulphurea var. sulphurea	I	2.66±0.30	1.64±0.17	4.30±0.38	-	1.62±0.22	38.14±3.25	m
	II	1.84±0.18	0.97±0.08	3.97±0.22	1.16±0.24	1.90±0.26	24.43±1.63	sm
	III	2.35±0.26	1.31±0.25	3.66±0.25	-	1.79±0.66	35.79±6.29	sm
	IV	2.06±0.35	1.30±0.12	3.36±0.35	-	1.58±0.34	38.69±5.17	m
	V	1.84±0.19	1.34±0.20	3.18±0.30	-	1.37±0.23	42.14±3.97	m
	VI	1.66±0.13	1.37±0.14	3.03±0.22	-	1.21±0.15	45.21±2.95	m
	VII	1.50±0.17	1.27±0.13	2.77±0.30	-	1.18±0.04	45.85±0.86	m
O.cilicica	I	2.33±0.32	1.15±0.25	4.92±0.56	1.44±0.18	2.03±0.31	23.37±2.50	sm
	II	3.19±0.64	1.55±0.31	4.74±0.47	-	2.06±1.08	32.70±7.57	sm
	III	2.74±0.27	1.65±0.30	4.39±0.37	-	1.66±0.36	37.59±5.25	m
	IV	2.40±0.20	1.40±0.30	3.80±0.41	-	1.71±0.34	36.84±4.72	sm
	V	2.13±0.22	1.49±0.22	3.62±0.38	-	1.43±0.19	41.16±3.28	m
	VI	2.00±0.36	1.43±0.18	3.43±0.48	-	1.40±0.23	41.69±3.96	m
	VII	1.94±0.29	1.37±0.19	3.31±0.47	-	1.42±0.05	41.39±0.93	m
O.beata	I	2.13±0.39	1.60±0.09	3.73±0.41	-	1.33±0.23	42.90±3.85	m
	II	2.02±0.14	1.37±0.20	3.39±0.22	-	1.47±0.26	40.41±4.13	m
	III	1.82±0.15	1.33±0.20	3.15±0.17	-	1.37±0.35	42.22±5.14	m
	IV	1.81±0.11	1.22±0.09	3.03±0.15	-	1.48±0.14	40.26±2.33	m
	V	1.67±0.10	1.20±0.08	2.87±0.14	-	1.39±0.11	41.81±1.88	m
	VI	1.50±0.09	1.18±0.10	2.68±0.05	-	1.27±0.18	44.03±3.51	m
	VII	1.45±0.08	1.17±0.08	2.62±0.09	-	1.24±0.13	44.66±2.59	m
O.podperae	I	2.70±0.17	1.40±0.30	4.10±0.38	-	1.93±0.40	34.15±4.61	sm
	II	2.59±0.58	1.26±0.15	3.85±0.64	-	2.06±0.47	32.73±4.87	sm
	III	2.16±0.36	1.28±0.12	3.44±0.36	-	1.69±0.36	37.21±5.38	m
	IV	1.93±0.31	1.30±0.11	3.23±0.42	-	1.48±0.13	40.25±2.12	m
	V	1.62±0.23	1.32±0.06	2.94±0.27	-	1.23±0.16	44.90±3.36	m
	VI	1.51±0.07	1.13±0.12	2.64±0.17	-	1.34±0.12	42.80±2.22	m
	VII	1.40±0.16	1.04±0.16	2.44±0.24	-	1.35±0.23	42.62±4.21	m
O.lasistanica	I	2.41±0.26	1.69±0.29	4.11±0.22	-	1.42±0.42	41.25±6.00	m
	II	2.18±0.18	1.60±0.26	3.79±0.37	-	1.36±0.18	42.33±3.45	m
	III	2.12±0.31	1.48±0.21	3.60±0.47	-	1.43±0.13	41.18±2.42	m
	IV	1.94±0.20	1.40±0.26	3.36±0.40	-	1.39±0.21	41.84±3.55	m
	V	1.76±0.33	1.36±0.19	3.12±0.38	-	1.29±0.28	43.68±4.82	m
	VI	1.73±0.40	1.23±0.17	2.96±0.37	-	1.40±0.42	41.63±6.33	m
	VII	1.55±0.30	1.26±0.07	2.81±0.30	-	1.23±0.24	44.82±4.16	m
O.viciifolia	I	2.46±0.37	1.62±0.22	4.08±0.60	-	1.53±0.13	39.60±1.97	m
	II	2.24±0.23	1.47±0.25	3.71±0.46	-	1.53±0.20	39.59±2.92	m
	III	2.04±0.18	1.50±0.30	3.54±0.40	-	1.37±0.26	42.45±4.15	m
	IV	1.96±0.21	1.37±0.12	3.33±0.27	-	1.43±0.17	41.22±3.01	m
	V	1.80±0.17	1.35±0.14	3.14±0.27	-	1.34±0.18	42.82±2.99	m
	VI	1.59±0.18	1.28±0.14	2.87±0.31	-	1.24±0.09	44.64±1.71	m
	VII	1.54±0.31	1.14±0.13	2.68±0.40	-	1.36±0.26	42.43±4.41	m

CNTN: Chromosome number, CT: Chromosome type, ±: Standart deviation

Dendrogram for *Onobrychis* taxa was generated via hierarchical cluster analysis and *Onobrychis* taxa comprised of three groups (Figure 5). While *O. fallax* var. *longifolia*, and *O. podperae* were in the first group, *O. sulphurea and O. cilicica* were in the second group. *O. beata*, *O. lasistanica*, and *O. viciifolia* were included as a separate group. Avci et al. (2014) indicated that *O. beata* and *O. lasistanica* were very close together by using SSR markers and they were in the same group with *O. viciifolia* similar to our chromosome findings. In the study about phylogenetic relationship of *Onobrychis* taxa with the help of morphological and molecular markers by Avci et al. (2016), *O. fallax*, *O. sulphurea* and *O. podperae* included in same group, *O. beata* and *O. viciifolia* were in another group.

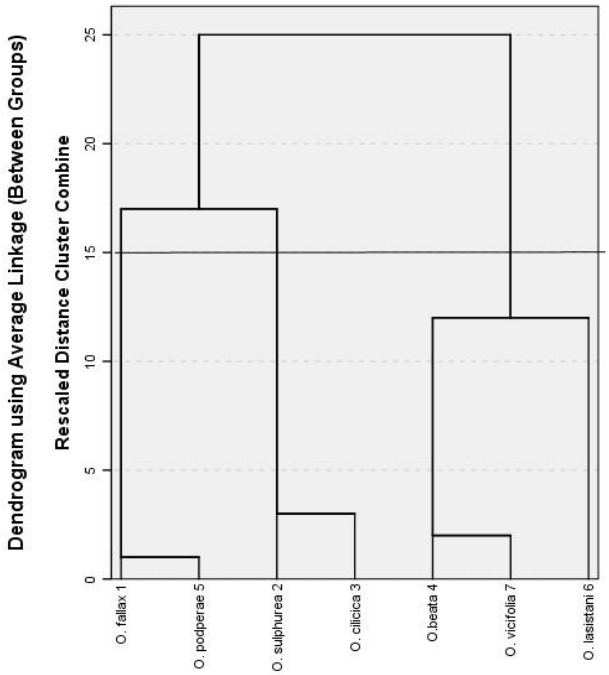

Figure 5 Dendrogram of the investigated Onobrychis taxa created by using five chromosome characteristics (Long and short arm lengths, centromeric index, relative and total chromosome lengths)

Conclusion

The detailed chromosome characteristics were investigated in six endemic *Onobrychis* taxa and relation of them to cultivated relative was determined. Karyological properties of six endemic *Onobrychis* taxa were revealed and there was not any difference with regard to chromosome properties between *O. fallax* var. *fallax* and *O. fallax* var. *longifolia*. Basic chromosome number was observed x=7 as expected and ploidy level was generally diploid (2n=14) except *O. viciifolia* and *O. lasistanica* (2n=28). Chromosomes of *Onobrychis* taxa are symmetric because of median to submedian in point of centromeric position. Although *O. fallax* var. *longifolia* and *O. viciifolia* taxa were similar with respect to morphological and molecular markers in some research (Avci et al., 2014; Avci et al., 2016), they are classified as separate groups in this study. However, our study showed that *O. lasistanica and O. beata* are similar to each other and they have high potential to be relative with *O. viciifolia*. This information should be useful in determining taxonomic status of *Onobrychis* taxa and breeding studies of *Onobrychis* in the future.

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