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Karyological Properties of Some *Onobrychis* Taxa Belonging to A *Hymenobrychis* Section Growing Naturally in Turkey

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Abstract: In this study, chromosome numbers and morphologies of six *Onobrychis* taxa [*Onobrychis tournefortii* (Willd.) Desv., *Onobrychis albiflora* Hub-Mor., *Onobrychis hypargyrea* Boiss., *Onobrychis radiata* (Desf.) Bieb., *Onobrychis meschetica* Grossh, and *Onobrychis galegifolia* Boiss.] were investigated with the squash preparation method. These taxa belong to the *Hymenobrychis* section, including two endemic species naturally grown in Turkey. First, karyotype analysis of the *Onobrychis meschetica* was performed. Then mitotic metaphase and basic chromosome numbers of the taxa were determined in 2n=14 and x= 7, respectively. Chromosomes showed differences as median to submedian according to centromer position. Except *O. meschetica*, all of the taxa generally included a satelite on chromosome I or IV.

Keywords: *Onobrychis*, chromosome, karyotype, centromer position

Türkiye'de Doğal Olarak Yetişen Hymenobrychis Seksiyonuna Ait Bazı *Onobrychis* Taksonlarının Karyolojik Özellikleri

Öz: Bu çalışmada, altı *Onobrychis* taksonunun [*Onobrychis tournefortii* (Willd.) Desv., *Onobrychis albiflora* Hub-Mor., *Onobrychis hypargyrea* Boiss., *Onobrychis radiata* (Desf.) Bieb., *Onobrychis meschetica* Grossh. ve *Onobrychis galegifolia* Boiss.] kromozom sayıları ve morfolojileri ezme preparat yöntemi ile araştırılmıştır. Türkiye'de doğal olarak yetişen ve iki tanesi endemik olan bu taksonlar, Hymenobrychis seksiyonuna dahildir. Bu çalışmayla, *Onobrychis meschetica* taksonunda ilk kez karyotip analizi yapılmıştır. Taksonların mitotik metafaz ve temel kromozom sayıları sırasıyla, 2n = 14 ve x = 7 olarak belirlenmiştir. Kromozomlar, sentromer pozisyonuna göre median özellikten submediana kadar farklılık göstermiştir. *O. meschetica* dışında, incelenen tüm taksonlar genellikle birinci ve dördüncü kromozom üzerinde bir satalit içermektedir.

Anahtar Kelimeler: *Onobrychis*, kromozom, karyotip, sentromer pozisyonu

1. Introduction

The *Onobryhis* genus is a member of Fabaceae family and it has 170 annual and perennial taxa which dispersed in the interior of minor Asia, which includes Central Anatolia (Turkey), the entire Transcaucasus, the higher parts of Iran, and Turkmenistan. Turkey, one of the most important diversity center of the genus, has 55 *Onobrychis* taxa and 28 of them are endemic (Hedge, 1970; Aktoklu, 2001; Avcı and Kaya, 2013). This taxa is well-adapted in arid and semi-arid lands of Turkey because of its deep roots. They can be safely grazed since their hay does not cause bloat in ruminant animals contrary to alfalfa (Pupilli et al., 1989; Li et al., 1993; Çeliktaş et al., 2006).

Phylogenetic relationships have not been clearly disclosed by using morphological parameters in taxonomic studies due to closely related and excessive number of taxa and cross pollination (Avcı et al., 2016). The genus is obviously subdivided in two subgenus namely *Onobrychis* with three sections and *Sisyrosema* with two sections based on morphological and molecular DNA techniques in Turkey (Davis et al., 1988; Aktoklu, 2001; Emre et al., 2007; Arslan and Ertugrul, 2010; Avcı et al., 2014; Avcı

et.al., 2016). Therefore, there are still some problems about grouping of sections under subgenus and the distribution of taxa. It is important to confirm previous studies with detailed cytotaxonomic studies.

There are limited karyological studies on studied taxa of *Hymenobrychis* section. Akçelik et al. (2012); Arslan et al. (2012); Sepet et al. (2011); Hejazi et al. (2010); Tekin et al. (2016) performed karyological properties of *O. tournefortii*, *O. hypargyrea*; *O. galegifolia; O. radiata* and on *O. albiflora*. However, there is no studies on *O. meschetica*. The aim of this study is to reveal of cytotaxonomic properties of these taxa belonging to *Hymenobrychis* section by using different populations unlike previous studies. Thus, the taxonomic status and phlogenetic relationship of taxa were defined more clearly.

2. Material and Method

The root tips were used from seedling obtained by germinating ripe seeds which were collected from natural terrains of Turkey and they are listed in Table 1.

Table 1. The taxa used in the study and their locations and coordinates
Çizelge 1. *Araştırmada kullanılan taksonlar, konumları ve koordinatları*

N	Species	Locations	Latitude	Longitude	Height (m)	Endemics
1	*O. tournefortii*	Sivas, Taşlıdere	39°37'03"	37º01'04"	1312	*
2	*O. albiflora*	Sivas, between Sincan to Karaman villages	39°27'34"	37°49'14"	1246	*
3	*O. hpargyrea*	Between Karabük to Araç road.	41º12'35"	32º48'49"	365	-
4	*O. radiata*	Kars, between Kötek to Paslı	40°45'25"	42°58'00"	1609	-
5	*O. meschetica*	Kars, Akyaka	40°45'10"	43°38'00"	1536	-
6	*O. galegifolia*	Adıyaman, Gölbaşı	37º50᾽44"	37º18᾽57"	897	-

The ripe seeds with hard characteristics were abraded by mechanical scarification with sandpaper and were germinated at 20 ± 1 °C on wet filter paper, as described by Avcı and Kaya (2013). Root tips of 1-2 cm in length were pretreated with 0.5% α-Bromonaphthalene at room temperature for 4 h. A fixation, 10% formaldehyde:1% chromic acid (1:1) solution was used at room temperature for 16 hours. Hydrolysis was performed with 1 N NaOH solution at 60°C and hydrolysis time changed between 7 to 13 minutes depending on taxa. The root tips were dyed with hematoxylin-iron for 3-4 h and then treated with 1% cellulase enzyme (Celulase Onozuka RS) to soften hardened root tips for two minutes and squashed in a droplet of 45% acetic acid and lactic acid (10:1) (Hejazi et al., 2010).

The best 5 images obtained from different metaphase plates, were captured with a Zeiss Aixo Vision microscope integrated camera and constriction (Table 3). The basic chromosome number of *Onobrychis* genus was observed as either x=7 or x=8 in many studies (Abou-El-

carpological measurements were performed with Zeiss Axio Vision software. The following parameters were measured to determine the karyotype characteristics of the taxa: long arm (LA), short arm (SA), satellite (SAT), total chromosome length (TCL) [LA+SA], relative length percentage (RL%) [100xTotal length of each chromosome/total length of all chromosomes], arm ratio (AR) [LA/SA], centromeric index (CI%) [100xSA/ (LA+SA)]. Karyotype formula was determined as described by Levan et al. (1964). Karyograms were arranged based on the size of chromosomes.

3. Results and Discussion

According to obtained results, the somatic chromosome number was 2n=14 and the basic chromosome number was x=7 (Table 2) and all chromosomes were varied from metacentric to submetacentric in terms of position of centromeric enain, 2002; Akçelik et al., 2012; Ghanavati et al., 2010; Hejazi et al., 2010; Massoud et al., 2010; Sepet et al., 2011).

Table 2. Karyotype characteristics of 6 taxa of *Onobrychis*. 2n, somatic chromosome number; x, basic chromosome number; TLC, total chromosome lenght; KF, Karyotype formula; m, metacentric; sm, submetacentric

Çizelge 2. *6 Onobrychis taksonunun karyotip özellikleri. 2n, somatik kromozom sayısı; x, temel kromozom sayısı; TLC, toplam kromozom uzunluğu; KF, karyotip formülü; m, metasentrik; sm, submetasentrik*

Species	2n	x	TCL (µm)	KF
O. tournefortii	14	7	15.73	7 m
O. albiflora	14	7	23.58	4 m+3 sm
O. hpargyrea	14	7	27.35	4 m+3 sm
O. radiata	14	7	21.84	7 m
O. meschetica	14	7	22.76	7 m
O. galegifolia	14	7	20.33	6 m+1 sm

While, the lowest mean values of long arm, short arm and total chromosome length were 1.24, 0.88 and 2.25 µm in *O. tournefortii*, the highest averages of them were 2.32, 1.40 and 3.91 µm in *O. hypergyrea*. However, the arm ratio mean value was varied from 1.32 µm in *O. radiata* to 1.66 µm in *O. hypergyrea.* When, the lowest centromeric index average was 35.35 in *O. hypergyrea* unlike other averages, the highest value was 59.74 in *O. meschetica*. Although all of the taxa generally included a satelite on chromosome I (*O. tournefortii*, *O. hypergyrea* and *O. radiata*) or IV (*O. albiflora*), a satelite was not found in *O. meschetica* (Table 3).

All chromosomes of *O. tournefortii* were median type according to position of centromeric constriction, and chromosome 1 had a satellite (Fig 1). The somatic chromosome number (2n=14) of this taxon was confirmed by Akçelik et al. (2012) and Arslan et al. (2012). However, chromosome types varied between median to submedian and satellite was not exist in their studies.

Table 3. Detailed karyological parameters of investigated *Onobrychis* taxa. LA, long arm; SA, short arm; CL, chromosome length; SAT, satellite; AR, arm ratio; CI, centromeric index; m, metacentric; sm, submetacentric

Çizelge 3. *İncelenen Onobrychis taksonlarının detaylı karyolojik özellikleri. LA, uzun kol; SA, kısa kol; CL, kromozom uzunluğu; SAT, sat-elit; AR, kol oranı; CI, sentromerik indeks; m, metasentrik; sm, submetasentrik*

Chromsome numbers and taxa names	Chromosome arms (µm)		CL (µm)	SAT	AR	CI	Chromosome type
	LA	SA					
O. tournefortii							
I	1.21±0.11	0.88±0.08	2.91±0.25	0.82±0.09	1.38±0.06	30.22±1.66	m
II	1.46±0.31	0.97±0.09	2.56±0.24	-	1.50±0.24	38.38±5.46	m
III	1.47±0.24	1.00±0.19	2.40±0.29	-	1.47±0.19	41.41±3.30	m
IV	1.26±0.05	0.92±0.20	2.17±0.20	-	1.36±0.27	41.80±6.17	m
V	1.21±0.23	0.87±0.06	2.08±0.19	-	1.39±0.33	42.40±5.59	m
VI	1.07±0.14	0.87±0.15	1.94±0.23	-	1.22±0.23	44.71±5.04	m
VII	0.99±0.11	0.67±0.05	1.67±0.12	-	1.47±0.21	40.33±3.20	m
Mean	**1.24±0.18**	**0.88±0.10**	**2.25±0.41**		**1.39±0.09**	**39.89±4.68**	
O. albiflora							
I	2.86±0.69	1.39±0.27	4.25±0.86	-	2.05±0.42	33.08±4.12	sm
II	2.38±0.83	1.44±0.32	3.81±1.03	-	1.65±0.50	38.71±6.93	m
III	2.18±0.59	1.27±0.33	3.45±0.88	-	1.71±0.25	37.00±3.75	sm
IV	1.32±0.35	1.06±0.32	3.34±0.80	0.95±0.17	1.24±0.21	31.49±2.95	m
V	2.03±0.70	1.14±0.28	3.17±0.88	-	1.78±0.56	36.83±6.50	sm
VI	1.68±0.33	1.22±0.44	2.90±0.70	-	1.37±0.40	41.17±6.88	m
VII	1.67±0.45	1.00±0.21	2.66±0.61	-	1.67±0.42	37.94±6.43	m
Mean	**2.02±0.51**	**1.22±0.16**	**3.37±0.53**		**1.63±0.26**	**36.60±3.31**	
O. hpargyrea							
I	1.95±0.40	1.30±035	4.60±0.93	1.33±0.31	1.54±0.28	25.67±7.44	m
II	2.57±0.59	1.92±0.68	4.49±0.93	-	1.33±0.40	41.59±7.38	m
III	2.80±0.53	1.55±0.56	4.35±0.99	-	1.80±0.42	34.94±5.90	sm
IV	2.65±0.69	1.37±0.24	4.01±0.91	-	1.93±0.26	34.41±3.17	sm
V	2.22±0.65	1.30±0.30	3.52±0.78	-	1.70±0.47	37.35±7.31	sm
VI	2.14±0.47	1.26±0.47	3.40±0.93	-	1.69±0.35	36.18±4.35	m
VII	1.87±0.64	1.11±0.43	2.98±1.04	-	1.68±0.26	37.30±3.74	m
Mean	**2.31±0.36**	**1.40±0.26**	**3.91±0.61**		**1.66±0.19**	**35.35±4.86**	
O. radiata							
I	1.56±0.41	1.16±0.37	4.06±0.81	1.33±0.29	1.34±0.65	28.45±5.56	m
II	2.11±0.42	1.67±0.42	3.78±0.79	-	1.26±0.28	44.05±4.86	m
III	2.05±0.56	1.41±0.20	3.46±0.71	-	1.45±0.27	49.84±5.02	m
IV	1.89±0.50	1.37±0.15	3.26±0.58	-	1.37±0.31	45.59±5.39	m
V	1.59±0.36	1.21±0.29	2.80±0.66	-	1.31±0.10	43.33±1.84	m
VI	1.42±0.31	1.05±0.18	2.47±0.49	-	1.35±0.11	33.88±1.88	m
VII	1.10±0.30	0.91±0.17	2.01±0.46	-	1.20±0.15	45.74±2.84	m
Mean	**1.67±0.36**	**1.25±0.25**	**3.12±0.73**		**1.32±0.08**	**41.55±7.55**	

Table 3. (Contunue) Detailed karyological parameters of investigated *Onobrychis* taxa. LA, long arm; SA, short arm; CL, chromosome length; SAT, satellite; AR, arm ratio; CI, centromeric index; m, metacentric; sm, submetacentric

Çizelge 3. *(Devam) İncelenen Onobrychis taksonlarının detaylı karyolojik özellikleri. LA, uzun kol; SA, kısa kol; CL, kromozom uzunluğu; SAT, sat-elit; AR, kol oranı; CI, sentromerik indeks; m, metasentrik; sm, submetasentrik*

Chromsome numbers and taxa names	Chromosome arms (µm)		CL (µm)	SAT	AR	CI	Chromosome type
	LA	SA					
O. meschetica							
I	2.49±0.41	1.55±0.14	4.06±0.53	-	1.60±0.18	61.32±2.75	m
II	2.30±0.21	1.39±0.29	3.69±0.42	-	1.65±0.32	62.47±4.45	m
III	2.09±0.34	1.26±0.13	3.35±0.47	-	1.65±0.11	62.14±1.58	m
IV	1.90±0.22	1.24±0.27	3.14±0.29	-	1.53±0.38	60.70±6.34	m
V	1.73±0.12	1.31±0.20	3.04±0.28	-	1.32±0.17	57.18±3.35	m
VI	1.73±0.12	1.17±0.23	2.89±0.26	-	1.47±0.28	59.81±5.05	m
VII	1.42±0.19	1.17±0.12	2.59±0.29	-	1.21±0.10	54.59±2.14	m
Mean	**1.95±0.36**	**1.30±0.13**	**3.25±0.49**		**1.49±0.16**	**59.74±2.87**	
O.galegifolia							
I	1.58±0.32	1.03±0.27	3.80±0.73	1.19±0.24	1.53±0.40	26.95±3.36	m
II	2.23±0.42	1.30±0.21	3.54±0.62	-	1.71±0.16	36.91±1.78	sm
III	2.03±0.37	1.30±0.26	3.33±0.52	-	1.56±0.36	39.16±5.55	m
IV	1.68±0.42	1.09±0.18	2.97±0.19	-	1.57±0.38	36.73±5.64	m
V	1.49±0.24	1.11±0.15	2.60±0.31	-	1.34±0.26	42.70±4.81	m
VI	1.22±0.28	0.97±0.10	2.19±0.38	-	1.25±0.18	44.71±3.41	m
VII	1.02±0.20	0.78±0.16	1.80±0.34	-	1.30±0.15	43.20±3.25	m
Mean	**1.61±0.42**	**1.08±0.18**	**2.89±0.73**		**1.46±0.20**	**38.62±6.02**	

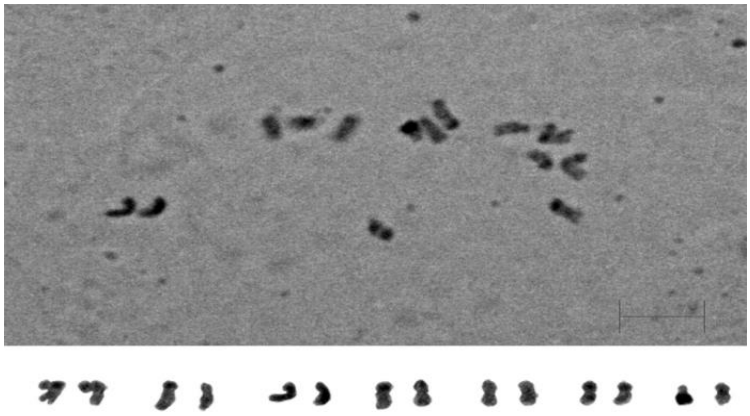

Figure 1. Mitotic metaphase chromosomes and karyogram of *Onobrychis tournefortii*
Şekil 1. *Onobrychis tournefortii taksonunun mitotik metafaz kromozomları ve karyogramı*

O. albiflora is an endangered endemic species that is commen in a local area in Turkey. Tekin et al. (2016) observed that the chromosome number was 2n=14 and chromosomes varied median and submedian similar to our findings. However, chromosome 4 had a satellite (Fig 2).

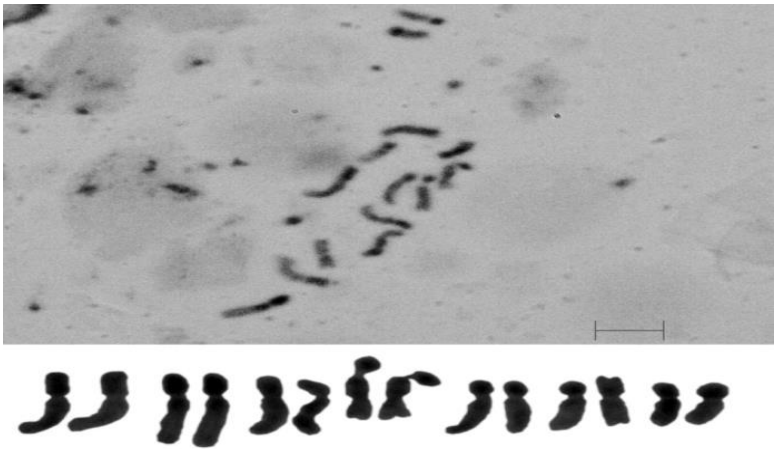

Figure 2. Mitotic metaphase chromosomes and karyogram of *Onobrychis albiflora.*
Şekil 2. *Onobrychis albiflora taksonunun mitotik metafaz kromozomları ve karyogramı*

The total chromosome length of *O. hypergyrea* varied from 2.98-.4.60 µm and the total chromosome length was 27.35 µm (Table 3, Table 2). This species had 4 median (4 m) and 3 submedian (3 sm) chromosomes and chromosome 1 included a satellite (Fig 3). Akçelik et al. (2012) and Sepet et al. (2011) were confirmed in terms of somatic chromosome number, centromeric position and satellite in this study.

O. radiata and *O. meschetica* species are morphologically very similar to each other. *O. meschetica* was recorded as a new species in Turkey first time by Aktoklu (2001). In this study, there were some similarity in terms of somatic chromosome number (2n=14) and total chromosome length.

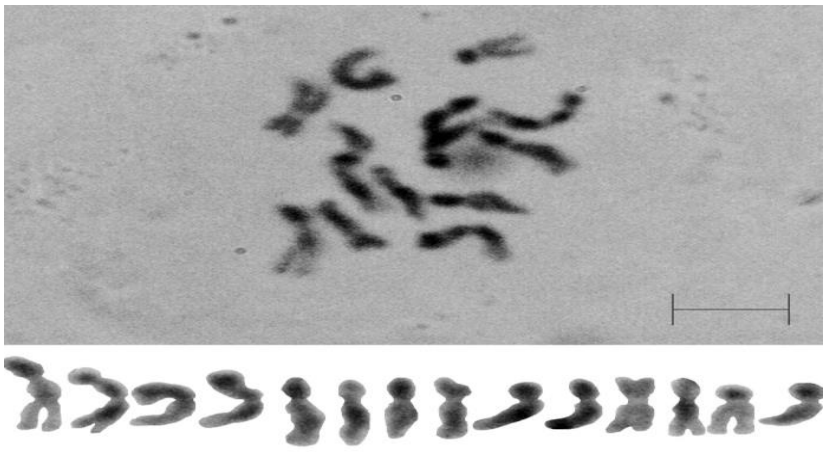

Figure 3. Mitotic metaphase chromosomes and karyogram of *Onobrychis hypargyrea.*
Şekil 3. *Onobrychis hypargyrea taksonunun mitotik metafaz kromozomları ve karyogramı*

The total chromosome lengths varied from 2.01-4.06 µm and 2.59-4.06 (Table 3) in *O. radiata* and *O. meschetica*, respectively. However, while all chromosomes of these taxa were median and chromosome 1 had a satellite in *O. radiata* (Fig 4), *O. meschetica* had no satellite (Fig 5).

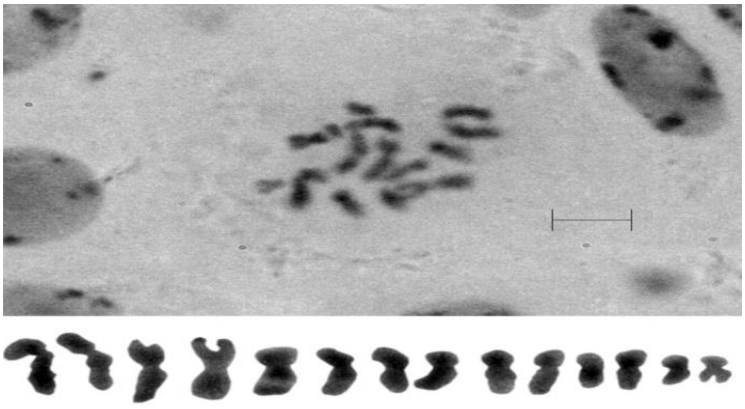

Figure 4. Mitotic metaphase chromosomes and karyogram of *Onobrychis radiata.*
Şekil 4. *Onobrychis radiata taksonunun mitotik metafaz kromozomları ve karyogramı.*

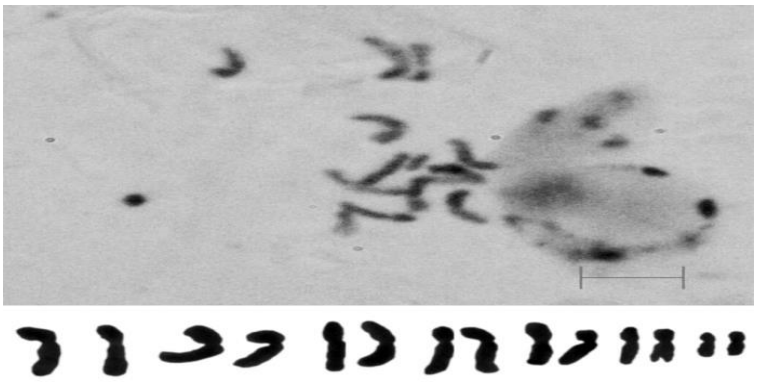

Figure 5. Mitotic metaphase chromosomes and karyogram of *Onobrychis meschetica.*
Şekil 5. *Onobrychis meschetica taksonunun mitotik metafaz kromozomları ve karyogramı*

Hejazi et al. (2010) had similar results in terms of somatic chromosome number (2n=14) in the Iranian population of *O. radiata*. But, chromosomes of this population varied from median to submedian and no satellites were observed. Total chromosome lengths of *O. galegifolia* varied from 1.80-3.80 µm and total chromosome length was 20.33 µm (Table 3, Table 2). Also, chromosomes of this species varied from median (6 m) to submedian (1 sm) according to centromeric position, and chromosome 1 had a satellite (Fig 6).

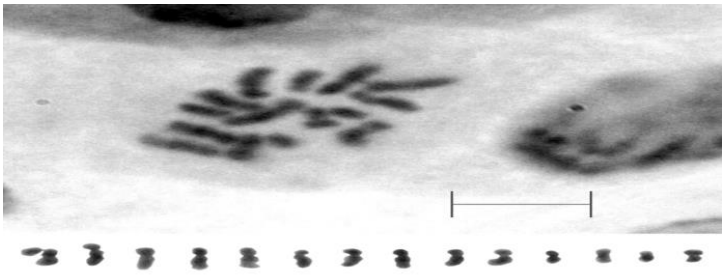

Figure 6. Mitotic metaphase chromosomes and karyogram of *Onobrychis galegifolia*
Şekil 6. *Onobrychis galegifolia taksonunun mitotik metafaz kromozomları ve karyogramı*

Arslan et al. (2012) indicated that the somatic chromosome number of *O. galegifolia,* collected from a different locality in Turkey, was 2n=16. Also, no satellites were observed and the chromosomes varied from median, submedian, and subterminal.

4. Conclusions

In conclusion, differences were observed in presence and location of satellite and chromosome symbol according to centromeric position among *Onobrychis* taxa. The basic chromosome number was x=7 and the ploidy level was diploid (2n=14) in all investigated species. The karyotype characteristic of the genus was symmetrical, because all chromosomes of the species varied from median to submedian according to centromeric position. While *O. tournefortii*, *O. hypargyrea*, *O. radiata,* and *O. galegifolia* had a satellite on chromosome 1, *O. albiflora* had a satellite on chromosome 4. The more diversified populations within the same taxa must be studied to reveal obvious karyological features.

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