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KARYOTYPE ANALYSIS OF SOME *ONOBRYCHIS* (SAINFOIN) SPECIES IN TURKEY

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Abstract — In this study, karyotype analysis of the species of *Onobrychis* sp. was undertaken using the squash method. The results showed that the chromosome number of *Onobrychis tournefortii* (Willd.) Desv., *O. gracilis* Besser, *O. hypargyrea* Boiss. is 2n = 14 and *O. argyrea* Boiss. subsp. *argyrea* Boiss., 2n = 16. The karyotype formula of *O. tournefortii* (Willd.) Desv. is 4m+3sm, of *O. hypargyrea Boiss.* and *O. gracilis* Besser 3m+4sm, and of *O. argyrea* Boiss. subsp. *argyrea* Boiss. 2m+ 5sm+1 st.

Key words: Cytogenetis, chromosome morphology, chromosome number, karyotype, sainfoin

INTRODUCTION

The *Onobrychis* genus is represented throughout the world with 162 species and in Turkey there are 52 species, 27 of which are endemic in Turkey (Aktoklu 1995). Studies on the chromosome number and morphology have been conducted on the *Onobrychis* genus. According to Abou-El-Enain (2002), the chromosome numbers of the genus *Onobrychis* are reported as 2n = 14, 28, 56, 16, 32, and 2 basic chromosome numbers, x=7 and x=8, are present in the genus.

The karyotype analysis and chromosome counting of *Onobrychis tournefortii* (Willd.) Desv.*, Onobrychis argyrea* subsp. *argyrea* Boiss.*, Onobrychis hypergyrea* Boiss.*, Onobrychis gracilis* Besser. (the chromosome number of this last species was not counted) was conducted for the first time in the present study.

MATERIALS AND METHODS

The four *Onobrychis* specimens were collected and identified in 2005. Voucher specimens and seeds are being conserved in the Herbarium of Ankara University, Faculty of Agriculture, Department of Field Crops. These four species, endemic in Turkey, were collected from the following areas: *Onobrychis tournefortii* (Willd.) Desv., Nevşehir, Turkey, 38˚ 40 09K , 35˚ 51 49 D, 1060 m. Collector: C. Sancak, S. Avcı, defining: A. Duran; *Onobrychis gracilis* Besser, Pozantı, Adana,Turkey, 37˚ 24 31K, 31˚ 52 44D, 780 m., Collector: C. Sancak, S. Avcı, defining: A.Duran;, *Onobrychis hypargyrea* Boiss. Çubuk-II dam, Ankara, Turkey, 40˚ 17 27K, 33˚ 00 57D, 1161 m. Collector: C. Sancak, S. Avcı, defining: A.Duran, *Onobrychis argyrea* Boiss. subsp. argyrea Boiss., Darende, Malatya, Turkey, 38˚ 23 50K, 37˚ 35 25D, 1408 m. Collector: C. Sancak, S. Avcı, defining: A.Duran.

In order to define the karyotype and chromosome number, the root tips of 2-3 day-old seedlings were used for accumulation of cells in metaphase as described by Elçi (1965). The roots were cut and kept for 12 h at 0ºC. Afterwards, the roots were determined in a 3:1 absolute alcohol, glacial acetic acid mixture for 30 min, hydrolyzed for 12 min in 1 N HCl at 60ºC and stained with Feulgen for 30 min

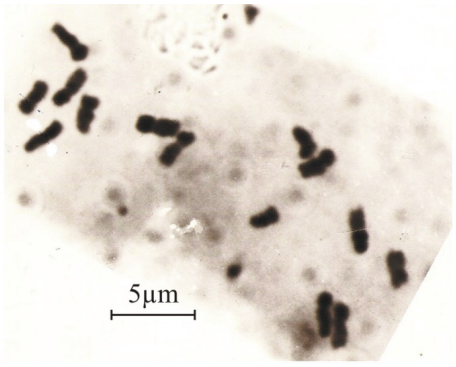




Fig. 1. Mitotic metaphase chromosomes and karyogram of *Onobrychis tournefortii* (Willd.) Desv. (2n=14).

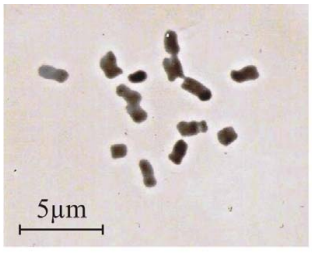
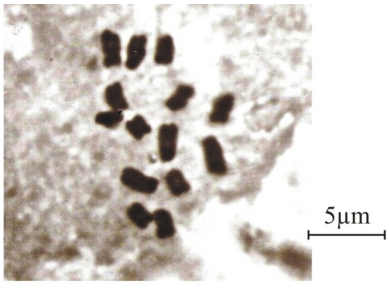




Fig. 2. Mitotic metaphase chromosomes and karyogram of of *O. hypargyrea* Boiss (2n=14).



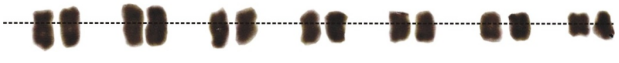
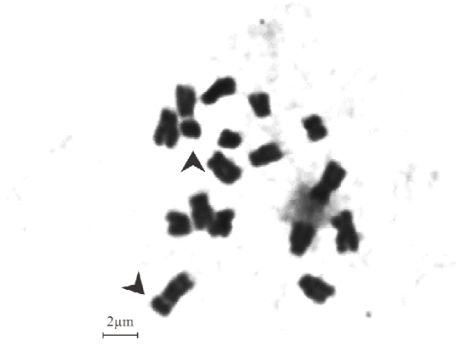


Fig. 3. Mitotic metaphase chromosomes and karyogram of *O. gracilis* Besser (2n=14).



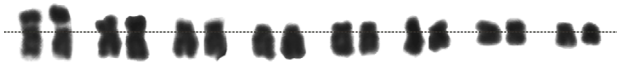


Fig. 4. Mitotic metaphase chromosomes and karyogram of *O. argyrea* Boiss. subsp. *argyrea* Boiss. (2n=16).

the dark at room temperature before making the microscopic preparations. Only the roots of *O. argyrea* Boiss. subsp. *argyrea* Boiss. were stained with Aceto-Iron-Hematoxilin for 20 min at 30°C (Zarifi 2004). Synchronized root tips were then squashed in 1% lactopropionic orcein for chromosome preparations as described by Busch et al. (1996). The counting of the chromosomes, measuring their lengths, and conducting the karyotype analysis, were performed using permanent slides which contain the chromosomes at the metaphase stage of mitosis. Photographs, enlarged 10x100, were taken using a camera attached to the microscope. Only the slides with a good spread, clearly observable morphologies and somatic root tips on the same plane were used. At least 8 complete metaphase figures with clearly distinguishable chromosomes were measured using LUCIA software and the average values of the relative sizes of the chromosomes and their arms were used to construct ideograms. The total chromosome length was measured with the computer system. The rules for numbering chromosomes within ideograms generally followed those used by Verma (1978), and Lavania and Sharma (1980).

Relative chromosome length = (chromosome length/total chromosome lenghts)x100; arm ratio = length of long arm / length of short arm; centromeric

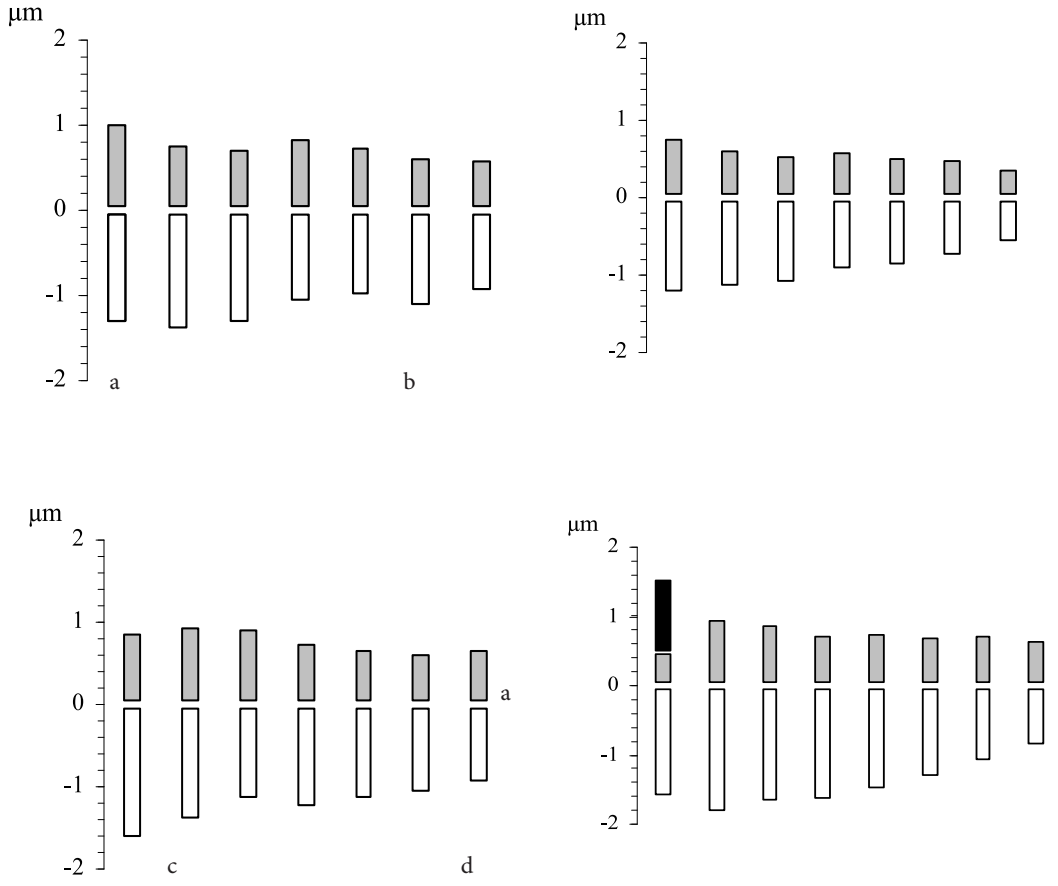


Fig. 5. İdeograms of a) *Onobrychis tournefortii* (Willd.) Desv., (2n=14), b) *O. hypargyrea* Boiss. (2n=14), c) *Onobrychis gracilis* Besser, (2n=14), d) *Onobrychis. argyrea* Boiss. subsp. *argyrea* Boiss. (2n=16).

index = [(length of long arm / (length of short arm + length of short arm)]x100. The position of centromeric constriction was recorded as median (m: 1.0-1.7), submedian (sm: 1.7-3.0) and subterminal (st: 3.0-7.0) by the arm ratio (Martin et al., 2008).

RESULTS AND DISCUSSION

In this study, 4 species were studied: *Onobrychis tournefortii* (Willd.) Desv., *O. gracilis* Besser, *O. hypargyrea* Boiss., and *O. argyrea* Boiss. subsp. *argyrea* Boiss.

The chromosome number was 2n=14 in *O. tournefortii* (Willd.) Desv., (Fig. 1 and 5a). Total chromosome lengths varied between 2.19-1.40 µ. Arm ratios were 1.32-1.97. The karyotype of *O. tournefortii* (Willd.) Desv. has 4 pairs of metacentric chromosomes and 3 pairs of submetacentric (Table 1).

The chromosome number was 2n=14 in *O. hypargyrea* Boiss. (Fig. 2 and 5b). Chromosomes ranged from 0.80 to 1.83 µ in this species. Arm ratios were between 1.41-2.22 µ. The centromeric index was 31.24-38.21. 3 pairs of chromosomes were metacentric, 4 chromosome pairs were submetacentric.

The somatic chromosome number was observed as 2n= 14 in *O. gracilis* Besser (Fig. 3, and 5c). Total chromosome length was 1.46-2.34 µ. Arm ra-

Table 1. Detailed features of somatic metaphase chromosome of *O. tournefortii* (Willd.) Desv.

Chromsome No.	Chromosome arms (μ)		Total lenght (μ)	Arm ratio (L/S)	Centromeric index*	Chromosome type
	Long arm (L)	Short arm (S)				
1	1.25±0.06	0.94±0.05	2.19 ±0.09	1.35±0.05	42.79	m
2	1.33±0.06	0.71±0.03	2.03±0.09	1.89±0.04	34.75	sm
3	1.25±0.06	0.66±0.04	1.91±0.09	1.95±0.10	34.32	sm
4	1.00±0.06	0.77±0.04	1.78±0.09	1.32±0.08	43.68	m
5	0.92±0.05	0.68±0.04	1.59±0.09	1.37±0.05	42.39	m
6	1.05±0.05	0.54±0.03	1.60±0.08	1.97±0.08	34.01	sm
7	0.87±0.03	0.53±0.03	1.40±0.04	1.68±0.09	37.82	m

*: [(S/(L+S)]x 100

Table 2. Detailed features of somatic metaphase chromosome of *O. hypargyrea* Boiss.

Chromsome No.	Chromosome arms (μ)		Total lenght (μ)	Arm ratio (L/S)	Centromeric index*	Chromosome type
	Long arm (L)	Short arm (S)				
1	1.14±0.10	0.69±0.05	1.83 ±0.06	1.74±0.28	38.26	sm
2	1.08±0.06	0.54±0.03	1.62±0.05	2.04±0.17	33.35	sm
3	1.02±0.05	0.46±0.02	1.48±0.05	2.22±0.14	31.43	sm
4	0.84±0.04	0.52±0.02	1.36±0.03	1.65±0.16	38.43	m
5	0.79±0.03	0.46±0.04	1.25±0.03	1.80±0.19	36.54	sm
6	0.67±0.04	0.43±0.02	1.10±0.05	1.58±0.13	39.21	m
7	0.50±0.11	0.30±0.06	0.80±0.16	1.41±0.30	31.24	m

*: [(S/(L+S)]x 100

Table 3. Detailed features of somatic metaphase chromosome of *O. gracilis* Besser.

Chromsome No.	Chromosome arms (μ)		Total lenght (μ)	Arm ratio (L/S)	Centromeric index*	Chromosome type
	Long arm (L)	Short arm (S)				
1	1.55±0.04	0.79±0.04	2.34 ±0.01	1.99±0.17	33.74	sm
2	1.32±0.03	0.88±0.11	2.20±0.11	1.60±0.25	39.49	m
3	1.08±0.06	0.86±0.09	1.94±0.14	1.29±0.11	43.95	m
4	1.18±0.11	0.66±0.04	1.85±0.08	1.83±0.29	36.28	sm
5	1.09±0.05	0.60±0.04	1.68±0.01	1.86±0.21	35.51	sm
6	1.00±0.00	0.56±0.04	1.56±0.04	1.80±0.12	35.88	sm
7	0.87±0.04	0.59±0.06	1.46±0.07	1.55±0.20	39.90	m

*: [(S/(L+S)]x 100

Table 4. Detailed features of somatic metaphase chromosome of *O. argyrea* Boiss. subsp. *argyrea* Boiss.

Chromsome No.	Chromosome arms (μ)		Total lenght (μ)	Arm ratio (L/S)	Centromeric index*	Chromosome type
	Long arm (L)	Short arm (S)				
1	1.52±0.05	0.40±0.06	2.94 ±0.08	4.74±0.89	13.48	st
2	1.74±0.08	0.89±0.05	2.63±0.10	1.99±0.12	33.79	sm
3	1.60±0.04	0.80±0.05	2.40±0.07	2.05±0.14	33.25	sm
4	1.58±0.05	0.67±0.04	2.25±0.07	2.41±0.15	29.75	sm
5	1.43±0.06	0.68±0.02	2.10±0.05	2.13±0.15	32.42	sm
6	1.24±0.06	0.64±0.03	1.88±0.08	1.95±0.09	34.14	sm
7	1.00±0.06	0.65±0.02	1.65±0.06	1.58±0.14	39.63	m
8	0.80±0.02	0.59±0.02	1.39±0.03	1.37±0.08	42.60	m

*: [(S/(L+S)]x 100

tios were 1.29-1.99 and the centromeric index was 33.74-39.90. The karyotype of *O. gracilis* Besser has 3 metacentric chromosome pairs and 4 submetacentric chromosome pairs (Table 3).

The total chromosome number was observed as 2n=16 in *O. argyrea* Boiss. subsp. *argyrea* (Figs. 3 and 5d) and showed agreement with the literature (Öztürk et al., 2009). The karyotype formula was 1st +2m + 5sm. Total chromosome length was 1.39-2.94. The arm ratio varied between 1.37-4.74 and the centromeric index was 13.48-42.60 (Table 4). *O. argyrea* Boiss. subsp. argyrea is endemic for Turkey. The chromosome number was counted (2n = 16) by Öztürk et al. (2009), but this is the first time the chromosome morphology of the species was studied.

Most of the cytological studies in the genus have concentrated on chromosome count. In this study, we determined the chromosome numbers and detailed measurements of four species of *Onobrychis* as 2n=14, 16 for the first time in Turkey. When the results provided from this study are compared with previous studies of the chromosome numbers of the *Onobrychis* genus, they do not show variation. Previous reports show that investigated members of *Onobrychis* have chromosomes with 2n=14, 16, 28, 32 (Ansariasl et al. 2001; Abou-El-Enain, 2002; Ansari et al. 2002; Hoşgören, 2006; Hatami and Nasırzadeh, 2007; Hesamzadeh Hejaji and Ziaeinasab, 2008). This study together with former results shows that the basic haploid chromosome numbers of *Onobrychis* may be n=7, 8.

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