Research article/Araştırma makalesi

Karyological study on endemic *Sonchus erzincanicus* Matthew (Asteraceae) in Turkey

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Abstract

Sonchus erzincanicus Matthews (Asteraceae) species is endemic to Turkey and distributing naturally in Erzincan. In this research, *Sonchus erzincanicus* was collected from Erzincan, its somatic chromosome number and karyotype was determined. The somatic chromosome number was determined as 2n=18 in *S. erzincanicus*. The karyotype analysis of this species was examined using Image Analysis System (Bs200Pro). The total haploid chromosome lengths 20.11 μm and the shortest chromosome length is 1.63 μm, the longest is 2.97 μm. The karyotype formulae of this species consist of six median chromosome pairs and three submedian chromosome pairs. The karyotype analysis of this species was first carried out in our study.

Key words: Karyotype, *Sonchus erzincanicus*, Asteraceae, Turkey

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Endemik *Sonchus erzincanicus* Matthews (Asteraceae) (Erzincan sütotu) türünün üzerinde karyolojik çalışma

Özet

Sonchus erzincanicus Matthews (Asteraceae) Türkiye için endemiktir ve doğal olarak Erzincan'da yayılış gösterir. Bu çalışmada, Erzincan'dan toplanan *Sonchus erzincanicus* türünün somatik kromozom sayısı belirlendi ve karyotip analizi yapıldı. Türün kromozom sayısı 2n=18 olarak tespit edildi. Bu türün Görüntü Analiz Sistemi aracılığı ile Karyotip Analizi yapıldı. Haploit kromozom uzunluğu 20.11 μm'dir ve en küçük kromozom 1.63 μm en büyük kromozom ise 2.97 μm uzunluğundadır. Türün karyotip formülü altı median ve üç submedian kromozom tiplerinden oluşmaktadır. Bu türün karyotip analizi ilk defa çalışmamızda analiz edildi.

Anahtar kelimeler: Karyotip, *Sonchus erzincanicus*, Asteraceae, Türkiye

1. Introduction

Asteraceae is the largest family of flowering plants, with approximately 1620 genera and more than 23.600 species (Stevens, 2007). Asteraceae is widely distributed within diverse regions ranging from the Southwestern United States, Mexico, and Southern Brazil to South Africa, Middle and Southwestern Asia, and Australia. South America is acknowledged as the geographic origin of the family phylogenetically (Bremer, 1994). Asteraceae represents the largest number of species in the flora of Turkey and the East Aegean Islands, with the total number of recorded species at 1209. Of these, 447 species are endemic, making the endemism rate 37%. This family is the second largest family of the Turkish Flora, with a total of 134 genera (Davis et al.,1988; Özhatay and Kültür, 2006).

The genus *Sonchus* L. has a worldwide distribution except for Central and South America (Heywood, 1978). It is commonly considered to be related to *Aetheorhiza* Cass., *Reichardia* Roth and *Launaea* Cass. (Stebbins, 1953), but it is a more isolated and distinct genus (Sell, 1975). In recent years, this genus has been the subject of chemical studies (Mahmoud et al., 1984; Shimizu et al., 1989; Manez et al.,1994). *Sonchus* is represented with seven taxa in Turkey (Davis, 1975). This taxa; *Sonchus asper* L. subsp. *glaucescens* (Jordan) Ball, *S. oleraceus* L., *S. tenerrimus* L., *S. arvensis* L. subsp. *arvensis*, *S. arvensis* L. subsp. *uliginosus* (Bieb.) Bég., *S. palustris* L., *S. erzincanicus* Matthews.

Sonchus arvensis subsp. *uliginosus* species is represented to Erzincan. *Sonchus* species are variously known as "sütlük", "kuzu gevreği", and "eşek marulu" in Turkey. Also, some of which are used as a food in Turkey (Akartürk, 2001). Flowers are perfect and generally self-incompatible. Perennial sowthistle can reproduce by seed and vegetatively is pollinated by insects including honeybees and other bees, hover flies and blister beetles (Derscheid and Schultz, 1960).

The reported chromosome numbers are 2n=14, 18, 20, 27, 30, 32, 36, 37, 54, 64 and 188 in the genus *Sonchus* (http://www.tropicos.org/NameSearch.aspx?projectid=9). There were a lot of cytological studies on the genus *Sonchus*, However, only a few karyotype had been made on this genus. In this study chromosome numbers and chromosome morphology of this species were determined. Karyological knowledge needs to be used in conjunction with other sources of data to achieve a better understanding of the cytologic relationship of *Sonchus* species, leading to their natural classification. In this regard, karyotypes were determined in *Sonchus erzincanicus* species of *Sonchus* growing naturally in Turkey, and karyological attributes of selected species were evaluated for the first time.

2. Materials and methods

2.1. *Plant material*

Sonchus erzincanicus, (Sütlük) a very rare endemic species restricted to B7 Erzincan, E of Turkey. The plant materials were identified by Kandemir and the voucher specimens were preserved at Department of Biological Education, Erzincan University, Erzincan, Turkey. It has been included in the Red Data Book of Turkish Plant (Ekim et al., 2000) as an endangered species, but very little is known about its detailed distribution and ecology. The plant is a perennial forb. It has an erect growth form, and the stem is 80 cm long, 0.3-10 mm thick and milky latex. The plant basal leaves are rosette and oblong-elliptic and cauline leaves are few. The capitula is 1.5-2 cm wide. The involucre is cylindrical and has sparse glandular hair. The flowering period is July-August. It grows in slightly saline marsh at altitudes of 1250 m. (Davis, 1975), (Figure 1).

Figure 1. General appearance of *Sonchus erzincanicus*

Sonchus erzincanicus samples were collected during the field studies of the project titled "Important Plant Areas along the BTC pipeline" from B7 square (Erzincan: Ekşisu, 39° 43´ 98´´ N, 39° 37´ 55´´ E, 1154 m, 20.08.2004) in Turkey, (Figure 2).

2.2. *Chromosome analysis*

Karyomorphological observations were made on mitotic metaphase cells of root-tips obtained from germinated achenes. Root tips were pretreated for 16h in α-monobromonaphthalene at 4°C and washed with distilled water and finally fixed in Carnoys solution (3:1) absolute ethanol:glacial acetic acid, overnight. The root tips were hydrolysed for 10 min in 1 N HCl at room temperature, washed and stained in 2% (w/v) aceto-orcein for 2 h. Stained root tips were then squashed in a drop of 45% acetic acid and permanent slides were made by mounting in Depex. The photographs, enlarged 10x100, were taken using a camera attached to the microscope.
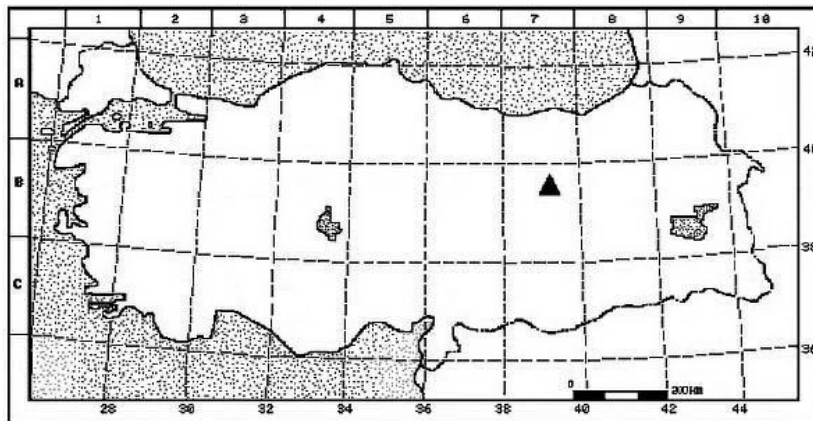
Figure 2. The distribution map (▲) of *Sonchus erzincanicus* in Turkey

Only the slides with a good spread, with clearly observable morphologies and with somatic root tips on the same plane were used. Chromosome measurements were made in at least five well-spread metaphases, bearing the same chromosome contraction. The karyotype characterizations were measured, ideogram and karyogram were designed by using an Image Analysis System (Duran et al., 2010; Çetin et al., 2010; Martin et al., 2011a, 2011b).

2.3. Karyotype characterization

The following parameters were measured in each metaphase plate to characterize the karyotypes numerically: diploid number of chromosomes (2n), long arm (LA), short arm (SA), total length (TL = LA+SA), arm ratio (AR = LA/SA), centromeric index [CI = SA / (LA+SA)], difference of range relative length (DRL = MaxRL% - MinRL%), value of relative chromatin (VRC = ΣTL/n), karyotype formula (KF) according to Levan's method (Levan et al., 1964). Total form percentage (TF%) = [(ΣSA / ΣTL) × 100] (Huziwara, 1962). Percent of symmetry index (%SI) = [(length of smallest chromosome/length of longest chromosome) × 100]; centromeric gradient value (CG) = [(length of median short arm/length of median chromosome) × 100 (Lavania and Srivastava, 1992). Also, Asymmetry index was indicated in this study.

According to Romero Zarco (1986), interachromosomal asymmetry index (**A1**) = 1-[$\frac{\sum_{i=1}^{n}\frac{bi}{Bi}}{n}$], (bi = the average length for short arms in every homologous chromosome pair or group; Bi = the average length for long arms in every homologous chromosome pair or group; n = the number ofhomologous chromosome pairs or groups), interchromosomal asymmetry index (**A2**) = s / x, (s = standard deviation of the chromosome length; x = the mean of chromosome length)..

3. Results

The chromosome number of *Sonchus erzincanicus* was determined to be 2n=18. This species was determined 2n=2x=18=6m+3sm with a basic chromosome number of x=9 (Figure 3). The shortest chromosome length is 1.63 µm, the longest is 2.97 µm, and haploid chromosome length is 20.11 µm. Chromosome arm ratios are measured as 1.16–2.55 µm. Centromeric index varies between 3.15 µm and 6.26 µm, and relative lengths vary from 8.11 µm to 14.79 µm. The karyotype formulae consisted of six median chromosomes and three submedian chromosomes (Table 1). Also relative length (DRL = MaxRL% - MinRL%) was found 6.68 µm, value of relative chromatin (VRC = ΣTL/n) is 1.11 µm, total form percentage (TF%) is 39.98 µm, percent of symmetry index (%SI) is 54.82, centromeric gradient value (CG) is 68.70, asymmetry index is 0,0004, A1 index is 0,32 and A2 index is 0,18 (Table 2).The chromosome number and morphologies were reported for the first time in this study, respectively. Karyogram of the best metaphases of this species and ideogram were arranged in this study (Figure 4, 5).

4. Conclusions

According to literature, 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 this species of *Sonchus erzincanicus* as 2n=18 for the first time in Turkey. The reported chromosome numbers are 2n=14, 18, 20, 27, 30, 32, 36, 37, 54, 64 and 188 in the genus *Sonchus* (Table 3).

Table 1. Measurements (μm) of somatic chromosomes in *Sonchus erzincanicus* (m = median, sm= submedian)

Chromosome pair no	Long arm	Short arm	Total length	Arm ratio	Centromeric index	Relative length	Chromosome type
1	1,710	1,26	2,97	1,36	6,26	14,79	m
2	1,740	0,94	2,68	1,85	4,66	13,30	sm
3	1,310	1,10	2,41	1,19	5,48	11,98	m
4	1,620	0,63	2,25	2,55	3,15	11,19	sm
5	1,340	0,90	2,24	1,49	4,47	11,15	m
6	1,140	0,99	2,12	1,16	4,90	10,56	m
7	1,250	0,71	1,95	1,77	3,51	9,70	sm
8	1,030	0,82	1,86	1,25	4,10	9,22	m
9	0,940	0,69	1,63	1,36	3,44	8,11	m

Table 2. Karyotype characteristics of *Sonchus erzincanicus* (2n - somatic chromosome number, long and short arm in each chromosome, AR - arm ratio, CI - centeromeric index, TL - total length, TF% - total form percentage, DRL - difference of relative length, VRC - value of relative chromatin, SI - symmetry Index percentage, CG - centromeric gradient, Asymmetry index, KF - karyotype Formula (m: metacentric, sm: submetacentric), A1 - Intrachromosomal asymmetry index, A2 - Interchromosomal asymmetry index)

| Chromosome number	2n=18
Long arm in each chromosome	1.74 μm
Short arm in each chromosome	1.26 μm
Total length (**TL** = LA+SA)	1.63 μm - 2.97 μm
Arm ratio (**AR** = LA/SA)	1.16 μm - 2.55 μm
Centromeric index [**CI** = SA/(LA+SA)]	3.15 μm – 6.26 μm
Difference of relative length (**DRL** = MaxRL% - MinRL%)	6.68 μm
Value of relative chromatin (**VRC** = ΣTL/n)	1.11 μm
Karyotype formula (**KF**)	6m + 3sm
Intrachromosomal asymmetry index (**A1**)	0.32
Interchromosomal asymmetry index (**A2**)	0.18
Total form percentage (**TF**%)	39.98
Percent of symmetry index (%**SI**)	54.82
Centromeric gradient value (**CG**)	68.70
Asymmetry index	0.0004
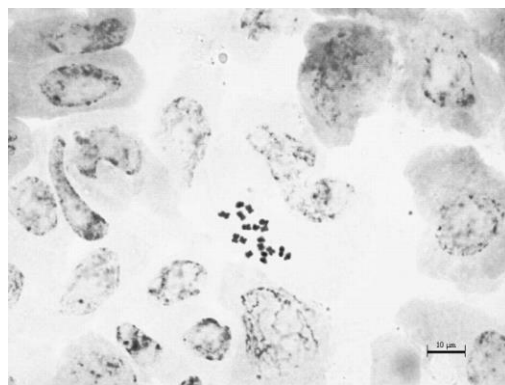
Figure 3. Mitotic metaphase chromosomes in *Sonchus erzincanicus*, Bar: 10 μm

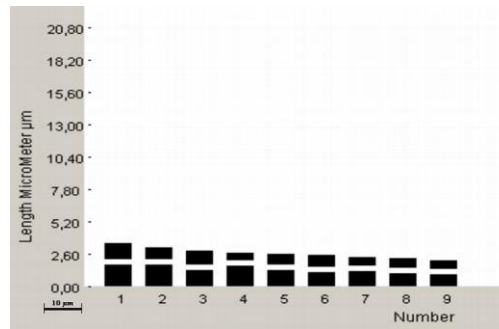Figure 4. Karyogram of *Sonchus erzincanicus*, Bar: 10 μm

Figure 5. Ideogram of *Sonchus erzincanicus*, Bar: 10 μm

Table 3. Studied taxa of *Sonchus* and chromosome counts

Taxa	Chromosome counts	References
Sonchus angustissimus Hook. f. *S. aquatilis* Pourr. *S. araraticus* Nazarova & Barsegyan, *S. brachyotus* DC. *S. capillaris* Svent. *S. crassifolius* Pourr. ex Willd. *S. daltonii* Webb *S. elliotianus* Hiern *S. asper* L. Hill subsp. *asper* *S. asper* L. subsp. *glaucescens* (Jord.) Ball *S. maritimus* L. *S. maritimus* L.subsp. *aquatilis* (Pourr.) Nyman *S. novocastellanus* Cirujano *S. pinnatus* Aiton *S. pustulatus* Willk. *S. schweinfurthii* Oliv. & Hiern *S. sosnowskyi* Schchian *S. wightianus* DC. *S. palustris* L.	2n=18	Renard et al., 1983 Silvestre, 1984 Dalgaard, 1985 Kilian, 1988 Nazarova, 1989 Gill and Omoigui, 1992 Morton, 1993 Razaq et al., 1994 Probatova et al., 1998 Lövkvist and Hultgård, 1999 Nazarova, 2004 Suda et al., 2005
S. tenerrimus L.	2n=14	Mejías, 1988 Ruíz,1990 Oberprieler and Vogt, 1993
S. lachnocephalus Rech. f.	2n=20	Razaq et al.,1994
S. microcephalus Mejías	2n=30	Díaz et al.,1992
S. macrocarpus Boulos & C. Jeffrey	2n=32	Kamel, 2004
S. oleraceus L.	2n=32, 36, 64	Lavrenko and Serditov, 1991 Al-Bermani et al., 1993 Oberprieler and Vogt, 1993 Carr et al., 1999 Probatova, 2006 Gemeinholzer, 2005
S. uliginosus M. Bieb.	2n=27, 36, 188	Mulligan, 1984
S. arvensis L. var. *maritimus* Wahlenb.	2n=54	Jalas and Pellinen, 1985
S. kirkii Hamlin *S. arvensis* L. var. *glabrescens* Günther, Grab & Wimm. *S. arvensis* subsp. *uliginosus* (M. Bieb.) Nyman *S. arvensis* L. subsp. *arvensis*	2n=36	Morton, 1981 Jalas and Pellinen, 1985 Kiehn et al.,1991 Lövkvist and Hultgård, 1999
S. arvensis L.	2n=18, 36, 37, 54	Nazarova, 1989 Hong and Zhang, 1990 Lavrenko and Serditov, 1991 Singh, 1993 Dempsey et al., 1994 Dmitrieva, 2000 Volkova and Basargin, 2002

According to Mejías & Andrés, their karyotype studies about *S. oleraceus*, *S. microcephalus*, *S.asper* subsp. *glaucescens* and *S. asper* subsp. *asper* indicate similarity with *S. erzincanicus.* While in our study, intrachromosomal

asymmetry index (A1) of *S. erzincanicus* was reported 0.32, according to Mejías & Andrés, the A1 index of intrachromosomal variability is 0.35 or 0.37 in the three populations of *S. oleraceus*. Also very similar value, A1 = 0.35, is shown in the plants of *S. microcephalus*. In *S. asper*, the index ranges from 0.40 to 0.43 (Mejías and Andrés, 2004).

While in our study, interchromosomal asymmetry index (A2) of *S. erzincanicus* was reported 0.18, according to Mejías & Andrés, the interchromosomal variability index shows considerable variation in *S. oleraceus*, ranging from A2 = 0.21 in the plants from Cullera − Favareta and A2 = 0.34 in the plants from Sevilla. The single karyotype of *S. microcephalus* studied shows A2 = 0.26. In the karyotypes of *S. asper*, the A2 index ranges from 0.17 to 0.23, differences not having been detected between subspecies. Also according to Mejías & Andrés, A1 index of other *Sonchus* species; *S. tenerrimus* was reported 0.29-0.33, *S. maritimus* was reported 0.34–0.47, *S. aquatilis* was reported 0.22–0.25, *S. crassifolius* was reported 0.34–0.36, *S. novocastellanus* was reported 0.43, *S. pustulatus* was reported 0.32. A2 indexes of other *Sonchus* species were reported respectively, 0.20–0.22, 0.10–0.17, 0.12, 0.10–0.14, 0.16, 0.14. On the other hand, in our study mean chromosome length range from 1.63 to 2.97 while *S. oleraceus* 1.31–2.60, *S. microcephalus* 1.22–2.56, *S. tenerrimus* 1.81–3.11, *S. asper* 1.27–2.21, *S. asper* subsp. *glaucescens* 0.93–1.86, *S. maritimus* 1.72–2.57, *S. aquatilis* 1.61–2.68, *S. crassifolius* 2.05–3.08, *S. novocastellanus* 1.72–3.43, *S. pustulatus* 1.95–2.97 (Mejías and Andrés, 2004).

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