

Karyotype Analysis of *Silene salsuginea* Hub.-Mor. and *Silene doganii* A.Duran & Y. Menemen Local Endemic to for Turkey

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Abstract

In this study the somatic chromosome numbers and karyotype of two *Silene* L. species growing naturally in Turkey are examined. These species are *Silene salsuginea* Hub.-Mor. and *Silene doganii* A.Duran & Y. Menemen. The somatic chromosome studies were conducted using squash preparation method. The studies revealed that the chromosome numbers of *S. salsuginea* and *S. doganii* are $2n = 24$. The basic chromosome number of both species is determined as $x = 12$. The karyotype formula of *S. salsuginea* is $11m+1sm$ and of *S. doganii* $3m+9sm$. The total length of the metaphase chromosomes of *S. salsuginea* ranges between $1.16 \mu\text{m}$ - $2.65 \mu\text{m}$ and of *S. doganii* between $0.88 \mu\text{m}$ - $1.75 \mu\text{m}$. The total haploid chromosome length of *S. salsuginea* is measured as $20.32 \mu\text{m}$, and of *S. doganii* as $42.08 \mu\text{m}$. Their chromosome morphologies, karyogram and ideograms are made using Image Analysis System.

Key words: Chromosome number, karyotype, *Silene*.

INTRODUCTION

There are approximately 700 species in *Silene* L. (Caryophyllaceae) in the world. The genus is distributed in the Northern hemisphere and is especially concentrated in the Mediterranean region [1]. It is represented with 136 species in Turkey with an endemism ratio about 40 % [2-6].

Succulentae Boiss. section is represented in Turkey with two species. These are *Silene thymifolia* Sibth. & Sm. and *Silene salsuginea*. The typical species of the *Succulentae* section is *S. salsuginea*. The classification of *S. thymifolia* in section *Spergulifoliae* Boiss. rather than section *Succulentae* is more appropriate as given in the Flora of Turkey [2]. *Silene salsuginea* with succulent leaves is a rare local endemic species in the continental salty steps of Anatolia. Due to this feature *S. salsuginea* is considered to be the best species representing section *Succulentae*.

Silene doganii is a local endemic species distributed at the Amanos Mountains [5]. *S. doganii* species is represented in *Sclerocalycinae* Boiss. section. *Sclerocalycinae* section is represented with 16 species in Turkey [2]. In this section, *Silene caramanica* Boiss. & Heldr., has been divided into two subspecies recently [7].

Some taxonomical [8-9], morphological [10], molecular [11-12], palinological [13], chemical [14-17] and cytotoxicological studies on *Silene* species have been carried out by different researchers [18-24]. However, there were not any karyological studies concerning *S. salsuginea* and *S. doganii*.

The chromosome numbers of the genus *Silene* are determined in previous studies as $2n = 20, 24$ and 28 in 40 taxa as reported in the Turkish Flora [2-3, 25]. The karyomorphological and taxonomic studies on genus *Silene* are important as these studies help us to overcome taxonomic problems of genus *Silene*. There are some species of genus *Silene* that are monoic

and others that are dioic. This feature can only be determined in karyological studies of the relevant species. The karyological features of *S. salsuginea* and *S. doganii* species have not been studied previously. The present study, determining the karyomorphological features of these two species which are local endemic to Turkey, will be of use to overcome problems in the classification of these two species. Furthermore, as these species are endangered a detailed classification can lead to the preservation of the genetic sources by means of cell culture. In addition, a karyomorphological study contributes to the improvement of a genetic strain by selection.

The aim of this study is to determine the chromosome number and karyological features of these two *Silene* species.

MATERIALS and METHODS

The two *Silene* specimens were collected and identified in 2005. Voucher specimen and their seeds are being conserved in the Herbarium of Selçuk University Faculty of Education. These two taxa were collected from the following areas:

Silene salsuginea Hub.-Mor.; B5 Aksaray: Eskil, 950 m, 15.10.2005, salty steppe, M.Dinç 2523 & A.Duran.

Silene doganii A.Duran & Y.Menemen; C6 Osmaniye: Zorkun, Keldaz Tepe, 1750 m, 18.08.2005, forest clearings, M.Dinç 2478 & A.Duran.

In order to determine the chromosome number and karyological features of these two *Silene* species, all cytological observations were made on the root tips. Root tips were germinated on wet filter paper in petri dishes. After germination, fresh root tips about 1cm long were cut, pretreated in α -monobromonaphthalene at 4°C for 16 hours, and then fixed with glacial acetic acid: absolute alcohol (1:3) at 4°C for 24 hours. These were deposited in 70% ethanol at 4°C . The root tips were hydrolyzed in 1N HCl at room temperature for 10-12 minutes.

Finally they were squashed and stained in 2 % aceto-orcein. Permanent slides were prepared using standard liquid nitrogen method. Chromosome measurements were based on at least five metaphase plates.

The length of each chromosome was determined using software Image Analysis System on a personal computer. Measurements were conducted by the use of five metaphase plates for each taxon. The total and relative length of each chromosome was measured, using Image Analysis System. Afterwards, each chromosome was paired with its homolog. Total length of haploid chromosome complements was determined both for *S. salsuginea* and *S. doganii*. Each chromosome was measured with 0.0001 sensitivity. The karyogram of the best metaphases and ideogram of *S. salsuginea* and *S. doganii* were sequenced according to their decreasing length.

RESULTS

Silene salsuginea and *S. doganii* was examined for their chromosome number and morphology for the first time in the present study. The chromosome number of *S. salsuginea* and *S. doganii* were determined as $2n = 24$ (Figure 1). Ideograms and karyograms of the studied species were prepared using Image Analysis System (Figures 2-3).

The chromosome length of *Silene salsuginea* ranges from 1.16 μm to 2.65 μm . The chromosome arm ratio ranges from 1.09 μm to 1.83 μm . Eleven chromosome pairs of *S. salsuginea*, out of the twelve, are median. One chromosome pair is submedian. Total haploid chromosome length is 20.32 μm (Table 1). The ideogram of *S. salsuginea* is given in Figure 2a. The karyogram of *S. salsuginea* is given in Figure 3a.

The chromosome length of *Silene doganii* is between 2.54 μm and 5.27 μm . The haploid chromosome length is 42.08 μm (Table 2). According to the arm ratio, three chromosome pairs are median, nine chromosome pairs are submedian. The ideogram of this species is given in Figure 2b. The karyogram of *S. doganii* is given in Figure 3b.

Table1. Measurements (μm) of somatic chromosomes in *Silene salsuginea*

Chromosome Pair no.	Chromosome arms (μm)		Total length (μm)	Arm ratio (L/S)	Relative length (%)	Chromosome type
	Long arm (L)	Short arm (S)				
1	1.49	1.16	2.65 \pm 0.03	1.29	13.02	m
2	1.12	0.88	2.00 \pm 0.05	1.28	9.81	m
3	1.06	0.81	1.87 \pm 0.07	1.31	9.22	m
4	1.08	0.70	1.78 \pm 0.08	1.54	8.75	m
5	0.92	0.82	1.74 \pm 0.11	1.12	8.56	m
6	0.98	0.68	1.66 \pm 0.08	1.45	8.19	m
7	1.02	0.60	1.62 \pm 0.03	1.69	8.01	m
8	0.84	0.74	1.58 \pm 0.09	1.14	7.77	m
9	0.88	0.57	1.45 \pm 0.03	1.52	7.13	m
10	0.74	0.68	1.42 \pm 0.01	1.09	6.98	m
11	0.82	0.57	1.39 \pm 0.04	1.43	6.86	m
12	0.75	0.41	1.16 \pm 0.02	1.83	5.70	sm

Total length of haploid complement: 20.32 μm

Table 2. Measurements (μm) of somatic chromosomes in *Silene doganii*

Chromosome Pair no.	Chromosome arms (μm)		Total length (μm)	Arm ratio (L/S)	Relative length (%)	Chromosome type
	Long arm (L)	Short arm (S)				
1	3.52	1.75	5.27 \pm 0.11	2.02	12.51	sm
2	3.02	1.29	4.31 \pm 0.04	2.35	10.22	sm
3	2.56	1.61	4.17 \pm 0.04	1.59	9.90	m
4	2.47	1.35	3.82 \pm 0.05	1.83	9.08	sm
5	2.30	1.29	3.59 \pm 0.02	1.78	8.55	sm
6	1.84	1.50	3.34 \pm 0.05	1.22	7.92	m
7	2.18	1.02	3.20 \pm 0.01	2.13	7.62	sm
8	1.85	1.27	3.12 \pm 0.01	1.46	7.42	m
9	2.11	0.89	3.00 \pm 0.08	2.37	7.12	sm
10	2.03	0.93	2.96 \pm 0.06	2.17	7.04	sm
11	2.05	0.71	2.76 \pm 0.04	2.87	6.57	sm
12	1.66	0.88	2.54 \pm 0.03	1.90	6.03	sm

Total length of haploid complement: 42.08 μm

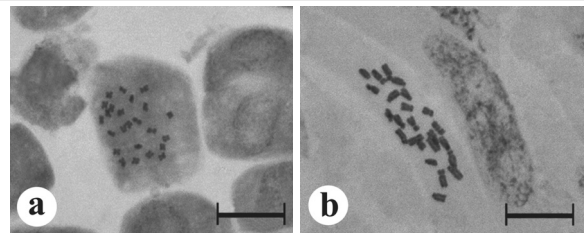
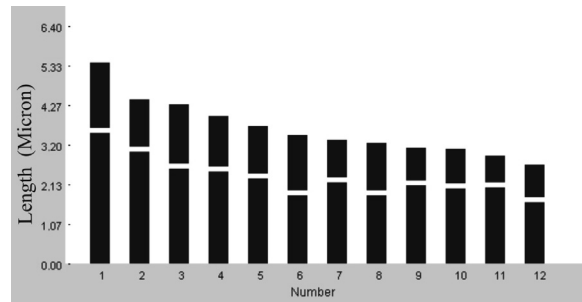


Figure 1. Mitotic metaphase chromosomes in *Silene* taxa. a. *S. salsuginea*



2n = 24, b. *S. doganii* 2n = 24. Scale Bar = 10 μm .

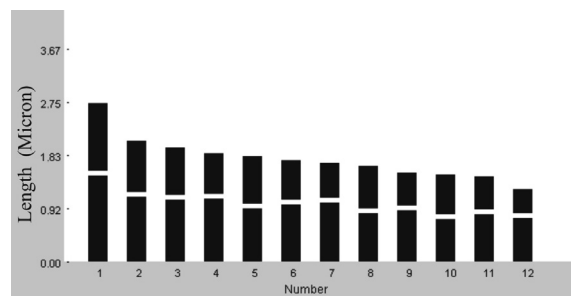


Figure 2. Ideograms of *Silene* species. a. *S. salsuginea* 2n = 24 b. *S. doganii* 2n = 24. Scale Bar = 10 μm .

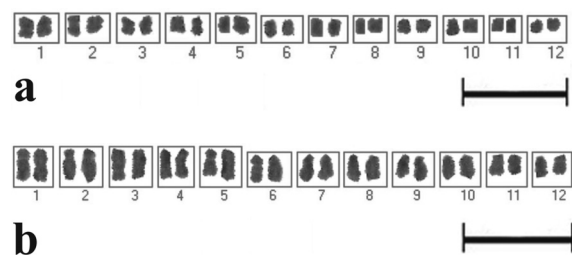


Figure 3. Karyograms of *Silene* species. a. *S. salsuginea* 2n = 24 b. *S. doganii* 2n = 24. Scale Bar = 10 μm .

DISCUSSION

The detailed morphological features of chromosomes obtained from these two taxa indicated that the somatic chromosome numbers of *Silene salsuginea* and *S. doganii* species are the same ($2n = 24$). However, their chromosome morphologies are different. The chromosome length of *S. salsuginea* ranges between 1.16 μm and 2.65 μm . Its karyotype formula is $11m+1sm$. There were not any cytotaxonomical studies conducted regarding these Turkish local endemic species identified recently. The mitotic chromosome length of *S. doganii* ranges from 2.54 μm to 5.27 μm . Its karyotype formula is defined as $3m+9sm$.

The chromosome number and morphology of the species and subspecies of *Silene* in our studies are similar to the taxa analyzed in the previous studies [22]. In a karyological study made by Yıldız and Çırpıcı (1996) on 19 species of genus *Silene*, the chromosome number of 15 species are determined as $2n = 24$, of four species as $2n = 48$, and the basic chromosome number as $x = 12$. The average chromosome length of the taxa mentioned in the above study was reported to range between 1.00 μm and 4.54 μm , while in our study they were ranging between 1.16 μm and 5.27 μm for the species *S. salsuginea* and *S. doganii*.

In the karyological study that Yıldız and Çırpıcı conducted in 1996, the chromosomes of *Silene paphlagonica*, *S. vulgaris* var. *vulgaris* and *S. gallica* were all reported to be metacentric; but of *S. sangaria* with $2n = 48$ chromosomes, 32 chromosomes to be metacentric and 10 chromosomes as submetacentric. Within the same study, it had been reported that most of the chromosome numbers of *S. bellidifolia* (12 chromosomes), *S. compacta* (10 chromosomes) and *S. olympica* (10 chromosomes) are submetacentric. Acrocentric chromosomes were reported only for *S. chlorifolia* (18 chromosomes), *S. sangaria* (2 chromosomes) and *S. compacta* (4 chromosomes). In the species analyzed in the present study, there are 11 metacentric chromosomes and one submetacentric chromosome in *S. salsuginea*. In *S. doganii* three chromosomes are metacentric and nine are submetacentric [22]. Yıldız and Çırpıcı, (1996) reported of *S. italica* and *S. dichotoma* the chromosome morphology to be metacentric (11 chromosomes) and one as submetacentric [22]. The chromosome morphology of *S. salsuginea* is compatible with both the above mentioned studies and the literature.

Some taxa of genus *Silene* are dioic and have sex chromosomes; such as *S. otites* (L.)Wib. and *S. alba* (Miller) Krause subsp. *ericalycina* (Boiss.)Walters [22]. The taxa of the genus *Silene* in the present study are monoic.

Another cytological study on the genus *Silene*, namely of *S. latifolia* and *S. dioica*, the chromosome numbers were reported as $2n = 24$ [26]. Our findings regarding the chromosome numbers of the species analyzed are compatible with the above mentioned. The karyomorphological study on the species of *S. latifolia* and *S. dioica* revealed that these species are dioic and have sex chromosomes. However, there were no sex chromosomes in the species *S. salsuginea* and *S. doganii*, indicating that they are monoic.

According to Malallah et al., (2003) the chromosome number of *Silene villosa* in the Kuwait Flora is as $2n = 24$ [27]. Our findings are similar to the findings of Malallah et al., (2003).

Constantinidis et al., (2002) conducted a karyological study on 20 Angiospermae taxa and they identified the chromosome numbers of *Silene fabarioides*, *S. salamandra*, *S. fabaria* subsp. *domokina* and *S. haussknechtii* to be $2n = 24$ [28]. The finding of the present study is similar to the study conducted by Constantinidis et al., (2002) previously.

In another cytological study on the European *Silene* taxa, it was reported that the basic chromosome number is $x = 10$ and $x = 12$. The somatic chromosome numbers of the *Silene* species analyzed in the present study are similar to the findings of previous studies. However, the *Silene* species in *Conomorpha* section were given as $2n = 20$ [29]. The findings of our study differ from the study of Abdel Bari, (1973) in regard to chromosome numbers.

A similar cytological study was carried out with Image Analysis Systems on 31 taxa belonging to 14 different Angiosperm families in Saudi Arabia. In that study, *Silene apetala* was found to have $2n = 24$ [30].

In this study the karyograms and ideograms of the studied species were prepared using Image Analysis Software. If Image Analysis System is to be compared to the traditional methods of measurement, it is less time consuming. Furthermore it is more practical and more sensitive.

The chromosome numbers and morphologies of the *Silene doganii* and *S. salsuginea* species, narrow local Turkish endemics, were determined for the first time in this study. We hope that this study will contribute to future cytotaxonomical studies of the genus *Silene*.

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