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Karyomorphological studies in seven taxa of the genus *Salvia* (Lamiaceae) in Turkey

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In this study, the karyotypes of mitotic chromosomes were determined of seven taxa of *Salvia* (Lamiaceae) collected from their natural habitats in Turkey: *S. viridis* ($2n = 16$), *S. candidissima* subsp. *occidentalis* ($2n = 20$), *S. sclarea*, *S. ceratophylla*, *S. chionantha* ($2n = 22$), *S. viscosa* and *S. verticillata* subsp. *amasiaca* ($2n = 32$). The karyotype formulae were $5m+3sm$ in *S. viridis*, $2M+5m+3sm$ in *S. candidissima* subsp. *occidentalis*, $1M+10m$ in *S. sclarea*, $8m+3sm$ in *S. ceratophylla*, $7m+4sm$ in *S. chionantha*, $9m+5sm+2st$ in *S. viscosa*, and $15m+1sm$ in *S. verticillata* subsp. *amasiaca* by the karyotype image analysis system. Somatic chromosome numbers ranged from $2n = 16$ to $2n = 32$. The ideograms were drawn based on centromeric index and arranged in decreasing size order. The present results were compared with the previous cytological studies in the genus.

Keywords: chromosome; image analysis; karyotype; *Salvia*; Turkey

Introduction

The genus *Salvia* L. (Lamiaceae) encompasses about 1000 species, approximately two thirds of which are in the New World (Wester and Claßen-Bockhoff 2011). It is distributed widely in temperate areas of the Old and New World, with three distinct regions: Central and South America, Western Asia and Eastern Asia (Alziar 1988–1993).

The genus *Salvia* has 105 taxa, 57 of which are endemic to Turkey (Celep and Kahraman 2012). SW Asia (Turkey to Iran and Afghanistan) is one centre of diversity. There is no distinct border between the countries and Flora Iranica (Hedge 1982) includes nearly as many *Salvia* species as the Flora of Turkey.

Several reports are available on the chromosome numbers and the karyotypes of the genus *Salvia*. Most of these studies have only been based on chromosome counts (Yakovleva 1933; Patudin et al. 1975; Afzal-Rafii 1976, 1980, 1981; Markova and Ivanova 1982; Diez et al. 1984; Palomino et al. 1986; Rosúa and Blanca 1988; Mercado et al. 1989; Nakipoğlu 1993a, 1993b; Murin 1997). The chromosome number is an important character in plant cytotaxonomy and may provide information on polyploidy and other significant genome changes (Murin 1997; Özdemir and Şenel 1999). Plant chromosome number databases are a useful tool for systematic comparisons of geographical or taxonomical groups of plants (Tunamoto et al. 2000). In addition, chromosome counts can increase our understanding of phylogenetic relationships at different taxonomic levels (Diez et al. 1984). Beside the chromosome number, chromosome morphology is commonly used in plant taxonomy. These data are also useful for clarifying the

origin, speciation and phylogenetic relationships of plants (Alberto et al. 2003). The centromere position and the relative chromosome length are the most important karyotypic features, and have allowed assessment of chromosomal affinities based on the concept of symmetry and asymmetry (Yang et al. 2004).

According to karyological studies of different species of *Salvia*, the basic chromosome numbers are $n = 6, 7, 8, 9, 10, 11, 13, 15, 16, 17, 19$ and 22 . (Yakovleva 1933; Haque 1981). The basic numbers of 6, 7 and 8 may be considered as primary and the higher numbers seem to be of secondary origin (Haque 1981). In a cytomorphological study on some taxa of *Salvia* growing in Turkey, diploid chromosome numbers were reported as $2n = 14, 18, 20, 22, 28, 32$ and 60 (Martin et al. 2011). Many *Salvia* species show extreme variations in both their somatic and meiotic chromosomes (Haque and Ghoshal 1980; Haque 1981), which may have various causes, including genotypic and environmental differences (Haque 1981). Moreover, changes in the chromosome number and variation of karyotype structure can be highlighted as the principal mechanism of species diversification. In other words, these chromosomal variations may be due to the presence of distinct chromosomal races, which thus behave as a new variety. It may be that as *S. viscosa* is highly cross-pollinating, hybridisation among the different varieties along with polyploidy may have played a great role in the origin of these new chromosomal races (Haque 1981).

Karyological data on the genus *Salvia* are abundant, however, those on species growing in Turkey are little known (Yakovleva 1933; Patudin et al. 1975; Afzal-Rafii

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1976, 1979, 1980, 1981; Haque and Ghoshal 1980; Markova and Ivanova 1982; Diez et al. 1984; Palomino et al. 1986; Rosúa and Blanca 1988; Haque 1982; Mercado et al. 1989; Baltisberger 1991; Nakipoğlu 1993a, 1993b; Murin 1997; Özdemir and Şenel 1999; Tunamoto et al. 2000; Alberto et al. 2003; Yang et al. 2004; Martin et al. 2011).

Therefore, in this study observations were made on chromosomes of seven taxa belonging to four sections of *Salvia* in Turkey: *Salvia viridis* L. (in sect. *Horminum*), *S. candidissima* Vahl subsp. *occidentalis* Hedge, *S. sclarea* L., *S. ceratophylla* L., *S. chionantha* Boiss. (in sect. *Aethiopsis*), *S. viscosa* Jacq. (in sect. *Plethiosphace*), and *S. verticillata* subsp. *amasiaca* (Freyn & Bornm.) Bornm. (in sect. *Hemisphace*).

The present study aims to provide the chromosome numbers and the karyotypes of the several representative species of *Salvia* from different localities in Turkey, to verify previous reports or show numbers which are different from those cited previously.

Materials and methods

Plant materials

Endemism and collection data of seven taxa collected from different localities in Turkey for karyological analysis are listed (Table 1). Voucher specimens were deposited in Laboratory of Plant Systematics, Department of Biological Sciences, Middle East Technical University (METU), Ankara, Turkey.

Preparation of karyotypes

The karyological study was conducted on root tips germinated on wet filter paper in Petri dishes. After germination, the fresh root tip meristems were pretreated in α -monobromonaphthalene at 4°C for 16 h, and then fixed in glacial acetic acid and absolute alcohol (1:3) at 4°C for 24 h. These samples were deposited in 70% ethanol at 4°C. The root tips were hydrolysed in 1 N HCl at room temperature for 12 min. Finally, they were squashed and stained in 2% aceto-orcein. Permanent slides were prepared using standard liquid nitrogen method (Martin et al. 2011). Karyotypes were determined using Image Analysis System (Bs200Pro, http://www.bab.com.tr/prgdis.php?prog_id=bssito&dilsec=1) on a personal computer. Chromosomes were classified using the nomenclature of Levan et al. (1964) as shown in Table 2. Mitotic metaphase chromosomes are given in Figure 1. Ideograms of these taxa were arranged in order of decreasing length of homologue chromosome pairs (Figure 2).

Results

In this study, the detailed karyotypes of the chromosomes are provided for each taxon of four sections (*Horminum*, *Aethiopsis*, *Plethiosphace* and *Hemisphace*)

Table 1. Collection data of the *Salvia* taxa examined. (ANK: Ankara University, Faculty of Science, Department of Biology Herbarium)

Taxon	Sections	Collection data
<i>S. viridis</i>	<i>Horminum</i>	Siirt: between Siirt and Pervari, 26 km from Siirt, 5 June 2008, A. Kahraman 1536 (ANK!)
<i>S. sclarea</i>	<i>Aethiopsis</i>	Erzincan: Erzincan-Kemah road, 8–9 km to Kemah, 1282 m, 15 July 2007, A. Kahraman 1480 (ANK!)
<i>S. ceratophylla</i>	<i>Aethiopsis</i>	Hakkari: at the junction with Hakkari-Van road, 1625 m, 7 June 2008, A. Kahraman 1566 (ANK!)
<i>S. chionantha</i> *	<i>Aethiopsis</i>	Antalya: Elmalı to Korkuteli, 21 June 2007, F. Celep 1258 (ANK!)
<i>S. candidissima</i> subsp. <i>occidentalis</i>	<i>Aethiopsis</i>	Antalya: Elmalı, Entrance of Cedar Research Forest, 7 July 2007, F. Celep 1326 (ANK!)
<i>S. verticillata</i> subsp. <i>amasiaca</i>	<i>Hemisphace</i>	Konya: Beysehir to Seydisehir, 5–7 km, 6 June 2008, F. Celep 1431 (ANK!)
<i>S. viscosa</i>	<i>Plethiosphace</i>	Hatay: Samandag to Yayladag, around Aydinbahce. 2 June 2009, F. Celep 1647 (ANK!)

*Endemic taxa.

Table 2. The nomenclature method of Levan et al. (1964).

Term	Location	r (arm ratio)
M	Median point	1.0
m	Median region	1.0–1.7
sm	Submedian region	1.7–3.0
st	Subterminal region	3.0–7.0
t	Terminal region	7.0– ∞
T	Terminal point	∞

within *Salvia*. The diploid chromosome numbers were $2n = 16, 20, 22$ and 32 .

Sect. Horminum

S. viridis

The chromosome number of *S. viridis* was found to be $2n = 16$ (Figure 1a). The shortest chromosome length is 1.04 μm , the longest is 1.51 μm , and the haploid chromosome length is 10.23 μm . Chromosome arm ratios are 1.05–2.47. The centromeric index varies between 3.33 and 6.55 and relative lengths vary from 10.17 to 14.77. The karyotype formulae of this species consist of five median pairs and three submedian pairs. The ideograms of this species are shown in Figure 2a.

Sect. Aethiopsis

S. sclarea

The chromosome number of *S. sclarea* was found to be $2n = 22$ (Figure 1b). The shortest chromosome length is

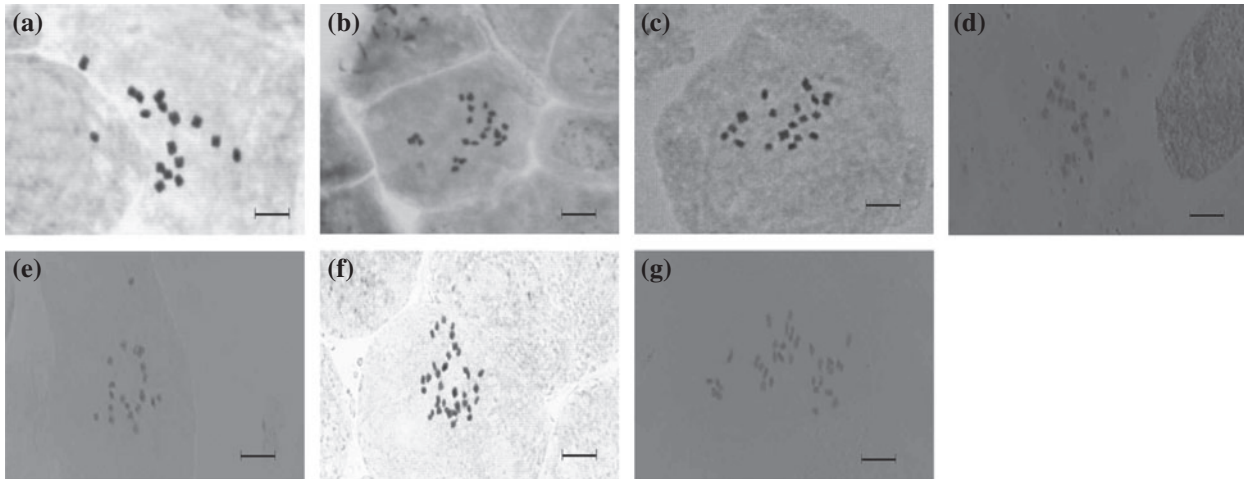


Figure 1. Somatic chromosomes in studied taxa. (a) *Salvia viridis*; (b) *S. sclarea*; (c) *S. ceratophylla*; (d) *S. chionantha*; (e) *S. candidissima* subsp. *occidentalis*; (f) *S. viscosa*; (g) *S. verticillata* subsp. *amasiaca*. Bar = 10 μm .

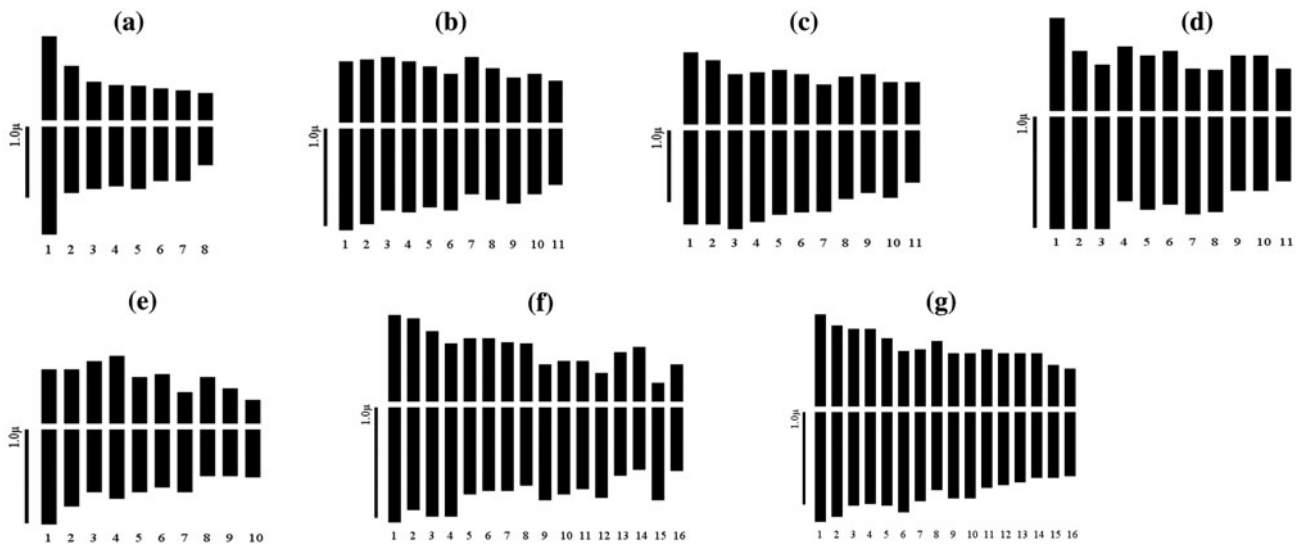


Figure 2. Ideograms in studied taxa. (a) *Salvia viridis*; (b) *S. sclarea*; (c) *S. ceratophylla*; (d) *S. chionantha*; (e) *S. candidissima* subsp. *occidentalis*; (f) *S. viscosa*; (g) *S. verticillata* subsp. *amasiaca*.

1.02 μm , the longest is 1.67 μm , and the haploid chromosome length is 15.09 μm . Chromosome arm ratios are 1.00–1.67. The centromeric index varies between 2.87 and 4.44 and relative lengths vary from 6.74 to 11.08. The karyotype formulae of this species consist of one median (M) pair and 10 median pairs (m). The ideograms of this species are shown in Figure 2b.

S. ceratophylla

The chromosome number of *S. ceratophylla* was found to be $2n = 22$ (Figure 1c). The shortest chromosome length is 1.33 μm , the longest is 2.33 μm , and the haploid chromosome length is 20.25 μm . Chromosome arm ratios are 1.21–2.03. The centromeric index varies between 2.77 and 4.99 and relative lengths vary from

6.58 to 11.51. The karyotype formulae of this species consist of eight median pairs and three submedian pairs. The ideograms of this species are shown in Figure 2c.

S. chionantha

The chromosome number of *S. chionantha* was found to be $2n = 22$ (Figure 1d). The shortest chromosome length is 0.96 μm , the longest is 1.85 μm , and the haploid chromosome length is 14.62 μm . Chromosome arm ratios are 1.20–2.40. The centromeric index varies between 2.53 and 5.75 and relative lengths vary from 6.57 to 12.65. The karyotype formulae of this species consist of seven median pairs and four submedian pairs. The ideograms of this species are shown in Figure 2d.

S. candidissima subsp. *occidentalis*

The chromosome number of *S. candidissima* subsp. *occidentalis* was found to be $2n = 20$ (Figure 1e). The shortest chromosome length is 0.76 μm , the longest is 1.59 μm , and the haploid chromosome length is 11.59 μm . Chromosome arm ratios are 1.00–1.98. The centromeric index varies between 2.20 and 5.78 and relative lengths vary from 6.56 to 13.76. The karyotype formulae of this species consist of two median pairs (M), five median pairs (m) and three submedian pairs. The ideograms of this species are shown in Figure 2e.

Sect. Plethiosphace*S. viscosa*

The chromosome number of *S. viscosa* was found to be $2n = 32$ (Figure 1f). The shortest chromosome length is 0.92 μm , the longest is 1.83 μm , and the haploid chromosome length is 20.54 μm . Chromosome arm ratios are 1.14–4.94. The centromeric index varies between 0.83 and 3.85 and relative lengths vary from 4.48 to 8.94. The karyotype formulae of this species consist of nine median pairs, five submedian pairs and two subterminal pairs. The ideograms of this species are shown in Figure 2f.

Sect. Hemisphace*S. verticillata* subsp. *amasiaca*

The chromosome number of *S. verticillata* subsp. *amasiaca* was found to be $2n = 32$ (Figure 1g). The shortest chromosome length is 1.10 μm , the longest is 2.18 μm , and the haploid chromosome length is 25.16 μm . Chromosome arm ratios are 1.18–1.79. The centromeric index varies between 1.63 and 3.96 and relative lengths vary from 4.37 to 8.65. The karyotype formulae of this species consist of 15 median pairs and one submedian pair. The ideograms of this species are shown in Figure 2g.

Discussion

Chromosome numbers are distinctive characters among the sections of the studied *Salvia* taxa. The current paper presents the somatic chromosome numbers and karyotypes of seven taxa in four sections of the genus *Salvia* growing in Turkey. The chromosome number of $2n = 16$ was determined only in *Salvia viridis* from sect. *Horminum*. *S. candidissima* subsp. *occidentalis* from sect. *Aethiopsis* has a chromosome number of $2n = 20$, three taxa (*S. sclarea*, *S. ceratophylla* and *S. chionantha*) from sect. *Aethiopsis* have a chromosome number of $2n = 22$. *Salvia verticillata* subsp. *amasiaca* from sect. *Hemisphace* and *S. viscosa* from sect. *Plethiosphace* have $2n = 32$.

In the *Flora of Turkey and the East Aegean Islands*, Hedge (1982) reported that *Salvia* had a great variety of chromosome numbers: $2n = 8, 14, 15, 16, 18, 20, 21,$

22, 24, 32, and 44. In the *Flora of Europe*, Hedge (1972) also presented chromosome numbers ranging from $2n = 12$ to $2n = 64$. The chromosome counts ($2n = 16, 20, 22, 32$) of the taxa studied here confirm these previous reports.

The Chinese *Salvia* examined by Yang et al. (2004, 2009) showed tetraploids with the chromosome number of $2n = 4x = 32$ in only three taxa, – *S. brevilabra* Franch., *S. evansiana* Hand.-Mazz. and *S. przewalskii* Maxim. var. *przewalskii*, – others were diploids with $2n = 2x = 16$. The chromosome counts ($2n = 4x = 32$) of *S. viscosa* and *S. verticillata* subsp. *amasiaca* confirm previous data. The chromosome number of *Salvia verticillata* was reported as $2n = 16$ in previous studies (Van Loon and Snelders 1979; Afzal-Rafii 1980; Guinochet and Lefranc 1981; Markova and Ivanova 1982; Magulaev 1976; Strid and Franzen 1983; Sekovski and Jovonovska 1983; Lövkist and Hultgard 1999). Patudin et al. (1975) reported both diploid and tetraploid chromosome numbers as $2n = 2x = 16, 2n = 4x = 32$ in *S. verticillata*. For this species, Gill (1974) reported a chromosome number of $2n = 16+0-1B$, whereas we found $2n = 4x = 32$. According to Peruzzi et al. (2011), polyploidization increases proportionally to both distance from the Equator and latitudinal ranges. We suggest here on that latitudinal gradients might account for a polyploidy increase.

B chromosomes, which are also known as supernumerary or accessory chromosomes, have been often detected before in the karyotypes of *Salvia*. Yang et al. (2009) presented the first report of the B chromosomes for one species (*S. tricuspis* Franch.) of *Salvia* from China. However, in our study, B chromosomes are not found in the investigated taxa. B chromosomes are about adaptive role. For some Chinese species B chromosomes occur, but generally polyploidization occurs for adaptive role for Turkish species, polyploidization at geographic scale may be found in their number rather than B chromosomes.

The chromosome morphology shows important differences among some taxa in sections of *Salvia*. The smallest chromosome length (0.76 μm) observed in *S. candidissima* subsp. *occidentalis* contrasts with the largest (2.33 μm) length observed in *S. ceratophylla*. *S. viridis* (10.23 μm) has the shortest haploid chromosome length and *S. verticillata* subsp. *amasiaca* (25.16 μm) has the largest. The smallest arm ratio was observed in *S. sclarea* and *S. candidissima* subsp. *occidentalis* (1.00) and the largest was observed in *S. viscosa* (4.94). The smallest centromeric index was measured in *S. viscosa* (0.83) and the largest in *S. viridis* (6.55). The smallest relative value was measured in *S. verticillata* subsp. *amasiaca* (4.37) and the largest in *S. viridis* (14.77). The karyotype formulae were obtained as 5m+3sm for *Salvia viridis*, 1M+10m for *S. sclarea*, 8m+3sm for *S. ceratophylla*, 7m+4sm for *S. chionantha*, 2M+5m+4sm for *S. candidissima* subsp. *occidentalis*, 15m+1sm for *S. verticillata* L. subsp. *amasiaca* and

9m+5sm+2st for *S. viscosa*. The morphologies of metaphase chromosomes were different in the species analysed. The metaphase chromosome pairs were usually of median and submedian type.

In section *Aethiopsis*, the smallest chromosome length was found in *S. candidissima* subsp. *occidentalis* (0.76 µm) and the largest in *S. ceratophylla* (2.33 µm). The smallest total haploid chromosome length was obtained in *S. candidissima* subsp. *occidentalis* (11.59 µm) and the largest in *S. ceratophylla* (20.25 µm). The smallest arm ratio (1.00) was seen in both *S. sclarea* and *S. candidissima* subsp. *occidentalis* and the largest (2.40) in *S. chionantha*. Both the smallest (2.20) and the largest (5.78) centromeric index were exhibited by *S. candidissima* subsp. *occidentalis*. *S. sclarea* had the lowest value of relative length (6.74) and *S. candidissima* subsp. *occidentalis* the highest (13.76). The karyotype formulae were 1M+10m for *S. sclarea*, 8m+3sm for *S. ceratophylla*, 7m+4sm for *S. chionantha* and 2M+5m+3sm for *S. candidissima* subsp. *occidentalis*. It was confirmed that chromosome morphologies among species are specific to each taxa.

A large number of studies have been carried out on the cytology of *S. sclarea*. Özdemir and Şenel (1999) reported that the somatic chromosome number of *S. sclarea* is $2n = 22$, which agrees with our result. Karyotypic features of *S. sclarea* from Iran were reported by Kharazian (2011) including a chromosome number of $2n = 22$, and the smallest and largest chromosome lengths varied between 0.3 µm and 0.7 µm. The karyotype formula was determined as 2M+m+sm+3st+3t+T by Kharazian (2011). In our study, the shortest chromosome length was found to be 1.02 µm, the longest was 1.67 µm and the karyotype formula was obtained as 1M+7m for *S. sclarea*. The results of Kharazian (2011) and ours are not congruent. These differences may result from populations growing in different regions and chromosome preparation treatments. Kharazian (2011) also reported that the chromosome number of *S. ceratophylla* was $2n = 14$ and the chromosome lengths were in the range of 0.25 to 1.1 µm. In contrast, we found for the same species a chromosome number of $2n = 22$, the shortest chromosome length was 1.33 µm and the longest was 2.33 µm. The chromosome number results of Kharazian (2011) do not agree with our findings because different ecological, geological and climatological regions may lead to significant differences in chromosome numbers. In addition both diploids and tetraploids with the chromosome number of $2n = 4x = 44$ were reported in *S. ceratophylla* (Afzal-Rafii 1976, 1981; Markova and Ivanova 1982).

In previous cytological studies on *S. viridis*, chromosome numbers were reported as $2n = 16$ (Magulaev 1976; Afzal-Rafii 1976, 1981; Markova and Ivanova 1982; Díez et al. 1984; Nakipoğlu 1993a, 1993b; Özkan 2006). The same is true for the present study. However, karyotype analysis has been performed for the first time in this study. Both chromosome number and karyotype

properties of *S. chionantha* have been reported for the first time in this study.

Afzal-Rafii (1980) reported that *S. viscosa* had a chromosome number of $2n = 32$. We determined the same chromosome number and we conducted the first karyotype analysis of *S. viscosa*.

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