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Chromosome number of some *Satureja* species from Turkey

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Abstract. The genus *Satureja* belonging to the Lamiaceae family includes about 200 species, generally aromatic, distributed in the Mediterranean basin. In genus *Satureja*, the chromosomal data were reported from only 26 species. In this study, it is aimed to eliminate the deficiencies in the chromosomal data of *Satureja* species, which are distributed in Turkey, which is center of origin and diversity of the genus *Satureja*. It was reported only one chromosome number ($2n = 30$), the first report for chromosome numbers of three taxa, the same chromosome count (excluding B-chromosomes) with previous report in only one species, and the new chromosome number in only one species. In conclusion, this study presented new data into the chromosomal records of genus *Satureja* that might be useful for interpreting or understanding relationships among the species. In addition, dysploidy and polyploidy variations might probably have played an important role in speciation. In this regard, the results contributed to some missing data in *Satureja* cytotaxonomy.

Keywords: *Satureja*, chromosome, dysploidy, polyploidy, Turkey.

INTRODUCTION

The genus *Satureja* L. belonging to the Lamiaceae family includes about 200 species, generally aromatic, distributed in the Mediterranean basin. *Satureja* species are distributed in Morocco, Libya, Saudi Arabia, Caucasus, Iran, Iraq and Turkey, which are mostly Mediterranean countries from Europe, Asia and North Africa. Turkey is center of origin and diversity of the genus *Satureja* (Harley et al. 2004; Dirmenci et al. 2019).

According to Flora of Turkey records, the genus *Satureja* is represented by a total of 15 species, five of which are endemic. *Satureja* species, popularly known as "pointed thyme" or "stone thyme", have a wide range of uses in the food, pharmaceutical and cosmetic industries due to high amounts of thymol

and carvacrol (Momtaz and Abdollahi 2010; Dirmenci et al. 2019). The dried leaves of *Satureja* species are used as spice, food additive and herbal tea (Babajafari et al. 2014). The genus *Satureja* has been the focus of many studies due to its biological activities such as antimicrobial, antioxidant and anti-HIV-1. It has been stated that the rich essential oil (eg mono- and sesquiterpenes) and phenolic content (eg phenolic acids, catechins, and flavonoids) of *Satureja* species are responsible for these activities (Eminağaoğlu et al. 2007; Bektaş 2020).

Cytotaxonomy is a branch of taxonomy that uses karyological parameters to classify organisms. In cytotaxonomy, chromosomal configuration is the most widely used parameter to understand the relationship between organisms. Inference of species relationships is based on the assumption that closely related species share similar features in their chromosomal arrangement. By analyzing similarities and differences in chromosomes, karyotype evolution and species evolution can be reconstructed. The number, structure and behaviour of chromosomes are very valuable in taxonomy and the chromosome numbers (x, $2n$) are the most commonly used characters (Guerra 2008; Eroğlu et al. 2020; Martin et al. 2020; Eroğlu et al. 2021). In genus *Satureja*, the chromosomal data were reported from only 26 species. Eighteen species were only diploid; however, they revealed two different basic numbers: $x = 13$ ($2n = 26$) and $x = 15$ ($2n = 30$). Seven species were polyploid and revealed two different polyploidy levels: tetraploidy ($2n = 4x = 24$, 28, 44, and 60) and hexaploidy ($2n = 6x = 48$). The polyploid species revealed four different basic numbers such as $x = 6$, 7, 11, and 15. *Satureja sahendica* Bornm. had diploid and polyploid records. In addition, *S. hortensis* L. presented dysploidy, which is an alteration in basic number, generally by fusion, without the significant loss or gain of genetic material (Shariat et al. 2013; Irani et al. 2014; Bordbar et al. 2021; Chromosome Counts Database 2022; Vozhdehnazari et al. 2022).

In Turkish *Satureja*, the chromosomal data were reported from eight species, which were *S. macrantha* C.A. Mey. ($2n = 24$), *S. coerulea* Janka, *S. cuneifolia* Ten., *S. pilosa* Velen. *S. thymbra* L. ($2n = 30$), *S. spinosa* L. ($2n = 30 + 2B$), *S. spicigera* Boiss. ($2n = 44$, 60), and *S. hortensis* ($2n = 45$-48) (Shariat et al. 2013; Irani et al. 2014; Chromosome Counts Database 2022; Vozhdehnazari et al. 2022). There was no record of the chromosome number of seven species, which were *S. aintabensis* P.H.Davis, *S. amani* P.H.Davis, *S. boissieri* Hausskn. ex Boiss., *S. cilicica* P.H.Davis, *S. icarica* P.H.Davis, *S. parnassica* Heldr. & Sartori ex Boiss., and *S. wiedemanniana* Lall. ex Velen. Therefore, the lack of some chromosomal reports from Turkey, which is located in the Mediterranean Basin, may lead to some uncertainties in the cytotaxonomy. In this study, it is aimed to eliminate the deficiencies in the chromosomal data of *Satureja* species, which are distributed in Turkey, which is center of origin and diversity of the genus.

MATERIALS AND METHODS

Plant material

Within the scope of this study, *S. aintabensis*, *S. boissieri*, *S. icarica*, *S. macrantha* and *S. spinosa* distributed in different localities of Turkey were examined by chromosome numbers. The examined plant samples were collected from their natural habitats and identified by Prof. Dr. Tuncay Dirmenci et al (Figure 1). The collected plant samples were preserved in Balıkesir University, Necatibey Faculty of Education, Department of Biology Education. The distribution regions and collection information are given in Figure 2 and Table 1.

Cytogenetic procedure

The seeds of the collected plant samples were kept at -40°C for 1 month and then planted in petri dishes. The samples were kept in the dark at 4°C for 21 days, and at the end of 21 days, the samples placed in the climate cabinet were kept until the root tip tissue reached a few centimeters in length. Germinated root tips were kept in α-monobromonaphthalene for 16 hours at 4°C for the first treatment. Afterwards, root tips were fixed in 3:1 absolute alcohol:glacial acetic acid and stored in 70% alcohol in the refrigerator. The root tips were removed from the refrigerator, hydrolyzed in 1N HCl at room temperature for 10 minutes and stained with 2% aceto-orcein for 2 hours at room temperature. Then, squash preparations were prepared with 45% acetic acid. After the preparations were frozen in liquid nitrogen, they were dried at room temperature and stabilized with Depex medium (Martin et al. 2018; Eroğlu et al. 2021).

Ten metaphase plates were used for counting the somatic chromosomes of each species. After the mitotic chromosomes, which were well distributed, had good morphology, and were on the same plane, were detected, their photographs were taken at 1000× magnification with a camera attached to the microscope (Olympus BX51). The chromosome photographs were analyzed using the Image Analysis System (Bs200ProP).

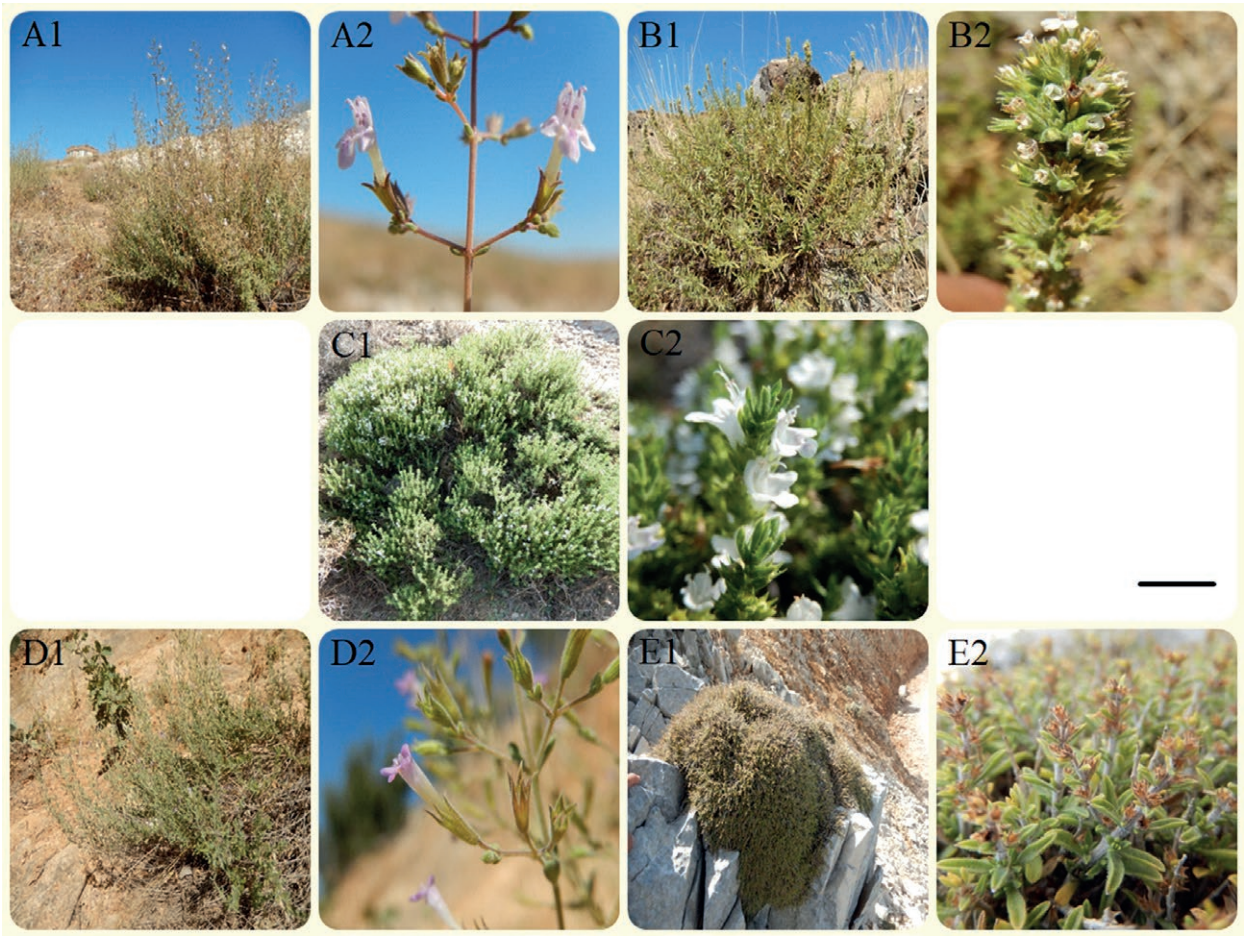

Figure 1. Habitat (1) and flowers (2) of *Satureja* species. (A) *S. aintabensis*; (B) *S. boissieri*; (C) *S. icarica*; (D) *S. macrantha*; and (E) *S. spinosa*. Scale bar, 10 cm for habitat (1) and 5 mm for flowers (2).

Figure 2. Distribution map of the studied species in Turkey. (A) *S. aintabensis*; (B) *S. boissieri*; (C) *S. icarica*; (D) *S. macrantha*; and (E) *S. spinosa*.

Table 1. The collection information and localities of studied *Satureja* species.

Species	Collection cite	Altitude	Date	Voucher number
S. aintabensis	Gaziantep, Samköy, Behind Dülükbaba promenade.	1000 m	03.09.2018	Dirmenci 5210 & Arabacı
S. boissieri	Adıyaman, Çelikhan, between Yazıbaşı village and Ulubaba mountain, 8th km.	1700 m	02.09.2018	Dirmenci 5207 & Arabacı
S. icarica	Çanakkale, between Gökçeada and Aydıncık, 2nd-3rd km.	300 m	15.08.2018	Dirmenci 5166
S. macrantha	Ardahan, between Göle and Şenkaya, 10th km.	1800 m	31.08.2018	Dirmenci 5196 & Arabacı
S. spinosa	Muğla, Fethiye, Babadağ, above telpher.	1900 m	11.09.2018	Dirmenci 5224 & Yıldız

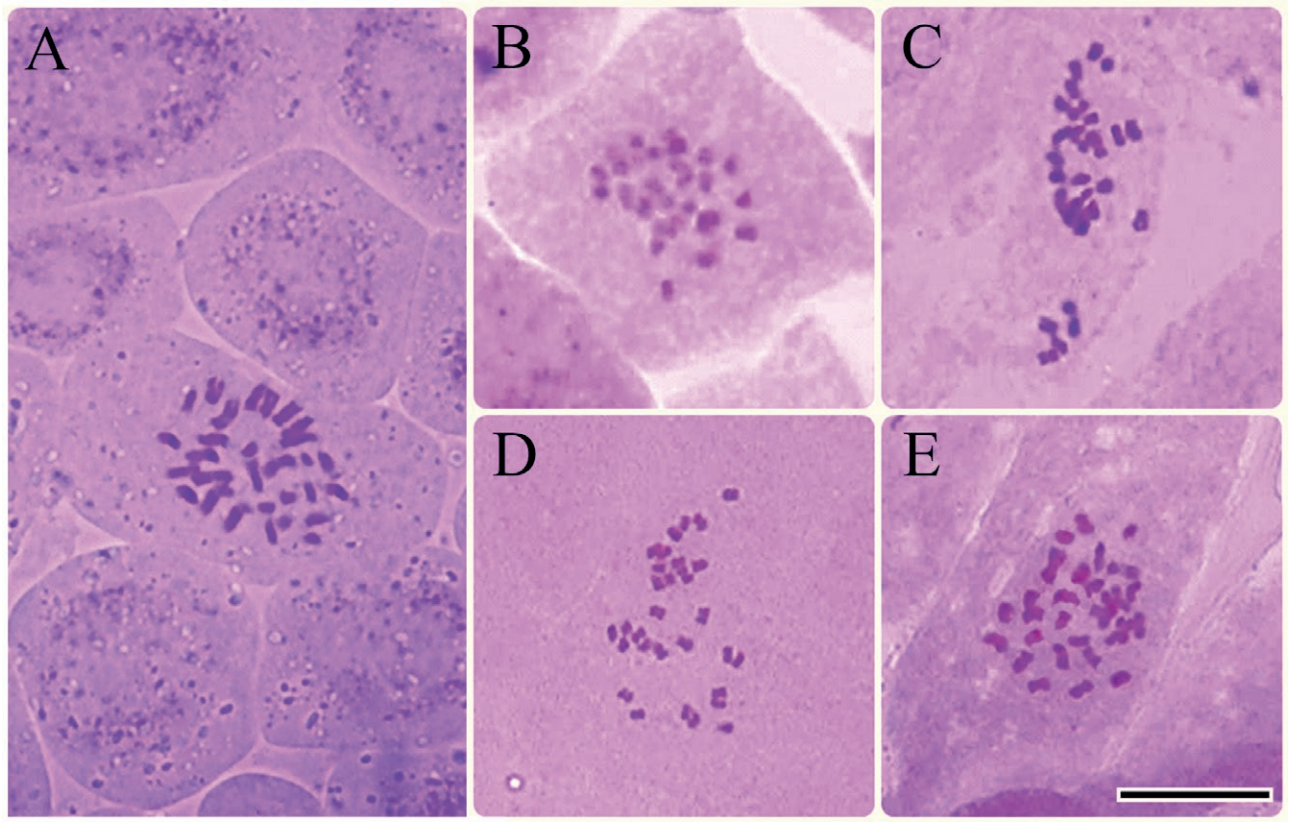

Figure 3. Metaphase chromosomes of *Satureja* species. (A) *S. aintabensis*; (B) *S. boissieri*; (C) *S. icarica*; (D) *S. macrantha*; and (E) *S. spinosa*. Scale bar 10 μm.

RESULTS

Figure 3 presented the mitotic metaphase chromosomes of five *Satureja* species. The chromosome numbers of studied *Satureja* species were given in Table 2. In all species, the diploid number was $2n = 30$, three of which were reported for the first time and the basic number was $x = 15$. In genus *Satureja*, because the chromosomes were very small and the centromere region is unclear, detailed chromosomal measurements were not made.

Table 2. The chromosome numbers of studied *Satureja* species.

Species	x = basic number, $2n$ (ploidy level)
S. aintabensis	$x = 15$, $2n = 30$ (diploid)
S. boissieri	$x = 15$, $2n = 30$ (diploid)
S. icarica	$x = 15$, $2n = 30$ (diploid)
S. macrantha	$x = 15$, $2n = 30$ (diploid)
S. spinosa	$x = 15$, $2n = 30$ (diploid)

Table 3. The chromosome numbers of Turkish *Satureja* in present and previous studies.

Species (alphabetically)	x = basic number, $2n$ (ploidy level)	References	Observation
S. aintabensis	$x = 15$, $2n = 30$ (diploid)	Present study	First report
S. boissieri	$x = 15$, $2n = 30$ (diploid)	Present study	First report
S. coerulea	$x = 15$, $2n = 30$ (diploid)	Chromosome Count Database 2022	Previous report
S. cuneifolia	$x = 15$, $2n = 30$ (diploid)	Chromosome Count Database 2022	Previous report
S. hortensis	$x = 12$, $2n = 48$ (tetraploid) 45-47 (probably dysploidy)	Chromosome Count Database 2022	Previous report
S. icarica	$x = 15$, $2n = 30$ (diploid)	Present study	First report
S. macrantha	$x = 15$, $2n = 30$ (diploid) $x = 12$, $2n = 24$ (diploid)	Present study Vozhdehnazari et al. 2022	New count
S. pilosa	$x = 15$, $2n = 30$ (diploid)	Chromosome Count Database 2022	Previous report
S. spicigera	$x = 15$, $2n = 60$ (tetraploid) $x = 11$, $2n = 44$ (tetraploid)	Shariat et al. 2013 Irani et al. 2014	Previous report
S. spinosa	$x = 15$, $2n = 30$ (diploid) $x = 15$, $2n = 30 + 2B$ (diploid)	Present study Chromosome Count Database 2022	B-chromosomes not observed
S. thymbra	$x = 15$, $2n = 30$ (diploid)	Chromosome Count Database 2022	Previous report

DISCUSSION

The genus *Satureja* was represented by a total of 15 species in Turkey. Eleven Turkish *Satureja* whose chromosome numbers had been reported with present and previous studies were given in the Table 3 for comparison. In the present study, the diploid number of all species was $2n = 30$, three of which were reported for the first time: *S. aintabensis*, *S. boissieri*, and *S. icarica*. The chromosome number represented new cytotype in only one species, which was *S. macrantha* ($2n = 30$). Vozhdehnazari et al. (2022) reported that the chromosome number of *S. macrantha* was $2n = 24$. The chromosome number of *S. spinosa* agreed with the previous report excluding B-chromosomes.

In Turkish *Satureja*, five different chromosome numbers were recorded such as $2n = 24$, 30, 44, 48, 60 and $2n = 30$ was the most common diploid number. *S. spinosa* was the only species to have B-chromosomes. Montmollin (1986) reported that the karyotype of *S. spinosa* was $2n = 30 + 2B$, which were small supernumerary chromosomes other than A-chromosomes. B-chromosomes originated from the A-chromosomes and were a basic source of intraspecific variations of nuclear DNA (Heneidak et al. 2019). Although we obtained the same diploid number, we did not observe B-chromosomes. This was probably due to the locality difference.

In Table 3, *Satureja* was a polybasic genus by $x = 11$, 12, 15 with ploidy levels of $2x$ and $4x$. Nine species were diploid with $2n = 2x = 30$. *S. hortensis* and *S. spinosa* were polyploid, which revealed only one polyploidy level of tetraploidy ($2n = 4x = 44$, 48, and 60).

A basic number of $x = 15$ dominated in reported *Satureja* species (all species excluding *S. hortensis* in Table 3), which were *S. aintabensis*, *S. boissieri*, *S. coerulea*, *S. cuneifolia*, *S. icarica*, *S. macrantha* (only in this study), *S. pilosa*, *S. spicigera* (only in this study), *S. spinosa*, and *S. thymbra*. In addition, the basic numbers of $x = 11$ and 12 were recorded. However, different basic numbers were reported, such as $x = 6$ for *S. multiflora* Briq. and $x = 7$ for *S. sahendica* (Krogulevich 1978; Irani et al. 2014). In detecting karyotype evolution and speciation processes, basic chromosome number is one of the most important parameter. In genus *Satureja*, basic number variations were probably caused by descending or ascending dysploidy and dibasic polyploidy.

In Table 3, nine species were diploid with $2x = 30$ (81.82% of the species) and two species were polyploid with $4x = 44$, 48, and 60 (18.18% of the species). Polyploidy was probably one of the important mechanisms in the karyotype evolution of the genus, as it occurred at a non-negligible rate (Chromosome Counts Database 2022) in the genus *Satureja*.

In the present study, it was reported only one chromosome number ($2n = 30$), the first report for chromosome numbers of three taxa, the same chromosome count (excluding B-chromosomes) with previous report in only one species, and the new chromosome number in only one species. In conclusion, this study presented new data into the chromosomal records of genus *Satureja* that might be useful for interpreting or understanding relationships among the species. In addition, dysploidy and polyploidy variations might probably have played an important role in speciation. In this regard, the results contributed to some missing data in *Satureja* cytotaxon-

omy. However, all taxa should be investigated to elucidate the relationships between *Satureja* species and the chromosomal data should be supported by molecular data.

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