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*Original research*

# The detailed chromosome measurements and b-chromosomes in *Salvia virgata* Jacq.

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**Abstract:** *Salvia virgata* Jacq. (Lamiaceae), popularly known as fatmanaotu, is used as wound healing and spreads naturally in Turkey. The purpose of this study is to determine the diploid chromosome number, detailed chromosome measurements, B chromosomes and karyotype asymmetry of *S. virgata*. The results showed that the diploid chromosome number is $2n = 32 + 0\text{-}2B$ in *S. virgata*. The karyotype formula is $2n = 4x = 32 = 24m + 6sm + 2st$. The length of the chromosomes ranges varied from 0.56 to 1.65µm. Total haploid and mean haploid length are 18.10 and 1.13 µm, respectively. The centromeric index values varied from 19.54 to 48.57. The intrachromosomal asymmetry value ($M_{CA}$) and interchromosomal asymmetry value ($CV_{CL}$) are 20.28 and 28.68, respectively. The karyotype of *S. virgata* close to symmetric type. In this study, detailed chromosome measurements and 0-2B chromosomes of *S. virgata* were showed for the first time. The species has small chromosomes shaped in median, submedian and subterminal.

**Keywords:** Asymmetry index, B chromosome, Karyotype, Lamiaceae, *Salvia virgata*

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## Introduction

The genus *Salvia* L. (sage) belongs to the subfamily Nepetoideae of Lamiaceae. It is the largest genus of Lamiaceae with approximately 1000 species in the world. The species which are herbaceous, shrubby or woody and annual, biennial or perennial, spread mainly in temperature and tropical areas of Eastern Asia, Western Asia, Central America and South America. The distribution centers of the species are Mediterranean region, Central Asia, Mediterranean region, Andean mountains of South America and highlands of Mexico (Alberto et al., 2003; Martin et al., 2011; Gedik et al., 2016).

The *Salvia* species are used in traditional folk medicine as herbal tea and food in perfumery, cosmetics and pharmaceutical industry due to antibacterial, antitumoral, antidiabetic, antiseptic and antioxidant activities (Alberto et al., 2003; Martin et al., 2011; Gedik et al., 2016; Ben Khedher et al., 2017).

*S. virgata* Jacq., popularly known as fatmanaotu, is used as wound healing and spreads naturally in Turkey. It grows at altitudes from 1 to 2400 m. in all regions of

Turkey (Martin et al., 2011). This species is used against the skin diseases and blood cancer (Bayram et al., 2016).

Karyological studies may contain valuable information for phylogenetic and morphological studies in organisms. It was reported that genus *Salvia* had diverse chromosome numbers ranging from $2n = 12$ to $2n = 88$ (Patudin et al., 1975; Markova and Ivanova, 1982; Nakipoğlu, 1993; Alberto et al., 2003; Martin et al., 2011; Gedik et al., 2016). Some species of Salvia have 0-3 B chromosome (Nakipoğlu, 1993; Alberto et al., 2003; Martin et al., 2011). B chromosomes are supernumerary or accessory chromosomes, which do not follow Mendelian rules of inheritance. The purpose of this study is to determine the diploid chromosome number, detailed chromosome measurements, B chromosomes and karyotype asymmetry of *S. virgata*.

## Materials and Methods

The seeds of *S. virgata* were collected from in Yozgat (Turkey). The information regarding the collection is given below.

*Salvia virgata*-TURKEY. Yozgat: Yozgat-Ankara way, 1216 m, 05-VII-2017, 39° 48' 346" N; 34° 46' 408" E.

The seeds of different *S. virgata* samples were germinated at room temperature in petri dishes. The root tips were fixed in a fixative solution (3 part glacial acetic acid-1 part ethanol at 24 h. The roots were stored at 4ºC in ethanol (70%). The samples were hydrolysed for 10 min at 60ºC in HCl (1N). The samples were stained in aceto-orcein for 2 h and squashed in acetic acid (45%) (Eroğlu et al., 2013; Martin et al., 2015; Şahin et al., 2016; Altay et al., 2017).

Qualified 10 metaphase plate was selected and photographed with an Olympus BX53 microscope Karyotypes were determined using KaryoType software on a personal computer (Altınordu et al., 2016). The long arm length (L), short arm length (S), total chromosome length (TCL = L + S), arm ratio (L/S), centromeric index (CI = S/[L + S] × 100) were measured. The karyotype formula was determined using the nomenclature of Levan et al. (1964).

For analysis of karyotype asymmetry, two known methods were used. Peruzzi and Eroğlu (2013) reported the $M_{CA}$ parameter for intrachromosomal asymmetry index, as shown in Eqs. (1) - (2) below.

$$A = mean\ \frac{(L-S)}{(L+S)} \quad (1)$$

$$M_{CA} = A \times 100 \quad (2)$$

Paszko (2006) reported that the $CV_{CL}$ parameters for interchromosomal asymmetry index, as shown in Eqs. (3) - (4) below.

$$A2 = \frac{standard\ deviation}{mean\ chromosome\ length} \quad (3)$$

$$CV_{CL} = A2 \times 100 \quad (4)$$

## Results

The metaphase chromosomes, monoploid ideogram and chromosomal measurement data are given in Figure 1A-B, Figure 2 and Table 1, respectively. Karyotype formula, ploidy level, $M_{CA}$ and $CV_{CL}$ are given in Table 2. The measurement reports showed that the diploid chromosome number is $2n = 32 + 0\text{-}2B$ in *S. virgata*. The karyotype formula is $2n = 4x = 32 = 24m + 6sm + 2st$. All chromosomes are almost median type outside submedian chromosomes 12, 15, 16 and subtelocentric chromosome 13. In the chromosomes, no satellite was observed.

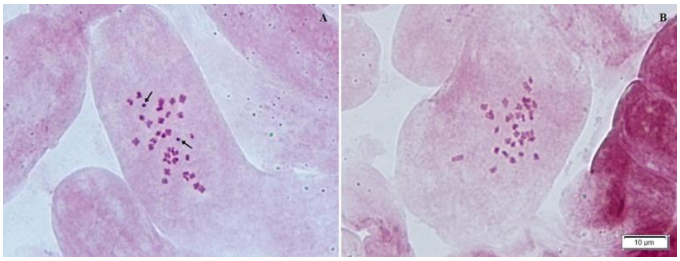

**Figure1.** Metaphase chromosomes of *Salvia virgata*: (**A**) $2n = 32 + 2B$ (arrowhead) (**B**) $2n = 32$.

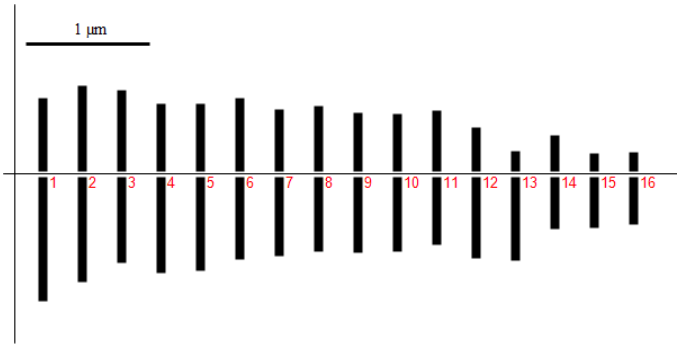


**Figure 2.** Monoploid ideogram of *Salvia virgata.*

**Table 1.** The measurement data of chromosomes of *Salvia virgata.*

| Chr. Pair | TCL (µm) | L (µm) | S (µm) | L/S | Chr. Type | CI |
|---|---|---|---|---|---|---|
| 1 | 1.65 | 1.04 | 0.61 | 1.70 | m | 36.97 |
| 2 | 1.60 | 0.88 | 0.72 | 1.22 | m | 45.00 |
| 3 | 1.40 | 0.72 | 0.68 | 1.06 | m | 48.57 |
| 4 | 1.37 | 0.80 | 0.57 | 1.40 | m | 41.61 |
| 5 | 1.35 | 0.78 | 0.57 | 1.37 | m | 42.22 |
| 6 | 1.30 | 0.69 | 0.61 | 1.13 | m | 46.92 |
| 7 | 1.18 | 0.66 | 0.52 | 1.27 | m | 44.07 |
| 8 | 1.17 | 0.62 | 0.55 | 1.13 | m | 47.01 |
| 9 | 1.12 | 0.63 | 0.49 | 1.29 | m | 43.75 |
| 10 | 1.10 | 0.62 | 0.48 | 1.29 | m | 43.64 |
| 11 | 1.08 | 0.57 | 0.51 | 1.12 | m | 47.22 |
| 12 | 1.05 | 0.68 | 0.37 | 1.84 | sm | 35.24 |
| 13 | 0.87 | 0.70 | 0.17 | 4.12 | st | 19.54 |
| 14 | 0.73 | 0.43 | 0.30 | 1.43 | m | 41.10 |
| 15 | 0.57 | 0.42 | 0.15 | 2.80 | sm | 26.32 |
| 16 | 0.56 | 0.40 | 0.16 | 2.50 | sm | 28.57 |

Abbreviations: total chromosome length (TCL), long arm length (L), short arm length (S), arm ratio (L/S), centromeric index (CI), median (m), submedian (sm), subterminal (st), chromosome (Chr.)

**Table 2.** The karyotype formula, ploidy level and asymmetry values of *Salvia virgata.*

| | |
|---|---|
| Karyotype formula | $2n = 32 + 0\text{-}2B$<br>24m + 6sm + 2st |
| Ploidy level | $4x$ |
| $M_{CA}$ | 20.28 |
| $CV_{CL}$ | 28.68 |

The length of chromosomes varied from 0.56 to 1.65µm. Total haploid length and mean haploid length are 18.10 and 1.13 µm, respectively. The centromeric index values varied from 19.54 to 48.57. A centromere with median zone has low centromeric index, unlike a centromere with telocentric zone has high centromeric index.

The intrachromosomal asymmetry value ($M_{CA}$) and interchromosomal asymmetry value ($CV_{CL}$) are 20.28 and 28.68, respectively. The values of $M_{CA}$ and $CV_{CL}$ increase up to 100 with centromeric shift. This value refers to the most asymmetrical karyotype. Unlike the values of $M_{CA}$ and $CV_{CL}$ can be at least zero. This value refers to the most symmetrical karyotype. The karyotype of *S. virgata* close to symmetric type.

## Discussion

The results showed that the chromosome number is $2n = 32$ and basic chromosome number is $x = 8$ in *S. virgata*. It was reported that the basic chromosome number is $x = 8$ in *S. virgata* (Patudin et al., 1975; Markova and Ivanova, 1982; Nakipoğlu, 1993; Martin et al., 2011). The *Salvia* species have the different levels of ploidy as $x = 7, 8, 9, 10, 11, 13$ (Alberto et al., 2003; Martin et al., 2011). Also, it was reported that the diploid chromosome numbers are $2n = 16$ (Markova and Ivanova, 1982; Nakipoğlu, 1993) and $2n = 32$ (Patudin et al., 1975; Martin et al., 2011) in *S. virgata*. In this study, the detailed chromosome measurements of *S. virgata* were given for the first time.

In genus *Salvia*, one of the most common chromosome numbers is $2n = 32$. According to the Chromosome Count Database, the chromosome numbers of *S. californica* Brandegee, *S. bucharica* Popov, *S. carduacea* Benth., *S. japonica* Thunb., *S. napifolia* Jacq. and *S. viscosa* Jacq. are $2n = 32$ (CCDB, 2018). Besides genus *Salvia* had diverse chromosome numbers ranging from $2n = 12$ to $2n = 88$. For example, the chromosome numbers are $2n = 12$ in *S. hispanica* L. and $2n = 88$ in *S. pallida* Benth. (CCDB, 2018).

B chromosomes, which are also known as supernumerary chromosomes, are a major source of intraspecific variation in nuclear DNA. In natural plant populations, B chromosomes support large genomes and cause DNA polymorphisms (Jones et al., 2008). The B chromosomes have been observed in *Salvia* species (Nakipoğlu, 1993; Alberto et al., 2003; Martin et al., 2011). In this study, we observed B chromosomes in *S. virgata* (Figure 1A), which is the first report of *S. virgata*.

At the present time, the data can be obtained with chromosome studies: the diploid chromosome number, detailed karyotype analysis, karyotype asymmetry, chromosome banding and painting. Among them, one of the most cheap, popular and widely used by especially botanists is karyotype asymmetry. The intrachromosomal asymmetry is characterized by predominance chromosomes with terminal/subterminal centromeres and the interchromosomal asymmetry is characterized with highly heterogeneous chromosome sizes (Peruzzi and Eroğlu, 2013). Karyotype asymmetry was determined by using $M_{CA}$ (intrachromosomal) and $CV_{CL}$ (interchromosomal) methods. According to the $M_{CA}$ and

$CV_{CL}$ values, the karyotype of *S. virgata* close to symmetric type.

## Conclusion

As a result of this study, detailed chromosome measurements and 0-2B chromosomes of *S. virgata* were showed for the first time. The species has small chromosomes between 0.56-1.65 µm shaped median, submedian and subterminal. In this study it was understood that the chromosomal data could support morphological characters. The *Salvia* genus contains many taxa with unknown detailed chromosomal data. More detailed chromosomal data are needed to contribute to the cytotaxonomy of *Salvia*.

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