J Genet Resour2021;7(1): 29-35 *Homepage: http://sc.journals.umz.ac.ir/*

RESEARCH ARTICLE *DOI: 10.22080/jgr.2020.19758.1201*

J|G|R

A New Chromosome Number Report in *Stachys* L. Species by Use of Karyological Analysis

Akram Rajabi Mazaher[1], Seied Mehdi Miri[1*] and Abdollah Mohammadi[2]

[1] Department of Horticulture, Karaj Branch, Islamic Azad University, Karaj, Iran
[2] Department of Plant Breeding, Karaj Branch, Islamic Azad University, Karaj, Iran

ARTICLE INFO

Article history:
Received 27 August 2020
Accepted 26 October 2020
Available online 6 November 2020

Keywords:
Asymmetry index
Chromosome numbers
Karyotype
Stachys

****Corresponding authors:***
✉ SM. Miri
smmiri@kiau.ac.ir

p-ISSN 2423-4257
e-ISSN 2588-2589

ABSTRACT

The somatic chromosome numbers and karyotype features of seven populations representing three species of *Stachys* L. (Lamiaceae), which are naturally distributed across Iran, were described. The results confirm the presence of different basic chromosome numbers including x = 15 and 17 within the genus. All the studied taxa were diploid and the chromosome counts of two species including *S. benthamiana* and *S. setifera* (2n = 34) are reported for the first time, while the chromosome number of *S. byzantina* (2n = 30) is confirmed. The chromosomes in the studied taxa of *Stachys* were generally small, as the longest chromosome length was detected on *S. setifera* (18713) (2.26 µm), whereas *S. setifera* (23354) demonstrated the shortest length (1.46 µm). The karyotypes were symmetrical composing of metacentric chromosomes as indicated by their mean arm ratio (AR) that ranged between 1.11 in *S. setifera* (23354) and 1.29 in *S. byzantina* (37985), so it was classified as class 1A according to Stebbins' categories. Based on the values of total form percentage (TF%, 47.1%), Arano index of karyotype asymmetry (AsK%, 52.5%), symmetry index (S%, 94.0%) and differences of range relative length (DRL, 0.36), *S. setifera* (23354) had the most inter-and intra-chromosomal symmetric karyotype. Also, *S. byzantina* (37985) had the most inter-and intra-chromosomal asymmetric karyotype based on the values of TF% (42.0%), AsK% (56.1%), and relative length of chromosome (RL%, 6.6%). The results of cluster analysis based on chromosomal parameters divided the taxa into two main groups using the Ward method. Group I included taxa with x = 17 and group II contained *S. byzantina* (x = 15).

Please cite this paper as: Rajabi Mazaher A, Miri SM, Mohammadi A. 2021. A New Chromosome Number Report in *Stachys* L. Species by Use of Karyological Analysis. *J Genet Resour* 7(1): 29-35. doi: 10.22080/jgr.2020.19758.1201

Introduction

Stachys L., with approximately 300 species worldwide, is the largest genus of subfamily Lamioideae and among the largest genera of Lamiaceae (Labiatae). It has been shown in an existing phylogeny to be polyphyletic, and several related genera should be included within *Stachys* (e.g., *Sideritis* with about 140 species), so the genus *Stachys* would grow to more than 500 species (Marhold, 2011). Species of the genus are mainly found in the warm temperate climate of the Mediterranean and Irano-Turanian regions, with secondary centers in North America, South America, and southern Africa (Akçiçek *et al*., 2012; Salmaki *et al*., 2012). Reportedly, 23 species, 9 subspecies, and 2 hybrids have been recognized in Iran, of which 17 taxa are endemic (Salmaki *et al*., 2012).

The genus *Stachys* consists of annual or perennial herbs or subshrubs that are hispid or soft-pubescent (Salmaki *et al*., 2012; Bilušić Vundać, 2019). Leaves are sessile or stipulate, their blades are oblong to ovate, with serrate to crenate margins. Flowers are sessile or short-stalked, with two or more clustered in the axils of the leaves on the upper part of the stem. Corolla is narrow, generally with a short, pouched spur on the lower side of the tube. The upper lip is erect or generally parallel to the tube axis, concave, entire (notched), and generally hairy, and the lower lip is perpendicular to the tube axis or reflexed, three (two)-lobed and glabrous to hairy (Bilušić Vundać, 2019). Several *Stachys* species are exploited in traditional medicine as astringent, wound-healing, anti-diarrhoeal, anti-nephritic, and anti-inflammatory agents (Tundis *et al*., 2014). Moreover, antimicrobial, antianxiety, antioxidant, antinephritic, and cytotoxic activities of some *Stachys* species are documented (Goren, 2014; Tundis *et al*., 2014). Iridoids, flavonoids, phenolic acids, and diterpenoids are reported as secondary metabolites of different species of this genus (Keshavarzi *et al*., 2016; Tundis *et al*., 2014). In Mediterranean regions and Iran, the species are consumed as herbal remedies and wild tea (mountain tea) (Goren, 2014).

Stachys shows a wide range of variability that is difficult to define (Akçiçek, 2020). The main taxonomic problems of *Stachys* are the nomenclatural of some taxa and delimitation of taxa within natural groups defined as formal sections (Khadivi-Khub and Aghaei, 2015). Karyotype analysis and chromosome counting provide valuable information in identifying species and inferring some closely related taxa, especially to resolve the taxonomy of complex groups (Fallahi *et al*., 2020; Oroji Salmasi *et al*., 2019). Also, an investigation on karyotypes supplied important information for plant breeders (Tarinejad and Mirshekari, 2009). *Stachys* species and populations show extensive variation in morphological and cytological characters (Keshavarzi *et al*., 2016; Khadivi-Khub and Aghaei, 2015). Karyological research conducted on taxa of the genus *Stachys* has shown that chromosome numbers vary from 2n=10 to 102, although it is generally 2n = 30, 34, or 66 (Martin *et al*., 2016). There is little data on the karyology of the genus *Stachys*, and these data state only the chromosome numbers of the taxa because the lengths of the chromosomes were not suitable for karyotype analysis, and the centromere positions could not be observed (Martin *et al*., 2011). This study aims to characterize the karyotype features of seven populations of three *Stachys* species, in which the karyotype of two species is reported for the first time.

Materials and Methods

Plant materials

A karyotype study was performed on seven *Stachys* populations belonging to three species from the collections of Natural Resources Gene Bank of Iran, Research Institute of Forests and Rangelands (RIFR), Tehran, Iran (Table 1). The seeds were disinfected by 0.2% carboxin thiram fungicide for 3 min and placed in Petri dishes on moist filter paper at 4 °C for two weeks. Then, they were germinated at 25 °C in a germinator under dark conditions.

Table 1. Geographical information of the *Stachys* species investigated in the present study.

Species	Location	Latitude	Longitude	Altitude (m)	Gene bank code
S. benthamiana Boiss.	Ilam- Darreh Shahr	N 33º 08' 38"	E 47º 22' 28"	650	13425
S. byzantina K. Koch	Ardabil- Ardabil	N 38º 15' 28"	E 48º 17' 59"	1351	37985
S. byzantina K. Koch	Ardabil- Ardabil	N 38º 27' 47"	E 48º 31' 66"	1332	37992
S. setifera C. A. Mey.	Yazd-Yazd	N 31º 53' 50"	E 54º 22' 04"	1216	18713
S. setifera C. A. Mey.	Yazd-Taft	N 31º 44' 50"	E 54º 12' 32"	1560	23354
S. setifera C. A. Mey.	Yazd-Taft	N 31º 75' 90"	E 54º 12' 17"	1601	23356
S. setifera C. A. Mey.	Kerman-Bardsir	N 29º 93' 84"	E 56º 57' 28"	2035	41181

Cytological studies

For the karyotype study, 1-2 cm freshly grown root tips were collected from the germinated seeds. Different protocols were tested for pretreatment including (1) ice-cold water (0 °C) for 24 h, (2) 0.05% colchicine for 3 h at 25 °C, (3) α-bromonaphthalene for 16 h at 4 °C, (5) 2 mM 8-hydroxyquinoline for 120, 135 and 150 min at 4 °C, 16 °C, and 25 °C, and the best result was obtained from 2 mM 8-hydroxyquinoline at 16 °C for 135 min. The root tips were then fixed in Carnoy I (3 ethanol: 1 acetic acid) for 24 h at room temperature. The fixed root tips were then washed thoroughly in distilled water and macerated in 1N HCl at 60 °C for 10 min. The roots were stained with 1% aqueous aceto-orcein for 3-4 hours and squashed in 45% acetic acid. For each taxon, the chromosomes of 25 mitotic metaphase cells were observed and photographed using a light microscope (CX52 Olympus) at 1000 magnification supplemented Canon digital camera (Powershot G1X). The somatic chromosome number and karyotype details were studied in at least 25 well-prepared metaphase plates.

Statistical analyses

The long arm (LA) and short arm (SA) length of the chromosomes were measured using Micromeasure 3.3 software, and other chromosomal parameters such as mean chromosome length (CL), arm ratio (AR), the ratio of short to long arms (r-value), relative length of chromosome (RL%) and centromeric index (CI) were calculated by Excel. The chromosomes were identified by Levan *et al*. (1964) procedure. Karyotype symmetry was determined according to Stebbins (1971), while other karyotype parameters or asymmetry indices like haploid total chromosome length (HCL), total form percentage (TF%) (Huziwara, 1962), Arano index of karyotype asymmetry (AsK%) (Arano, 1963), symmetry index (S%) and differences of range relative length (DRL) were determined. The species studied were grouped based on karyotype features by the Ward clustering method.

Results

The studied taxa were diploid and had two basic chromosome numbers including 2n = 2x = 30 (*S. byzantina*) and 2n = 2x = 34 (*S. benthamiana* and *S. setifera*) (Table 2), whose chromosome number of the two latter species were revealed for the first time.

The longest chromosomal parameters among the taxa including HCL (38.3 µm), CL (2.2 µm), LA (1.2 µm), and SA (1.0 µm) were detected in *S. setifera* (18713). The lowest of these parameters (24.9 µm, 1.46 µm, 0.77 µm, and 0.69 µm, respectively) were in *S. setifera* (23354) (Table 2). HCL and CL of *S. benthamiana* were 25.8 µm and 1.5 µm, while in *S. byzantine* they varied between 25.3-28.5 µm and 1.7-1.9 µm, respectively. *S. setifera* (23354) had the lowest AR (1.11) and the highest CI (0.47) and r-value (0.89), while the highest AR and the lowest CI and r-value (1.29, 0.43, and 0.77) were observed in *S. byzantina* (37985). The mean value of RL% in *S. byzantina* (6.6%) was more than the other two species (5.8%). There were no significant Pearson correlations between chromosome length with altitude and geographic coordinates (data not shown).

Fig. 1 illustrates karyotypes, karyograms, and ideograms obtained for the studied taxa. One or two pairs of satellites were observed on the short arm of the chromosomes of all taxa except for *S. setifera* (18713). *S. benthamiana*, *S. byzantina* (37985), *S. byzantina* (37992), and *S. setifera* (23356) had two pairs of satellites on chromosomes no. 14, 12, 9, and 11, respectively, while *S. setifera* (23354), and *S. setifera* (41181) had one pair of satellites on chromosomes no. 4 and 17, respectively. All taxa had metacentric (m) chromosomes and their karyotypes were classified in the 1A Stebbins classes (Table 2).

S. setifera (23354) had the highest values of TF% (47.1%) and S% (94.0%), as well as the lowest values of AsK% (52.5%) and DRL (0.36), so it possessed the most intra- and interchromosomal symmetry karyotype. The lowest value of TF% (42.0%) and the highest value of AsK% (56.1%) belonged to *S. byzantina* (37985). Based on the results of interchromosomal asymmetry, *S. byzantina* (37992) with the highest DRL (3.47) had the most asymmetrical karyotype. TF%, AsK%, S% and DRL in *S. benthamiana* were 46.0%, 53.9%, 81.0% and 1.24, respectively. Among the populations of *S. setifera*, the lowest TF% (44.1%) and S% (70.2%) as well as the highest AsK% (55.4%), and DRL (2.0) were identified in the populations of 41181, 23356, 18713, and 23356, respectively (Table 2).

The genetic relationship among taxa based on karyotype similarities was assessed by cluster analysis. The Ward phenogram assigned the accessions into two main groups (Fig. 2). The first group is comprised of *S. benthamiana* and *S. setifera* (2n = 34), while the second consists of *S. byzantina* (2n = 30).

Discussion

The results of karyological characteristics of the seven populations representing three species of *Stachys* L. showed that the basic chromosome number was x = 15 (*S. byzantina*), and x = 17 (*S. benthamiana* and *S. setifera*), and all the populations were diploid.

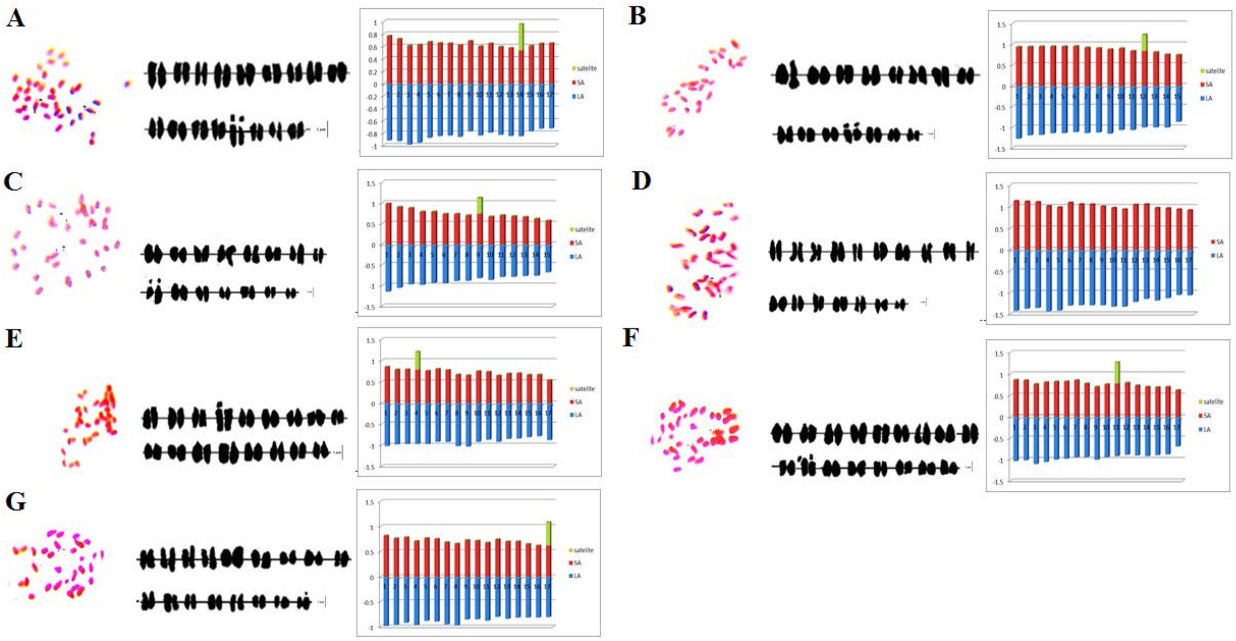

Fig 1. Karyotypes, idiograms and karyograms of A) *S. benthamiana* (13425); B) *S. byzantina* (37985); C) *S. byzantina* (37992); D) *S. setifera* (18713); E) *S. setifera* (23354); F) *S. setifera* (23356); G) *S. setifera* (41181). Scale bar = 1 µm.

Table 2. Chromosome, karyotype, and asymmetry parameters were measured on the seven populations of the studied *Stachys* species.

Species	2n	HCL (µm)	CL (µm)	LA (µm)	SA (µm)	AR	CI	r-value	RL%	TF%	AsK%	S%	DRL	SC	KF
S. benthamiana (13425)	2x=34	25.84	1.52	0.82	0.70	1.17	0.46	0.85	5.88	46.05	53.94	81.06	1.24	1A	$32m+2m^{sat}$
S. byzantina (37985)	2x=30	28.57	1.90	1.07	0.83	1.29	0.43	0.77	6.65	42.00	56.17	76.36	1.82	1A	$28m+2m^{sat}$
S. byzantina (37992)	2x=30	25.35	1.69	0.91	0.77	1.18	0.45	0.84	6.62	45.56	53.84	58.68	3.47	1A	$28m+2m^{sat}$
S. setifera (18713)	2x=34	38.32	2.26	1.25	1.01	1.23	0.44	0.80	5.89	44.80	55.45	77.34	1.51	1A	34m
S. setifera (23354)	2x=34	24.90	1.46	0.77	0.69	1.11	0.47	0.89	5.86	47.10	52.57	94.03	0.36	1A	$33m+1m^{sat}$
S. setifera (23356)	2x=34	27.20	1.60	0.86	0.73	1.18	0.45	0.84	5.84	45.62	53.75	70.21	2.06	1A	$32m+2m^{sat}$
S. setifera (41181)	2x=34	27.38	1.61	0.89	0.71	1.25	0.44	0.79	5.84	44.08	55.26	78.88	1.38	1A	$33m+1m^{sat}$

2n: somatic chromosome number, HCL: haploid total chromosome length, CL: chromosome length, LA: long arm, SA: short arm, AR: arm ratio, CI: centromeric index, r-value; the ratio of short to long arms, RL%: the relative length of the chromosome, TF%: total form percentage, AsK%; Arano index of karyotype asymmetry, S%: symmetry index, DRL: differences of range relative length, SC: symmetry classes of Stebbins, KF: karyotype formula

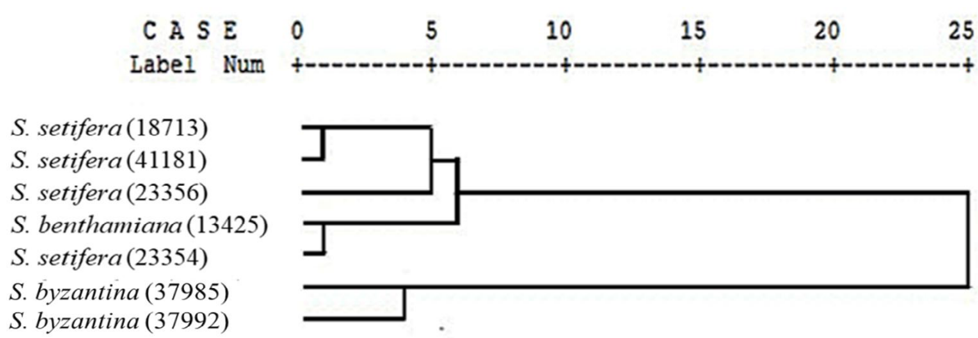

Fig. 2. Ward cluster dendrogram based on all the studied chromosomal traits of seven populations of the studied *Stachys* species.

The chromosome counts of *S. benthamiana* and *S. setifera* (2n = 34) are reported for the first time. Dirmenci *et al*. (2011) and Techio *et al*. (2017) showed that *S. byzantina* had 2n = 30 chromosomes. The most common basic chromosome number in genus *Stachys* are x = 15 (Strid, 1965; Gill, 1980; Baltisberger, 2002, 2006; Dirmenci *et al*., 2011; Martin *et al*., 2011, 2016; Khadivi-Khub and Aghaei, 2015) and x = 17 (Strid, 1965; Baltisberger, 2002, 2006; Baltisberger and Widmer, 2009; Samaropoulou *et al*., 2013; Floden, 2016; Martin *et al*., 2016; Güner *et al*., 2019; Gedik and Kocabaş, 2020), which is consistent with our results. In the previous researches, it was revealed that most species of *Stachys* are diploid (Strid, 1965; Baltisberger, 2002, 2006; Dirmenci *et al*., 2011; Martin *et al*., 2011, 2016; Samaropoulou *et al*., 2013), but Rad *et al*. (2012) found di-, tri- and tetraploid individuals in *S. inflate*. Zakaria and Zare (2013) and Khadivi-Khub and Aghaei (2015) reported *S. lavandulifolia* to be tetraploid (2n = 4x = 60).

Stachys has small chromosomes (mostly less than 2 µm) with hardly visible centromeres (Marhold, 2011). The length of chromosomes in the present study are congruent with reports of Samaropoulou *et al*. (2013) in *S. parolinii* (1.1-2.2 µm) and Zakaria and Zare (2013) in *S. lavandulifolia* (0.9-2.4 µm), and almost medium in size compared to other *Stachys* species. Khadivi-Khub and Aghaei (2015) and Güner *et al*. (2019) reported that the chromosome size of *S. lavandulifolia* and *S. kurdica* were small and varied between 1.30-1.48 µm and 0.95-1.51 µm, respectively. Martin *et al*. (2016) studied the karyology of six endemic species of *Stachys* from Turkey and reported that the length of chromosomes was in the range of 0.9 µm (*S. buttleri*) to 2.1 µm (*S. pinardii*). However, Gedik and Kocabaş (2020) found that the chromosome lengths of *S. marashica* varied in the range of 2.3-4.5 µm.

Population diversity existed in the number of satellites and their positions on the chromosomes. It has been determined that one or two pairs of the chromosomes of these taxa (except *S. setifera* (18713)) have satellites. Strid (1965) reported that some species of *Stachys* had one or two pairs of satellites. On the other hand, Tarinejad and Mirshekari (2009), Zakaria and Zare (2013), Khadivi-Khub and Aghaei (2015), Martin *et al*. (2016), Güner *et al*. (2019), and Gedik and Kocabaş (2020) did not observe satellites in the karyotype of the studied *Stachys* species.

The chromosomes of these species are of the metacentric type. Meanwhile, Tarinejad and Mirshekari (2009) found that *S. byzantina* had metacentric and sub-metacentric chromosomes. Khadivi-Khub and Aghaei (2015) reported that all chromosome pairs of *S. lavandulifolia* were metacentric, while Martin *et al*. (2016), Güner *et al*. (2019), and Gedik and Kocabaş (2020) found that the chromosome types of *Stachys* species were metacentric and sub-metacentric. Karyotype formulae and quantitative analysis have a great uniformity among populations of any species; however, the karyotypes of *S. setifera* populations are not as fully constant. At the interspecific level, quantitative and qualitative data did not allow the differentiation of *S. benthamiana* and *S. setifera*. Changes in the morphology of the chromosomes have been frequently related to evolution in higher plants (Zuo and Yuan, 2011). The similarity in karyotype formulae may indicate that if the mechanisms of speciation involved chromosome rearrangements, these may not have been large structural mutations, but small or cryptic changes. Alternatively, if speciation has occurred as a consequence of large chromosome modification, these may have been changes that did not modify the karyotype morphology, such as paracentric inversions or reciprocal translocations with segments of equal size (Seijo and Fernández, 2003).

According to Stebbin's classification, all taxa were in class 1A. This indicated chromosome symmetry between the populations. Khadivi-Khub and Aghaei (2015) also indicated a 1A symmetrical karyotype in *S. lavandulifolia*, while Güner *et al*. (2019) classified *S. kurdica* chromosomes in class 2A. Stebbin's classification could not determine the most symmetrical or asymmetrical karyotype. Thus, we resorted to other indices. The TF% and S% values decreased with increasing asymmetry, while the AsK% value increased with increasing asymmetry (Güner *et al*., 2019). Having the highest rates of TF% and S% and the lowest rates of AsK% and DRL, *S. setifera* (23354) was considered the most symmetrical karyotype. Interchromosomal asymmetry is due to heterogeneity among chromosome sizes in a complement (Peruzzi and Eroğlu, 2013). The highly asymmetric karyotypes could be the result of chromosome rearrangements due to the loss of chromosome segments, unequal translocations, differential amplification of heterochromatic regions, or even the hybridization between species with different chromosome sizes. All these events increase the interchromosomal asymmetry by increasing the morphological discontinuities between chromosomes in a karyotype (Medeiros-Neto *et al*., 2017).

Ward cluster analysis according to karyological traits classified the seven studied populations into two main groups based on their basic chromosome numbers. Cluster I included *S. benthamiana* and *S. setifera*, and the populations of *S. byzantina* were classified in Cluster II. *S. benthamiana* and *S. setifera* (x = 17) were placed in the same group, indicating that they are more closely related species, while *S. byzantine* (x = 15) had the highest distance from the other two species. This may be associated with the different evolutionary history of cytological and morphological characters in *Stachys* species.

In conclusion, the present study showed that the basic chromosome numbers of the studied *Stachys* taxa are x=15 (*S. byzantina*) and x=17 (*S. benthamiana* and *S. setifera*). Chromosome numbers correspond to the diploid level. Also, all chromosomes of three species were metacentric, and the karyotype in these species was symmetric. Higher values of the TF% and S%, as well as lower values of AsK% and DRL indices, revealed that *S. setifera* (23354) is a taxon with the highest level of karyotypic symmetry. Our results could be important for identifying the taxonomy and breeding programs of *Stachys* species.

Conflicts of interest

The authors declare that they have no conflicts of interest.

References

Akçiçek E, Dirmenci T, Dündar E. 2012. Taxonomical notes on *Stachys* sect. *Eriostomum* (Lamiaceae) in Turkey. *Turk J Bot* 36: 217-234.

Akçiçek E. 2020. Taxonomic revision of *Stachys* sect. *Olisia* (Lamiaceae: Lamioideae) in Turkey. *Phytotaxa* 449: 109-148.

Arano H. 1963. Cytological studies in subfamily Carduoideae (Compositae) of Japan. IX. The karyotype analysis and phylogenic considerations on *Pertya* and *Ainsliaea*. *Bot Mag (Tokyo)* 76: 32-39.

Baltisberger M, Widmer A. 2009. Karyological data of some angiosperms from Romania. *Willdenowia* 39: 353-363.

Baltisberger M. 2002. Cytological investigations on some Albanian plant species. *Candollea* 56: 245-260.

Baltisberger M. 2006. Cytological investigations on Bulgarian phanerogams. *Willdenowia* 36 (Special Issue): 205-216.

Bilušić Vundać V. 2019. Taxonomical and phytochemical characterisation of 10 *Stachys* taxa recorded in the Balkan Peninsula flora: A review. *Plants* 8:32.

Dirmenci T, Yıldız B, Akçiçek E, Martin E, Dündar E. 2011. *Stachys vuralii* (Lamiaceae), a new species from north Anatolia, Turkey. *Ann Bot Fenn* 48: 401-408.

Fallahi M, Mohammadi A, Miri SM. 2020. The natural variation in six populations of *Calendula officinalis* L.: A karyotype study. *J Genet Resour* 6:34-40.

Floden AA. 2016. A new endemic hedgenettle (*Stachys*: Lamiaceae) from Tennessee. *Phytoneuron* 53: 1-6.

Gedik O, Kocabaş YZ. 2020. Karyological characteristics of five endemic species with a natural spread in Kahramanmaraş flora. *Acta Bio Turcica* 33: 132-139.

Gill LS. 1980. Cytotaxonomy of the genus *Stachys* L. in Canada. *Caryologia* 33: 473-481.

Goren AC. 2014. Use of *Stachys* species (mountain tea) as herbal tea and food. *Rec Nat Prod* 8: 71-82.

Güner Ö, Selvi S, Altınordu F, Martin E. 2019. The analysis of some biological data on *Stachys kurdica* (Lamiaceae) in Turkey. *Phytol Balc* 25: 271-280.

Huziwara Y. 1962. Karyotype analysis in some genera of Compositeae. VIII. Further studies on the chromosome of Aster. *Am J Bot* 49: 116-119.

Keshavarzi M, Rezaei MB, Miri SM. 2016. The comparison of morphological and phytochemical evaluation in some population of *Stachys lavandulifolia* Vahl. in different provinces under field conditions. *Eco-phytochem J Med Plant* 4: 78-87.

Khadivi-Khub A, Aghaei Y. 2015. Chromosome counts and karyotypic study of *Stachys lavandulifolia* Vahl. *Braz J Bot* 38: 113-118.

Levan A, Fredga K, Sandberg AA. 1964. Nomenclature for centromeric position on chromosomes. *Hereditas* 52: 201-220.

Marhold K. 2011. IAPT/IOPB chromosome data 11. *Taxon* 60: 1220-1223.

Martin E, Altınordu F, Güner Ö, Akçiçek E. 2016. Karyological studies of six endemic species of *Stachys* (Lamiaceae) subsect. *Fragiles* from Turkey. *Cytologia* 81: 231-236.

Martin E, Çetin Ö, Akçiçek E, Dirmenci T. 2011. New chromosome counts of genus *Stachys* (Lamiaceae) from Turkey. *Turk J Bot* 35: 671-680.

Medeiros-Neto E, Nollet F, Moraes AP, Felix LP. 2017. Intrachromosomal karyotype asymmetry in Orchidaceae. *Genet Mol Biol* 40: 610-619.

Oroji Salmasi K, Javadi H, Miri SM. 2019. Karyotype analysis of some *Allium* species in Iran. *J Plant Physiol Breed* 9: 115-127.

Peruzzi L, Eroğlu HE. 2013. Karyotype asymmetry: again, how to measure and what to measure? *Comp Cytogen* 7: 1-9.

Rad AC, Atri M, Mohsenzadeh F, Jahandideh E. 2012. Chromosome counts in *Stachys inflata* Benth (Laminoideae): Chromosome number variation in different populations from Iran. *Chrom Bot* 7:67-71.

Salmaki Y, Zarre S, Govaerts R, Bräuchler C. 2012. A taxonomic revision of the genus *Stachys* (Lamiaceae: Lamioideae) in Iran. *Bot J Linn Soc* 170: 573-617.

Samaropoulou S, Bareka P, Artelari R, Kamari G. 2013. Karyological studies on some endemic and rare species of Kephalonia, Ionian Islands, Greece. *Fl Medit* 23: 215-221.

Seijo JG, Fernández A. 2003. Karyotype analysis and chromosome evolution in South American species of *Lathyrus* (Leguminosae). *Am J Bot* 90: 980-987.

Stebbins GL. 1971. Chromosomal evolution in higher plants. Edward Arnold LTD, London, 87-89.

Strid A. 1965. Studies in the Aegean flora: VI. Notes on some genera of Labiatae. In: Nordenstam B, ed. *Botaniska notiser, Sweden Lund Bot* 118: 104-122.

Tarinejad AR, Mirshekari B. 2009. Study on variation of karyotypes between and within species of *Salvia officinalis* L., *Stachys byzantine* L. and *Dracocephalum molarica* L. *Acta Hortic* 853: 39-46.

Techio VH, Resende LV, Braz GT, Resende KF, Samartini CQ. 2017. Unconventional vegetables collected in Brazil: chromosome number and description of nuclear DNA content. *Crop Breed Appl Biotechnol* 17: 320-326.

Tundis R, Peruzzi L, Menichini F. 2014. Phytochemical and biological studies of *Stachys* species in relation to chemotaxonomy: A review. *Phytochemistry* 102: 7-39.

Zakaria RA, Zare N. 2013. Karyotypic analysis on *Stachys lavandulifolia* Vahl from Northwest of Iran. *Iran J Rangeland Forest Plant Breed Genet Res* 21: 132-139.

Zuo L, Yuan Q. 2011. The difference between the heterogeneity of the centromeric index and intrachromosomal asymmetry. *Plant Syst Evol* 297: 141-145.