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Research article

Karyological characteristics of five endemic species with a natural spread in Kahramanmaras flora

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Abstract: Karyological characteristics of five endemic plant species with a natural spread in flora of Kahramanmaras province were investigated in this study. Chromosome number of investigated species was identified as 2n=14 for *Salvia marashica* İlçim, Celep & Doğan, 2n=34 for *Stachys marashica* İlçim, Çenet & Dadandi, 2n=16 for *Alyssum filiforme* Nyar, 2n=18 for *Hyacinthella acutiloba* K. Perss. & Wendelbo and 2n=20 for *Iris schachtii* Markgr. Karyotype characteristics of these five endemic species were determined and total chromosome lengths, karyotype formulas, centromere positions, centromere index and relative lengths were measured. Asymmetric index values were also calculated, and ideograms were generated for chromosomes of the species. Of these endemic species, karyological characteristics of *Salvia marashica*, *Stachys marashica* and *Alyssum filiforme* species were determined and reported for the first time with this study.

Keywords: *Salvia*, *Stachys, Alyssum, Hyacinthella,* karyotype

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Introduction

In this study, five endemic plant species with a natural spread in the flora of Kahramanmaras province were investigated. Kahramanmaras province with different climate transition zones, close vicinity to different phytogeographical regions and position on Anatolian Diagonal hosts a rich plant and habitat diversity. With regard to intensity of endangered species, the province is also located within the boundaries of Mediterranean Biodiversity Hot Spots (Uzun et al., 2018; Kocabaş et al., 2017). *Salvia* and *Stachys* species belong to *Lamiaceae* family. The genus *Salvia* is represented with about 900 taxa worldwide. The gene centre of the genus is America and South Asia (Hedge, 1960, 1992). In Turkey, 87 species have natural spread (Hedge, 1982; Davis, 1988; Vural and Adıgüzel, 1996). According to Kursat et al. (2018) chromosome numbers in *Salvia* genus are varied as n = 6, 7, 8, 9, 10, 11, 12, 13, 15, 16, 18 and 32 (Afzal-Rafii, 1976; Haque, 1981; Yang et al., 2009; Masoudi et al., 2010; Song et al., 2010; Martin et al., 2011b). *Salvia marashica* species are local-endemic species with ligneous bottom sections, 30-70 cm plant heights, pink flowers and leaves and stems with long secretion hairs (Ilcim et al., 2009). *Stachys* genus has a sub-composite structure and natural spread in different regions and climate zones of the world. The genus has about 300 taxa and there are 82 *Stachys* species in Turkish flora (Davis et al., 1988; Sumbul, 1990; Gemici and Leblebici, 1998).

According to Martin et al. (2011a) chromosome numbers of the species in *Stachys* genus range from 2n=10 to 2n=102. *Stachys marashica* is a local endemic non-branching species with 9.5-25 cm plant heights, non-hairy stem and yellow flowers (Ilcim et al., 2008). *Alyssum filiformes*pecies belong to *Brassicaceae* family. *Alyssum* genus has 200 taxa worldwide and represented with 107 taxa in Turkey. Kamari et al. (2011) reported chromosome

number of *Alyssum* species as 2n= 14, 16, 24, 32 and 40. *Alyssum filiforme* has an upright branchy stem with plants heights of 15-35 cm and yellow flowers (Davis, 1985; Dudley et al., 1997). *Hyacinthella acutiloba* is endemic species of *Asparagaceae* family. *Asparagaceae* family has 143 genera and about 3700 species worldwide and represented with 182 species in Turkey. In previous literatures, chromosome numbers of species of *Hyacinthella* genus were reported as 2n=16, 18, 20, 22, 24 and 60. This genus has plant heights of 8-15 cm, generally with 3 leaves in violet color (Güner et al., 2012). *Iris schactii* is an endemic species of *Iridaceae* family with plant heights of 10-20 cm and cream color flowers. *Iris* genus is represented with 43 taxa in Turkey (Mathew, 1984). According to Yu et al. (2009), chromosome numbers of *Iris* genus vary from species to species generally as 2n=26, 28, 32, 36, 40, 42 and 44. Kocyigit et al. (2013) reported chromosome numbers as n=8, 10, 11, 12 *Iris* sub-genus *iris* and as n= 10, 12, 13 for sub-genus *scorpiris*.

Materials and methods

Seed samples used in present study were collected from the localities where these species have a natural spread in the flora of Kahramanmaras province. These localities are provided in Table 1. Karyotype studies were conducted on root-tip meristem cells of germinated seeds. Seeds were germinated in petri dishes over two-fold moist filter papers at 24°C in a climate cabin. Actively growing root tips were treated with aquos colchine (0.05%) solution at room temperature for 2 hours (*Alyssum filiforme, Hyacinthella acutiloba, Iris schachtii*), paradichlorobenzene saturated solution at room temperature for 4 hours (*Salvia marashica*) and α-monobromonaphtaline solution at +4°C for 16 hours (*Stachys marashica*). Root tips were removed from pre-treatment solutions and fixated in 1:3 glacial acetic acid-ethanol solution at +4°C for 24 hours in a fridge. Fixated root tips were stored in 70% alcohol in a fridge. For staining, samples were hydrolyzed in 1 N HCl acid in an oven at 60°C. At the end of the hydrolysis process, root tips were stained in Feulgen die for an hour at dark (Elci and Sancak 2013). Chromosome centromere positions were determined in accordance with Levan et al. (1964). In determination of karyotype asymmetry; Syi and rec index values were determined in accordance with Huziwara (1962) (TF %), Arano (1963) (As K %), Greilhuber and Speta (1976); A index values were determined in accordance with Watanabe et al. (1999) and finally A_1 and A_2 index values were determined in accordance with Romero (1986). Also, the online chromosome number databases, Index to Plant Chromosome Numbers (http://www.tropicos.org/Project/IPCN) were checked. The pictures of the species in natural flora are given in Figure 1.

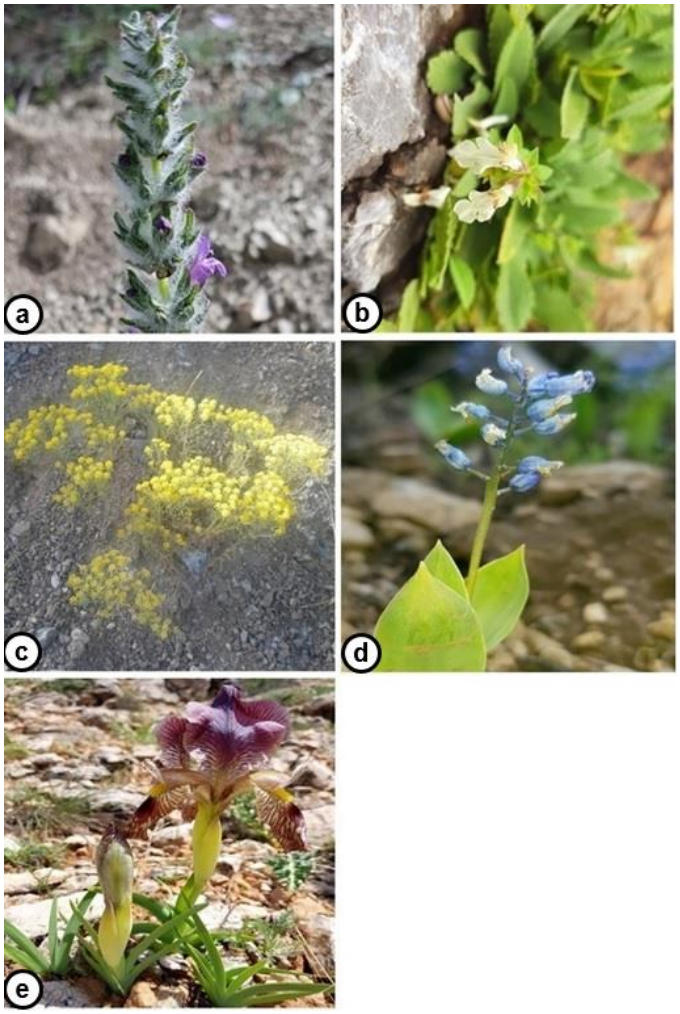

Figure 1. The images from species natural habitat: a-*Salvia marashica*, b-*Stachys marashica,* c-*Alyssum filiforme,* d-*Hyacinthella acutiloba,* e-*Iris schachtii*

Results

Karyological characteristics of five endemic species with a natural spread in Kahramanmaras flora were investigated in detail. Data on karyology of these five endemic species are provided below.

Salvia marashica Ilcim, Celep & Doğan

Somatic chromosome number of *Salvia marashica* species was identified as 2n=2x=14. Chromosome lengths of the species varied between 2.86-5.22 µm and total karyotype

length was 28.70 μm. Species chromosomes had 2 median (m), 3 sub-median (sm) and 2 sub-terminal (st) centromeres. Chromosome arm ratios varied between 1.32-3.90, centromere index values varied between 20.38-43.09 and relative lengths varied between 9.96-18.21 (Table 2 and 3). Somatic metaphase chromosomes and ideograms are presented in Figure 2a and 3a.

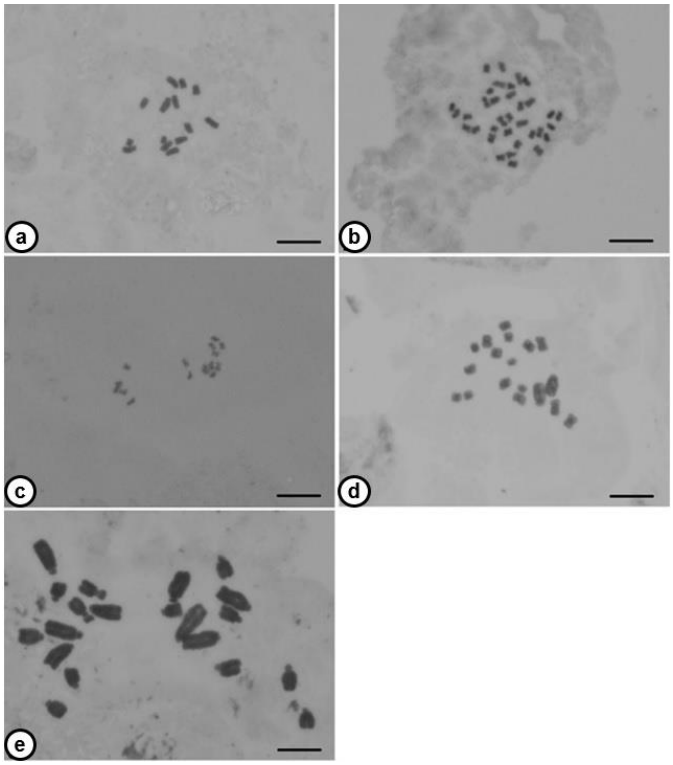

Figure 2. Metaphase images of the species: a-*Salvia marashica*, b-*Stachys marashica,* c-*Alyssum filiforme,* d-*Hyacinthella acutiloba,* e-*Iris schachtii.* (Scale bar 10 μm)

Stachys marashica Ilcim, Cenet & Dadandi

Somatic chromosome number of *Stachys marashica* species was identified as 2n=2x=34. Chromosome lengths of the species varied between 2.35-4.50 μm and total karyotype length was 58.45 μm. Species chromosomes had 15 median (m) and 2 sub-median (sm) centromeres. Chromosome arm ratios varied between 1.17-1.92, centromere index values varied between 34.19-46.03 and relative lengths varied between 4.03-7.70 (Tables 2 and 3). Somatic metaphase chromosomes and ideograms are presented in Figure 2b and 3e.

Alyssum filiforme Nyar.

Somatic chromosome number of *Alyssum filiforme* species was identified as 2n=2x=16. Chromosome lengths of the species varied between 1.60-2.67 μm and total karyotype length was 16.92 μm. Species chromosomes had 7 median (m) and 1 sub-median (sm) centromeres. Chromosome arm ratios varied between 1.23-1.73, centromere index values varied between 36.52-44.70 and relative lengths varied between 9.46-15.81 (Table 2 and 3). Somatic metaphase chromosomes and ideograms are presented in Figure 2c and 3b.

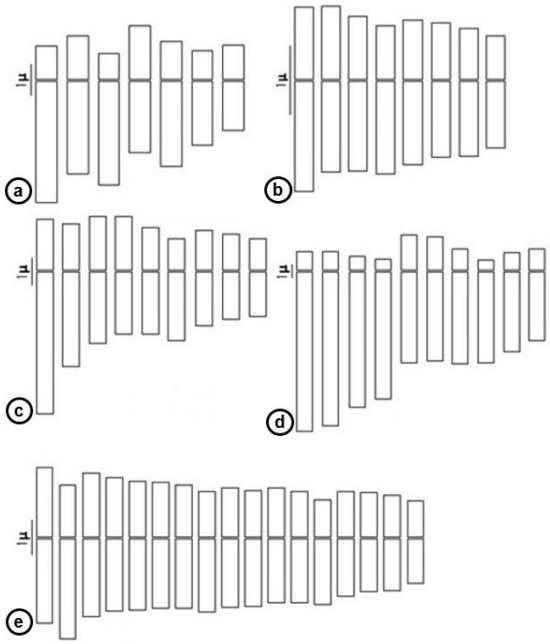

Figure 3. Haploid ideogram: a-*Salvia marashica*, b-*Alyssum filiforme*, c-*Hyacinthella acutiloba,* d-*Iris schachtii,* e-*Stachys marashica*

Table 1. Localities and collector numbers of five endemic taxa investigated

Taxa	Localities	Collector No
Salvia marashica	C6 Kahramanmaraş: Upper Ceyhan valley, Ahır Mt., around Maksutlu village, 1450-1600 m, rocky places, S 316464.22 *D* 4172537.76	Yzk 2200
Stachys marashica	C6 Kahramanmaraş, Andırın, Harboğazı, on lime-stone rocks, 385 m, S 265296.98 D 4157844.12	Yzk 2201
Alyssum filiforme	C6 Kahramanmaraş: Türkoğlu, Ceceli village, 550 m. *S 304647.46 D 4136383.67*	Yzk 1796
Hyacinthella acutiloba	B6 Kahramanmaraş: Göksun, *Esenköy village, S 299583.50 D 4232874.64, 1885 m.*	Yzk 2153
Iris schachtii	B6 Kahramanmaraş: Göksun, *Büyükkızılcık village S 299342.16 D 4233912.90, 1600 m.*	Yzk 2167

Table 2. Somatic chromosome number (2n), ploidy level, karyotype formula, ranges of chromosome length, total karyotype length (TKL) for the studied taxa

Taxa	2n	Ploidy Level	Karyotype formula	Chromosome length range (μm)	TKL (μm)
Salvia marashica	14	2x	2m+3sm+2st	2.86-5.22	28.70
Stachys marashica	34	2x	15m+2sm	2.35-4.50	58.45
Alyssum filiforme	16	2x	7m+1sm	1.60-2.67	16.92
Hyacinthella acutiloba	18	2x	6m+3sm	2.86-7.22	38.72
Iris schachtii	20	2x	3sm+2st+5T	6.90-13.24	98.07

Table 3. Karyomorphological parameters of five endemic species

Salvia marashica (2n=14)					*Stachys marashica* (2n=34)				
Pair No	RL	AR	CI	Type	Pair No	RL	AR	CI	Type
1	18.21	3.57	21.89	st	1	7.70	1.21	45.20	m
2	16.09	2.09	32.37	sm	2	7.59	1.92	34.19	sm
3	15.33	3.91	20.39	st	3	7.07	1.22	45.04	m
4	14.72	1.32	43.09	m	4	6.60	1.23	44.88	m
5	14.57	2.23	30.98	sm	5	6.33	1.29	43.71	m
6	11.10	2.16	31.63	sm	6	6.22	1.29	43.65	m
7	9.97	1.42	41.29	m	7	6.09	1.35	42.63	m
Alyssum filiforme (2n=16)					8	5.91	1.63	38.07	m
Pair No	RL	AR	CI	Type	9	5.84	1.40	41.66	m
1	15.82	1.53	39.59	m	10	5.70	1.48	40.35	m
2	14.20	1.24	44.70	m	11	5.66	1.30	43.42	m
3	13.15	1.43	41.13	m	12	5.45	1.42	41.25	m
4	12.61	1.74	36.53	sm	13	5.16	1.78	36.03	sm
5	12.41	1.41	41.48	m	14	5.11	1.27	44.00	m
6	11.43	1.36	42.37	m	15	4.86	1.17	46.03	m
7	10.91	1.46	40.60	m	16	4.67	1.24	44.59	m
8	9.47	1.54	39.40	m	17	4.04	1.25	44.44	m
Iris schachtii (2n=20)					*Hyacinthella acutiloba* (2n=18)				
Pair No	RL	AR	CI	Type	Pair No	RL	AR	CI	Type
1	13.74	8.54	10.48	T	1	18.66	2.76	26.62	sm
2	13.29	8.27	10.79	T	2	13.66	2.02	33.10	sm
3	11.57	9.13	9.87	T	3	12.08	1.32	43.09	m
4	10.71	10.53	8.68	T	4	11.22	1.14	46.81	m
5	9.84	2.58	27.91	sm	5	10.21	1.44	40.99	m
6	9.55	2.65	27.40	sm	6	9.66	2.15	31.78	sm
7	8.76	4.22	19.15	st	7	9.03	1.33	42.98	m
8	7.89	8.45	10.58	T	8	8.07	1.29	43.74	m
9	7.63	4.45	18.33	st	9	7.41	1.37	42.21	m
10	7.03	3.16	24.06	sm					

Abbreviation: RL: Relative length, AR: Arm ratio, CI: Centromer index, Type: Centromere status

Hyacinthella acutiloba K. Perss. & Wendelbo

Somatic chromosome number of *Hyacinthella acutiloba* species was identified as 2n=2x=18. Chromosome lengths of the species varied between 2.86-7.22 μm and total karyotype length was 38.72 μm. Species chromosomes had 6 median (m) and 3 sub-median (sm) centromeres. Chromosome arm ratios varied between 1.13-2.75, centromere index values varied between 26.62-46.81 and relative lengths varied between 7.40-18.66 (Table 2 and 3). Somatic metaphase chromosomes and ideograms are presented in Figure 2d and 3c.

Iris schachtii Markgr.

Somatic chromosome number of *Iris schachtii* species was identified as 2n=2x=20. Chromosome lengths of the species varied between 6.90-13.24 μm and total karyotype length was 98.07 μm. Species chromosomes had 3 sub-median (sm), 2 sub-terminal (st) and 5 terminal point (T) centromeres. Chromosome arm ratios varied between 2.58-10.52, centromere index values varied between 8.67-27.40 and relative lengths varied between 7.03-13.73 (Table 2 and 3). Somatic metaphase chromosomes and ideograms are presented in Figure 2e and 3d.

Karyotype asymmetry was determined by using several methods; TF (%), As K (%), Syi, Rec and the degree of asymmetry A, A_1 and A_2. Present findings revealed that TF% values varied between 15.91-42.21, AsK% values varied between 57.78-84.08, Syi values varied between 18.92-73.05 and Rec values varied between 59.53-78.43. While A_1 index values varied between 0.31-0.94, A_2 index values varied between 0.15-0.30 and A index values varied between 0.16-0.42 (Table 4).

Table 4. The values of asymmetry indices (Rec, Syi, TF (%), As K (%), A, A1, A2) in the working taxa.

Taxa	Rec	Syi	TF (%)	As K (%)	A	A1	A2
Salvia marashica	78.43	44.52	30.80	69.19	0.42	0.51	0.19
Stachys marashica	76.38	73.05	42.21	57.78	0.16	0.94	0.16
Alyssum filiforme	79.03	68.84	40.77	59.22	0.18	0.31	0.15
Hyacinthella acutiloba	59.53	60.62	37.74	62.25	0.21	0.34	0.30
Iris schachtii	72.79	18.92	15.91	84.08	0.66	0.78	0.23

Discussion

Karyological characteristics of five endemic species were investigated in detail in this study. Diploid chromosome number of *Salvia marashica* species was identified as 2n=14. Previous karyological studies revealed that basic chromosome numbers of *Salvia* species as n=6, 7, 8, 9, 10, 11, 13, 15, 16, 17, 19 and 22 (Haque, 1981). Martin et al. (2015) conducted a study and reported chromosome number as 2n = 16 for *S. viridis*, 2n = 20 for *S. candidissima* subsp. *occidentalis*, 2n = 22 for *S. sclarea*, *S. ceratophylla*, *S. chionantha* and 2n = 32 for *S. viscosa* and *S. verticillata* subsp. *amasiaca*. Gedik et al. (2016) reported chromosome number as 2n = 18 for *S. ceratophylla*, 2n = 18 for *S. syriaca*, 2n = 22 for *S. palaestina*, 2n = 22 for *S. aethiopis*, 2n = 16 for *S. russellii*, 2n = 28 for *S. multicaulis* and 2n = 14 for *S. trichoclada.* Kursat et al. (2018) indicated chromose number as 2n = 14 for *S. euphratica* var. *euphratica*, 2n = 14 for *S. euphratica* var. *leicalycina*, 2n = 22 for *S. hypargeia*, 2n = 22 for *S. candidissima* subsp. *candidissima*, 2n = 18 for *S. frigida*, 2n = 18 for *S. virgata*, 2n = 14 for *S. suffruticosa* and 2n = 32 for *S. verticillata* subsp. *verticillata*. Chromosome number of *Salvia marashica* was similar with the chromosome number of several *Salvia* species. Chromosome number of this species was identified and reported for the first time in this study.

Chromosome number of *Stachys marashica* endemic species was identified as 2n=34. Previous studies on *Stachys* species indicated different diploid chromosome numbers for this species. Martin et al. (2011a) studied 26 *Stachys* taxa (14 species, 11 sub-species and 1 variety) and reported the chromosome number as 2n=30. Rad et al., (2012) conducted a study on 13 *Stachys inflata* Benth. population collected from western provinces of Iran and reported diploid chromosome numbers as between 2n=2x=16 and 2n=4x=32. Martin et al. (2016) worked on *Stachys* species and reported two different chromosome numbers 2n=30 for *Stachys chasmosericea* and *S. pinardii* species and 2n=34 for *S. euadenia, S. buttleri, S. pseudopinardii* and *S. longiflora* species. Chromosome number of *Stachys marashica* species was identified and reported for the first time in this study.

Chromosome number of *Alyssum filiforme* endemic species was identified as 2n=16. Previous studies revealed varying chromosome numbers for *Alyssum* species. Kamari et al. (1998) reported diploid chromosome number of *Alyssum meniocoides* Boiss and *Alyssum huetii* Boiss. as 2n=14. Ozturk et al. (2009) indicated diploid chromosome number of *Alyssum strigosum* subsp. *Strigosum* and *Alyssummurale* subsp. *murale* var. *murale* taxa as 2n = 16. As can be inferred from those studies, chromosome numbers vary from one species to another. Chromosome number of *Alyssum filiforme* species was identified and reported for the first time in this study and present reports comply with the findings of earlier studies.

In previous studies, chromosome number of *Hyacinthella acutiloba* species was reported as 2n= 18 (Persson and Persson, 1992; Johnson and Brandham, 1997). In present study, chromosome number was also identified as 2n=18. Species of *Hyacinthella* genus generally have varying basic chromosome numbers. According to literatures, *H. lineate* had 2n=16 (Persson and Wendelbo, 1982), *H. heldreichii* (Boiss.) Chouard had 2n=18 (Johnson and Brandham, 1997), *H. hispida* (J. Gay)

Chouard had 2n=18 (Persson and Persson, 1992), *H. glabrescens* (Boiss.) K. Persson & Wendelbo had 2n=18 (Persson and Persson, 1992) and 2n=20 (Johnson and Brandham, 1997), *H. leucophaea* (C. Koch) Schur had 2n=20 (Johnson and Brandham, 1997), *H. atchleyi* had 2n=20 (Persson and Wendelbo, 1982; Speta, 1982), *H. pallens* Schur had 2n=20 (Persson and Wendelbo, 1982), *H. pallasiana* (Stev.) Losinsk had 2n=20 (Persson and Wendelbo, 1982), *H. atropatana* (Grossh.) Mordak & Zakharyeva had 2n=22 (Pogosian, 1997), *H. lazulina* K. Perss. & J. Perss. had 2n=22 (Persson and Persson, 1992; Johnson and Brandham, 1997), *H. micrantha* had 2n=22 (Persson and Wendelbo, 1982), *H. millingenii* had 2n=22 (Persson and Wendelbo, 1982), *H. persica* had 2n=22 (Speta, 1982), *H. siirtensis* Mathew had 2n=22 (Persson and Persson, 1992), *H. venusta* K. Persson had 2n=22 (Persson, 2000), *H. nervosa* (Bertol.) Chouard had 2n=24 (Persson and Persson, 1992), *H. dalmatica* Chouard had 2n=60 (Persson and Persson, 2000) chromosomes.

Chromosome number of *Iris schachtii* species was reported as 2n=4x=48 (Koca, 1989). In present study, chromosome number of *Iris schachtii* was identified as 2n=20. Several previous karyology studies were conducted on *Iris* genus; according to Ferjani and Haouala (2015), *Iris juncea* had 2n = 32 = 30sm + 2st and a pair of satellite, *I. sisyrinchium* had 2n= 24 = 18 st + 6 sm karyotype formula. *I. acutiloba* C.A. Mey. had 2n=20 chromosomes (Avishi and Zohary, 1977), *I. afghanica* Wendelbo had 2n=22 (Johnson and Brandham, 1997), *I. alata* Poir. had 2n=24 (Colasante and Vosa, 1981), *I. alberti* Regel had 2n=24 (Zakirova and Nafanailova, 1990), *I. albicans* Lange had 2n=44 (Colasante and Sauer, 1993), *I. antilibanotica* Dinsm. had 2n=20 (Avishai and Zohary, 1977; Kliphuis and Barkoudah, 1977), *I. aucheri* (Baker) Sealy had 2n=24 (Kamari et al., 1999), *I. bicapitata* Colas. had 2n=40 (Colasante, 1996), *I. biglumis* Vahl had 2n=40 (Murin et al., 1980), *I. bloudowii* Ledeb. had 2n=26 (Doronkin and Krasnikov, 1984), *I. cycloglossa* Wendelbo had 2n=28 (Gustafsson and Wendelbo, 1975), *I. danfordiae* (Baker) Boiss. had 2n=18, 27 (Johnson and Brandham, 1997), *I. decora* Wall. had 2n=36 (Malla et al., 1981), *I. dichotoma* Pall. had 2n=32 (Sha et al., 1995), *I. ensata* Thunb. had 2n=22 (Mehra and Sachdeva, 1976) and 2n=40, 80 (Pandita and Mehra, 1982), *I. florentina* L. had 2n=44 (Colasante and Sauer 1993), *I. galatica* Siehe had 2n=24 (Ozkan et al., 2001), *I. histrioides* Foster ex Hayek had 2n=16+1B (Johnson and Brandham, 1997), *I. japonica* Thunb. had 2n=28, 34, 36, 54 (Colasante and Sauer, 1993), *I. japonica* Thunb. had 2n=30, 32, 36, 60 (Zhou et al., 2003), *I. junonia* had 2n=4x=48, *I. purpureobractea* had 2n=2x=24, 2n=4x=48, 2n=4x+1=49, 2n=6x=72; *I. taochia* had 2n=2x=24; *I. schachtii* had 2n=4x=48 chromosomes (Koca, 1989).

The present study was conducted to put forth karyological characteristics of five endemic species with a natural spread in Kahramanmaras flora. Among the investigated species, karyological characteristics of *Salvia marashica*, *Stachys marashica* and *Alyssum filiforme* were identified and reported for the first time in this study.

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