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Karyological study on four species of *satureja* (Lamiaceae) in Iran

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Abstract

A karyological study of four taxa (9 populations) of the genus *Satureja* L. from different geographic origins is presented. The haploid levels were different among species (n= 12, 13, 14, 22). Most of the numbers and all of the karyotypes analysis were reported for Iran for the first time. The karyotypic results showed the diversity among the species as mostly displayed median region (m) and sub median region (sm) and the chromosome lengths were determined between 1.81-2.05µm in *S. macrosiphonia* ,1.87- 2.31µm in *S. mutica*, 1.52-1.65 µm in *S. sahendica* and 1.57- 1.64 µm in *S. spicigera*. Detailed karyotype analysis allows us to postulate relationships among them.

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Introduction

Satureja is a genus of aromatic plants of the family Lamiaceae (the mint family). The genus has been a subject of much discussion among taxonomists and is variously treated. In Flora Europaea, (Heywood and Richardson, 1972) recognized 5 genera in the region including *Calamintha, Acinos, Clinopodium, Micromeria* and *Satureja*. In the Flora of USSR (Shishkin, 1954); China (Li and Wei, 1992); Turkey (Dirmenci, 2010) and Iranica (Rechinger, 1982) a similar classification was adopted considering to some specific genera which were included on the basis of geographical distribution. *Satureja* in its narrow concept is a genus comprising 30 species, mainly distributed in Mediterranean region but also extended to Irano-Turanian phytogeographical region. *Satureja* belongs to the tribe Mentheae within the subfamily Nepetoideae and includes about 284 species in the world, (Nixon, 2006). This genus is represented in Iranian flora by 16 species, nine of which are endemic for the country (*S. macrosiphonia ,S. bachtiarica ,S. rechingeri ,S. isophylla S. atropatana ,S. sahendica ,S. khuzistanica ,S. intermedia and S. edmondi*) (Jam Zad, 2009). There are about 30 species called savories, of which summer savory and winter savory are the most important in cultivation. *Satureja* species are native to warm temperate regions and may be annual or perennial. They are low-growing herbs and sub shrubs, reaching heights of 15– 50 cm. The leaves are 1 to 3 cm long, with flowers forming in whorls on the stem, white to pale pink-violet. Both summer savory and winter savory are used to flavor food. The former is preferred by cooks but as an annual is only available in summer, winter savory is an evergreen perennial. Certain organic chemicals are derived from these species, which are useful to humans. They have usually well known, and will be used by native inhabitants as spice, medicinal plant or source of essential oils. Medicinal properties and a large variety of specimens in the species increase the importance of diversity studies in this genus. Within the *Satureja* genus the genetic diversity has been dealt with using morphological characters, (Hadian, 2011 ; Kasyani, 2012) Enzyme electrophoresis, (Attar, 2006 ; Hadian, 2010) cytogenetic and molecular markers (RAPD, SAMPL and AFLP); (Hadian, 2010; Braüchler, 2005; Braüchler, 2006; Braüchler, 2008). There was no report to date on the detailed karyotype of these species. Thus the karyotype analysis of these species was first time counted in Iran. The chromosome count and karyotype studies are not only useful in predicting morphological similarity and diversity among *Satureja* species they are valuable sources of taxonomic and biosystematics information. There was no report to date on the detailed karyotype of these species. Thus, the karyotype analysis of these species was first time counted in Iran. Therefore, the objectives of this study are 1) to present karyological information, particularly the differences among them and 2) to determine the chromosome number and haploidy levels of these taxa.

Material and methods

Plant materials

The materials used in this study were collected in different areas of Iran. The localities, gene bank codes and species names are shown in Table 1. Vouchers are deposited in gene bank RIFR (Research Institute of Forest and Rangelands from Iran).

Chromosomal studies

The following procedures were followed, in carrying out the chromosomeal studies. For mitotic studies, the Root tip meristems obtained from seedlings were pretreated with 0.5% saturated α-Bromo naphthalene at 4 ^{o}C for 4h , fixed in 40% formaldehyde and 1% chromic acid (1:1) for at least 16 h at room temperature , then root tips were rinsed for 3 h in distilled water. Hydrolysis was carried out with 1N NaOH at 60 ^{o}C for 8 min, dyed with hematoxylin for 3-4 h and squashed in a droplet of 45 % acetic acid and lactic acid (10:1). The preparations were observed with an optical microscope (BH_2 Olympus supplemented digital color video camera) at a magnification of 1908x.

The best metaphysical plates were selected and measured by Micro measure 3.3 software (Reeves and Tear, 2000). In each mitotic metaphase (at least 5

plates) the arm's length of each chromosome was measured , according to the previous studies (Hesamzadeh Hejazi and Rasuli, 2006; Hesamzadeh and Ziaei Nasab, 2009, 2010).The following parameters were estimated in each metaphase plate to characterize the karyotypes numerically : Long Arm(LA) , Short Arm (SA), Total Length(TL)[LA+SA],Relative Length percentage (RL%), Arm Ratio(AR)[LA/SA], Centromeric Index (CI)[SA/(LA+SA)],Value of Relative chromatin (VRC)[ΣTL/n], relative length of long arm(LA%), relative length of short arm(SA%). Karyotype asymmetry was estimated by three different methods namely , Total Form percentage (TF%)[(ΣSA/ΣTL)×100] (Huziwara,1962), Difference of Relative Length (DRL)[$Max_{RL\%}$-$Min_{RL\%}$];Dispersion Index (DI)[%CV×CG] where CV represents the coefficient of variation for chromosome Length and CG represents the centromeric gradient value [(Length of median short arm / Length of median chromosome)×100] (Levania and Srivastava, 1992) ; intra chromosomal asymmetry index (A_1) $\left[1-\sum(\overline{SA}/\overline{LA})/n\right]$ and inter chromosomal asymmetry index (A_2) $[\,S\bar{d}\,/\,\overline{X}\,]$ where *n* is the number of homologues, $S\bar{d}$ is the average of standard deviation, and $\overline{X}$ is the mean chromosome length (Romero Zarco,1986). Both indices (A_1 and A_2) are independent to chromosome number and size. Also karyotypic evolution has been determined using the symmetry classes of Stebbins (SC) (Stebbins, 1971). Karyotype formula was determined by chromosome morphology based on centromere position according to classification of (Levan *et al.* 1964). For each species , karyograms and haploid idiograms were drawn based on length of chromosome size (arranged large to small). In order to determine the variation between species, one – way balanced ANOVA was performed on normal data and parameter means were compared by Duncan's test Numerical analyses were performed using (SAS, 1996) software ver.6.12.

Results

Karyotype analyses of the four species of *satureja* were determined. Somatic cells of investigated species have 2n= 24, 26, 28 and 44 chromosomes for *S. macrosiphonia, S. mutica, S. sahendica and S. spicigera* respectively. Average of chromosomal length of investigated species ranged from 1.52 to 2.31 µm.

Table 1. localities of species used in the study.

species	localities	Gene bank code (RIFR)
Satureja macrosiphonia Bornm.	Lorestan 1	201
S. macrosiphonia Bornm.	Lorestan2	202
S. mutica Fisch.&C.A.Mey.	Khorasan1	100
S. mutica Fisch.&C.A.Mey.	Gilan	44
S. mutica Fisch.&C.A.Mey.	lorestan	46
S. sahendica Bornm.	Azarbaijan 1	38
S. sahendica Bornm.	Azarbaijan 2	10
S. spicigera Boiss.	Gilan1	32
S. spicigera Boiss.	Gilan2	35

Among these, while *S. sahendica* (10) has the smallest chromosome (Fig.7), the biggest chromosome was observed on *S. mutica* (46) (Fig. 6).Detailed measurements of somatic chromosomes of nine different *Satureja* populations are given in Table 2-10.

Mean length of the haploid chromosome complement, recorded from at least three cells, were 21.77±0.42 µm, 24.63±0.51 µm, 24.38±0.55 µm, 27.27±0.46 µm, 30.05±0.60 µm, 21.39±0.40 µm, 23.10±0.44 µm, 36.20±0.53 µm, 34.66±0.58 µm respectively in *S. macrosiphonea*(201), *S.*

macrosiphonea(202), *S. mutica*(100), *S. mutica*(44), *S. mutica*(46), *S. sahendica*(10), *S. sahendica*(38) , *S. spicigera*(32) and *S. spicigera*(35).

Based on total length, chromosome pairs were arranged from 1 to 12, 1 to 13, 1 to 14 and 1 to 22 in order of decreasing length(Fig: 1-9). Chromosome length of the complement ranged from 0.95 to 3.49 µm, with a mean chromosome size of 1.81± 0.42µm observed in *S. macrosiphonea*(201).Based on the location of the primary constriction the following karyotypic formula was developed for *S. macrosiphonea*(201): K.F.(n=12) = 11m+1sm. Chromosome length of the complement ranged from 0.88 to 3.69 µm, with a mean chromosome size of 2.05± 0.51 µm observed in *S. macrosiphonea*(202). Based on the location of the primary constriction the following karyotypic formula was developed for *S. macrosiphonea*(202): K.F.(n=12) = 9m+3sm. Chromosome length of the complement ranged from 0.78 to 3.90 µm, with a mean chromosome size of 1.88±0.55 µm observed *S. mutica*(100). Based on the location of the primary constriction the following karyotypic formula was developed for *S. mutica*(100): K.F.(n=13) = 10m+3sm. Chromosome length of the complement ranged from 0.87 to 3.45 µm, with a mean chromosome size of 2.10±0.46 µm observed *S. mutica*(44). Based on the location of the primary constriction the following karyotypic formula was developed for *S. mutica*(44): K.F.(n=13) = 13m. Chromosome length of the complement ranged from 0.95 to 4.22 µm, with a mean chromosome size of 2.31±0.60 µm observed *S. mutica*(46). Based on the location of the primary constriction the following karyotypic formula was developed for *S. mutica*(46): K.F.(n=13) = 12m+1sm. Chromosome length of the complement ranged from 0.65 to 2.96 µm, with a mean chromosome size of 1.53±0.40 µm observed *S. sahendica*(10). Based on the location of the primary constriction the following karyotypic formula was developed for *S. sahendica*(10): K.F.(n=14) = 10m+4sm. Chromosome length of the complement ranged from 0.67 to 3.16 µm, with a mean chromosome size of 1.65± 0.44µm observed *S. sahendica*(38). Based on the location of the primary constriction the following karyotypic formula was developed for *S. sahendica*(38): K.F.(n=14) = 10m+4sm. Chromosome length of the complement ranged from.

Table 2. Measurements (µm) of somatic metaphase chromosomes of *satureja macrosiphonia*(201).

ch.	LA±SE	SA±SE	TL±SE	%LA	%SA	AR	CI	K.F.
1	1.91±0.14	1.58±0.22	3.49±0.14	8.79	7.26	1.21	0.45	m
2	1.57±0.16	0.99±0.26	2.57±0.23	7.22	4.59	1.57	0.39	m
3	1.36±0.08	0.92±0.27	2.29±0.18	6.26	4.24	1.48	0.40	m
4	1.28±0.09	0.85±0.26	2.14±0.17	5.91	3.91	1.51	0.39	m
5	1.15±0.10	0.77±0.22	1.93±0.15	5.30	3.57	1.49	0.40	m
6	1.07±0.21	0.69±0.21	1.76±0.15	4.91	3.16	1.55	0.39	m
7	0.92±0.19	0.73±0.25	1.65±0.08	4.23	3.34	1.26	0.44	m
8	0.85±0.09	0.58±0.19	1.43±0.11	3.93	2.65	1.48	0.40	m
9	0.73±0.14	0.59±0.19	1.33±0.06	3.38	2.72	1.24	0.44	m
10	0.73±0.16	0.44±0.10	1.17±0.12	3.35	2.02	1.66	0.37	m
11	0.68±0.14	0.37±0.06	1.06±0.12	3.13	1.72	1.82	0.35	sm
12	0.57±0.13	0.38±0.04	0.95±0.07	2.61	1.76	1.48	0.40	m

Long arm (LA), Short arm (SA), total length (TL), Long arm percentage (%LA), Short arm percentage (%SA), arm ratio (AR), Centromeric index (CI), Karyotype Formula (K.F.), (m: metacentric) (sm: submetacentric).

0.53 to 3.81 µm, with a mean chromosome size of 1.65±0.53 µm observed *S. spicigera*(32). Based on the location of the primary constriction the following karyotypic formula was developed for *S. spicigera*(32): K.F.(n=22) = 18m+4sm. Chromosome length of the complement ranged from 0.40 to 3.78 µm, with a mean chromosome size of 1.58±0.58 µm observed *S. spicigera*(35). Based on the location of the primary constriction the following karyotypic

formula was developed for *S. spicigera*(35): K.F.(n=22) = 18m+4sm.

Fundamentally, all of the studied species showed very similar karyotypic characters except on LA%, SA% and A_2 traits.The dispersion index is calculated as the proportionate measure of centromeric gradient to the coefficient of variation for chromosome length. The highest value of DI was found in *S. spicigera* (35) (25.62) and the lowest value of DI was found in *S .mutica*(44) (15.78) species. Symmetry type of Stebbins (1971) and asymmetry indices of Romero Zarco (1986) are given in (Table 11). A statistical comparison based on completely randomized design demonstrates that there are non significant differences among the species for all the measured traits except, LA% and SA% traits with ($p<1\%$) and A_2 trait with ($p<5\%$) (Table 12). The Duncan's test applied to the chromosome morphometric traits showed a non significant difference among the nine populations, except for LA%, SA% and A_2 traits (Table 13).

Table 3. Measurements (µm) of somatic metaphase chromosomes of *satureja macrosiphonia*(202).

ch.	LA±SE	SA±SE	TL±SE	%LA	%SA	AR	CI	K.F.
1	1.95±0.13	1.73±0.05	3.69±0.09	13.60	16.90	1.13	0.47	m
2	1.79±0.13	1.58±0.14	3.37±0.08	12.42	15.43	1.13	0.47	m
3	1.64±0.19	1.14±0.17	2.78±0.20	11.38	11.13	1.43	0.41	m
4	1.41±0.20	1.04±0.14	2.45±0.15	9.79	10.12	1.36	0.42	m
5	1.38±0.13	0.83±0.04	2.21±0.23	9.62	8.05	1.67	0.37	m
6	1.30±0.17	0.73±0.03	2.03±0.23	9.07	7.09	1.79	0.36	sm
7	1.12±0.20	0.77±0.05	1.89±0.14	7.80	7.51	1.46	0.41	m
8	0.97±0.09	0.55±0.08	1.52±0.17	6.73	5.42	1.74	0.36	sm
9	0.83±0.10	0.60±0.04	1.43±0.10	5.81	5.82	1.40	0.42	m
10	0.78±0.04	0.50±0.05	1.28±0.11	5.44	4.90	1.55	0.39	m
11	0.70±0.07	0.40±0.05	1.10±0.12	4.87	3.91	1.75	0.36	sm
12	0.50±0.06	0.38±0.06	0.88±0.05	3.45	3.70	1.30	0.43	m

Long arm (LA), Short arm (SA), total length (TL), Long arm percentage (%LA), Short arm percentage (%SA), arm ratio(AR),Centromeric index(CI) Karyotype Formula (K.F.), (m: metacentric)(sm: submetacentric).

Table 4. Measurements (µm) of somatic metaphase chromosomes of *satureja mutica* (100).

ch.	LA±SE	SA±SE	TL±SE	%LA	%SA	AR	CI	K.F.
1	2.28±0.33	1.61±0.18	3.90±0.27	9.37	6.62	1.41	0.41	m
2	1.88±0.23	1.32±0.01	3.20±0.23	7.70	5.40	1.42	0.41	m
3	1.55±0.14	1.18±0.09	2.74±0.15	6.37	4.85	1.31	0.43	m
4	1.53±0.19	0.93±0.05	2.46±0.24	6.28	3.83	1.64	0.38	m
5	1.34±0.09	0.72±0.04	2.06±0.25	5.48	2.97	1.85	0.35	sm
6	1.06±0.04	0.73±0.09	1.79±0.13	4.33	2.99	1.45	0.41	m
7	1.06±0.08	0.50±0.04	1.56±0.23	4.34	2.06	2.10	0.32	sm
8	0.88±0.05	0.50±0.03	1.38±0.15	3.60	2.06	1.74	0.36	sm
9	0.80±0.04	0.49±0.10	1.30±0.12	3.29	2.04	1.61	0.38	m
10	0.75±0.06	0.46±0.08	1.21±0.20	3.08	1.88	1.64	0.38	m
11	0.65±0.06	0.42±0.06	1.06±0.09	2.65	1.71	1.55	0.39	m
12	0.56±0.03	0.38±0.04	0.94±0.07	2.28	1.58	1.44	0.41	m
13	0.48±0.01	0.29±0.02	0.78±0.08	2.00	1.21	1.65	0.38	m

Long arm(LA), Short arm(SA),total length (TL), Long arm percentage (%LA), Short arm percentage (%SA), arm ratio(AR),Centromeric index(CI) Karyotype Formula(K.F.), (m: metacentric)(sm: submetacentric).

Discussion

In this study, chromosome numbers and detailed measurements of nine populations of *satureja* genus were determined for the first, time in Iran. A number of investigators have examined the chromosome numbers of the *satureja* genus .However, out of the

30 different species listed in Flora Europaeae, (Heywood and Richardson, 1972) and Iranika flora by 14 species (Rechinger, 1986), the chromosome numbers of only 12 species have been studied. All the so far studied *satureja* species have revealed a chromosome numbers of 2n=20, 24, 30, 48 and 60 (Cenci, 1969; Genzalez, 1982; Ozaydin, 2004).

Table 5. Measurements (µm) of somatic metaphase chromosomes of *satureja mutica*(44).

ch.	LA±SE	SA±SE	TL±SE	%LA	%SA	AR	CI	K.F.
1	2.12±0.15	1.33±0.34	3.45±0.32	7.75	4.87	1.59	0.39	m
2	1.81±0.15	1.25±0.21	3.05±0.23	6.62	4.57	1.45	0.41	m
3	1.71±0.18	1.15±0.24	2.86±0.23	6.25	4.22	1.48	0.40	m
4	1.55±0.13	1.06±0.22	2.62±0.20	5.68	3.90	1.46	0.41	m
5	1.40±0.15	1.10±0.19	2.50±0.13	5.15	4.02	1.28	0.44	m
6	1.27±0.11	1.03±0.23	2.30±0.09	4.67	3.77	1.24	0.45	m
7	1.14±0.21	0.91±0.08	2.05±0.09	4.15	3.35	1.24	0.44	m
8	1.07±0.16	0.81±0.07	1.88±0.10	3.19	2.97	1.32	0.43	m
9	1.03±0.15	0.75±0.09	1.78±0.11	3.78	2.74	1.37	0.42	m
10	0.95±0.10	0.66±0.09	1.60±0.12	3.47	2.41	1.44	0.41	m
11	0.76±0.13	0.53±0.04	1.29±0.09	2.79	1.96	1.42	0.41	m
12	0.61±0.12	0.41±0.04	1.02±0.08	2.23	1.50	1.48	0.40	m
13	0.51±0.09	0.36±0.02	0.87±0.06	1.85	1.32	1.43	0.41	m

Long arm(LA), Short arm(SA),total length (TL), Long arm percentage (%LA), Short arm percentage (%SA), arm ratio(AR),Centromeric index(CI) Karyotype Formula(K.F.), (m: metacentric)(sm: submetacentric).

Table 6. Measurements (µm) of somatic metaphase chromosomes of *satureja mutica*(46).

ch.	LA±SE	SA±SE	TL±SE	%LA	%SA	AR	CI	K.F.
1	2.16±0.14	2.06±0.12	4.22±0.04	7.17	6.86	1.05	0.49	m
2	2.09±0.08	1.72±0.15	3.81±0.15	6.97	5.72	1.22	0.45	m
3	1.91±0.12	1.46±0.15	3.37±0.18	6.36	4.86	1.31	0.43	m
4	1.60±0.05	1.46±0.08	3.05±0.06	5.32	4.85	1.10	0.48	m
5	1.44±0.17	1.16±0.18	2.60±0.11	4.79	3.86	1.24	0.45	m
6	1.39±0.14	0.95±0.15	2.34±0.18	4.62	3.16	1.46	0.41	m
7	1.38±0.18	0.75±0.16	2.13±0.25	4.58	2.51	1.83	0.35	sm
8	1.05±0.21	0.87±0.19	1.92±0.08	3.51	2.89	1.21	0.45	m
9	0.95±0.21	0.73±0.13	1.68±0.09	3.15	2.44	1.29	0.44	m
10	0.84±0.15	0.66±0.16	1.50±0.07	2.80	2.19	1.28	0.44	m
11	0.78±0.19	0.55±0.12	1.34±0.09	2.60	1.85	1.40	0.41	m
12	0.66±0.15	0.48±0.09	1.14±0.07	2.19	1.61	1.36	0.42	m
13	0.58±0.12	0.37±0.08	0.95±0.08	1.92	1.23	1.56	0.39	m

Long arm(LA), Short arm(SA),total length (TL), Long arm percentage (%LA), Short arm percentage (%SA), arm ratio(AR),Centromeric index(CI) Karyotype Formula(K.F.), (m: metacentric)(sm: submetacentric).

Table 7. Measurements (µm) of somatic metaphase chromosomes of *satureja sahendica*(10).

ch.	LA±SE	SA±SE	TL±SE	%LA	%SA	AR	CI	K.F.
1	1.73±0.19	1.23±0.27	2.96±0.21	8.10	5.73	1.41	0.41	m
2	1.47±0.18	0.98±0.18	2.45±0.20	6.86	4.57	1.50	0.40	m
3	1.38±0.17	0.82±0.19	2.20±0.23	6.43	3.84	1.67	0.37	m
4	1.28±0.16	0.77±0.13	2.05±0.21	5.98	3.58	1.67	0.37	m
5	1.14±0.09	0.64±0.04	1.78±0.20	5.33	2.98	1.79	0.36	sm
6	1.10±0.05	0.56±0.08	1.66±0.22	5.14	2.64	1.95	0.34	sm
7	0.91±0.07	0.50±0.07	1.42±0.17	4.27	2.35	1.82	0.35	sm
8	0.78±0.09	0.50±0.05	1.28±0.11	3.65	2.35	1.55	0.39	m
9	0.79±0.10	0.46±0.06	1.25±0.13	3.70	2.16	1.71	0.37	sm
10	0.71±0.09	0.44±0.07	1.15±0.11	3.31	2.06	1.60	0.38	m
11	0.54±0.04	0.41±0.07	0.95±0.05	2.54	1.92	1.32	0.43	m
12	0.50±0.07	0.36±0.05	0.86±0.06	2.35	1.68	1.40	0.42	m
13	0.38±0.04	0.35±0.03	0.73±0.01	1.78	1.63	1.09	0.48	m
14	0.39±0.05	0.26±0.06	0.65±0.05	1.83	1.20	1.52	0.40	m

Long arm (LA), Short arm(SA),total length (TL), Long arm percentage (%LA), Short arm percentage (%SA), arm ratio(AR),Centromeric index (CI), Karyotype Formula(K.F.), (m: metacentric)(sm: submetacentric).

Table 8. Measurements (µm) of somatic metaphase chromosomes of *satureja sahendica*(38).

ch.	LA±SE	SA±SE	TL±SE	%LA	%SA	AR	CI	K.F.
1	1.86±0.10	1.30±0.20	3.16±0.23	8.04	5.64	1.43	0.42	m
2	1.47±0.10	1.28±0.12	2.77±0.08	6.35	5.53	1.15	0.47	m
3	1.46±0.10	1.01±0.09	2.47±0.18	6.33	4.38	1.44	0.41	m
4	1.27±0.04	0.88±0.23	2.15±0.16	5.50	3.80	1.45	0.41	m
5	1.16±0.10	0.75±0.13	1.91±0.17	5.04	3.23	1.56	0.39	m
6	1.09±0.09	0.60±0.05	1.69±0.20	4.71	2.62	1.80	0.36	sm
7	1.09±0.10	0.52±0.06	1.61±0.23	4.71	2.27	2.09	0.32	sm
8	0.85±0.12	0.62±0.04	1.47±0.10	3.70	2.67	1.38	0.42	m
9	0.82±0.10	0.46±0.01	1.28±0.15	3.56	2.00	1.78	0.36	sm
10	0.74±0.09	0.41±0.03	1.15±0.13	3.20	1.78	1.80	0.36	sm
11	0.63±0.04	0.39±0.03	1.03±0.10	2.76	1.69	1.63	0.38	m
12	0.55±0.06	0.37±0.04	0.92±0.07	2.40	1.60	1.50	0.40	m
13	0.46±0.03	0.36±0.02	0.82±0.04	2.00	1.56	1.29	0.44	m
14	0.38±0.02	0.29±0.01	0.67±0.04	1.65	1.24	1.33	0.43	m

Long arm(LA), Short arm(SA),total length (TL), Long arm percentage (%LA), Short arm percentage (%SA), arm ratio(AR),Centromeric index(CI) Karyotype Formula(K.F.), (m: metacentric)(sm: submetacentric).

Table 9. Measurements (µm) of somatic metaphase chromosomes of *satureja spicigera*(32).

ch.	LA±SE	SA±SE	TL±SE	%LA	%SA	AR	CI	K.F.
1	1.97±0.02	1.85±0.08	3.81±0.05	5.43	5.10	1.06	0.48	m
2	1.75±0.05	1.65±0.03	3.40±0.04	4.84	4.56	1.06	0.48	m
3	1.64±0.10	1.25±0.17	2.90±0.16	4.54	3.46	1.31	0.43	m
4	1.54±0.08	1.15±0.17	2.69±0.16	4.25	3.17	1.34	0.43	m
5	1.42±0.04	1.07±0.15	2.49±0.14	3.93	2.96	1.32	0.43	m
6	1.28±0.13	1.08±0.12	2.36±0.08	3.52	2.99	1.18	0.46	m
7	1.30±0.18	0.75±0.15	2.06±0.22	3.59	2.08	1.72	0.37	sm
8	1.17±0.14	0.72±0.16	1.89±0.18	3.22	1.99	1.62	0.38	m
9	1.04±0.06	0.70±0.12	1.75±0.14	2.89	1.95	1.48	0.40	m
10	0.96±0.08	0.70±0.15	1.65±0.11	2.64	1.92	1.37	0.42	m
11	0.98±0.10	0.51±0.07	1.49±0.19	2.69	1.42	1.90	0.34	sm
12	0.88±0.06	0.48±0.05	1.37±0.16	2.44	1.33	1.83	0.35	sm
13	0.76±0.10	0.48±0.04	1.24±0.11	2.10	1.33	1.57	0.39	m
14	0.77±0.12	0.38±0.03	1.15±0.16	2.13	1.05	2.03	0.33	sm
15	0.62±0.06	0.43±0.00	1.05±0.07	1.70	1.19	1.43	0.41	m
16	0.49±0.03	0.41±0.03	0.90±0.03	1.36	1.13	1.20	0.45	m
17	0.46±0.00	0.36±0.02	0.82±0.04	1.27	0.99	1.29	0.44	m
18	0.43±0.02	0.33±0.03	0.76±0.04	1.19	0.90	1.31	0.43	m
19	0.38±0.03	0.31±0.02	0.69±0.03	1.05	0.85	1.23	0.45	m
20	0.36±0.02	0.28±0.02	0.64±0.03	0.99	0.77	1.29	0.43	m
21	0.30±0.01	0.27±0.02	0.56±0.01	0.82	0.74	1.12	0.47	m
22	0.30±0.01	0.24±0.01	0.53±0.02	0.82	0.65	1.26	0.44	m

Long arm(LA), Short arm(SA),total length (TL), Long arm percentage (%LA), Short arm percentage (%SA), arm ratio(AR),Centromeric index(CI) Karyotype Formula(K.F.), (m: metacentric)(sm: submetacentric).

Table 10. Measurements (µm) of somatic metaphase chromosomes of *satureja spicigera* (35).

ch.	LA±SE	SA±SE	TL±SE	%LA	%SA	AR	CI	K.F.
1	2.16±0.18	1.62±0.06	3.78±0.22	6.25	4.67	1.34	0.43	m
2	1.94±0.13	1.34±0.08	3.28±0.24	5.60	3.88	1.44	0.41	m
3	1.85±012	1.27±0.07	3.12±0.24	5.34	3.67	1.45	0.41	m
4	1.69±0.19	1.05±0.11	2.74±0.26	4.89	3.02	1.62	0.38	m

5	1.34±0.04	1.06±0.05	2.41±0.11	3.87	3.07	1.26	0.44	m
6	1.29±0.02	0.89±0.09	2.19±0.16	3.73	2.57	1.41	0.41	m
7	1.29±0.11	0.76±0.06	2.06±0.21	3.73	2.21	1.69	0.37	m
8	1.10±0.10	0.77±0.05	1.87±0.13	3.17	2.22	1.43	0.41	m
9	1.15±0.15	0.63±0.03	1.78±0.21	3.32	1.81	1.84	0.35	sm
10	0.94±0.09	0.68±0.08	1.62±0.11	2.73	1.96	1.39	0.42	m
11	0.85±0.08	0.59±0.09	1.45±0.10	2.46	1.72	1.43	0.41	m
12	0.79±0.05	0.56±0.10	1.36±0.09	2.28	1.63	1.40	0.42	m
13	0.73±0.11	0.40±0.07	1.13±0.13	2.10	1.16	1.82	0.35	sm
14	0.56±0.05	0.45±0.06	1.02±0.05	1.63	1.30	1.25	0.44	m
15	0.50±0.04	0.35±0.04	0.85±0.06	1.45	1.01	1.44	0.41	m
16	0.45±0.02	0.32±0.03	0.77±0.05	1.30	0.92	1.42	0.41	m
17	0.44±0.03	0.25±0.02	0.69±0.08	1.27	0.71	1.79	0.36	sm
18	0.33±0.02	0.28±0.02	0.61±0.02	0.95	0.80	1.18	0.46	m
19	0.33±0.01	0.25±0.05	0.57±0.03	0.95	0.71	1.33	0.43	m
20	0.30±0.02	0.20±0.10	0.50±0.04	0.86	0.59	1.44	0.41	m
21	0.27±0.01	0.20±0.03	0.46±0.03	0.77	0.57	1.36	0.42	m
22	0.21±0.02	0.18±0.00	0.40±0.01	0.62	0.53	1.17	0.46	m

Long arm(LA), Short arm(SA),total length (TL), Long arm percentage (%LA), Short arm percentage (%SA), arm ratio(AR),Centromeric index(CI) Karyotype Formula(K.F.), (m: metacentric)(sm: submetacentric).

Table 11. Karyotype characteristics of nine populations of *satureja*.

POPULATION	2n	TL	%TF	DRL	VRC	A1	A2	DI	SC	K.F.
S. macrosiphonia (201)	24	1.814	40.081	12.145	1.814	0.316	0.426	17.633	1A	11m+1sm
S. macrosiphonia (202)	24	2.054	41.807	11.515	2.054	0.295	0.438	16.542	1A	9m+3sm
S. mutica(100)	26	1.876	39.231	12.717	1.876	0.363	0.514	17.251	1A	10m+3sm
S. mutica(44)	26	2.099	41.287	9.383	2.099	0.272	0.377	15.785	1A	13m
S. mutica(46)	26	2.312	43.927	12.310	2.312	0.233	0.473	18.356	1A	12m+1sm
S. sahendica(10)	28	1.529	38.657	7.537	1.529	0.352	0.452	16.622	1A	10m+4sm
S. sahendica (38)	28	1.650	39.989	11.180	1.650	0.330	0.469	17.286	1A	10m+4sm
S. spicigera (32)	44	1.647	42.536	9.108	1.647	0.261	0.587	21.068	1A	18m+4sm
S. spicigera (35)	44	1.576	40.834	9.757	1.576	0.292	0.642	25.625	1A	18m+4sm

2n: somatic chromosome number, TL: total length, %TF: total form percentage, DRL: Difference of Relative Length, VRC: Value of Relative chromatin, A1: Intrachromosome asymmetry index, A_2: Interchromosome asymmetry index, DI: Dispersion Index, SC: Symmetry classes of Stebbins, K.F.: Karyotype Formula (m: metacentric, sm: submetacentric).

Table 12. The results of analysis of variance for karyotypic data based on CRD design.

		Mean of squares												
Source of variation	Degrees of freedom	TL	LA	SA	AR	CI	LA%	SA%	TF%	DRL	VRC	A_1	A_2	DI
species	8	0.0027^{ns}	0.012^{ns}	0.016^{ns}	0.0045^{ns}	0.0004^{ns}	0.16^{**}	0.10^{**}	0.05^{ns}	0.11^{ns}	0.027^{ns}	0.005^{ns}	0.010^{*}	0.35^{ns}
Error	18	0.012	0.0006	0.0078	0.0039	0.0003	0.002	0.003	0.041	0.085	0.012	0.004	0.004	0.17
CV%		8.44	7.712	10.27	5.09	2.79	2.25	3.24	3.18	8.85	8.43	11.15	9.11	9.70

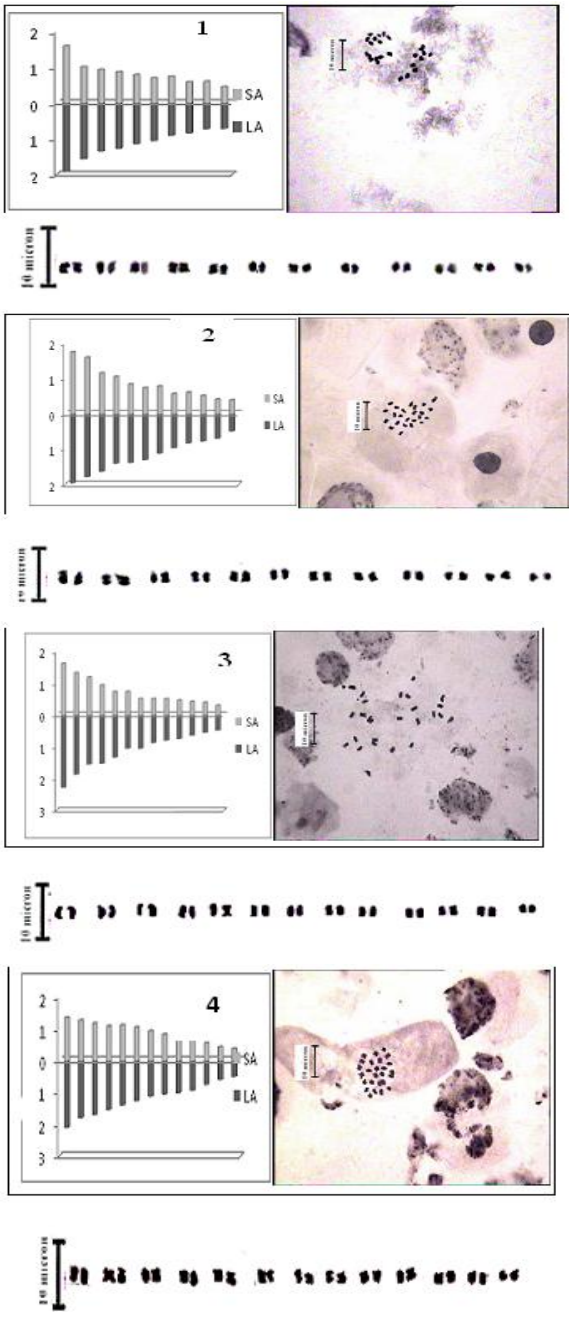
*-significant at 5% level of probability, **- significant at 1% level of probability, ns-Non significant.

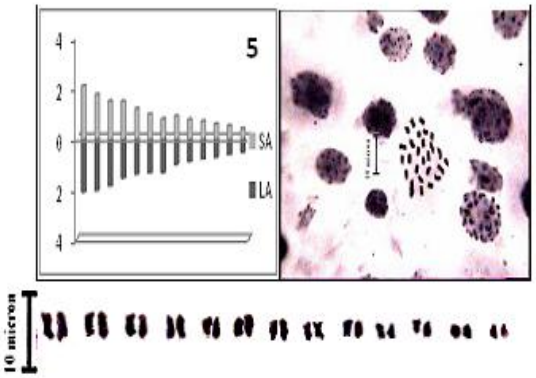
Table 13. Mean of chromosomes analysis of *Satureja* Species.

population	TL	LA	SA	AR	CI	%LA	%SA	%TF	DRL	VRC	A_1	A_2	DI
S. Mutica (46)	1.51 ± 0.26^{a}	1.13 ± 0.14^{a}	1.00 ± 0.12^{a}	1.16 ± 0.03^{a}	0.65 ± 0.00^{a}	2.07 ± 0.03^{d}	1.83 ± 0.03^{ab}	6.62 ± 0.42^{a}	3.49 ± 1.61^{a}	1.51 ± 0.26^{a}	0.48 ± 0.01^{a}	0.68 ± 0.08^{abc}	4.27 ± 0.26^{b}
S. Mutica(44)	1.44 ± 0.26^{ab}	1.10 ± 0.13^{ab}	0.92 ± 0.14^{ab}	1.20 ± 0.11^{a}	0.64 ± 0.12^{a}	2.12 ± 0.16^{cd}	1.78 ± 0.16^{abc}	6.42 ± 2.15^{ab}	3.06 ± 0.27^{a}	1.44 ± 0.27^{ab}	0.51 ± 0.05^{a}	0.61 ± 0.00^{c}	3.97 ± 0.27^{b}
S. Mutica(100)	1.36 ± 0.12^{ab}	1.06 ± 0.08^{ab}	0.85 ± 0.05^{ab}	1.27 ± 0.04^{a}	0.62 ± 0.00^{a}	2.16 ± 0.01^{abc}	1.73 ± 0.01^{cd}	6.26 ± 0.22^{ab}	3.55 ± 1.56^{a}	1.36 ± 0.12^{ab}	0.60 ± 0.01^{a}	0.71 ± 0.03^{abc}	4.15 ± 0.12^{b}
S. Macrosiphonea(201)	1.33 ± 0.30^{ab}	1.03 ± 0.13^{ab}	0.85 ± 0.18^{ab}	1.27 ± 0.15^{a}	0.62 ± 0.02^{a}	2.23 ± 0.22^{a}	1.82 ± 0.22^{ab}	6.32 ± 2.67^{ab}	3.46 ± 1.83^{a}	1.33 ± 0.30^{ab}	0.55 ± 0.06^{a}	0.64 ± 0.06^{bc}	$4.160.30\pm^{b}$
S. Macrosiphonea(20	1.43 ± 0.16^{ab}	1.09 ± 0.12^{ab}	0.92 ± 0.04^{ab}	1.22 ± 0.06^{a}	0.63 ± 0.01^{a}	2.20 ± 0.08^{ab}	1.86 ± 0.08^{a}	6.46 ± 1.07^{ab}	3.39 ± 0.60^{a}	1.43 ± 0.16^{ab}	0.54 ± 0.03^{a}	0.66 ± 0.01^{bc}	4.05 ± 0.16^{b}

2)													
S. Sahendica (38)	1.28±0.07^{b}	0.99±0.05^{ab}	0.81±0.04^{b}	1.25±0.07^{a}	0.63±0.01^{a}	2.06±0.12^{d}	1.68±0.12^{cd}	6.32±1.04^{ab}	3.34±0.20^{a}	1.28±0.07^{b}	0.57±0.03^{a}	0.68±0.04^{abc}	4.14±0.07^{b}
S. Sahendica (10)	1.23±0.12^{b}	0.96±0.07^{b}	0.76±0.05^{b}	1.27±0.04^{a}	0.62±0.00^{a}	2.09±0.02^{c} ^{d}	1.66±0.02^{d}	6.21±0.34^{b}	3.25±4.03^{a}	1.23±0.12^{b}	0.59±0.01^{a}	0.66±0.08^{bc}	4.04±0.12^{b}
S.Spiciger a(32)	1.28±0.09^{b}	0.97±0.05^{b}	0.83±0.04^{ab}	1.20±0.07^{a}	0.64±0.00^{a}	1.61±0.04^{e}	1.39±0.04^{e}	6.52±1.04^{ab}	3.01±0.58^{a}	1.28±0.08^{b}	0.50±0.02^{a}	0.76±0.00^{ab}	4.58±0.08^{ab}
S. Spicigera(35)	1.25±0.07^{b}	0.96±0.06^{b}	0.80±0.02^{b}	1.21±0.12^{a}	0.63±0.01^{a}	1.63±0.08^{e}	1.36±0.08^{e}	6.38±1.79^{ab}	3.12±0.37^{a}	1.25±0.06^{b}	0.53±0.04^{a}	0.80±0.02^{a}	5.06±0.06^{a}

The karyotype concept has been extensively used in characterizing and distinguishing chromosomes of different species. Mitotic karyotype analyses are also helpful in studying evolutionary problems (Gottschalk, 1972).





The results showed only minor differences in gross morphology of the karyotypes of *S. macrosiphonia*, *S. mutica*, *S. sahendica* and *S. spicigera* species. In order to refine the measure of karyotype asymmetry, we used Dispersion Index (DI) that has the potential to decipher even the minor karyotypic variations. The DI index plays an important role in arranging the species within the same class of karyotype asymmetry in an advancing order of specialization by permitting further gradations, as depicted by species arrangement within sections. Higher values of DI index would mean an enhanced order of karyotypic specialization. Karyotype of *S. mutica*(44) is characterized by symmetrical karyotypes and comparatively smaller mean haploid chromosome complement lengths and mean chromosome sizes. Only significant effect of LA%, SA% or A_2 among species proved that the nine populations have karyotypes that resemble very closely to each other except on total length of chromosomes. In view of the fact that, fewer DRL values illustrated more symmetry of karyotype, *S. sahendica*(10) and *S. mutica*(100) with DRL =7.53 and 12.71 have the most symmetric and asymmetric karyotypes respectively. Similarly, High DRL value leads to more changes in the construction of chromosomes; therefore there must be more changes in *S. mutica* (100) chromosomal construction.

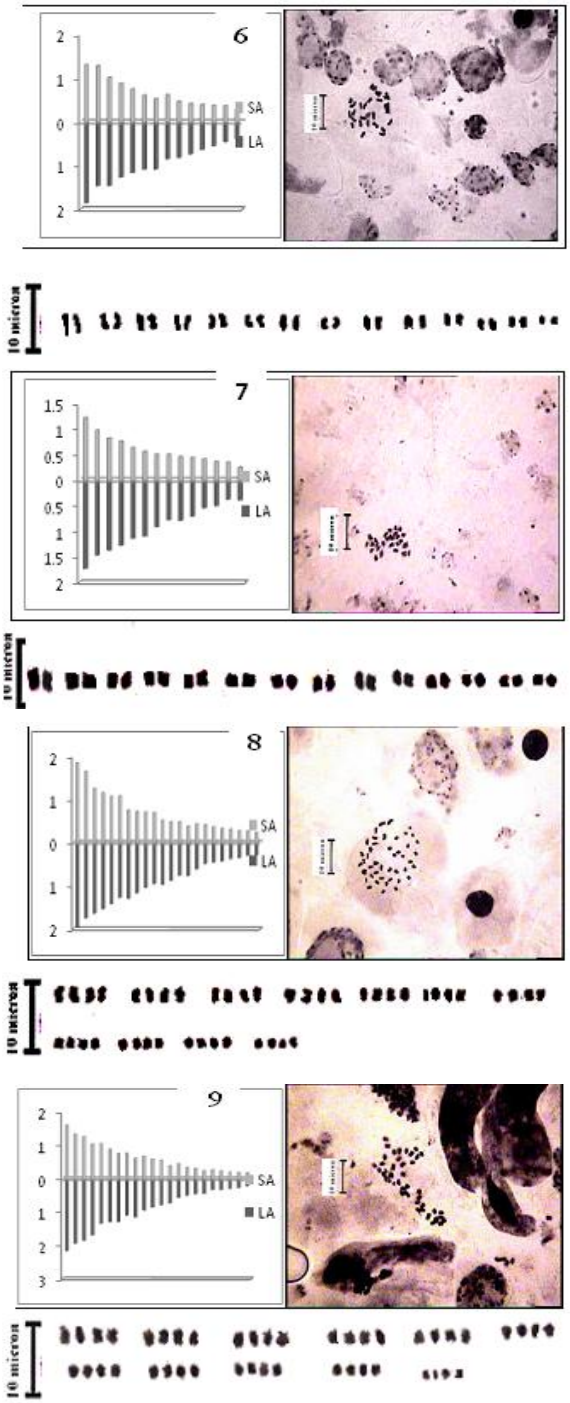


Fig. 1-9. Metaphase plates, ideogram and karyogram of somatic chromosomes of *S .macrosiphonia*(201) , *S. macrosiphonia* (202).), *S. mutica*(100), *S. mutica*(44), *S. sahendica* (10), *S. sahendica*(38) , *S. Spicigera* (35) , *S .spicigera* (32) respectively.

In conclusion, from the examination of chromosome number of the different species of *Satureja* it seems logical to conclude that the ploidy levels are different and the basic chromosome numbers should be differ among species. Such karyotypic similarities and cross ability/ genetic relationships raise an important curiosity as to whether there is any valid relationship between karyotypic similarity and cross ability in the genus *Satureja* and if this could give us any insight into genetic relationships. Thus, it is important that other endemic *Satureja* species also be studied to gain further understanding regarding genetic relationships and phylogeny in the genus.

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