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## Original Article

# Karyotype Analysis of Populations in Five *Satureja* Species from Iran

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**ABSTRACT**

A karyological study of five taxa (15 populations) of the genus *Satureja* L. from different geographic origins is presented. The haploid levels were different among species (n= 12 and 15). The work deals with chromosome number and morphometry. We found two of the usual basic chromosome numbers in this study for *satureja* species, x= 12 and x= 15. All populations were diploid and were located in 1B or 2B Stebbins classes. The karyotypic results showed the diversity among the species as mostly displayed median region (m) and submedian region (sm) and the chromosome lengths were determined between 0.60 µm in *Satureja sahendica* Bornm. (1041) to 2.27 µm in *S. macrantha* C.A.Mey. (3). Detailed karyotype analysis allows us to group the different populations and determine the relationships among them. It also pointed out the possibility of inter and intra-species cross to improve plants.

## INTRODUCTION

The genus *Satureja* is one of the most important genera of the Lamiaceae family. It is described in the Mentheae tribe (sub-tribe Menthinae ) belonging to the Nepetoideae subfamily and includes about 480 species in the world [1].This genus is represented in Iranian flora by 16 species, 10 of which are endemic for the country (*S. sahendica* Bornm., *S. edmondii* Briquet, *S. kallarica* Jamzad, *S. kermanshahensis* Jamzad, *S. khuzistanica* Jamzad, *S. bachtiarica* Bunge, *S. intermedia* C. A. Mey., *S. isophylla* Rech.f., *S. atropatana* Bunge and *S. rechingeri* Jamzad [2].

*Satureja* species are native to warm temperate regions and may be annual or perennial. They are subshrubs and low-growing herbs, reaching heights of 20- 50 cm, with flowers forming in whorls on the stem, white to pale pink-violet. They are commonly well known by native residents and are used by native inhabitants as medicinal plant, spice or source of essential oils [3-5].

In this study five different (four of which were endemic) taxa (15 populations) of the *Satureja* species including *S. macrantha* C. A. Mey, *S. sahendica*, *S. edmondi*, *S. kermanshahensis and S. khuzistanica* were examined.

*S. macrantha* is a small shrub distributed in western and northwestern parts of Iran. *S. sahendica* is a late flowering species (late summer and fall), growing on rock walls and rocky slopes and distributed in western and northwestern parts of Iran [6]. *S. edmondi* is mainly perennials and occupy mountainous habitats in northern and western parts of Iran. *S. khuzistanica*, is one of the important medicinal plants among the nomadic residents of southwestern of Iran and grows on dry, limestone

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rocky slopes poor in mineral content. This is a traditional herbal that uses a decoction of the aerial parts (mainly dried leaves) as toothpaste and oral disinfectant [7]. Finally, *S. kermanshahensis* species grows in crevices of rocks in the Kermanshah province in the west of Iran.

A large number of samples in each species and their drug trustees increase the importance of diversity studies in this genus and species. Within the *Satureja* genus the genetic diversity has been investigated using morphological characters [8,9] Enzyme electrophoresis [10,11] and molecular markers such as RAPD, SAMPL and AFLP [11-16]. Studying karyotypes and knowing the number of chromosomes is not only useful in predicting morphological similarity and diversity among *Satureja* species, but they are also valuable sources of taxonomic and biosystematics information.

The basic chromosome numbers that have been reported for the *Satureja* species is vary as follows: *S. multiflora,* x= 6 [17]; *S. acinos,* x=9 [18]; *S. vulgaris,* x= 10 [18]; *S. bulgarica* and *S. spicigera*, x=11, [19,20]; *S.macrosiphonia*, x=12 [20]*; S. mutica,* x=13 [20]; *S. sahendica,* x=14 [20]*; S. cristata, S. cuneifolia, S. bakhtiarica, S.rechingeri, S.spicigera***,** *S. montana*, x= 15 [19, 21-23]; *S. robusta*, x= 21 [24] and *S. hortensis,* x= 24 [25].

There was rarely a report to date on the detailed karyotype of these species in this research, also different and even inaccurate reports on the number of chromosomes of some of the studied species have been observed. Therefore, we have been tried to resolve the ambiguities in this research. Thus the karyotype analysis of some species in this report was the first time counted in Iran. 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, herbarium codes, geographical situation and species names are shown in Table 1. Vouchers are deposited in gene bank RIFR (Research Institute of Forest and Rangelands from Iran).

**Table 1** Geographical characteristics of *Satureja* species localities

| species | localities | Herbarium Code (RIFR) | Geographical Status | Altitude (m, a.s.l ) |
|---|---|---|---|---|
| *S. macrantha* C.A.Mey | Arasbaran | 3 | Northwest of Iran, North of East Azerbaijan province | 536 |
| *S. macrantha* C.A.Mey | Azerbayejan1 | 4 | Northwest of Iran | 657 |
| *S. macrantha* C.A.Mey | Azerbayejan2 | 17 | Northwest of Iran | 832 |
| *S. sahendica* Bornm. | Bijar | 1041 | Northeastof Kordestan province | 2200 |
| *S. sahendica* Bornm. | Gheydar | 1042 | South of Zanjan province | 2470 |
| *S. sahendica* Bornm. | Golabir | 1043 | Southwest of Zanjan province | 1805 |
| *S. sahendica* Bornm. | Soltanieh1 | 1044 | South of Zanjan | 1919 |
| *S. sahendica* Bornm. | Soltanieh2 | 1045 | South of Zanjan | 2032 |
| *S. sahendica* Bornm. | Sahand | 7 | South of Tabriz city | 2200 |
| *S. sahendica* Bornm. | Songhor | 8 | Northeast of Kermanshah | 2470 |
| *S. edmondi* Briq. | Kamyara | 770 | South of Sanandaj | 1320 |
| *S. edmondi* Briq. | Perav mountain | 771 | North of Kermanshah | 1480 |
| *S. edmondi* Briq. | Songhor | 772 | Northeast of Kermanshah | 1365 |
| *S. kermanshahensis* Jam. | Sumar | 601 | West of Kermanshah province | 1180 |
| *S. khuzistanica* Jam. | Lorestan | 705 | West of Iran | 486 |



### Chromosomal studies and data analysis

Root tip meristems obtained from seedlings were pretreated with 0.5% saturated α-Bromo naphthalene at 40C for 2.5h, fixed in 40% formaldehyde and 1% chromic acid (1:1) for at least 15 h at room temperature, then root tips were rinsed for 1 h in tap water. Hydrolysis was carried out with 1N NaOH at 60 ºC for nine minutes, dyed with Aceto- Iron-hematoxylin for 7-8h and squashed in a droplet of 45% acetic acid and lactic acid (10:1). Each of the prepared samples was observed with an optical microscope (BH2Olympus supplemented digital color video camera) at a magnification of 2020x. The best metaphase plates were selected and measured by Micro measure 3.3 software [26]. In each mitotic metaphase (at least five or six plates) the arm's length of each chromosome was measured and all parameters were estimated to characterize the karyotypes, according to the previous studies [20, 27-32].

Karyotype asymmetry was estimated by four different methods namely, the difference of relative length (DRL); total form percentage (TF%) [33]; intra-chromosomal asymmetry index ($A_1$) and inter-chromosomal asymmetry index ($A_2$) [34]. Both indices ($A_1$ and $A_2$) are independent of chromosome number and size. Dispersion index (DI) [35] and also karyotypic evolution by using the symmetry classes of Stebbins (SC) [36] has been determined. The karyotype formula was determined according to the classification of Levan [37]. For each population, Metaphase plates and karyogram of somatic chromosomes were adjusted based on length of chromosome size (arranged largely to small).

To determine the diversity between populations, one-way balanced ANOVA was performed on normal data and parameters mean were compared by Duncan's test.To evaluate the contribution of each karyotypic parameter to the ordination of species, principal component analysis (PCA) was performed.

Clustering was performed using Ward's cluster analysis method after calculation of the Cophenetic correlation coefficient (r) to examine karyotype similarity among populations. Numerical analyses were performed using JMP ver. 3.1.2, 1995 [38]; Statisti*XL* ver 1.8, 2007 [39] and SAS ver. 6.12, 1996 [40] soft wares.

## RESULTS

With relation to the plant materials assayed, Karyotype analyses of five species (15 populations) of *Satureja* were determined. Somatic cells of investigated species had 2n= 2x= 24 chromosomes for *S. kermanshahensis; S. macrantha* and 2n= 2x= 30 chromosomes for *S. sahendica; S. edmondi* and *S. khuzistanica* species. Metaphase plates and karyogram of somatic chromosomes for five species are illustrated in Figure 1. The average total chromosomal length of investigated species ranged from 0.60 to 2.27 µm. The somatic chromosome numbers (2n), ploidy levels, ranges of chromosome length, symmetry index percentage, intra and inter-asymmetry indices, the difference of range relative length, total form percentage, symmetry classes, total karyotype length and karyotype formula of the investigated taxa and populations are summarized in (Table 2).

The mean value of the chromosome's long arm was varied from 0.52 µm in *S. edmondi* (770) to 0.76 µm in *S. edmondi* (771). The averages of the chromosome's short arm were different from 0.39 µm in *S. sahendica* (1041) to 0.63 µm in *S. edmondi* (771). The mean value of chromosome's total length was varied from 0.95µm in *S. edmondi* (770) to 1.39 µm in *S. edmondi* (771) and finally, the mean value of chromosome's arm ratio was changing from 1.21 in *S. edmondi* (770) to 1.69 in *S. sahendica* (7) (Table 4).

All chromosomes were metacentric (m) in populations of *S. macrantha* (17) and *S. edmondi* (770,771 and 772) while some chromosomes in populations of *S. macrantha* (3 and 4), *S sahendica* (1041, 1042 1043,1044,1045,7 and 8), *S. kermanshahensis* and *S. khuzistanica* were metacentric (m) or sub-metacentric (sm) (Table 2).The dispersion index (DI) is calculated as a proportional measurement of the centromeric gradient to the coefficient of variation for chromosome length. The highest value of DI was found in *S. macrantha* (17) (14.81) and the lowest value of DI was found in *S. sahendica* (1042) (7.62) (Table 4). Symmetry type of (Stebbins 1971) [36] and asymmetry indices of (Romero-Zarco 1986) [34] are given in (Table 2). In terms of the Stebbins' system, the karyotype of populations seizes 1B and 2B classes, which are considered majorly primitive classes in this system. All populations of *S. macrantha, S. sahendica, S. edmondi* and *S. kermanshahensis* are classified as 1B group except of *S. macrantha* (3), *S. sahendica* (7) and *S. khuzistanica* species that stand as 2B category (Table 2).

Using the Romero-Zarco asymmetry indices of $A_1$ and $A_2$ we determined the more asymmetric karyotype among the populations which have similar Stebbins classes of symmetry. In the populations with 2B classes, *S. sahendica* (7) possesses the highest $A_1$ value (0.38) and the lowest TF% value (37.47). In the populations with 1B class, *S. edmondi* (770) possesses the lowest $A_1$ value (0.16) and the highest TF% value (45.40) (Table 2).

To analyze the variability of the karyotypes among populations, length of the chromosome, long and short arms of the chromosome, arm ratio values, centromeric index, long and short arm percent, relative length percent, the difference of relative length, total form percentage and intra-chromosome asymmetry index were compared by one-way analysis of variance based on completely randomized design (CRD). Also, the Duncan test was carried out to test the differences between each

pair of means. The results of variance analysis revealed significant differences between the populations based on eleven karyotypic characteristics ($p< 0.05$ and $p< 0.01$) (Table 3). The Duncan test applied to the chromosome morphometric traits showed a highly significant difference among all examined populations (Table 4). Therefore, mean values of chromosome total length varied from 1.40 µm in populations *S. edmondi* (771) to 0.95 µm in populations *S. edmondi* (770).

The mean values of chromosome long arms varied from 0.764µm in *S. edmondi* (771) to 0.518µm in *S. edmondi* (770). Also, the mean values of chromosome short arms were different from 0.632 µm in *S. edmondi* (771) to 0.365 µm in *S. sahendica* (7). Using principal components analysis (PCA), the first two independent components accounted for about 81.28% of the total variation.

The first component emphasized total length, long and short arms, arm ratio, centromere index, short arm percent, total form percentage and intra-chromosome asymmetry index which had the highest coefficients of Eigenvectors and were important characters for classification of populations with about 50.24% of total variation. Long arm percent, relative length percent and difference of relative length were important traits in the second component (31.04%) (Table 5).

Grouping of investigated populations was based on their karyotypic traits (Fig. 3). The results showed that populations of *Satureja* genus have been grouped in a separate cluster.

By cutting the dendrogram resulting from cluster analysis by Ward's method with cophenetic correlation coefficient ($r= 0.85$) with a metric distance of 3.11, the populations were classified into six groups.

The highest metric distance (8.99) was obtained between *S. macrantha* (3) and *S. sahendica* (1041), which implies the least affinity between them. The lowest metric distance (0.55) was obtained between *S. sahendica* (1041) and *S. khuzistanica*, which implies the least karyotypic difference between them. Due to the single population of the two species *S. kermanshahensis* and *S. khuzistanica.* In this paper, their position in the cluster ((Table 6) is based on the similarity of chromosome number and chromosome size are located next to the *S. macrantha* and *S. sahendic* species (Fig. 3).

The diagram of population dispersion, based on the first two components, showed that the populations were separated into six groups, which completely fits with results obtained through the grouping analysis by Ward's method (Fig. 2). In Figure 2, as can be seen, the species are classified into different categories and separated from each other based on about 81% of variance and the coefficients of the first and second components. *S. edmondi* are located in the lowest right area of the plot due to factors such as TL, LA, SA, AR, CI, SA%, TF% (based on traits with high coefficients in the first component compared to the second component). Also, *S. macrantha,* due to the relatively high trait coefficients in both, the first and second components, are located on the right and at the top of the plot. *S. sahendica* and *S. khuzistanica* are located on the left side of the plot according to the traits of LA%, RL % and DRL (traits with higher coefficients in the second component than the first component) (Table 5, Fig. 2). Populations of *S. macrantha* (17) and *S. edmondi* (770) are classified as separate groups. Also three populations of *S. macrantha* (3), *S. macrantha* (4) and *S. kermanshahens* together they formed a separate group, three populations of *S. sahendica* (1041), *S. sahendica* (7) and *S. khuzistanica* together they formed other separate group.

Two populations of *S. edmondi* (771) and *S. edmondi* (772) together they formed a separate group and finally, the remaining five *S. sahendica* (8, 1042, 1043, 1044 and 1045) together they were classified into separate groups. The population of *S. macrantha* (17) due to having the highest values of SA%, RL%, TF% and DI and the population of *S. edmondi*(770) due to having the highest values of CI, TF% and the lowest values of TL, LA, AR and $A_1$ were grouped into separate classes. The populations of *S. sahendia* (7 and 1041) were isolated from other populations of *S. sahendia* due to having significant differences in the amount of AR, TF% and DRL values and were grouped with *S. khuzistanica* species in the same cluster. The population of *S. edmondi* (770) has been separated from other populations of *S. edmondi* due to having significant differences in TL, LA and SA values (Table 4; Fig. 2).

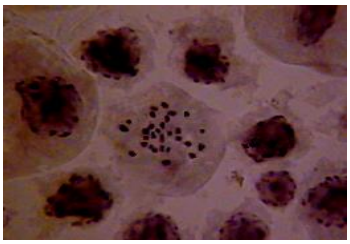
**Table 2** Somatic chromosome number (2n), ploidy level, ranges of chromosome length, Arm ratio (AR), asymmetry indices of Romero Zarco ($A_1$, $A_2$), Dispersion Index(DI), difference of range relative length (DRL), total form percentage (TF %), symmetry classes (SC) of Stebbins, total karyotype length (TKL) and Haploid karyotype formula (K.F.) (m: metacentric, sm: submetacentric)

| Population | 2n | Ploidy level | Chromosome length range | AR | $A_1$ | $A_2$ | DI | DRL | TF% | SC | TKL (µm) | K.F. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *S. macrantha*(3) | 24 | 2x | 0.72-2.27 | 1.51 | 0.35 | 0.37 | 14.49 | 10.45 | 38.90 | 2B | 28.92 | 10m + 2sm |
| *S. macrantha*(4) | 24 | 2x | 0.83-1.99 | 1.37 | 0.25 | 0.30 | 12.46 | 8.35 | 42.54 | 1B | 27.80 | 11m + 1sm |
| *S. macrantha*(17) | 24 | 2x | 0.73-2.21 | 1.26 | 0.19 | 0.34 | 14.81 | 9.60 | 44.75 | 1B | 30.86 | 12m |
| *S. sahendica* (1041) | 30 | 2x | 0.60-1.57 | 1.60 | 0.33 | 0.28 | 9.98 | 6.38 | 40.32 | 1B | 30.52 | 10m + 5sm |
| *S. sahendica* (1042) | 30 | 2x | 0.83-1.66 | 1.52 | 0.33 | 0.19 | 7.62 | 4.95 | 40.05 | 1B | 34.50 | 12m + 3sm |
| *S. sahendica* (1043) | 30 | 2x | 0.78-1.67 | 1.45 | 0.29 | 0.22 | 8.94 | 5.40 | 40.95 | 1B | 34.44 | 14m + 1sm |
| *S. sahendica* (1044) | 30 | 2x | 0.76-1.70 | 1.47 | 0.31 | 0.26 | 9.52 | 5.84 | 40.90 | 1B | 32.10 | 12m + 3sm |
| *S. sahendica* (1045) | 30 | 2x | 0.81-1.75 | 1.58 | 0.33 | 0.23 | 8.80 | 5.45 | 40.01 | 1B | 34.06 | 13m + 2sm |
| *S. sahendica* (7) | 30 | 2x | 0.66-1.58 | 1.67 | 0.38 | 0.27 | 9.97 | 6.28 | 37.47 | 2B | 29.10 | 8m + 7sm |
| *S. sahendica* (8) | 30 | 2x | 0.77-1.67 | 1.51 | 0.32 | 0.24 | 8.90 | 5.42 | 40.56 | 1B | 32.88 | 12m + 3sm |
| *S. edmondi* (770) | 30 | 2x | 0.64-1.59 | 1.21 | 0.16 | 0.27 | 11.31 | 6.68 | 45.40 | 1B | 28.48 | 15m |
| *S. edmondi* (771) | 30 | 2x | 0.92-2.07 | 1.22 | 0.17 | 0.23 | 10.04 | 5.58 | 45.20 | 1B | 41.90 | 15m |
| *S. edmondi* (772) | 30 | 2x | 0.89-1.99 | 1.26 | 0.20 | 0.21 | 8.67 | 5.73 | 44.66 | 1B | 38.34 | 15m |
| *S. kermanshahensis*(601) | 24 | 2x | 0.68-1.68 | 1.41 | 0.29 | .31 | 13.17 | 7.83 | 41.89 | 1B | 25.54 | 11m + 1sm |
| *S. khuzistanica*(705) | 30 | 2x | 0.71-1.75 | 1.57 | 0.34 | 0.27 | 9.21 | 6.68 | 39.43 | 2B | 31.62 | 10m + 5sm |

**Table 3** The results of analysis of variance for karyotypic data based on CRD design

| S.O.V | D.F. | TL | LA | SA | AR | CI | LA% | SA% | RL% | DRL | TF% | $A_1$ | $A_2$ | DI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Population | 14 | 0.45 $^{**}$ | 0.011 $^{**}$ | 0.016 $^{**}$ | 0.069 $^{**}$ | 0.002 $^{**}$ | 0.430 $^{**}$ | 0.384 $^{**}$ | 1.348 $^{**}$ | 8.058 $^{*}$ | 18.628 $^{**}$ | 0.014 $^{**}$ | 0.006 $^{ns}$ | 0.323 $^{ns}$ |
| Error | 30 | 0.011 | 0.003 | 0.002 | 0.015 | 0.0003 | 0.017 | 0.019 | 0.0147 | 3.760 | 3.536 | 0.002 | 0.004 | 0.169 |
| %C.V. | | 9.23 | 8.99 | 11.04 | 8.56 | 4.58 | 3.24 | 4.95 | 1.76 | 13.90 | 4.53 | 8.92 | 13.02 | 12.83 |

$^{**}$ and $^{*}$ significant at 1% and 5% levels of probability respectively; $^{ns}$: no significant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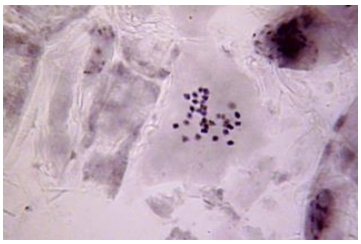
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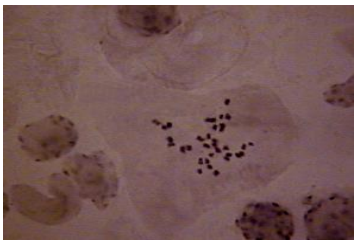
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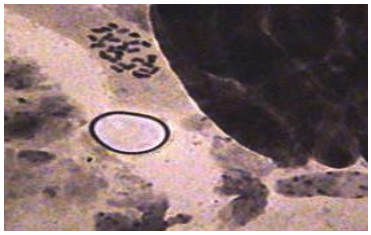
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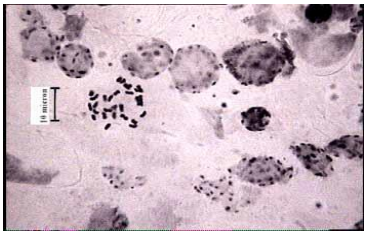
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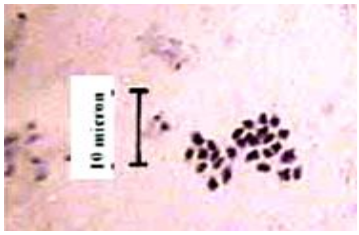
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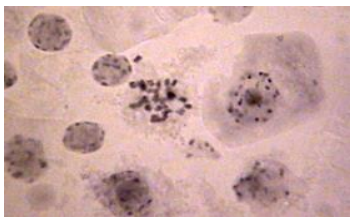
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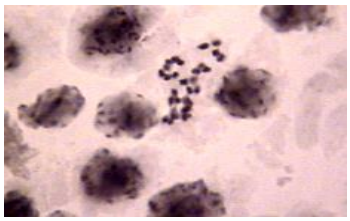
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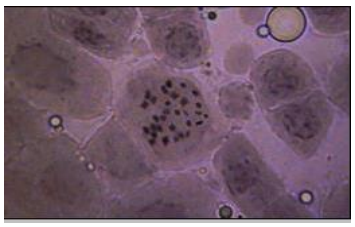
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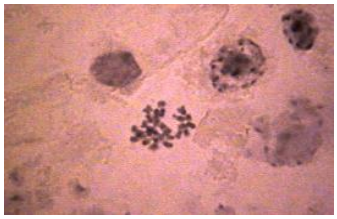
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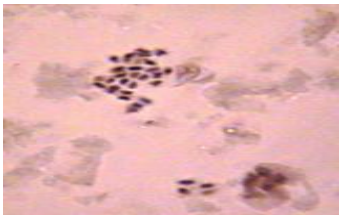
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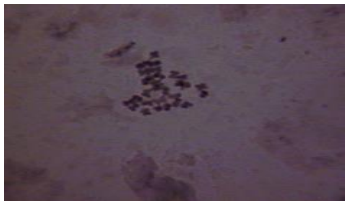
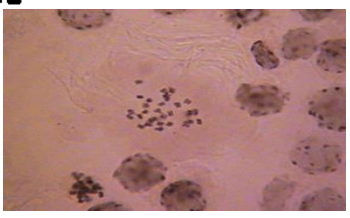
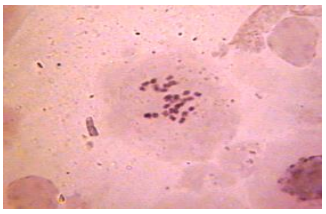

*S. edmondi* (771)


*S. edmondi* (772)


*S. kermanshahensis* (601)

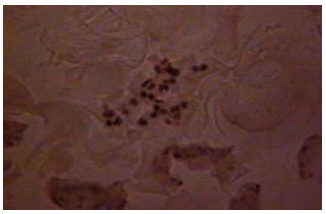

*S. khuzistanica* (705)

**Fig. 1** Mitotic metaphase of *Satureja* populations accompanied by karyograms.

**Table 4** Mean of parameters of chromosomes analysis of *Satureja* populations. TL: total length of chromosome, LA: long arm, SA: short arm, AR: arm ratio, CI: Centromeric index, LA%: long arm percent, SA%: short arm percent, RL%: Relative length percent; TF%: total form percentage, DRL: difference of relative length, $A_1$: intrachromosome asymmetry index, $A_2$: interchromosome asymmetry index, DI: Dispersion Index

In each column the same letters are not significantly different at P≤ 0.05

| Population | TL | LA | SA | AR | CI | LA% | SA% | RL% | TF% | DRL | A1 | A2 | DI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *S. macrantha*(3) | 1.205 bc | 0.719ab | 0.486bc | 1.507 ab | 0.396cde | 4.690a | 3.116bc | 7.862a | 38.909bc | 10.446a | 0.349ab | 0.369a | 14.492 ab |
| *S. macrantha*(4) | 1.158 bcd | 0.665ab | 0.492bc | 1.370bcde | 0.419abc | 4.615ab | 3.368a | 8.028a | 42.537ab | 8.346abc | 0.253bc | 0.304abc | 12.464abc |
| *S. macrantha*(17) | 1.286 ab | 0.710ab | 0.575ab | 1.260cde | 0.440ab | 4.402b | 3.496a | 7.928a | 44.753a | 9.604 ab | 0.193cd | 0.336ab | 14.807 a |
| *S. sahendica* (1041) | 1.017 cde | 0.621bc | 0.396cd | 1.605ab | 0.381de | 3.925c | 2.450d | 6.418b | 40.320bc | 6.378 bc | 0.326a | 0.278abc | 9.978abc |
| *S. sahendica* (1042) | 1.150 bcde | 0.689ab | 0.460cd | 1.525ab | 0.393cde | 3.943c | 2.585d | 6.563b | 40.065bc | 4.952c | 0.329ab | 0.186c | 7.616c |
| *S. sahendica* (1043) | 1.147 bcde | 0.674ab | 0.473c | 1.447bcd | 0.407bcde | 3.834cd | 2.655d | 6.521b | 40.972bc | 5.400c | 0.293ab | 0.216bc | 8.944bc |
| *S. sahendica* (1044) | 1.069 cde | 0.632b | 0.437 cd | 1.473abc | 0.402cde | 3.841cd | 2.607d | 6.481b | 40.900bc | 5.837c | 0.307ab | 0.261abc | 9.523abc |
| *S. sahendica* (1045) | 1.135 bcde | 0.692ab | 0.442cd | 1.583ab | 0.386cde | 3.964c | 2.517d | 6.515b | 40.010bc | 5.450c | 0.339ab | 0.231abc | 8.799bc |
| *S. sahendica* (7) | 0.969 de | 0.604bc | 0.365d | 1.692a | 0.372e | 4.015c | 2.410d | 6.464b | 37.669c | 6.284bc | 0.378a | 0.268abc | 9.968abc |
| *S. sahendica* (8) | 1.095 bcde | 0.649ab | 0.445cd | 1.516ab | 0.400cde | 3.901c | 2.618d | 6.534b | 40.630bc | 5.420c | 0.318ab | 0.235abc | 8.896 bc |
| *S. edmondi* (770) | 0.949 e | 0.518c | 0.430cd | 1.211e | 0.450a | 3.594e | 2.940c | 6.453b | 45.399a | 6.684bc | 0.163d | 0.273abc | 11.306abc |
| *S. edmondi* (771) | 1.396 a | 0.764a | 0.632a | 1.218de | 0.450a | 3.562e | 2.926c | 6.499b | 45.204a | 5.583c | 0.172d | 0.232abc | 10.038abc |
| *S. edmondi* (772) | 1.278 ab | 0.707ab | 0.570ab | 1.258cde | 0.441ab | 3.614ed | 2.911c | 6.540b | 44.663a | 5.732c | 0.207cd | 0.211bc | 8.674bc |
| *S.kermanshahensis*(601) | 1.064 cde | 0.618bc | 0.445cd | 1.406bcde | 0.413bcd | 4.646a | 3.305ab | 7.994b | 41.893ab | 7.831abc | 0.297ab | 0.307abc | 13.169ab |
| *S. khuzistanica*(705) | 1.053 cde | 0.636 b | 0.416cd | 1.579ab | 0.385cde | 3.904c | 2.482d | 6.429b | 39.460bc | 6.679bc | 0.345ab | 0.273abc | 9.213bc |

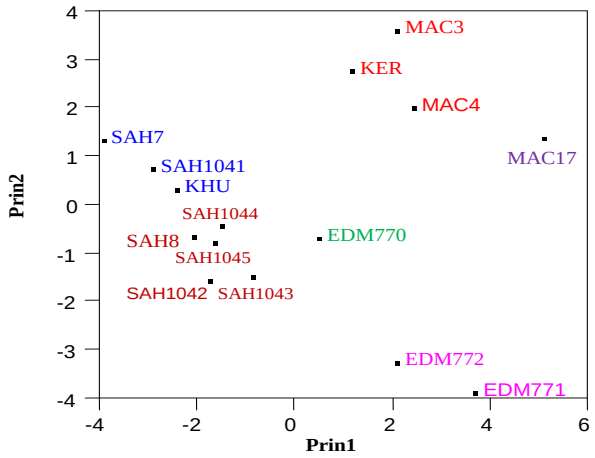



**Fig. 2** Scatter plot of 15 populations for the first two principal components

MAC: *S. macrantha*; EDM: *S. edmondi*; SAH: *S. sahendica*;

KER*: S. kermanshahensis*;KHU: *S. Khuzistanica*

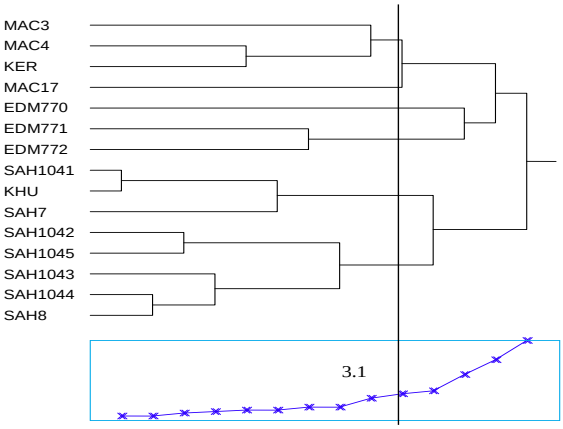



**Fig. 3** Dendrogram of 15 populations of *Satureja* species by analyzing 11 karyotipic parameters using

Ward's cluster analysis method. Cophenetic correlation r=0.85. MAC: *S. macrantha*; EDM: *S. edmondi*;

SAH: *S.sahendica*; KER*: S. kermanshahensis*;KHU: *S. khuzistanica*

**Table 5** Eigenvectors from the first two Principal components for 11 karyotype parameters to classify 15 populations of *Satureja* species.

| Parameters | First component | Second component |
|---|---|---|
| TL | 0.2798 | -0.1831 |
| LA | 0.1842 | -0.1287 |
| SA | 0.3257 | -0.2063 |
| AR | -0.3157 | 0.1713 |
| CI | 0.3103 | -0.1831 |
| LA% | 0.1137 | 0.4215 |
| SA% | 0.3386 | 0.1706 |
| RL% | 0.2418 | 0.3393 |
| TF% | 0.3148 | -0.1712 |
| DRL | 0.2134 | 0.3752 |
| $A_1$ | -0.3072 | 0.1805 |
| Eigen Value | 7.0337 | 4.3456 |
| Percentage of variance | 50.2407 | 31.0403 |
| Cum Percentage of variance | 50.24 | 81.28 |

**Table 6** Hierarchical Clustering, Method=Ward, Clustering History

| Number of Clusters | Distance | Leader | Joiner |
|---|---|---|---|
| 14 | 0.554562983 | SAH1041 | KHU |
| 13 | 0.6121416236 | SAH1044 | SAH8 |
| 12 | 0.9576944508 | SAH1042 | SAH1045 |
| 11 | 1.0888036049 | SAH1043 | SAH1044 |
| 10 | 1.2424650466 | MAC4 | KER |
| 9 | 1.2652000348 | SAH1041 | SAH7 |
| 8 | 1.5111192552 | EDM771 | EDM772 |
| 7 | 1.6540846005 | SAH1042 | SAH1043 |
| 6 | 2.5654195888 | MAC3 | MAC4 |
| 5 | **3.105471259** | **MAC3** | **MAC17** |
| 4 | 3.4513031617 | SAH1041 | SAH1042 |
| 3 | 5.2685470453 | EDM770 | EDM771 |
| 2 | 6.9404424054 | MAC3 | EDM770 |
| 1 | 8.9992772553 | MAC3 | SAH1041 |

## DISCUSSION

In this study, chromosome numbers and detailed measurements of five native different species (15populations) of *Satureja* genus were examined in Iran. The concept of karyotype has been used to identify and differentiate chromosomes from different populations. Mitotic karyotype analysis is also useful in studying evolutionary problems.

Contrary to the report [20], *S. sahendica* species of the same origin that was declared to have the number of 2n=28 chromosomes, this species has 2n=30 chromosome along with its different populations. Also, in this report, chromosomal details such as karyotype formula and symmetry classes of Stebbins of two *S. khuzistanica* and *S. sahendica* species are different from similar collection areas reported by (Shariat *et al*. 2013) [22]. In order to refine the measure of karyotype asymmetry, we used the DI value, which can decode decipher even minor karyotype changes. The DI index plays an important role in sorting species within the same class of karyotype asymmetry in a specialty advancing order by allowing further grading, as illustrated by the arrangement of species within sections.

The results of variance analysis based on karyotypic features indicated the occurrence of quantitative variation in chromosome size of the studied populations. A significant effect of chromosomal traits proved karyotype changes between populations. This shows the importance of chromosome study to distinguish the state of evolution and affinity between different populations. This study showed that populations of a particular species also have diversity within themselves. The important factors in the separation of populations were LA%, SA%, RL% and DRL values. The present study shows the change in chromosomal traits as one of the mechanisms of inter and intra-species diversification in the *Satureja* genus, as well as the earlier cytological reports [20, 22, 30, 41-43].

In conclusion, from the examination of the 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 differ among species. Investigation of different populations of different *Satureja* species showed that because different populations grow in different places with varying climate, height, soil, slope etc, therefore, geographical effects can probably be effective on the classification of karyotypic traits on species and populations belonging to them. The results of the molecular markers used among the 15 populations also showed a high diversity as well as the amounts of components of essential oils (data not shown). These genomic differences could be used for breeding purposes.Thus, these studies could greatly help us in classification and taxonomic studies.

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