Karyology of Iranian Endemic *Satureja* (Lamiaceae) Species

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Summary Savory is an annual or perennial semi-bushy aromatic plant cultivated for medicinal purposes. The karyology of five Iranian endemic *Satureja* (Lamiaceae) species collected from different locations was analyzed for the first time. In all species, the chromosome types were determined as mostly "m" and "sm". Four species (*Satureja bakhtiarica*, *S. khuzestanica*, *S. rechingeri*, and *S. sahandica*) were diploids with a karyotype formula of either 2n=2x=30m or 28m+2sm, falling into categories 1A and 1B according to Stebbins, while *S. specigera* was tetraploid (2n=4x=58m+2sm), falling into category 1B. Satellites were observed in all species. The mean chromosome length (TL) in diploids was 1.56 μm, and 1.35 μm in tetraploids. This small size (<3 μm) of the chromosomes, along with the presence of only two morphometric chromosome types and the predominance of metacentrics (m), confirm a relatively high degree of karyotypic symmetry. We believe that such data will enhance the karyological knowledge of the genus *Satureja*, and will prove to be an important source of information for new research concerning this genus.

Key words Chromosome, Cytogenetics, Karyotype, *Satureja*, Iran.

The *Satureja* species is known as "Marzeh" in Persian, and is commonly used as a spice and traditionally as a muscle pain reliever, tonic, and carminative in treating stomach and intestinal disorders such as cramps, nausea, indigestion, and diarrhea (Zargari 1990). The genus *Satureja* belongs to the family Lamiaceae, sub-family Nepetoideae, and the tribe Mentheae. Over 30 species of this genus are distributed in the eastern parts of the Mediterranean area (Cantino et al. 1992). They are annual or perennial semi-bushy aromatic plants that inhabit arid, sunny, stony, and rocky habitats (Slavkovska et al. 2001). Different species of *Satureja* are famous for their analgesic, antiseptic, antimicrobial, antiviral, antioxidant, antiproliferative, antiprotozoal, antifungal, antiarrheal, anti-inflammatory, antinociceptive, anticholinesterase, and vasodilatory activities. The valuable therapeutic aspects of this genus are mostly correlated with the existence of essential oils, flavonoids, and triterpenoids (Momtaz and Abdollahi 2010). The major constituents of the essential oils are phenols, carvacrole, and thymol (Sefidkon et al. 2004). The basic chromosome numbers that have been reported for the *Satureja* species vary as follows: x=6 for *Satureja multiflora* Briq (Krogulevich 1978), x=9 for *Satureja acinos* (Arohonka 1982), x=10 for *S. douglasii* Briq (Gill 1981), x=11 for *S. bulgarica* (Markova and Goranova 1995), x=15 for *S. coerulea* Janka (Markova 1989), x=21 for *S. robusta* Brenan (Morton 1993), and x=24 for *S. hortensis* L. (Gill 1981). No detailed information on the karyotype analysis of the investigated species was found in the literature. The present study was aimed to describe the number and morphological properties of the chromosomes of the Iranian endemic *Satureja* species.

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Materials and methods

Seeds of five Iranian endemic savory (Satureja) species were collected during the growing season of their natural habitats from different locations of Iran as described in Table 1.

Cytogenetic analysis

For the study of somatic chromosomes, root tips were obtained from germinated seeds in sterilized petri dishes. They were pre-treated in α-monobromonaphtalene (2h) and then fixed in ethanol: glacial acetic acid (3 : 1, v/v) for 24h (Karimzadeh et al. 2010, 2011). The root tips were then hydrolyzed with 1 N HCl at 60°C for 10 min, stained in hematoxilin reagent for 4h at 60°C, and finally squashed in 45% (v/v) acetic acid. Chromosome measurements were based on five metaphase plates. Slides were examined and microscopic photographs were taken using a Nikon Coolpix P90 digital camera interfaced to a BH2-RFCA Olympus microscope. Five mitotic metaphases were selected at random from each species (Paknia and Karimzadeh 2011). Nine chromosomal parameters were determined; we measured the long arm (L) and short arm (S) lengths, and calculated the total length of chromosomes (TL), form percentage of chromosome (F%=S/ΣTL×100), arm ratio (AR; L/S), r-value (S/L), relative length of chromosome (RL%=TL/ΣTL×100), total chromosome volume (TCV=πr²TL), where r=average chromosome radius, and the centromeric index (CI%=S/TL). Idiograms were drawn from mean values and chromosome types were determined using the formula of Levan et al. (1964). For karyotypic analysis, the following 10 parameters were calculated: number and location of satellite (SAT) pair, karyotype form total percentage (TF%=ΣS/ΣTL×100), total chromatin length (X=2ΣTL), symmetry index (S%=TLmin/TLmax×100), difference range of relative length (DRL=RLmax–RLmin), coefficient of variation [CV%=(total TL standard deviation/total TL mean)×100], dispersion index (DI=mean CI/CV), karyotype formula (KF=sum of chromosome types), Stebbins (1971) asymmetry categories (ST), and Romero-Zarco (1986) indices, which are intrachromosome asymmetry index (A1) and interchromosome asymmetry index (A2).

Statistical analysis

The karyotypic data were tested for normality and then were analyzed based on completely randomized design (CRD) with five replicate cells. Tukey’s test for mean comparisons and correlation analysis were carried out. Multivariate statistical analysis (Srivastava 2002) was performed on standardized data (mean=0, variance=1) using the Minitab 16 statistical software (Ryan and Joiner 2001). To evaluate the contribution of each chromosomal parameter to the ordination of genotypes, they were also subjected to principal components analysis (PCA) based on data matrix (Mirzaghaderi et al. 2010). Cluster analysis was carried out to examine chromosomal similarity among the genotypes. Clustering was performed using the unweighted pair-group method (UPGMA). Phenogram distortion was measured by computing the cophenetic correlation coefficient (r). Karyotype analysis was carried out according to the method described by Levan et al. (1964).
Results

Of the five Satureja species examined, four [S. bakhtiarica (S1), S. khuzestanica (S2), S. rechingeri (S3) and S. sahandica (S4)] were diploid (2n=2x=30), while the one [S. specigera (S5)] was tetraploid (2n=4x=60). ANOVA shows significant differences between the diploid species for all of the studied parameters except RL% (Table 2). Karyotypes of the somatic complement and the idiograms of the haploid complement of the studied Satureja species are shown in Figs. 2 and 3, respectively. There were significant differences among the species in their long and short arms of the chromosomes, total chromosome length (TL), r-value, form percentage, total chromosome volume (TCV), and centromeric index (CI) (Table 2). In the diploid species, the mean TL was 1.56 μm, varying from 1.35 μm (S4) to 1.95 μm (S3). The mean TCV was 0.75 μm³, ranging from 0.28 μm³ (S1) to 1.2 μm³ (S2). The mean CI of the complement varied from 42.2% (S2) to 45.68% (S1). In the tetraploid species (S5), the mean TL, TCV, and CI were 1.35 μm, 0.54 μm³, and 45.1%, respectively. The relationship between TL and CI of each species was plotted and illustrated in Fig. 4, showing three groups: S2, S3, and (S1, S4, S5). In all species, the types of chromosomes were determined to be mostly “m” (centromere at median region) and “sm” (centromere at sub median region), following the chromosome nomenclature of Levan et al. (1964). Hence, the karyotype formula was determined to be either 2n=2x=30m (S1, S3, S4) or 28m+2sm (S2) for the diploid species, and 58m+2sm for the tetraploid species (S5). According to various karyotypic symmetrical indices tested, the Satureja species presently studied showed different symmetrical groups. For exam-

<table>
<thead>
<tr>
<th>S.O.V.</th>
<th>Df</th>
<th>MS S</th>
<th>S L</th>
<th>TL</th>
<th>AR</th>
<th>r-Value</th>
<th>RL%</th>
<th>F%</th>
<th>TCV</th>
<th>CI%</th>
</tr>
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<tbody>
<tr>
<td>Species</td>
<td>3</td>
<td>50.204***</td>
<td>60.860***</td>
<td>58.545***</td>
<td>19.374***</td>
<td>19.135***</td>
<td>0.0583ns</td>
<td>5.2273***</td>
<td>98.865***</td>
<td>16.908***</td>
</tr>
<tr>
<td>Error</td>
<td>596</td>
<td>0.747</td>
<td>0.694</td>
<td>0.705</td>
<td>0.903</td>
<td>0.904</td>
<td>0.9997</td>
<td>0.9737</td>
<td>0.502</td>
<td>0.915</td>
</tr>
<tr>
<td>Total</td>
<td>599</td>
<td>CV% 29</td>
<td>27</td>
<td>28</td>
<td>30</td>
<td>31</td>
<td>33</td>
<td>32</td>
<td>23.5</td>
<td>31</td>
</tr>
</tbody>
</table>

ns: non-significant difference (p>0.05) and *** significant difference (p<0.001).

Fig. 1. Collection sites of Iranian endemic Satureja species on the map of Iran designed using GIS Microsoft.
ple, the highest value of total form percentage of karyotype (TF%) was detected in S1 (45.8%; Table 3; the most symmetric) and the lowest value was observed in S2 (41.98%; the most asymmetric). Meanwhile, the highest value of symmetry index (S%) was identified in S4 (49.85%; the most symmetric) while S3 demonstrated the lowest value (36.68%; the most asymmetric). The highest and the lowest values of difference range of relative length (DRL) were distinguished in S3 (12.57%; the most asymmetric) and S5 (10.7%; the most symmetric), respectively. The highest and
the lowest values of coefficient of variation (CV%) were identified in S5 (26.11%; the most asymmetric) and S3 (9.16%; the most symmetric), respectively. Similar to the results of S% and CV%, the highest value of dispersion index (DI) was found in S3 (4.89%; the most asymmetric) while S5 showed the lowest value (1.73%; the most symmetric). In conclusion, three (S%, DRL%, DI) among the five karyotypic symmetrical indices examined confirmed that among all five Satureja species examined, S3 and S5 appear to have the most asymmetrical and symmetrical karyotypes, respectively.

The karyotypes of three species (S1, S3, S4) were classified as 1A, and that of two species (S2, S5) were classified as 1B according to the Stebbins classification (Stebbins 1971) (Table 3). The Romero-Zarco (1986) indices, A1 and A2, were also calculated for detailed studies of asymmetry, showing three groups of species in the scatter diagram illustrated in Fig. 5. To determine the total variation in species and the parameters quota in total variation, the principal component analysis (PCA) of the karyotypic parameters was also performed, showing that the first three principal components account for 99.6% of the cumulative variation, and they were projected in a 2-dimensional graphic (Fig. 6). The UPGMA phenogram constructed on the basis of karyotype similarities (Fig. 7) shows three major clusters. The first cluster is comprised of S2, the second cluster of S1, S3, and S4, and S5 is separated in the third cluster. The PCA resulting species arrangement from this test fully fits that obtained with the UPGMA grouping analysis. Therefore, the results of this study suggest that the species within one cluster have the most homology in chromosomal variations.
Fig. 5. Scatter diagram of the Romero-Zarco asymmetry indices (A1, A2) of five *Satureja* species.

Fig. 6. Diagram resulting from principal components analysis one (highly related with the position of centromere) and two (strongly related with the relative chromosome length) of the studied *Satureja* species.

Fig. 7. Dendrogram showing the phenetic relationships among the studied *Satureja* species, constructed using unweighted pair group method with arithmetic averaging (UPGMA); cophenetic correlation=0.959.
Discussion

The chromosome number and detailed karyotypic characteristics of five Iranian endemic Savory (*Satureja*) species are being reported for the first time. The amount of data on chromosome numbers and karyotype of these species found in existing literature is scarce. In the present work, four of the *Satureja* species examined were diploid ($2n=2x=30$), which is in agreement with previous studies. These known diploid species reported were as follows: *S. horvatii* Silic (Papes and Silic 1981), *S. innota* (Lopez Gonzalez 1981), *S. macedonica* Form (Strid and Franzen 1981), *S. coerulea* Janka (Markova 1983), *S. cuneifolia* Ten (Markova 1989), *S. salzmannii* P.W. Ball (Morales 1990), *S. rouyana* Briq (Cardona 1991), *S. cristata* Nyman (Markova and Goranova 1995), *S. juliana* L. (Markova and Goranova 1995), *S. kitaibeli* Heuff (Markova and Goranova 1995), and *S. montana* L. (Boscaiu et al. 2000). In the present report, another *Satureja* species (*S. specigera*; S5) examined was tetraploid ($2n=4x=60$), which is in agreement with *S. hortensis* L. (Ferakova and Murin 1974; Gill 1981) but with a different chromosome number ($2n=4x=48$). Tetraploidy was the only polyploid level found in one of the species examined, which is considered to be the most successful condition among polyploids (De Wet 1980). The results of our present study prove that the basic chromosome number of the Iranian endemic *Satureja* species studied is $x=15$, which is in contrast to other *Satureja* species previously reported (Krogulevich 1978; Gill 1981; Arohonka 1982; Morton 1993), showing basic chromosome numbers of $x=9$, 11, 21 and 24.

The mean chromosome lengths of the Iranian endemic *Satureja* species ranged from $1.35 \mu m$ (*S. sahandica*; S4) to $1.95 \mu m$ (*S. rechingeri*; S3). The largest chromosome ($2.85 \mu m$) was observed in S3, and the smallest in *S. specigera* (S5, $0.85 \mu m$, Fig. 3). There were significant differences among the species in long length (TL), arm ratio (AR), $r$-value, total chromosome volume (TCV), and centromeric index (CI) (Table 2). Only two types of chromosomes ("m" and "sm") were obvious, showing karyotype formulas as typically "30m" in three diploid *Satureja* species (S1, S3 and S4), "28m+2sm" in one diploid species (S2), and "58m+2sm" in a tetraploid species (S5). Karyotypes of all species were symmetrical and fell in 1A and 1B of Stebbins classification. The asymmetric karyotype depending on the chromosome morphology can be linked to the evolutionary history of the taxa (Romero-Zarco 1986). Generally, the high intrachromosomal asymmetry index (A1) is considered as a specialized adaptation, while the interchromosomal asymmetry index (A2) may be directly related to the relative taxonomic distance between different taxa (Romero-Zarco 1986). The A1 index is variable between zero (all metacentric chromosomes) and one (all telocentric chromosomes), while the A2 index can vary between zero and infinite. It is related to the diversity in dimension of the chromosomes complement. The species *S. sahandica* (S4) had the smallest values of Romero-Zarco indices (Fig. 5), which is probably linked to its high capability to adapt to its habitat conditions, and hence is not particularly specialized. Three species, *S. bakhtiarica* (S1), *S. rechingeri* (S3), and *S. specigera* (S5), had intermediate values, and *S. khuzestanica* (S2) showed the largest value of Romero-Zarco indices, indicating that S5 may be a well-specialized species. The present results obtained by the assessment of the chromosome number and morphology of some Iranian endemic species of the genus *Satureja* growing naturally in different regions of Iran provide a remarkable contribution to the revision of the genus, the cultivation of economical and medicinal valuable species, and to the protection of gene reservoirs of endemic and threatened species, and act as a guide for future studies.

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References


