Karyotype Analyses on the Genus *Lallemantia* Fisch. & C.A.Mey. (Lamiaceae) from Turkey

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**Summary** In this research, *Lallemantia iberica* (Bieb.) Fisch. & Mey., *L. canescens* (L.) Fisch. & Mey. and *L. peltata* (L.) Fisch. & Mey. belonging to the *Lallemantia* Fisch. & C.A.Mey. genus (Lamiaceae) which is represented by three taxa growing naturally in Turkey, were studied. The somatic chromosome numbers observed in each taxa was 2n=14. The classification of chromosomes, the length of long and short arms, haploid chromosome length, arm ratio, centromeric index and relative chromosomal length were measured by Software Image Analyses (Bs200Pro). Also their karyotype asymmetry indices (TF%, As K%, Syi, Rec, A, A1 and A2) were calculated. According to the asymmetry index, *L. peltata* has a more symmetrical karyotype than *L. canescens*. This is the first study for account of chromosome counts and karyotypes for the studied taxa.

**Key words** *Lallemantia*, Karyotype, Lamiaceae, Turkey.

*Lallemantia* Fisch. & C.A.Mey. is a Caucasian originated genus distributing from Turkey to the Himalayas (Li and Hedge 1994, Harley *et al.* 2004, Govaerts *et al.* 2010). *Lallemantia* is distinguished from *Dracocephalum* L. with its upper lip of corolla and bracteoles. Its upper lip of corolla has two internal longitudinal folds and the bracteoles have distinctively 15-veins which are aristate-dentate. This genus is represented with five species in these regions and three of them grow in Turkey. The genus *Lallemantia* comprises annual and perennial species. *L. peltata* Fisch. & C.A.Mey. and *L. iberica* Fisch. & C.A.Mey. are annual and *L. canescens* Fisch. & C.A.Mey. is a perennial plant (Edmondson 1982).

*L. iberica* is known as "Ajdarbaşı" in Turkey (Güner *et al.* 2012). The leaves, oil and seed of this plant have been used as a reconstituent, stimulant, diuretic and expectorant, traditionally (Hedrick 1972, Aynechi 1986, Naghibi *et al.* 2005). The vernacular name of *L. canescens* is "Topajdarbaşı" and that of *L. peltata* is "Kalkanbaşı" (Güner *et al.* 2012). As *L. iberica* is distributed in a long area (from W (except NW) to E (except western and central Black Sea regions and Konya region), *L. canescens* is distributed only in the eastern Black Sea, Erzurum, Van, Hakkari and some province next to this area (Edmondson 1982).

In systematics, chromosome number is an important character for plant evolutionary studies and may provide some information about polyploidy and other highly significant genome changes (Guerra 2008, Louzada *et al.* 2010) or the benefits of plant chromosome number databases are a useful tool for systematic comparisons of geographical and taxonomical groups of plants (Peruzzi *et al.* 2012). Also chromosome counts can increase our understanding of phylogenetic relationships.
at different taxonomic levels (Yang et al. 2009).

In plant taxonomy the chromosome number and the chromosome morphology are progressively being used. These data are also considerable to clarify the origin, speciation and phylogenetic relationships of plants. Fundamentally, Stebbins classification (1971) has been used frequently for assessing karyotype asymmetry and to establish karyotypic relationship between different taxa (Stebbins 1971, Cai et al. 2004, Pavlova and Tosheva 2005, Eroğlu et al. 2013). The centromere position and the relative chromosome length are the most important karyotypic features which have allowed reasonable assessment of chromosomal affinities based on the concept of symmetry and asymmetry (Lavania and Srivastava 1992).

At this time, the chromosome number of Lallemantia baldshuanica Gontsch. and Lallemantia royleana (Benth.) Benth. has been reported as 2n=14 (Astanova 1984). The basic chromosome number was reported as x=7 (Raven 1975).

The purpose of the present study was to newly discover the chromosome numbers, karyotypes and karyotype asymmetry indices of three Lallemantia taxa from Turkey which had not been studied previously. The present study was taken to observe the karyotype asymmetry and symmetry among the taxa of Lallemantia and we reported the relationship among the present taxa based on karyological data.

Materials and methods

Chromosome counts were obtained from three of the members of the Lallemantia genus which were collected during field trips to different localities in Turkey (Table 1), and only mature plants with nutlets were collected. All samples germinated on wet filter paper in petri dishes which were pretreated in distilled water at 20±1°C for several days in Necmettin Erbakan University, Faculty of Science.

Pretreatment and preparation

For the chromosome studies, root tip meristems are used as experimental materials. After the nutlets germinated (1–1.5 mm), the materials was pretreated in α-monobromonaphthalene at 4°C for 16 h, fixed in 3:1 absolute alcohol–glacial acetic acid, then the root tips were hydrolyzed with 1 N HCl for 12 min at 60°C overnight and stained with 2% aceto orcein for 2 h at room temperature. Stained root tips were squashed in a drop of 45% acetic acid and permanent slides were made with the standard liquid nitrogen method; slides were dried for 24 h at room temperature and mounted in depex. The best metaphase photographs enlarged 10×100 were taken using an Olympus BX51 microscope with a digital camera Pixera PVC 100C attachment, and chromosome counts in mitosis metaphase were obtained usually determined from five different root tips from each individual.

Karyotype analysis

The classification of chromosomes, the length of long and short arms, haploid chromosome length, arm ratio, centromeric index and relative chromosomal length were measured by Software
Karyotype Analyses on the Genus Lallemantia Fisch. & C.A.Mey. (Lamiaceae) from Turkey

Image Analyses (Bs200Pro) loaded on a personal computer. Chromosomes were classified using the nomenclature of Levan et al. (1964). The classification of chromosomes as median (m), submedian (sm), subterminal (st) and terminal point (T) was based on the analysis of metaphase chromosomes. Ideograms of these taxa were arranged in decreasing length.

Karyotype asymmetry

For analysis of karyotype asymmetry, the following methods were used. To describe karyotype asymmetry and to determine the karyotypic relationships between species, Huziwara (1962) developed the total form percent (TF%), and then Arano (1963) developed another karyotype asymmetry index, the As K%. Greilhuber and Speta (1976) developed two indices to evaluate karyotype asymmetry which were called the Syi index and the Rec index by Venora et al. (2002). Zarco (1986) provided a different method to measure karyotype asymmetry which is the intrachromosomal asymmetry index (A1) and the other the interchromosomal asymmetry index (A2). Watanabe et al. (1999) defined degree of asymmetry of karyotypes (A), (Table 2).

Also, Stebbins (1971) distinguished 12 categories with respect to karyotype asymmetry, only 10 of which were known to occur in higher plants. He established these by recognizing three degrees of difference (A–C) between the largest and smallest chromosome of the complement, and four degrees (1–4) with respect to the proportion of chromosomes which are median pair with an arm ratio of less than 2:1 (Table 3).

Results and discussion

Lallemantia iberica

The mitotic metaphase chromosome number of Lallemantia iberica was identified as 2n=14
Chromosome lengths are 1.40–2.66 μm and arm ratios are between 1.13 and 1.70 in *L. iberica*. Karyotype formula is 6m+1sm. Centromeric index is between 4.33 and 7.59 and the relative lengths of chromosomes vary between 10.81 and 20.50. The total haploid chromosome length of the species is 12.98 μm. The karyogram (Fig. 1b) and the ideogram (Fig. 1c) were drawn based on centromeric index and given arranged in decreasing size order.

As for karyotype asymmetry, the karyotype of this taxon is classified to symmetry classes of Stebbins as 2A. The other karyotype asymmetry indices are 41%, 59%, 70, 70, 0.17, 0.28 and 0.22 for TF%, As K%, Syi, Rec, A, A1 and A2, respectively (Table 4).

**Lallemantia canascens**

The mitotic metaphase chromosome number of *Lallemantia canascens* was determined as 2n=14 (Fig. 2b). Chromosome lengths are 1.73–2.89 μm and arm ratios are between 1.18 and 1.73 in *L. canascens*. Karyotype formula is 6m+1sm. Centromeric index is between 4.63 and 7.11 and the relative lengths of chromosomes range between 11.06 and 18.52. The total haploid chromosome length of the species is 15.60 μm. The karyogram (Fig. 2b) and the ideogram (Fig. 2c) were drawn based on centromeric index and given arranged in decreasing size order.

As for karyotype asymmetry, the karyotype of this taxon is classified to symmetry classes of Stebbins as 2A. The other karyotype asymmetry indices are 41%, 59%, 70, 77, 0.17, 0.29 and 0.19 for TF%, As K%, Syi, Rec, A, A1 and A2, respectively (Table 5).

**Lallemantia peltata**

The mitotic metaphase chromosome number of *Lallemantia peltata* was determined as 2n=14 (Fig. 3b). Chromosome lengths are 0.99–2.10 μm and arm ratios are between 1.11 and 2.43 in *L. peltata*. Karyotype formula is 6m+1sm. Centromeric index is between 2.76 and 8.81 and the relative lengths of chromosomes range between 9.47 and 19.99. The total haploid chromosome length of the species is 10.51 μm. The karyogram (Fig. 3b) and the ideogram (Fig. 3c) were drawn based on centromeric index and given arranged in decreasing size order.

As for karyotype asymmetry, the karyotype of this taxon is classified to symmetry classes of

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**Table 4. Values of asymmetry indices in *L. iberica*.**

<table>
<thead>
<tr>
<th>Stebbins’ classification</th>
<th>TF%</th>
<th>As K%</th>
<th>Syi</th>
<th>Rec</th>
<th>A</th>
<th>A1</th>
<th>A2</th>
</tr>
</thead>
<tbody>
<tr>
<td>2A</td>
<td>41</td>
<td>59</td>
<td>70</td>
<td>70</td>
<td>0.17</td>
<td>0.28</td>
<td>0.22</td>
</tr>
</tbody>
</table>

(Fig. 1a).
Stebbins as 2B. The other karyotype asymmetry indices are 42%, 58%, 73, 71, 0.18, 0.29 and 0.25 for TF%, As K%, Syi, Rec, A, A1 and A2, respectively (Table 6).

In present study, three taxa were investigated belonging to *Lallemantia* which represented three taxa in Turkey. The basic chromosome number was determined as $x=7$ for the three taxa, and also the other two taxa were reported as $x=7$ (Raven 1975). By this time, the chromosome counts of two taxa belong to *Lallemantia* have been studied and have already been published in the world; *Lallemantia baldshuanica* and *L. royleana* were reported as $2n=14$ (Astanova 1984, Khatoon and Ali 1993). So our reports about *Lallemantia iberica*, *L. canascens* and *L. peltata* are in accordance with the previous reports.
with the basic chromosome number that supports this opinion is the same as the basic chromosome number and diploid chromosome number.

When we compare the karyotype asymmetry in genus *Lallemantia*, according to Stebbins (1971) classification, *L. iberica* and *L. canescens* are classified to symmetry classes of Stebbins as 2A. On the other hand, *L. peltata* is classified to symmetry classes of Stebbins as 2B. We can say that 2A class is more asymmetrical than 2B class or 2B class is more symmetrical than 2C class but we can’t determine which 2A class has higher symmetry. So Stebbins classification doesn’t clarify this situation and to determine the most symmetrical or asymmetrical karyotype we used other indices. The karyotype asymmetry was evaluated based on eight different parameters. Among these parameters, there are seven different quantitative indices. The TF%, Syi and Rec values decrease with increasing asymmetry and the As K%, the A1–A2 and the A values increase with increasing asymmetry (Zuo and Yuan 2011). According to the As K%, TF% and Syi indices, *L. peltata* has the most symmetrical karyotype. According to A and A1 indices, *L. iberica* has the most symmetrical karyotype and *L. peltata* has the most asymmetrical karyotype. The scatter diagram of populations’ dispersion was determined based on two components (A1–A2) (Fig. 4).

With this cytogenetical study we determined the chromosome morphology of *Lallemantia* taxa. The smallest chromosome length (0.99 μm) was observed in the taxon of *L. peltata*. In contrast, the biggest (2.89 μm) one was observed in the taxon of *L. canescens*. When it comes to the haploid chromosome length, *L. peltata* (10.51 μm) has the shortest and *L. canescens* (15.60 μm) has the largest one. The smallest and longest arm ratio was observed in *L. peltata* as 1.11–2.43. The smallest relative length value was measured in *L. peltata* (9.47) and the biggest relative value was measured in *L. iberica* (20.50). The metaphase chromosome pairs were usually determined as median and submedian type. In this study, the karyotype formulae were obtained as 6m+1sm for three taxa.

In the present research, somatic chromosome numbers, karyotype analysis and karyotype asymmetries of three *Lallemantia* taxa from the family of Lamiaceae were defined for the first time. We believe this study will play a positive role to enlighten this taxonomically revision genus. Determination of the number of chromosomes in the genus *Lallemantia* taxa also shed light on the opinion that further studies in this regard.

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References


