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Chromosome number and morphology of some accessions of four *Lallemantia* Fisch. & C.A. Mey. (Lamiaceae) species from Iran

Alireza Dolatyari & Asghar Kamrani

Summary: *Lallemantia* is a small genus comprising only five species. The upper corolla lip with two internal longitudinal folds differentiates the genus from closely related taxa. Uniform chromosome number and ploidy level have already been reported for the genus. In order to provide chromosomal data and to trace chromosome number changes both at inter- and intra-specific levels [Referee: Why do you expect any chromosome number changes?], we studied 10 accessions of four *Lallemantia* species from Iran. In accordance with previous results, we found $2n = 2x = 14$ for all the accessions studied; B-chromosomes were only found in one accession of *L. iberica*. In addition, detailed karyotypic data are presented for each accession. The mean value of centromere indices, 0.398, demonstrated the symmetric karyotype for the whole genus. Altogether, our data indicate that *Lallemantia* is a karyologically stable genus.

Keywords: B-chromosome, karyotype, *Lallemantia,* life form, Nepetoideae

The mint family (Lamiaceae) has a world-wide distribution and includes 7200 species (Bräuchler et al. 2010), many of which are aromatic and medicinal. Jamzad (2012) reported from Iran 46 genera in 5 subfamilies: Ajugoideae Kostel., Lamioideae Harley, Nepetoideae (Dumort.) Luerss., Scutellarioideae (Dumort.) Caruel and Viticoideae Briq. The Nepetoideae subfamily is the largest, including 27 out of 46 Iranian genera and it is characterized by the presence of hexacolpate three-nucleate pollens (Cantino & Sanders 1986) as well as the presence of rosmarinic acid (Harley et al. 2004). Molecular analyses support the circumscription of both the subfamily and the Mentheae tribe as monophyletic groups (e.g. Wagstaff et al. 1995; Wagstaff & Olmstead 1997; Walker et al. 2004; Bräuchler et al. 2005; Bräuchler et al. 2010; Drew & Sytsma 2012).

Lallemantia Fisch. & C.A. Mey. belongs to Nepetoideae, tribe Mentheae, subtribe Nepetinae and comprises only 5 species (Harley et al. 2004). It includes one perennial, *L. canescens* (L.) Fisch. & C.A. Mey., and four annual species, viz. *L. royleana* (Benth. in Wall.) Benth., *L. iberica* (Stev.) Fisch. & C.A. Mey., *L. peltata* (L.) Fisch. & C.A. Mey. and *L. baldshuanica* Gontsch. Representatives of the genus are distributed in the Mediterranean and in SW to Central Asia. All species are indigenous in Iran (Rechinger 1982; Jamzad 2012): *L. canescens* and *L. baldshuanica* are restricted to NW and NE Iran, respectively, but the other three species have wider distribution in Iran.

Historically, *Lallemantia* was classified as a subgenus of *Dracocephalum* by Budantsev (1993), but palynological (Dinç et al. 2009), morphological and molecular data (Wagstaff 1992; Harley et al. 2004; Trusty et al. 2004) support defining *Lallemantia* as a distinct genus. An upper corolla lip with two internal longitudinal folds and the mostly annual growth habit differentiate *Lallemantia* from *Dracocephalum*, which is mostly perennial and without corolla folds. Recently,

in a molecular phylogenetic study Drew & Sytsma (2012) demonstrated that *Dracocephalum* is not monophyletic and that at least *Hyssopus* L. and possibly *Lallemantia* are embedded within it.

In *Lallemantia,* the shape and size of bracteoles and flowers are key taxonomic features. Corolla size varies from 6–35 mm, the largest (25–35 mm) being found in the perennial *L. canescens* and the smallest (6–8 mm) in *L. royleana*. Members of the genus, especially *L. iberica*, are cultivated as medicinal plants for their essential oils and nutlets' mucilage (Estilai & Hashemi 1990; Baytop 1999).

Chromosomal change is an important mode of diversification and speciation in flowering plants (Weiss-Schneeweiss & Schneeweiss 2013), even if chromosome number diversification does not necessarily correlate with morphological diversification or vice versa (Frello & Heslop-Harrison 2000; Mandáková et al. 2010). Although there are many compensatory mechanisms and checkpoints ensuring the faithful transfer of genetic material to the next generations, errors leading to numerical and structural karyotypic variations and therefore evolutionary changes are inevitable (Weiss-Schneeweiss & Schneeweiss 2013). In addition, several plant species have been shown to have intraspecific numerical and/or structural karyotypic variants (Ebert et al. 1996; Choi et al. 2008; Souza et al. 2009), which in fact may constitute cryptic species. These facts imply that chromosomal analyses at intraspecific or even individual level can provide valuable taxonomic and evolutionary information.

In contrast to karyology, the phytochemical aspect of the Iranian *Lallemantia* taxa has been well studied (Ghannadi & Zolfaghari 2003; Sefidkon et al. 2006; Nori-Shargh et al. 2009; Amanzadeh et al. 2011; Zelatanov et al. 2012), although in the majority of the works only one species has been analyzed. Although chromosome numbers for all *Lallemantia* species have already been reported (Matveeva & Tikhonova 1968a, b; Matveeva & Tikhonova 1968b; Mehra & Gill 1968; Gill 1969; Chuksanova & Kaplanbekova 1971; Astanova 1984; Gill 1984; Khatoon & Ali 1993; Özcan et al. 2014), except for one accession of *L. royleana* (Aryavand 1977) no other Iranian population has been studied karyologically yet. The base chromosome number is $x = 7$, and only diploids have been reported for the genus. As closely related genera show high chromosomal variation (e.g. *Nepeta* with $2n = 16, 18, 30, 32, 34, 36, 54$; *Dracocephalum* L. with $2n = 10, 12, 14, 16, 18, 20, 24, 30, 32, 34, 36, 54, 70$: http://www.tropicos.org/Project/IPCN; Harley et al. 2004), chromosomal variation in *Lallemantia* might have been underestimated.

This is the first comprehensive karyological work including 10 accessions (four species) of the genus *Lallemantia* in Iran. Our main objectives are to 1) provide the chromosome number and karyo-morphometric data for four species of the genus, 2) look for intra-specific karyological variations, with studying at least two accessions per each species, 3) trace the probable numerical chromosomal changes throughout a widespread distribution range and 4) estimate the karyological data variations of annual and perennial life forms. [Referee: This aspect is not mentioned before in the introduction]

Materials and methods

Seeds and herbarium vouchers of 10 studied accessions were collected from natural habitats (Table 1). The vouchers are deposited in Tehran University Herbarium [TUH]. Seeds were germinated on one layer of wet filter paper. Roots with 1–2 cm length pretreated in 0.002 mol

Table 1. Localities and habit, karyotype formula and chromosome number of the studied accessions of *Lallemantia* sp. [TUH = Tehran University Herbarium].

Taxon	Locality	Habit	Karyotype formula	2n/ploidy level	Voucher No. [TUH]
L. canescens	Qazvin: Alamut, Pich-e Boon Vilage. N 36° 24' 48.0" E 50° 47' 02.0". 3000 m	perennial	8 m + 6 sm	14/2x	45858
L. canescens	Azerbaijan: N 38° 24' 16.9" E 44° 36' 54.0". 2400 m	perennial	10 m + 4 sm	14/2x	45859
L. iberica	Azerbaijan: Varzaghan. N 38° 29' 23.0", E 46° 29' 39.0". 2300 m	annual	2 M + 6 m + 6 sm	14/2x	45860
L. iberica	Qazvin: Takestan. N 36° 07' 38.0", E 49° 36' 44.0". 1580 m	annual	2 M + 8 m + 4 sm + 2 Bs	14 + 2 B/2x	45861
L. peltata	Tehran: Meygoon-shemshak road. N 35° 59' 58.4", E 51° 28' 47.0". 2400 m	annual	10 m + 4 sm	14/2x	45862
L. peltata	Qazvin: Tarum sofla, Ali Abad Vilage. N 36° 22.6' 01.0", E 49° 10' 10.3". 2800 m	annual	2 M + 6 m + 6 sm	14/2x	45863
L. peltata	Zanjan: Zanjan towards Abhar. N 36° 34' 02.4", E 48° 44' 15.5". 1800 m	annual	2 M + 8 m + 4 sm ^{1st}	14/2x	45864
L. royleana	Tehran: Tehran towards Saveh. N 35° 25' 23.0", E 50° 52' 11.0". 1050 m	annual	4 M + 10 m	14/2x	45865
L. royleana	Razavi Khorassan: Mashhad, Toroogh. N 36° 12' 15.0", E 59° 31' 18.0". 1070 m	annual	12 m + 2 sm ^{1st}	14/2x	45866
L. royleana	Esfahan: Mooteh protected area. N 33° 41' 56.1", E 50° 55' 39.0". 1900 m	annual	8 m + 4 sm + 2 st	14/2x	45867

Hydroxyquinoline solution for 3 h, then fixed in Carnoy' s I solution (absolute ethanol and glacial acetic acid, 3v : 1v) for about 3 h. At the next fixation step fresh fixative was used and roots were kept in the solution for about 21 h. Hydrolysis was carried out in 1N HCl at 60°C for 10 min. Roots were stained in aceto-orcein 2% solution for 3–5 h. Except cap root, the 1–2 mm tip roots were cut and squashed in a drop of the dye. The best metaphase digital images were taken using BX61 Olymus microscope equipped with DP25 digital camera at magnification of 1000× and 2000×. The chromosomes of at least five metaphase spreads per each accession were measured by Micromeasure 3.3 (Reeves & Tear 2000). The measured parameters and procedures for karyotype description are the same as in Zarco (1986) and Dolatyari et al. (2013). Index to plant chromosome numbers of Missouri Botanical Garden (http://www.tropicos.org/Project/IPCN) was used to assess the previous chromosomal works on the genus. In addition, we found more other reports in two indexes to plant chromosome numbers (Moore 1973; Fedorov 1974).

Results and discussion

The list of studied materials, locality data, chromosome numbers and karyotype formula of

Table 2. Karyological data of 10 studied *Lallemantia* sp. accessions. Abbreviations: TKL – total karyotype length, MAR – mean arm ratio, MVCI – mean value of centromere indices, DLSCL – difference between the longest and the shortest chromosome length. A_1 and A_2 – intrachromosomal and interchromosomal karyotype asymmetry indices, respectively.

Taxon	Province	TKL (µm)	MAR	MVCI	DLSCL	A_1	A_2
L. canescens	Qazvin	32.05	1.81	0.36	1.69	0.42	0.22
L. canescens	Azerbaijan	30.73	1.60	0.39	1.49	0.34	0.22
L. iberica	Azerbaijan	39.84	1.67	0.39	2.66	0.32	0.30
L. iberica	Qazvin	33.47	1.45	0.42	2.72	0.26	0.32
L. peltata	Tehran	29.08	1.60	0.39	1.92	0.34	0.31
L. peltata	Qazvin	29.16	1.56	0.40	1.48	0.31	0.24
L. peltata	Zanjan	33.37	1.59	0.39	1.84	0.35	0.27
L. royleana	Tehran	39.05	1.24	0.45	3.69	0.18	0.34
L. royleana	Razavi Khorassan	32.37	1.41	0.42	2.06	0.27	0.24
L. royleana	Esfahan	41.84	1.86	0.37	2.62	0.39	0.27

10 studied accessions are given in Table 1. Karyomorphometric data are presented in Table 2. Metaphase spread images are arranged in Fig. 1.

All studied taxa are diploid with $2n = 2x = 14$ and are characterized by the presence of comparatively small size chromosomes. Based on karyotype formula determined by centromere position (Levan et al. 1964), the most prevalent chromosome morphologies are metacentric and submetacentric and in a rare case subtelocentric (Table 2). The smallest chromosome, 1.27 µm, was found in *L. iberica* (Qazvin accession) and the largest one, 5.57 µm, in *L. royleana* (Tehran accession). Total karyotype length (TKL) varied from 29.08 µm in *L. peltata* (Tehran accession) to 41.48 µm in *L. royleana* (Esfahan accession). The mean value of centromere indices, 0.398, showed the symmetric karyotype in all studied accessions (Table 2). The most asymmetric intrachromosomal karyotype belonged to *L. canescens* (Qazvin accession, $A_1 = 0.423$) and the most symmetric one belonged to *L. royleana* (Tehran accession, $A_1 = 0.178$). Our results confirm previous reports for non-Iranian populations (references cited above) and only one Iranian report (Aryavand 1977) for the genus. We report B-chromosomes for the first time in the genus (*L. iberica*, Qazvin accession) [Referee: Rather, the presumed B-chromosomes are detached satellites (i.e. elongated and barely/non-visible secondary constrictions): see Zanjan accessions of L. peltata, where the secondary constriction is not elongated]. We believe [Referee: That's not a question of believe: either you consider them too little magnified or not] that the magnification of metaphase images of Özcan et al. (2014) has not been enough to show exact karyotypic variations, so we avoided comparing our results to theirs, except for total karyotype length (TKL) which would not depend on the centromere position. At present, *L. baldshuanica* Gontsch is the only species without karyomorphometric data. The karyological results and chromosomal literature reviews for each species are presented in alphabetical order.

***L. canescens* (L.) Fisch. & C.A. Mey.**

$2n = 2x = 14$ is reported for two studied accessions (Table 1, Fig. 1a, b). Our count is in accordance with two previous reports of this species (Matveeva & Tikhonova 1968b, Özcan et al. 2014). Amongst the studied accessions, the most intrachromosomal asymmetry ($A_1 = 0.423$) and the

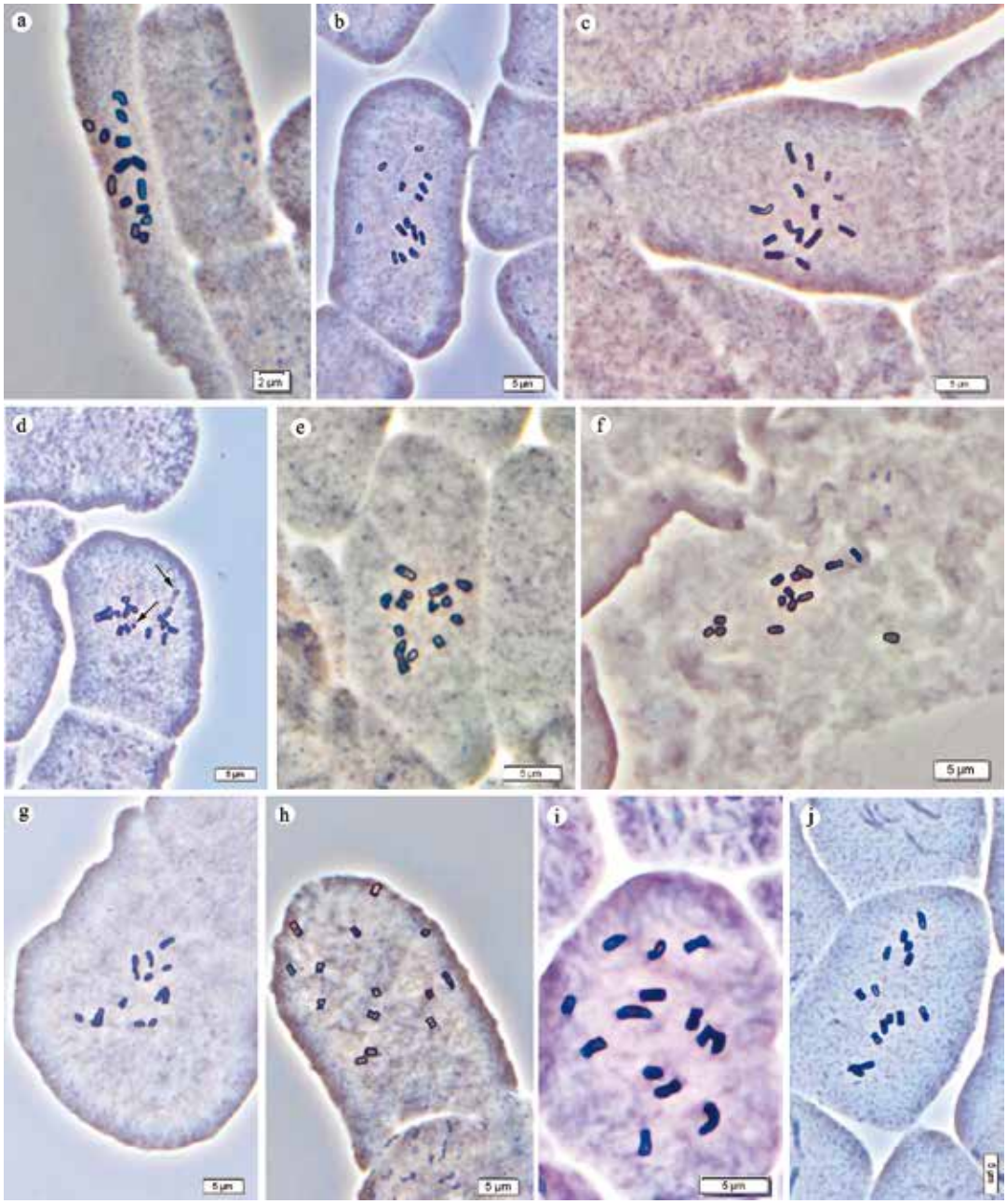

Figure 1. Metaphase plates of *Lallemantia*. All accessions show 2n = 2x = 14. a – *L. canescens* (Azerbaijan accession); b – *L. canescens* (Qazvin accession); c – *L. iberica* (Azerbaijan accession); d – *L. iberica* (Qazvin accession), arrows show B-chromosomes; e – *L. peltata* (Tehran accession); f – *L. peltata* (Qazvin accession); g – *L. peltata* (Zanjan accession); h – *L. royleana* (Razavi Khorassan accession); i – *L. royleana* (Esfahan accession); j – *L. royleana* (Tehran accession).

most interchromosomal asymmetry (A_2 = 0.216) were found in Qazvin accession of the species. This is the first chromosome report for the species from Iran. Total karyotype length (Table 2) of the perennial species is equal and even lower than annual species.

***L. iberica* (Stev.) Fisch. & C.A. Mey.**

We report $2n = 2x = 14 + 2Bs$ in Qazvin accession (Fig. 1d), and $2n = 2x = 14$ in Azerbaijan accession (Fig. 1c). Matveeva & Tikhonova (1968b) and Özcan et al. (2014) reported $2n = 14$ for the species, we report B-chromosomes for the first time in the species [Referee: See comment above: leaky no B's]. No Iranian population has been previously studied karyologically.

***L. peltata* (L.) Fisch. & C.A. Mey.**

We found the same chromosome number, $2n = 2x = 14$, in all three studied accessions (Table 1; Fig. 1e, f, g). In Zanjan accession one submetacentric chromosome with secondary constriction was found (Table 2). Our results confirm the two previous chromosome reports for non-Iranian samples of the species (Matveeva & Tikhonova 1968b, Özcan et al. 2014).

***L. royleana* (Benth. in Wall.) Benth. in DC.**

In three studied accessions, the same chromosome number, $2n = 2x = 14$, is reported (Table 1, Fig. 1h, i, j). Also, the identical chromosome number has been reported for other non-Iranian and Iranian populations of the species (Chuksanova & Kaplanbekova 1971; Aryavand 1977; Astanova 1984; Gill 1984; Khatoon & Ali 1993; Mehra & Gill 1968; Gill 1969; Matveeva & Tikhonova 1968a). In Razavi Khorassan accession, secondary chromosome constriction on one submetacentric chromosome was found.

Conclusions

In accordance to previous karyological works (Özcan et al. 2014 and other references cited above), no ploidy level variation or numerical chromosome changes (aneuploidy, B-chromosome and dysploidy) were found in spite of expanding sampling to the intraspecific level, except the occurrence of two B-chromosomes in one accession of *L. iberica*. The size of B-chromosomes is usually smaller than of A-chromosomes, at least much smaller than the largest A-chromosomes (Parker et al. 1991). The two B-chromosomes found were small (Fig. 1d), so it was impossible to identify chromosome arms via light microscopy. The mean number of centromeric indices, 0.398, demonstrated intrachromosomal symmetry for the whole genus. Altogether, our data indicate that *Lallemantia* is a karyologically stable genus in contrast to the closely related genus *Dracocephalum*, which shows extensive chromosomal number variation (Harley et al. 2004).

Based on uniform chromosome base number and level as well as on low potential of speciation (only 5 species), one can conclude that chromosomal changes would have a trifling role in the evolution of this genus. Short evolutionary history, self compatibility and small growth size, which probably caused these taxa less attractive for related pollinators, could be counted as major obstacles for high out-crossing, as one of the main factors of genetic diversification and therefore as the reason for such chromosomal stability. In the future, molecular taxonomical works would likely determine confidently the taxonomic position of the genus, probably in *Dracocephalum*, and so may change our view about the genus' stable chromosomal history. Molecular and pollination biological studies will be needed for testing each of these views.

We couldn't deduce meaningful relations between intraspecific variations in karyotypic parameters and morphological as well as biogeographical data. Beside the likely interpopulation chromosomal distinctions, we should keep in mind that these small intraspecific karyotypic variations observed can be due to unwanted measuring errors especially accompanying measurement of small size

chromosomes, somewhat subjective determination of the chromosome type especially in sub- (meta- or telocentric) configurations, and/or they can be due to differences in substages of the metaphase plates selected for analysis.

Our data show that TKL in perennial species (*L. canescens*) is a bit lower than in some annual species (Table 2). Özcan et al. (2014) estimated a slightly larger total haploid chromosome length in *L. canescens* than in the annual species. Comparing the amount of this parameter in the work to our results showed negligible differences between the annuals and the perennial species. We conclude that in the genus no considerable difference in TKL occur and that there is no relation between this parameter and life form change in contrast to the hypothesis of Rees & Naryan (1981) that a direct relationship exists between increasing genome size and life cycle duration. Dinç et al. (2009) indicated that the shape of *Lallemantia* pollen grains also has no taxonomic value and is not useful for species demarcation. However, both inter- and intra-chromosomal asymmetry indices and exine ornamentation of pollen grains (Dinç et al. 2009) distinguished *L. canescens* species from the annual species, although these features are more or less identical among the annuals.

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Addresses of the authors:

Dr Alireza Dolatyari
Department of Biology
Faculty of Science, University of Guilan
Rasht
Iran

Cytogenetics Laboratory
Plant Bank
Iranian Biological Resource Centre (IBRC)
ACECR Tehran
Iran

Dr Asghar Kamrani
The Center of Medicinal Plants Researches

Shahed University
Tehran
Iran
E-mail: kamrani@shahed.ac.ir