Araştırma Makalesi / Research Article

Karyotype Analysis of *Lallemantia* Fisch. & C.A.Mey. Species Grown in Turkey: A Detailed Karyotype Asymmetry Study

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Abstract
The study aimed to karyologically analyse three species [(*Lallemantia peltata* (L.) Fisch. & Mey., *Lallemantia iberica* (Bieb.) Fisch. & Mey. and *Lallemantia canescens* (L.) Fisch. & Mey.)] from *Lallemantia* Fisch. & C.A.Mey. (Lamiaceae) grown in Turkey. Also, it was calculated various karyotype asymmetry and S/A_I, CV_{CL} and M_C values in this study. Seed samples were given natural habitats and Feulgen staining method was used. The study showed that the chromosome numbers of *Lallemantia* species are 2n=14 and that they have median (m) and submedian (sm) centromeric chromosomes. The study also demonstrated karyotype analysis and asymmetry values and suggested that three species of *Lallemantia* genus were 2A based on Stebbins classifications. Furthermore, the study showed Pearson correlation using karyotype asymmetry values and a scatter diagram was formed using A_1 and A_2. The results obtained from the study were compared with the results of karyotype analysis performed by different literatures and it was concluded that there may be differences according to locality.

Keywords: Chromosome, *Lallemantia*, Karyotype Asymmetry, Karyotype Formula, Stebbins Classification.

Türkiye'de Yetişen *Lallemantia* Fisch. & C.A.Mey. Türlerinin Karyotip Analizleri: Detaylı Karyotip Asimetri Çalışması

Öz
Bu çalışma Türkiye'de yetişen *Lallemantia* Fisch. & C.A.Mey. cinsine ait üç türün [*Lallemantia peltata* (L.) Fisch. & Mey., *Lallemantia iberica* (Bieb.) Fisch. & Mey. and *Lallemantia canescens* (L.) Fisch. & Mey.)] karyolojik analizinin yapılmasını amaçlamıştır. Ayrıca, bu araştırmada çeşitli karyotip asimetrisi ve /A_I, CV_{CL} and M_C değerleri de hesaplanmıştır. Tohum örnekleri doğal habitatlarından toplanmış ve Feulgen boyama metodu kullanılmıştır. Bu çalışma, *Lallemantia* türlerinin kromozom sayılarının 2n=14 olduğunu ve median ve submedian sentromer kromozomlara sahip olduklarını bulmuştur. Ve ayrıca, bu çalışma *Lallemantia* cinsine ait üç türün Stebbbins sınıflandırmasına göre 2A grubunda olduğunu ileri sürmüş ve karyotip analizi ve asimetri değerlerini göstermiştir. Bundan başka, bu çalışma karyotip asimetri değerlerini kullanarak Pearson korelasyonunu ve A_1 and A_2'yi kullanarak saçılım grafiğini göstermiştir. Çalışmadan elde edilen sonuçlar, farklı literatürler tarafından yapılan karyotip analizlerinin sonuçlarıyla karşılaştırılmış ve lokailtelere göre farklılıklar olabileceği sonucuna ulaşılmıştır.

Anahtar Kelimeler: *Lallemnantia*, Karyotip Asimetrisi, Karyotip Formulü, Stebbins Sınıflandırması.

1. Introduction

Lamiaceae, represented by about 236 genera and 7000 taxa, is distributed throughout the world but especially in the Mediterranean. The endemism ratio of the family is 45% [1-3]. In the Flora of Turkey,

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the family has 45 genera and 735 taxa [1]. The subtribe Nepetinae comprises 12 genera and 350 taxa and is distributed in Eurasia and North America [4].

Lallemantia, a small genus from the Nepetinae, includes five species and is found in Europe, southwest Asia and the Himalayas [5]. The species from *Lallemantia* are annual or biennial plants that are used as food or for medicinal purposes [6]. In Turkey, *Lallemantia* (Ajdarbaşı) is represented by three species: *L. canescens* (topajbaşı), *L. peltata* (kalkanbaşı) and *L. iberica* (ajdarbaşı*)* [7]. *L. iberica,* known as Dragon's head, is cultivated for the high oil content of its seeds [8]. *L. canescens* has blue flowers and an attractive smell and is used ornamentally in gardens while *L. peltata* with its volatile oils is used as a medicinal plant [1,9]. *L. iberica* and *L. peltata* are annual herbs whilst *L. canescens* is perennial [10].

Chromosome studies are used in plant systematics to contribute to taxonomical knowledge. They can also be used for geographical and taxonomical comparisons [10]. Karyological studies showed that *Lallemantia* species have 2n=2x=14 chromosomes [11,12]. Similarly, Ozcan et al. [10] found that three *Lallemantia* species grown in Turkey at different localities to those in this study had 2n=14 chromosomes. This study aimed to karyologically analyse three species of *Lallemantia* (*L. peltata*, *L. iberica* and *L. canescens*) grown in Turkey and to compare the results with various studies from different localities.

2. Material and Method

2.1. Materials

The samples were gathered from natural habitats in Turkey in 2012-2013 and stored at the Bitlis Eren University Herbarium (BEUH) (Table 1, Figure 1).

Table 1. Localities of *Lallemantia* studied

Locality	Voucher number
B9, Bitlis, Bitlis Eren University, Rahva campus, north slopes, 2600 m, 12.08.2012	*Kursat* 6002
B7, Elazığ; Baskil, Bolucuk village, 1480 m, 12.09.2013.	*Kursat* 6005
B9, Bitlis, Nemrut mountain, steppes, 2290 m, 12.08.2012.	*Kursat* 6001

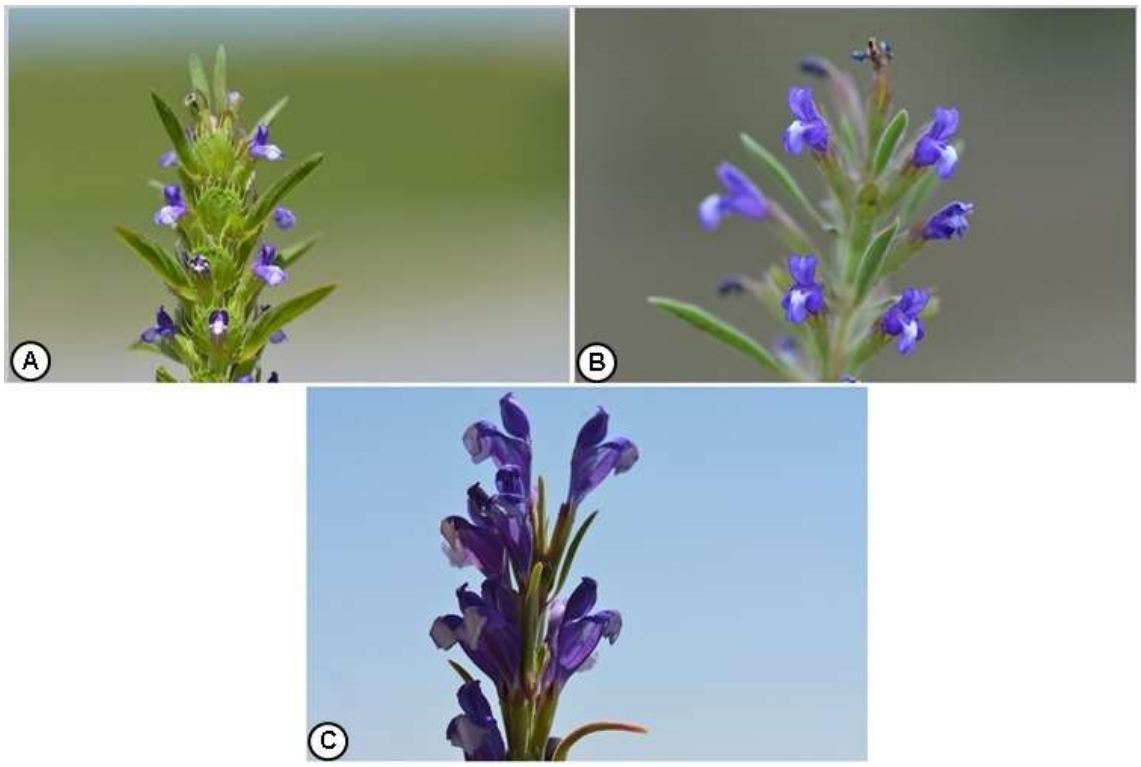


Figure 1. The Photographs of *Lallemantia* studied **(A:** *L. peltata***; B:** *L. iberica;* **C:** *L. canescens***)**

2.2. Method

The seeds were vegetated at 25 °C and the tips of the roots were treated with aqueous α-monobromonaftalin for 12 h at +4 ºC in a refrigerator and fixed with glacial acetic acid–absolute ethanol (1:3) for at least 24 h at 4 °C. Then, hyrolysed process was done (5 min., 1 N HCl, 60 °C) and rinsed in tap water for 3-5 min. Lastly, Feulgen was used for staining about 1h [13]. Photographs of metaphase chromosomes were taken from Olympus BX51 microscope and Olympus Camedia C-4000 digital camera.

2.2.1. Karyotype analysis

In this study, we measured, ploidy level, karyotype formula, total karyotype length (TKL), ranges of chromosome length, somatic chromosome number (2n), relative lengths (RL), arm ratios (AR), centromeric indices (CI), and Stebbins classification [14]. Classifications of centromeric positions and karyotype formulae were determined based on the methods of Levan et al. [15].

2.2.2. Karyotype asymmetry

This study used percent of symmetry index (SI%), index of karyotypic asymmetry (AsK%), total form percentage (TF%), value of relative chromatin (VRC), resemblance between chromosomes (Rec. index), symmetric indices (Syi index), dispersion index (DI) and difference of relative length (DRL) as the karyotype asymmetry [16-21]. The intra-chromosomal asymmetry index (A1) and interchromosomal asymmetry index (A2) were measured using the method of Romero Zarco [22] and the dispersion diagram was prepared using A_1 and A_2 (fig.4). Furthermore, this study also showed the S/A_I (karyotype symmetry/asymmetry index), CV_{CL} (coefficient of variation of chromosome length; interchromosomal asymmetry) and M_{CA} (mean centromeric asymmetry; intrachromosomal asymmetry) [23,24].

2.2.3. Statistical analysis

Pearson correlation was calculated based on karyotype asymmetry results using SPPS 21.0 (IBM Corporation, USA). 0.01 and 0.05 levels were used to compare the correlations.

3. Results and Discussion

The results of karyotype analysis [somatic chromosome number (2n), ploidy level, karyotype formula, ranges of chromosome length, TKL, A_1, A_2 indices and Stebbins classification] are given in Table 2 while RL, AR, CI] and Stebbins classification [14] are given in Table 3. Also, the findings of karyotype asymmetry [TF, SI%, AsK%, VRC, Syi index, Rec. index, DI, DRL, S/A_I, CV_{CL}, and $M_{CA,}$ A1, A2 are given in Table 4.

Table 2. Poidy level. Somatic chromosome number (2n), karyotype formula, ranges of chromosome length, total karyotype length (TKL) and Stebbins classification for the studied *Lallemantia* species

Taxa	***2n***	**Ploidy level**	**Karyotype formula**	**Chromosome length range (µm)**	**TKL (µm)**	**Stebbins classification**
L. peltata	14	2x	1m+6sm	1.82-2.87	15.84	2A
L. iberica	14	2x	3m+4sm	1.46-2.43	13.07	2A
L. canescens	14	2x	2m+5sm	1.56-2.18	12.88	2A

Table 3. Karyomorphological parameters of *Lallemantia* species

	Pair no	RL	AR	CI	Type
L. peltata	I	18.12	2.18	31.44	sm
	II	15.72	2.26	30.59	sm
	III	14.59	1.26	44.06	m
	IV	14.17	2.03	32.97	sm
	V	13.32	1.98	33.48	sm
	VI	12.59	2.12	32.04	sm
	VII	11.51	2.23	30.88	sm
L. canescens	I	16.99	2.03	32.90	sm
	II	15.30	1.64	37.87	m
	III	15.89	2.01	33.17	sm
	IV	13.81	2.06	32.66	sm
	V	13.06	1.46	40.60	m
	VI	12.79	2.04	32.86	sm
	VII	12.15	2.13	31.84	sm
L. iberica	I	18.60	2.07	32.49	sm
	II	15.83	1.56	38.95	m
	III	15.32	2.04	32.78	sm
	IV	13.95	1.98	33.55	sm
	V	12.89	1.44	40.82	m
	VI	12.21	2.02	33.05	sm
	VII	11.18	1.53	39.39	m

Table 4. Karyotype Assymetry of *Lallemantia* species

Species	**TF%**	**SI%**	**As.K%**	**VRC**	**Syi**	**Rec.**	**DI**	**DRL**	**S/A$_I$**	**CV$_{CL}$**	**M$_{CA}$**	**A$_1$**	**A$_2$**
L. peltata	32,82	62,71	67,11	0,31	48,99	88,8	2,39	3,36	1,85	7,24	32,70	0.48	0.15
L. iberica	35,63	60,08	64,39	0,26	55,0	85	3,08	3,7	1,57	8,83	28,76	0.43	0.17
L. canescens	34,67	68,88	65,21	0,26	52,94	93	1,94	2,21	1,71	5,43	30,9	0.46	0.12

***Lallemantia peltata* (L.) Fisch. & Mey.**

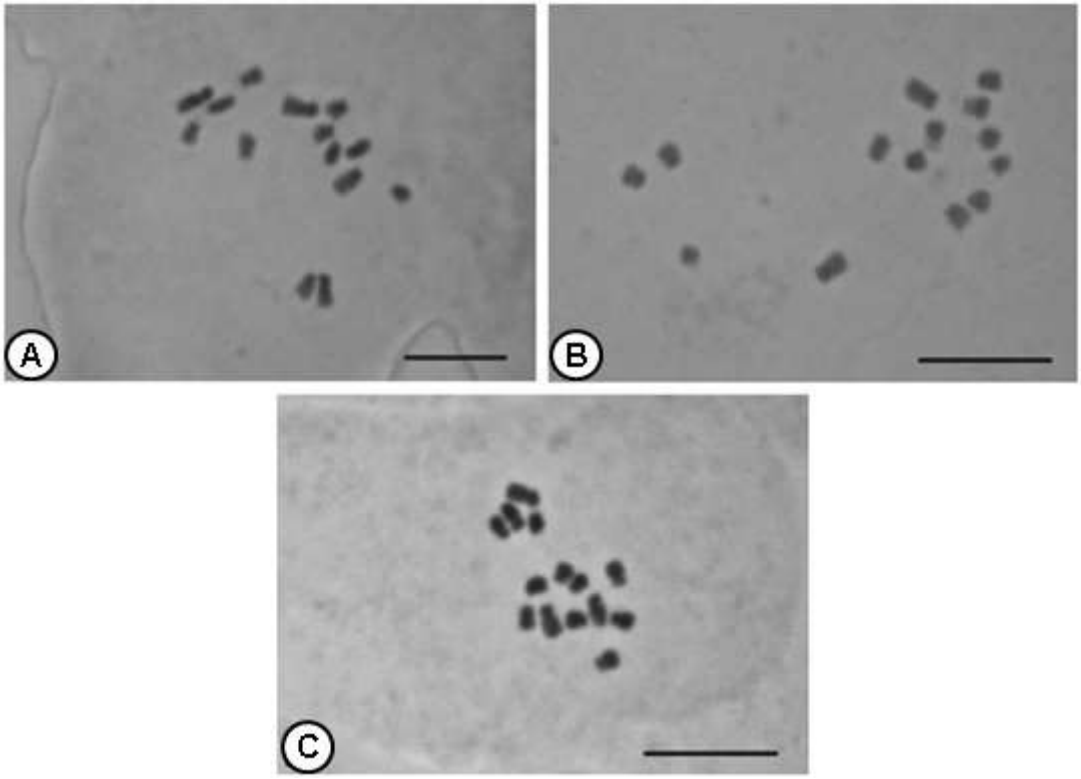
It has 2n=14 chromosome and includes one median (m) and six submedian (sm) chromosomes (Table 2, Figures 2A-3A). Lengths of the chromosomes ranged from 1.82 µm to 2.87 µm and TKL was 15.84 µm. The ratio of the longest to shortest chromosome was 1.5:1. This study found that AR values were between 1.26 and 2.26, CI values were between 30.59 and 44.06 and RL values were between 11.51% and 18.12% (Tables 2-3).

***Lallemantia iberica* (Bieb.) Fisch. & Mey.**

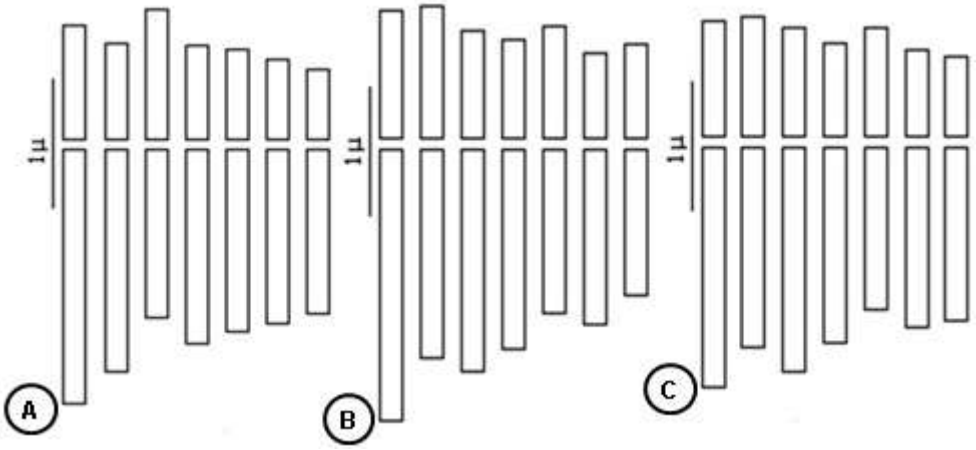
It has 2n=14 chromosome and includes three median (m) and four submedian (sm) chromosomes (Table 2; Figures 2B-3B). Lengths of the chromosomes ranged from 1.46 µm to 2.43 µm and TKL was 13.07 µm. The ratio of the longest to shortest chromosome was 1.6:1. (Tables 2-3). This study found that AR values were between 1.44 and 2.07, CI values were between 32.78 and 40.82 and RL values were between 11.18% and 18.60% (Tables 2-3).

***Lallemantia canescens* (L.) Fisch. & Mey.**

It has 2n=14 chromosome and includes two median (m) and five submedian (sm) chromosomes (Table 2, Figures 2C-3C). Lengths of the chromosomes ranged from 1.56 µm to 2.18 µm and TKL was 12.88 µm. The ratio of the longest to shortest chromosome was 1.3:1 (Tables 2-3). This study found that AR values were between 1.46 and 2.13, CI values were between 31.84 and 40.60 and RL values were between 12.15% and 16.99% (Tables 2-3).



Figures 2. Metaphase chromosomes of *Lallemantia* species. A: *Lallemantia peltata*, B: *Lallemantia iberica*, *Lallemantia canescens*



Figures 3. Haploid ideograms of *Lallemantia* studied. A: *Lallemantia peltata*; B: *Lallemantia iberica*.; C: *Lallemantia canescens*

Current data demonstrated that the karyotype formula of *L. peltata* is 1m+6sm, the karyotype formula of *L. iberia* is 3m+4 sm and the karyotype formula of *L. canescens* is 2m+ 5sm. However, Ozcan et al. [10] findings regarding the karyotype formula conflicted with the present study. They found that three *Lallemantia* species had a karyotype formula of 6m+1sm [10]. Also, Dolatyari and Kamrani [25] showed that various *Lallemantia* species including *L. iberica, L. canescens* and *L. peltata* have 2n=2x=14 chromosomes. They also found that accessions of *L. peltata* (1M+4m+2sm^{1st}; 5m+2sm; 1M+3m+3sm), *L. iberica* (1M+3m+3sm;1M+4m+2sm+2Bs) and *L. canescens* (4m+3sm; 5m+2sm) have karyotype formulae that differ from the present study and they observed two B-chromosomes in one accession of *L. iberica* [25]. This difference among *Lallemantia* accessions may be due to geography. A karyotype study done by Martin et al. [26] supported the theory that the karyotypes of species gathered from various areas might change. They explained that this resulted from infraspecific

and infrageneric variations such as climatological, geographical and ecological [26]. Reda et al. [27] also indicated that the chromosome structure and karyotype of the accessions might change because of significant adaptations.

On the other hand, the current study determined that the TF% varied from 32.82% to 35.63%; SI varied from 60.08 to 68.88; As.K% varied from 64.39 to 67.11; VRC varied from 0.26 to 0.31; Syi varied from 48.99 to 55; Rec. index varied from 85 to 93; DI varied from 1.94 to 3.08; DRL varied from 2.21 to 3.7; S/A_I varied from 1.57 to 1.85; CV_{CL} varied from 5.43 to 8.83, M_{CA} varied from 28.96 to 32.70; A1 varied from 0.43 to 0.48; and A2 varied from 0.12 to 0.17. (Table 4). This study demonstrated that *Lallemantia* species studied possess symmetric karyotypes ($1.0 < S/AI \leq 2.0$; [22] according to S/A_I. Also, the present study determined that *Lallemantia* species are 2A based on Stebbins classification. However, Ozcan et al. [10] demonstrated that *L. canescens* and *L. iberica* are 2A whereas *L. peltata* are 2B according to the Stebbins classification. In addition, the scatter diagram based on A1 and A2 showed that *Lallemantia* species exhibited close localisation (Figure 4). Furthermore, the Pearson correlation calculated using karyotype asymmetry values and correlation is significant at 0.01 and 0.05 (Table 5).

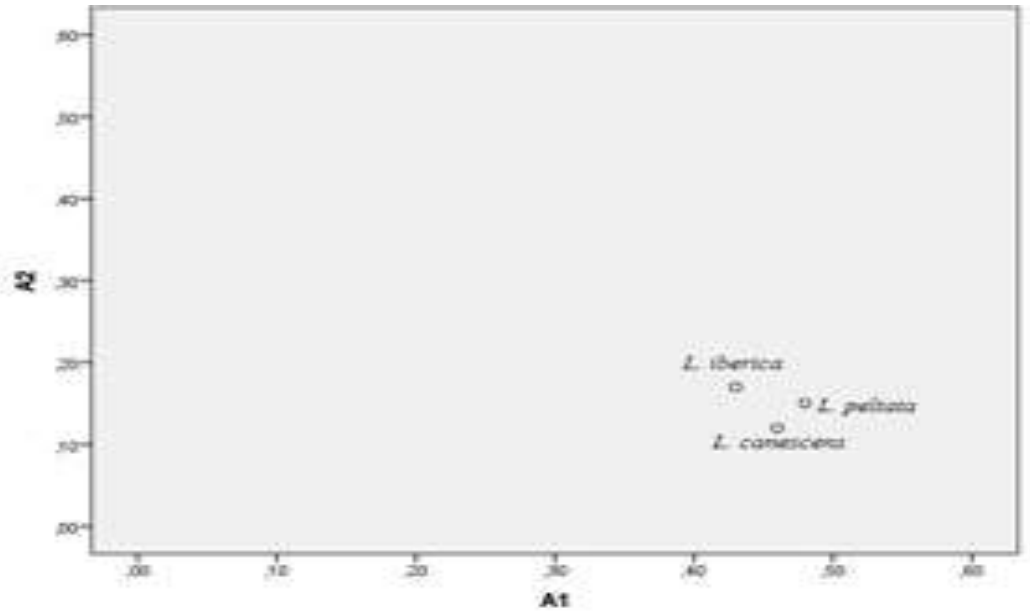


Figure 4. Scatter diagram based on A_1 and A_2

Table 5. Pearson correlation for karyotype asymmetry

	TF%	SI%	As.K%	VRC	Syi	Rec.	DI	DRL	S/A_I	CV_{CL}	M_{CA}	A_1	A_2
TF%	1	,540	,919	,762	1,000**	,363	,955	,992	,984	,990	,991	,998*	,998*
SI%	,540	1	,829	,957	,541	,980	,766	,433	,683	,655	,646	,595	,595
As.K%	,919	,829	1	,956	,919	,702	,995	,863	,975	,966	,962	,943	,943
VRC	,762	,957	,956	1	,763	,880	,920	,677	,866	,847	,840	,803	,803
Syi	1,00**	,541	,919	,763	1	,364	,955	,992	,984	,990	,992	,998*	,998*
Rec.	,363	,980	,702	,880	,364	1	,624	,246	,525	,493	,482	,424	,424
DI	,955	,766	,995	,920	,955	,624	1	,911	,993	,987	,985	,972	,972
DRL	,992	,433	,863	,677	,992	,246	,911	1	,954	,965	,968	,982	,982
S/A_I	,984	,683	,975	,866	,984	,525	,993	,954	1	,999*	,999*	,993	,993
CV_{CL}	,990	,655	,966	,847	,990	,493	,987	,965	,999*	1	1,00**	,997*	,997*
M_{CA}	,991	,646	,962	,840	,992	,482	,985	,968	,999*	1,00**	1	,998*	,998*
A_1	,998*	,595	,943	,803	,998*	,424	,972	,982	,993	,997*	,998*	1	1,00*
A_2	,998*	,595	,943	,803	,998*	,424	,972	,982	,993	,997*	,998*	1,00**	1

* Correlation is significant at 0.05 level. ** Correlation is significant at 0.01 level

4. Conclusion

This study demonstrated that the *Lallemantia* species grown in Turkey have 2n=2x=14 chromosomes. Also, the present study found that *L. peltata* has 1m+6sm karyotype formula, *L. iberia* has 3m+4 sm karyotype formula and *L. canescens* has 2m+ 5sm karyotype formula. The research also showed that three *Lallemantia* species are 2A based on Stebbins' classification. Furthermore, correlation was found based on karyotype asymmetry values and present results supported the contention that karyotypes display differences depending on locality.

Authors' Contributions

All authors contributed equally to the study

Statement of Conflicts of Interest

There is no conflict of interest between the authors.

Statement of Research and Publication Ethics

The author declares that this study complies with Research and Publication Ethics.

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