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Research Article

Cytotaxonomical analysis of eleven Turkish *Fritillaria* L. (Liliaceae) taxa

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Abstract

This paper reports the chromosome numbers of eleven Turkish *Fritillaria* taxa from seventeen populations along with their karyotypes and ideograms. They are *F. hermanis* subsp. *amana* (Rix) R.Wallis & R.B.Wallis 2n=24, *F. armena* Boiss. 2n=24 (endemic), *F. aurea* Schott 2n=24 (endemic), *F. crassifolia* Boiss. & A.Huet subsp. c*rassifolia* 2n=24 (endemic), *F. crassifolia* Boiss. & A.Huet subsp. *kurdica* (Boiss. & Noë) Rix 2n=24 (endemic), *F. imperialis* L. 2n=26+2B, 24+3B, 24, 26+3B, *F. minuta* Boiss. & Noë 2n=24, *F. persica* L. 2n=24, *F. pinardii* Boiss. 2n=24, 24+5B, *F. uva-vulpis* Rix 2n=36 and *F. viridiflora* Post 2n=24 (endemic). The *Fritillaria uva-vulpis* was the only triploid, others were diploid with some B chromosomes. The basic chromosome numbers were counted as x= 12 or 13. For all karyological characters of taxa, the correlation coefficient was defined and grouping was performed by cluster analysis (UPGMA, similarity) and by ordination based on principal-components analysis (PCA).

Keywords: *Fritillaria,* chromosome number, UPGMA, PCA, Turkey.

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Türkiye'deki 11 *Fritillaria* L. (Liliaceae) türünün Sitotaksonomik Analizi

Özet

Türkiye'deki 17 populasyondan 11 *Fritillaria* taksonunun karyotipleri ve idiyogramları verilmektedir. Bunlar; *F. hermanis* subsp. *amana* (Rix) R.Wallis & R.B.Wallis 2n=24, *F. armena* Boiss. 2n=24 (endemik), *F. aurea* Schott 2n=24 (endemik), *F. crassifolia* Boiss. & A.Huet subsp. c*rassifolia* 2n=24 (endemik), *F. crassifolia* Boiss. & A.Huet subsp. *kurdica* (Boiss. & Noë) Rix 2n=24 (endemik), *F. imperialis* L. 2n=26+2B, 24+3B, 24, 26+3B, *F. minuta* Boiss. & Noë 2n=24, *F. persica* L. 2n=24, *F. pinardii* Boiss. 2n=24, 24+5B, *F. uva-vulpis* Rix 2n=36 and *F. viridiflora* Post 2n=24 (endemik). Sadece *F. uva-vulpis* triploittir, diğerleri diploittir ve B kromozomu taşır. Bu sonuçlar temel kromozom sayısının x = 12 ve 13 olduğunu göstermektedir. Bütün taksonlara ait karyolojik özelliklerin korelasyon katsayısı belirlenmiş ve küme analizi (UPGMA) ve asıl bileşenler (PCA) yöntemleriyle gruplandırılmıştır.

Anahtar Kelimeler: *Fritillaria,* kromozom sayısı, UPGMA, PCA, Türkiye.

Introduction

The genus of *Fritillaria* L. (Liliaceae) comprises attractive, ornamental and medicinal species. Bulbous *Fritillaria* which is derived from bulbous of various *Fritillaria* species, has been used as one of the most important antitussive, expectorant and antihypertensive drugs in traditional Chines medicine (Kang 2002; 2004; Wang 2012). Also *F. ussuriensis* Maxim. was officially recorded in the 2005 edition of Chines Pharmacopoeia (Yang and Duan 2012). Additionally local people use some *Fritillaria* species for headache in Nepal (Rokaya 2010), for digestive problems, stomachache and kidney in Iran (Mosaddegh 2012).

Worldwide the genus *Fritillaria* comprises 165 taxa (Rix 2001) of which 122 have karyological data (IPCN 2014). In Turkey, the genus *Fritillaria* has represented 45 species of which 23 are endemics and of which only 10 species have karyological data (Rix 1984; Davis et al. 1988; Özhatay 2000; Özhatay and Kültür 2006; Özhatay et al. 2009; Özhatay et al. 2011). There are studies of the anatomical, morphological and palynological characteristics of few species belonging to the genus *Fritillaria* (Özler 2007; Alan 2008; Tekşen et al. 2010; Tekşen and Aytaç 2011). However, we suggest that karyological investigations are not enough on this medicinally and economically valuable genus in Turkey.

The chromosome numbers of examined taxa were given previously (Koçyiğit et al. 2014). The aim of this paper was to provide good cytological data to clearly define karyotypes for eleven taxa of the genus on based multivariate statistics.

Materials and Methods

Chromosome analysis

The material studied was collected in 2007-2008 by the authors. A list of the species studied, collection localities and voucher numbers are given in Table 1. The Chromosome counts were obtained from somatic metaphases using a standard squash technique (Özhatay 1984). Bulbs collected from the 17 populations were germinated on pots and root tip meristems were used for analysis. At least five metaphase plates were examined from different individuals for all the counts. Ideogram measurements were prepared with the aid of enlarged microphotographs of five well-spread metaphase plates of different individuals. The classification of chromosomes, length of the long (l) and short arms (s), arm ratio, centromeric index (s/l×100), and relative chromosomal length (a chromosome / total×100) were measured with image analysis systems KAMERAM & Canon A 640 camera. Chromosomes were classified according to the nomenclature of Levan 1964. Permanent slides and herbarium vouchers are retained at ISTE (The Herbarium of the Faculty of Pharmacy, Istanbul University). To evaluate karyological characters multivariate analysis was performed by use of PAST version 1.81. For all karyological characters of taxa, the correlation coefficient was defined and grouping was performed by cluster analysis (UPGMA, similarity) and by ordination based on principal-components analysis (PCA).

Results

Chromosome morphology

Morphometric data of karyotype in the *Fritillaria* studied taxa are summarized in Table 2 and added as attachment. Karyotypes are presented in Figure 1, respectively. The first two or three chromosome pairs usually are metacentric, metacentric – submetacentric or submetacentric. The other pairs are subtelocentric or acrocentric. The chromosome pairs are the most prominent characteristics at the karyotype of the genus *Fritillaria*. In all taxa, karyotype has subtelocentric and acrocentric chromosome.

Basic chromosome numbers are mostly well-defined within *Fritillaria* genus, and our results support previous investigations. The shortest chromosome (7.45 µm) was measured in *F.imperialis* population in Adıyaman, whereas *F. hermonis* subsp. *amana* had the longest chromosome (25.20 µm). The *F. persica* had the greatest arm ratio of 13.07 and the smallest centromeric index value of 7.11. On the other hand, *F. aurea* had the smallest arm ratio of

1.08 and the largest centromeric index value of 48.10. The *F. imperialis* from Adıyaman, (ISTE 0206) had the shortest total haploid complement length of 114.25 µm, *F. hermonis* subsp. *amana* had conversely the longest 225.42 µm. All the examined specimens were diploid with 2n=24 or 26, with the exception of *F. uva-vulpis,* it has 2n=3x=36.

Table 1: Localities and voucher numbers of *Fritillaria* taxa studied

Taxa	Voucher number (ISTE)	Locality	Altitude (m)
F. armena	93433	Adıyaman: Doğanlı village, Aksu field, *U. Rastgeldi 0202,* 23.04.2007	1288
F. aurea	93434	Adıyaman: Doğanlı village, Aksu field, *U. Rastgeldi, M. Gayberi 0203*, 23.04.2007	1294
F. crassifolia ssp. *crassifolia*	93343	Adıyaman: Doğanlı village, Aksu field, *U. Rastgeldi 0201*, 22.04.2007	1309
F. crassifolia subsp. *kurdica*	93443	Kahramanmaraş: Ahırdağı, Sulu Tarla, *U. Rastgeldi 4601*, 20.07.2007	1267
F. hermanis subsp. *amana*	93442	Gaziantep: İslahiye, Huzur upland, Tahtalı field, *U. Rastgeldi 2702,* 06.06.2007	1520
F.imperialis	0206	Adıyaman: Doğanlı village, Berzan Mountain, east slopes, *U. Rastgeldi 0206,* 02.05.2008	1719
	2101	Diyarbakır: Çermik, Kuyu village, Taşköprü field, *U. Rastgeldi 2101,* 30.04.2007	1230
	2102	Diyarbakır: Çüngüş, Mirgan Mountain, *U. Rastgeldi 2102,* 27.04.2008	1343
	2103	Diyarbakır: Kulp,Yaylak village, Mehmet kar field, *U. Rastgeldi, M.Koçyiğit 2103,* 27.09.2008	1672
F. minuta	93444	Adıyaman: Nemrut Mountain, west slopes, *U. Rastgeldi 4602,* 04.10.2007	1985
	93436a	Siirt: Baykan, Yarımca village, Mirgelo Mountain slopes, *U. Rastgeldi 5601*, 27.04.2007	1113
	93439	Siirt: Baykan, Günbuldu village, Klems Mountain, Şirantepe field, *U. Rastgeldi, M. Gayberi 5604,* 23.05.2007	1905
F. persica	93435	Şanlıurfa: Siverek, İleri village, *U. Rastgeldi 6301,* 25.04.2007	1277
F. pinardii	93446	Adıyaman: Esence village, *U. Rastgeldi, M.Koçyiğit 0209,* 19.04.2008	1349
	93447	Adıyaman: Yazıbaşı village, Ulubaba Mountain, Körte Kas field, *U. Rastgeldi 0211,* 24.08.2008	1900
F. uva-vulpis	93441	Şırnak: Cumhuriyet field, *U. Rastgeldi 7301,* 24.05.2007	1600
F. viridiflora	93431	Gaziantep: İslahiye, Katran Mountain, *U. Rastgeldi 2701,* 19.04.2007	470

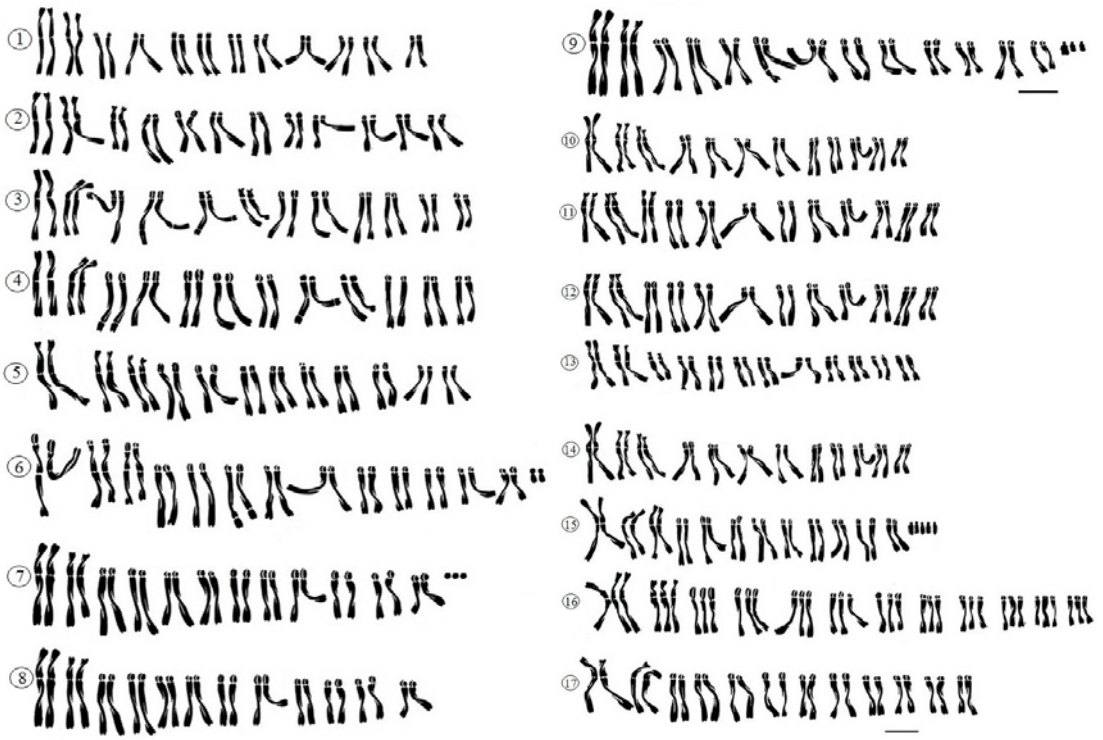

Figure 1. 1) *F. armena*, 2) *F. aurea*, 3) *F. crassifolia* subsp. *crassifolia*, 4) *F. crassifolia* subsp. *kurdica*, 5) *F. hermonis* subsp. *amana*, 6) *F. imperialis* (0206), 7) *F. imperialis* (2101), 8) *F. imperialis* (2102), 9) *F. imperialis* (2103) 10) *F. minuta* (93444), 11) *F. minuta* (93436a), 12) *F. minuta imperialis* (93439), 13) *F. persica*, 14) *F. pinardii* (93446), 15) *F. pinardii* (93447), 16) *F. uva-vulpis*, 17) *F. viridiflora* (Scale bar 10 µm).

Table 2. Somatic chromosome number (2n), ploidy level, karyotype formula and previous counts for the studied *Fritillaria* taxa

Taxa	Voucher number (ISTE)	Chromosome number (2n)	Ploidy level	Karyotypic formula	Chromosome size (µm)	THC (µm)	Previous counts
F. armena	93433	24	2x	2m + 2msm + 8t + 12st	11.46-21.91	178.03	n=12 (La Cour 1978) n=12 (La Cour 1978a)
F. aurea	93434	24	2x	2m + 2msm + 10t + 10st	11.68-20.81	178.55	n=12 (La Cour 1978) n=12 (La Cour 1978a)
F. crassifolia* subsp. *crassifolia	93343	24	2x	2m + 2msm + 2sm+ 8t +$2t_{SEC}$ + 6st + $2st_{SEC}$	11.50-23.34	189.95	n=12 (La Cour 1978) n=12 (La Cour 1978a) 2n=24 (Zonneveld 2010)
F. crassifolia* subsp. *kurdica	93443	24	2x	2m + 2msm + 2sm+ 8t +$2t_{SEC}$ + 6st + $2st_{SEC}$	13.09-25.10	206.83	
F.hermonis* subsp. *amana	93442	24	2x	2m + 4msm + 10t + 5st + $3st^{SAT}$	13.99-25.20	225.42	n=12 (La Cour 1978) n=12 (La Cour 1978a)
F.imperialis	0206	26+2B	2x	$2m^{SAT}$ + $2msm^{SAT}$ + $2sm^{SAT}$ + 6t +$2t_{SEC}$ +12st	7.45-15.47	114.25	2n=24 (Chatterjee 1971) n=12 (La Cour 1978) n=12 (La Cour 1978a) 2n=24 (Zonneveld 2010) 2n=36 (Özhatay 2002)
	2101	24+3B	2x	4sm + 14t + 6st	10.38-18.11	171.79	
	2102	24	2x	4sm + 18t + 4st	11.05-22.61	196.67	
	2103	26+3B	2x	2m + 2sm + 12t + 10st	9.57-22.93	204,13	
F. minuta	93444	24	2x	4msm + 2sm + 12t + 6st	10.43-20.21	164.79	2n=24 (Özhatay 2002)
	93436a	24	2x	6sm + 12t + 6st	9.65-16.72	150.75	
	93439	24	2x	4sm + 16t + 4st	10.29-18.12	152.93	
F. persica	93435	24	2x	2m + 4sm + 8t + 10st	11.25-22.44	173.08	2n=24 (Khanki 1997) n=12 (La Cour 1978) n=12 (La Cour 1978a) 2n=24 (Khanki 2002) 2n=24 (Özhatay 2002)
F. pinardii	93446	24	2x	2msm + 4sm + 8t + 10st	9.50-22.23	159.80	n=12 (La Cour 1978) n=12 (La Cour 1978a) 2n=24 (Zonneveld 2010)
	93447	24+5B	2x	2m + 2msm + 2sm + 6t + 12st	9.60-20.43	160.93	
F. uva-vulpis	93441	36	3x	2m + 2sm + 22t + 10st	8.24-16.00	134.96	2n=24 (Khanki 2002)
F. viridiflora	93431	24	2x	4sm + 10t + 10st	10.03-15.72	137.28	-

Two populations (ISTE 0206 and 2103) of *F. imperialis* and two subspecies of *F. crassifolia* have secondary constrictions on the long arm of the submetacentric (sm) and subtelocentric (t) chromosome pairs. The satellite in one acrocentric chromosome pair of *F. hermonis* subsp. *amana* is small and spherical whereas, the satellites in three metacentric chromosome pairs of *F. imperialis* (ISTE 0206) are big and long (Fig.1). Three populations (ISTE 0206, 2101 and 2103) of *F. imperialis* and one population (ISTE 93447) of *F. pinardii* have B-chromomosomes.

Multivariate analyses

The clustering dendrograms of UPGMA similarity and PCA ordination for *Fritillaria* are presented in Figures 2 and 3. In general, 3 major clusters are formed; the taxa of *F. minuta*, *F. imperialis* from two populations (ISTE 2101 and 2102), *F. uva-vulpis* and *F. viridiflora* comprise the first major cluster. The taxa of *F. persica*, *F. pinardii* and *F. imperialis* from other two populations (ISTE 0206 and 2103) form the second major cluster and the taxa of *F. armena*, *F. aurea*, *F. crassifolia* subsp. *crassifolia*, *F. crassifolia* subsp. *kurdica*, *F. hermonis* subsp. *amana* comprise the third cluster. Principal components analysis of karyological data has revealed that the first two PC factors accounted for 54.86% of the total variance. The eighth factor (number of acrocentric chromosome ≤ 8) has the highest negative correlation (-0.06) and the ninth factor (number of subtelocentric chromosome ≤ 8) has the highest positive correlation (0.95) (Table 3). Principal components analysis shows that some karyological characters as number of subtelocentric and acrocentric chromosomes seem to be important and explain most of the total variation among the taxa.

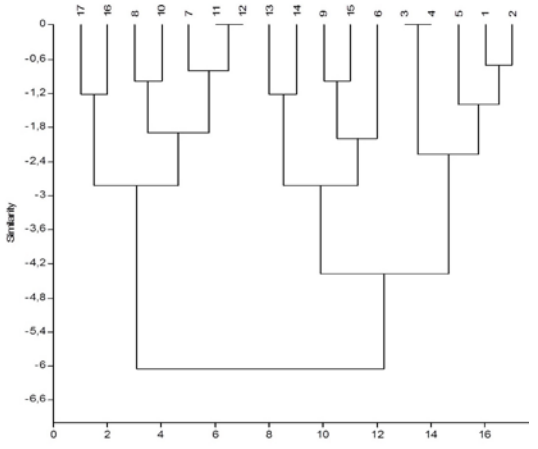

Figure 2. UPGMA clustering of *Fritillaria* taxa on the basis of karyological characters.

1) *F. armena*, 2) *F. aurea*, 3) *F. crassifolia* subsp. *crassifolia*, 4) *F. crassifolia* subsp. *kurdica*, 5) *F. hermonis* subsp. *amana*, 6) *F. imperialis* (0206), 7) *F. imperialis* (2101), 8) *F. imperialis* (2102), 9) *F. imperialis* (2103), 10) *F. minuta* (93444), 11) *F. minuta* (93436a), 12) *F. minuta imperialis* (93439), 13) *F. persica*, 14) *F. pinardii* (93446), 15) *F. pinardii* (93447), 16) *F. uva-vulpis*, 17) *F. viridiflora*

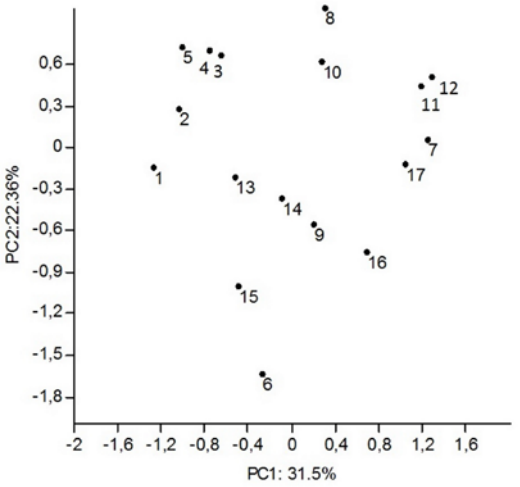

Figure 3. Principal components analysis of *Fritillaria* taxa based on karyological characters.

1) *F. armena*, 2) *F. aurea*, 3) *F. crassifolia* subsp. *crassifolia*, 4) *F. crassifolia* subsp. *kurdica*, 5) *F. hermonis* subsp. *amana*, 6) *F. imperialis* (0206), 7) *F. imperialis* (2101), 8) *F. imperialis* (2102), 9) *F. imperialis* (2103), 10) *F. minuta* (93444), 11) *F. minuta* (93436a), 12) *F. minuta imperialis* (93439), 13) *F. persica*, 14) *F. pinardii* (93446), 15) *F. pinardii* (93447), 16) *F. uva-vulpis*, 17) *F. viridiflora*

Table 3. Linear correlation among variables in *Fritillaria* taxa based on karyological characteristics

Source of Variation	Axis 1	Axis 2	Axis 3	Axis 4	Axis 5	Axis 6	Axis 7	Axis 8	Axis 9	Axis 10	Axis 11	Axis 12
1. Chromosome counts	0,00	0,38	0,23	0,08	0,23	0,52	0,93	0,18	0,53	0,04	0,23	0,00
2. Presence or absence of B-chromosome	-0,23	0,00	0,68	0,38	0,48	0,32	0,90	0,34	0,33	0,51	0,06	0,60
3. Presence or absence of secondary constriction	-0,31	0,11	0,00	0,23	0,12	0,41	0,08	0,48	0,88	0,94	0,35	0,66
4. Presence or absence of satellite	-0,43	0,23	0,31	0,00	0,23	0,23	0,18	0,93	0,53	0,67	0,80	0,73
5. Presence or absence of metacentric chromosome	-0,31	0,18	0,39	0,31	0,00	0,12	0,10	0,10	0,28	0,13	0,07	0,42
6. Presence or absence of submetacentric chromosome	-0,17	0,26	0,21	-0,31	-0,39	0,00	0,08	0,63	0,88	0,18	0,02	0,66
7. Presence or absence of metacentric-submetacentric chromosome	0,02	-0,03	0,44	0,34	0,41	-0,44	0,00	0,83	0,17	0,03	0,23	0,30
8. Number of acrocentric chromosome ≤ 8	0,34	-0,25	0,18	0,02	-0,41	0,13	-0,06	0,00	0,01	0,87	0,52	0,36
9. Number of subtelocentric chromosome ≤ 8	-0,16	0,25	0,04	0,16	0,28	-0,04	0,35	-0,61	0,00	0,43	0,95	0,54
10. Maximum chromosom lenght	-0,49	0,17	-0,02	0,11	-0,38	0,34	-0,54	0,04	-0,21	0,00	0,01	0,18
11. Minimum chromosom lenght	-0,31	0,46	-0,24	-0,07	-0,46	0,55	-0,31	-0,17	0,02	0,62	0,00	0,42
12. Ploidy	-0,68	-0,14	-0,12	-0,09	0,21	0,12	-0,27	-0,24	-0,16	0,34	0,21	0,00

Discussion

The family Liliaceae was divided into smaller families for a long time, the families of some genera were changed according APG III (Chase and Reveal 2009), but the genus *Fritillaria* has been conserved in the Liliaceae (Jussieu 1789). The chromosomes of Liliaceae have diversity in size, number and structure, but the chromosome numbers of *Fritillaria* are larger and more stable than other genera (Peruzzi et al. 2009). The karyology of *Fritillaria* has been studied by several researchers with reported chromosome numbers of n=9, 12, 13 (Noda 1975; La Cour 1978; Başak 1991; Kamari 1991; Özhatay 2002; Kamari and Phitos 2006; Tekşen and Aytaç 2008; Zonneveld 2010). Except for *F. imperialis*, karyomorphological data is completely lacking in Turkish *Fritillaria*, this study has completed the deficiency for the ten taxa. Also the chromosome numbers of *F. viridiflora* has been defined first time in this study.

The Southern Anatolia *Fritillaria* was revised by Tekşen and Aytaç (2011).

Nectaries in *Fritillaria* are large and well developed and have been used as basis for subgeneric classifications. Bakshi-Khaniki and Persson (1997) studied nectary morphology in 31 southwestern Asian *Fritillaria* species. Additionally, Rønsted et al. (2005) have used molecular phylogenetic evidence as basis for infrageneric classifications of *Fritillaria*. In the dendrogram (Fig 2), *Fritillaria* taxa are connected with each other at several levels, according to their chromosomal characteristics. The cluster analysis shows in the first major cluster that *F. minuta*, *F. imperialis* (2101 and 2102), *F. uva-vulpis* and *F. viridiflora* are close to each other. *F. uva-vulpis* and *F. viridiflora* already have not been studied, but both molecular data and morphological data do not agree about similarity between *F. minuta*, *F. imperialis*. On the other hand, the second major cluster presents that *F. persica* and *F. imperialis* are closely similar, also *F. aurea*, *F. crassifolia*, *F. hermonis* subsp. *amana* are related to each other in the third major cluster and these results have been supported by molecular and morphological data (Rønsted et al. 2005; Türktaş et al. 2012).

Polyploidy is common in flowering plants of cold climates with harsh and stressful environments (Meng et al. 2010; Yu et al. 2010; Schneider and Huertas 2010). One of the studied taxa is triploid, *F. uva-vulpis* has 2n=3x=36. About 74% of the 105 *Fritillaria* species with chromosome counts are diploid and only about 26% of the reported species are polyploidy (IPCN 2014). So it may not be said that polyploidy is common in the genus *Fritillaria*.

The structure of the satellites can be variable; also secondary constrictions can be seen in the chromosome of *Fritillaria*. Kamari (1991) have used the satellite shapes and secondary constrictions for a classification of the Greek *Fritillaria*. In this study, the satellites which are different size and shapes have been determined on the chromosomes of *F. hermonis* subsp. *amana* and *F. imperialis*. Furthermore, *F. imperialis* and *F. crassifolia* have secondary constrictions.

Among plants B-chromosomes are mostly distributed in monocots. The family Liliaceae is prominent for having a large number of species with B-chromosomes (Aquaro et al. 2007; Jojić et al. 2007). In this study, B-chromosomes have been observed on four *Fritillaria* specimens.

Conclusions

Chromosome numbers and morphology were evaluated from ten taxa (three endemics for Turkey), from 17 populations. Somatic chromosome count for *F. viridiflora* was determined for the first time. The basic chromosome number is invariable among taxa of *Fritillaria,* but in this study, some differences were observed in size and chromosome variation. Sometimes polyploidy, secondary constrictions and B-chromosomes may be observed. The subtelocentric and acrocentric chromosomes are important karyological characters for the *Fritillaria* taxa.

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