IRANIAN JOURNAL OF BOTANY 25 (2), 2019
DOI: 10.22092/ijb.2019.125656.1232

KARYOTYPIC VARIATION IN FIVE SPECIES OF THE GENUS FRITILLARIA (LILIACEAE)

F. Hazbavi, Sh. Hosseini, Gh. Mirzaghaderi & M. Advay

Received 2019. 03. 15; accepted for publication 2019.11.20

Hazbavi, F., Hosseini, Sh., Mirzaghaderi, Gh. & Advay M. 2019. 12. 30**:** Karyotypic variation in five species of the genus *Fritillaria (*Liliaceae). *-Iran. J. Bot. 25 (2): 127-134*. Tehran.

In this study, karyotypes of five *Fritillaria* species including *F. avromanica*, *F. assyriaca*, *F. chlorantha*, *F. persica* and *F. raddeana* belonging to three subgenera are presented. All species were diploid (2*n* = 2*x* = 24) with mean chromosome length of 15.86 µm. Four chromosome types (‘‘m’’, ‘‘sm’’, ‘‘st’’, ‘‘T’’) made five different karyotypic formula. Chromosome count of *F. avromanica (*2*n* = 2*x* = 24) is a new report. Analysis of variance for eight chromosomal parameters revealed inter specific chromosomal variation. Based on different karyotype asymmetry indices including TF %, CV %, A2, AI, AsK %, S%, *F. assyriaca* had the most asymmetrical karyotype. Based on Fluorescence in situ hybridization result, 4 and 6, 35S rDNA loci were observed for *F. raddeana* and *F. persica* respectively, which mainly located at distal or telomeric regions of chromosomes.

Fatemeh Hazbavi and Shahla Hosseini (correspondence< sh.hosseini@uok.ac.ir>) Department of Biological Science and Biotechnology, University of Kurdistan, Sanandaj, Iran. -Ghader Mirzaghaderi, Department of Agronomy and Plant Breeding, Faculty of Agriculture, University of Kurdistan, Sanandaj, Iran. -Mahfooz Advay, Department of Plant Sciences, School of Biology, College of Science, University of Tehran, Tehran, Iran.

Key words: Liliaceae; *Fritillaria*; Cytogenetic; FISH; NOR

بررسی تنوع کاریوتیپی در برخی از گونه‌های سرده لاله واژگون (تیره لاله)

فاطمه حزباوی: دانش آموخته کارشناسی ارشد زیست شناسی سلولی و مولکولی، گروه علوم زیستی، دانشکده علوم، دانشگاه کردستان، سنندج، ایران

شهلا حسینی: استادیار گروه علوم زیستی، دانشکده علوم، دانشگاه کردستان، سنندج، ایران

قادر میرزا قادری: دانشیار گروه زراعت و اصلاح نباتات، دانشکده کشاورزی، دانشگاه کردستان، سنندج، ایران

محفوظ ادوایی: دانشجوی دکتری سیستماتیک گیاهی، گروه علوم گیاهی، دانشکده زیست شناسی، دانشگاه تهران، تهران، ایران

در این مطالعه کاریوتیپ پنج گونه از سرده لاله واژگون شامل: Fritillaria avromanica، F. assyriaca، F. chlorantha، F. persica و F. raddeana، متعلق به سه زیر جنس، گردآوری شده از نواحی مختلف ایران ارائه شده است. همه گونه‌ها دیپلوئید (2n = 2x = 24) بوده و میانگین طول کروموزوم‌ها ۱۵/۸۶ میکرومتر بود. چهار نوع کروموزوم (‘‘m’’, ‘‘sm’’, ‘‘st’’, ‘‘T’’)، پنج فرمول کاریوتیپی متفاوت را ایجاد کردند. شمارش کروموزومی F. avromanica نخستین گزارش برای این گونه است. آنالیز واریانس هشت پارامتر کروموزومی تنوع درون گونه‌ایی را آشکار ساخت. براساس شاخص‌های متنوع عدم تقارن از جمله TF %، CV %، A2، AI، AsK %، S% ، گونه F. assyriaca، نامتقارن‌ترین کاریوتیپ را داشت. براساس نتایج هیبریداسیون فلورسنت درجا به ترتیب چهار و شش جایگاه 35S rDNA برای گونه‌های F. raddeana و F. persica مشاهده شد که عمدتاً در نواحی دیستال و تلومریک کروموزوم استقرار داشتند.

INTRODUCTION

Fritillaria L. (Liliaceae) with more than 165 taxa (130-140 species) are distributed through the temperate areas of the northern hemisphere (Day & al. 2014; Metin & al. 2013). They contain perennials, categorized by bisexual nodding flowers, campanulate to cupulate perianth of six tepals, marked with light/dark colored squares or with longitudinal stripes or fascia, and with nectaries at the base, or at the inflection (Rechinger 1990; Rix 1997). In the last review of the genus *Fritillaria*, Rix (2001), divided this genus into eight subgenera. However Iranian species belong to four subgenera including *Fritillaria*, *Theresia*, *Petilium* and *Rhinopetalum*. Most species are classified in subgenus *Fritillaria* (Rix 2001), which also include Iranian native species as well (*F. avromanica* Advay, Teksen & Maroofi, *F. chlorantha* Hausskn. & Bornm). *Fritillaria assyriaca* Baker as a reported species, contain the largest plant genome so far (1C = 127.4 pg), that Bennett and Smith (1976) has classified in *Fritillaria* subgenus. Molecular and morphological study (Sharifi-Tehrani and Advay 2015) revealed close relationship between *F. assyriaca* and *F. a vromanica*.

Chromosomes have been counted for more than 50 species of *Fritillaria* and most species have a basic chromosome number, of $x = 12$. Only a few species are exceptions to this, with $x = 9$ (3 species), $x = 11$ (2 species) and $x = 13$ (2 species), (Samaropoulou & al. 2016). Curiously enough that still no specific model for ploidy level and chromosome behavior has been marked for the genus (Fedorov 1969). In this regard, comparative karyotype analysis of related species can describe patterns and directions of chromosomal evolution in a group and estimate the possible evolutionary role (Peruzzi & al. 2008; Jafari & al. 2014 and Ahmadi-Roshan & al. 2016).

Khaniki (2002a, 2002b and 2005, Jafari & al. 2014, Ahmadi-Roshan & al. 2016) has reported chromosome number of some Iranian species belonging to subgenera *Petilium*, *Theresia*, *Rhinopetalum*, *Fritillaria* and *Fritillaria caucasica* group.

Substantial development in molecular cytogenetic have delivered more applications of karyotypes analysis. However, most of the previous cytological studies in the genus *Fritillaria* (as mentioned above) have concentrated on the classical cytogenetic analysis, while molecular cytogenetic aspects still remain to be addressed. With the development of FISH the sites of 35S rRNA genes can be more easily detected, and they have been extensively investigated as a complement to the classical cytogenetics.

The objectives of this study were to determine chromosome number, ploidy level and general information on karyotype characteristics of *F. avromanica* as a first report and *F. assyriaca, F. persica, F. chlorantha and F. raddeana* from different localities. FISH also was applied to identify the number and distribution of 35S rDNA loci in some of these species.

MATERIALS AND METHODS

Plant material

Seeds of *F. avromanica*, *F. assyriaca*, *F. chlorantha*, *F. persica and F. raddeana* were collected from natural habitats during 2015-2016. Vouchers were deposited at Herbarium of Kurdistan Research Center of Agriculture and Natural Resources (HOK), Sanandaj (IRAN). Collection data of the studied materials are presented in table 1.

Seed germination and root tip preparation

For the analysis of somatic chromosomes, 1–1.2 cm long fresh root tips were collected from the bulbs or seeds. For arresting the meristematic cells at metaphase, three different methods including root tip pretreatment in ice-cold water for 24 hours (h), pretreatment in aqueous solution of α-bromo naphthalene at room temperature and pretreatment in 0.05% colchicine solution at room temperature were tested. They were subsequently fixed in Carnoy's fixative (glacial acetic acid: ethanol, 3:1) overnight at 4°C. The roots were transferred to 70% (v/v) ethanol and kept at -20 °C.

The roots were hydrolyzed in 1 M HCl for 15 min at 60 °C and stained in Feulgen solution (Feulgen & Rossenbeck 1924) for 1 hour. The stained root tips were then squashed in a drop of 45 % (v/v) acetic acid. Good metaphase spreads were photographed, using a digital camera attached to a BX51 Olympus microscope. At least five metaphase spreads were analyzed from each species.

Fluorescence in situ hybridization (FISH)

Seeds of the studied species germinated on moist filter paper and root tips were collected as described above. For fluorescence in situ hybridization, the plasmid p*Ta*71 containing an EcoRI 35S rRNA repeat from wheat (Gerlach & Bedbrook 1979) used to detect the nucleolus organizer regions in the chromosomes of *Fritillaria* species. The clone was directly labeled with Atto488-dUTP using a nick translation kit (Jena Bioscience), according to the manufacturer's instructions. Slide preparation and fluorescence in situ hybridization were carried out as described by Mirzaghaderi & al. (2017).

RESULT AND DISCASSION

All five species of Iranian *Fritillaria* were diploid ($2n = 2x = 24$), which is corresponded well with the

previous studies (Lacour 1978; Khaniki 2002a; Jafari & al. 2014 and Ahmadi-Roshan & al. 2016). *Fritillaria avromanica* is a newly identified species (Advay & al. 2015) and here, we reported its chromosomes count and karyotype characteristics for the first time. Karyotypes and the idiograms of haploid complement of studied *Fritillaria* species are shown in figs. 1 and 2, respectively.

Subgenus *Theresia* showed shorter mean chromosomes (12.05 µm) than *Fritillaria* and *Petillium* subgenera (15.32 µm and 21.28 µm, respectively). The overall mean of total chromosome length (TL) was 15.86 µm ranging from 12.05 µm (for *F. persica*) to 21.28 µm (*F. raddeana*). The mean of total chromosome length of the haploid complement (HCL) was 190.472 µm, ranging from 144.65 µm (for *F. persica*) to 255.41 µm (in *F. raddeana*).

The mean CI of the chromosomes varied from 16 % (*F. persica*) to 23 % (*F. chlorantha*). Based on the chromosome terminology of Levan & al. (1964), four chromosome types of ''m'' (centromere at median region), ''sm'' (centromere at sub median region), ''st'' (centromere at sub terminal region), and ''T'' (centromere at terminal point) formed five different karyotypic formula (table 3). Two satellites pairs were detected on long arms of *F. assyriaca* with the size of 2.45–3.42 µm. Karyotypes of all five species were located in the 3A or 3B Stebbins categories (Stebbins 1971).

To evaluate the karyotype asymmetry, different indices were considered (table 2). Based on TF%, CV%, A2, AI, AsK, S% and A parameters, *F. assyriaca* (S2) was the most asymmetric species. As shown in table 2, the highest value of TF % (i.e. 25.29) was recorded for *F. chlorantha* (S3), (the most symmetric) and the lowest value (i.e. 18.6) was observed in *F. assyriaca* (S2), (the most asymmetric). The highest and the lowest values of coefficient of variation (CV %) were identified in *F. assyriaca* (S2), (31.19%; the most asymmetric) and *F. avromanica* (S1) (14.12 %; the most symmetric), respectively. Similar to the result of CV%, the AI varied from 5.22 µm (*F. avromanica*) to 17.34 µm (*F. assyriaca*). The highest value of A was identified in *F. assyriaca* (0.63) while *F. chlorantha* demonstrated the lowest value (0.5). The highest and the lowest values of S% (Symmetric index) were recorded in *F. persica* (58.25) and *F. assyriaca* (27.34), respectively. AsK% value varied from 81.39 in *F. assyriaca* to 74.70 in *F. chlorantha*. Analysis of variance of karyotypic parameters indicated significant differences in four chromosomal parameters, including L, S, CL, RL and F% ($p<0.05$ and $p<0.001$). Subtelocentric chromosome displayed the maximum frequency in all species with the exception of *F. raddeana* in which telocentric chromosome type was most frequent. The 'T'' chromosome type was present in species except for *F. chlorantha*, that might be an indication of more evolved species. All later report approved on 88 accessions and 35 distinct species of *Fritillaria*, the average of total length of chromosome was 15.16 µm (ranging 8.62–18.77 µm), which was rather in accordance with the present study (15.86 µm, ranging 14.76- 21.28 µm). Based on karyotype asymmetry data, the studied species were located in the 3A and 3B asymmetry groups of Stebbins (1971) implying they are relatively asymmetric. Furthermore, in case of TF %, CV %, A2, AI, AsK %, S% and A, all species were asymmetric, but the most asymmetric species was *F. assyriaca*.

Depending on species, 4 and 6 35S rDNA loci were observed which mainly located at the distal or telomeric regions of the chromosomes (fig.3). While only four rDNA loci were observed in *F. raddeana*, six loci were detected in *F. persica*. The locations of rDNA loci were also different in these species. While rDNA sites in *F. raddeana* were observed mainly at distal ends of the chromosomes, *F. persica* harbored them at the telomeric regions of the chromosomes.

Table 1. Sampling locations and chromosome numbers of *Fritillaria* Species.

Species	Species Code	Locality, collector and voucher ID
F. avromanica	S1	Prov. Kurdistan, avroman, Daravian, 46˚16′58.35″; 35°12′58.95″; 1800 m; M. advay, 1251, HOK.
F. assyriaca	S2	Prov. Kurdistan, Gardaneh salvatabad, 47˚7′5.63″; 35˚17′2.26″; 1865 m; M. advay; 1236, HOK.
F. chlorantha	S3	Prov. Kurdistan, Baneh, Babos Mountain, 45˚56′18.59″; 35˚59′13.15″; 2400 m; M. advay, 1229, HOK.
F. persica	S4	Prov. Kurdistan, Sarvabad, Bendol, 46˚15′7.95″; 35˚19′6.63″; 1900 m ; M. advay, 1278, HOK.
F. raddeana	S5	North Khorasan, Bojnord, Chaman bid, 56˚40′28.70″; 37˚26′18.04″; 1340 m; M. advay, 1272, HOK.

Table 2. Mean chromosomal and karyotypic parameters of *Fritillaria* spp. S1: *F. avromanica*, S2: *F. assyriaca*, S3: *F. chlorantha*, S4: *F. persica*, S5: *F. raddeana*.

Parameters	Species					Mean	Range	Species of range	
	S1	S2	S3	S4	S5			Min	Max
S (µm)	3.18	3.53	3.04	2.65	4.14	3.30	2.65- 4.14	S4	S5
L (µm)	11.58	15.50	9.004	9.52	17.13	12.54	9.004- 17.13	S3	S5
TL (µm)	14.76	19.03	12.18	12.05	21.28	15.86	14.76- 21.28	S4	S5
AR	4.3	5.84	4.09	4.55	6.27	5.01	4.09- 6.27	S3	S5
r value	0.27	0.25	0.36	0.28	0.24	0.28	0.24- 0.36	S5	S3
RL%	8.33	7.58	7.32	7.62	7.71	7.71	7.32- 8.33	S3	S1
F %	1.79	1.46	1.71	1.73	1.54	1.64	1.46- 1.79	S2	S1
CI	0.2	0.18	0.23	0.16	0.17	0.18	0.16- 0.23	S4	S3
HCL	177.15	228.89	146.26	144.65	255.41	190.472	144.65-255.41	S3	S5
TF %	21.55	18.6	25.29	21.82	19.47	21.346	18.6- 25.29	S2	S3
CV %	14.12	31.19	21.59	16.01	21.8	20.94	14.12- 31.19	S1	S2
A_1	0.723	0.74	0.63	0.71	0.75	0.71	0.63- 0.75	S3	S5
A_2	0.14	0.31	0.21	0.16	0.21	0.20	0.14- 0.31	S1	S2
AI	5.22	17.34	10.92	11.52	13.84	11.76	5.22- 17.34	S1	S2
AsK %	78.44	81.39	74.70	78.17	80.52	78.64	74.70- 81.39	S3	S2
S%	58.11	27.34	49.29	58.25	48.04	48.20	27.34- 58.25	S2	S4
A	0.58	0.63	0.50	0.56	0.62	0.58	0.50- 0.63	S3	S2
X_{CA}	58.29	62.6	50.31	58.43	63.92	58.71	50.31- 63.92	S3	S5
X_{CI}	0.20	0.18	0.24	0.20	0.18	0.2	0.18- 0.24	S5	S3
CV_{CI}	36.98	55.59	50.58	48.84	63.51	51.1	36.98- 63.51	S1	S5

Table 3. Stebbin's (1971) classification (ST) and Karyotype formula based on Levan & al (1964) nomenclature for *Fritillaria* spp.

Species	*F. avromanica* (S1)	*F. assyriaca* (S2)	*F. chlorantha* (S3)	*F. persica* (S4)	*F. raddeana* (S5)
ST	3A	3B	3B	3A	3B
Karyotype formula	2m + 4sm + 16st + 2 T	2m + 4sm+ 8st +10T	10m + 14st	4m+ 18st+ 2T	4m+ 2 sm+ 4st+ 14T

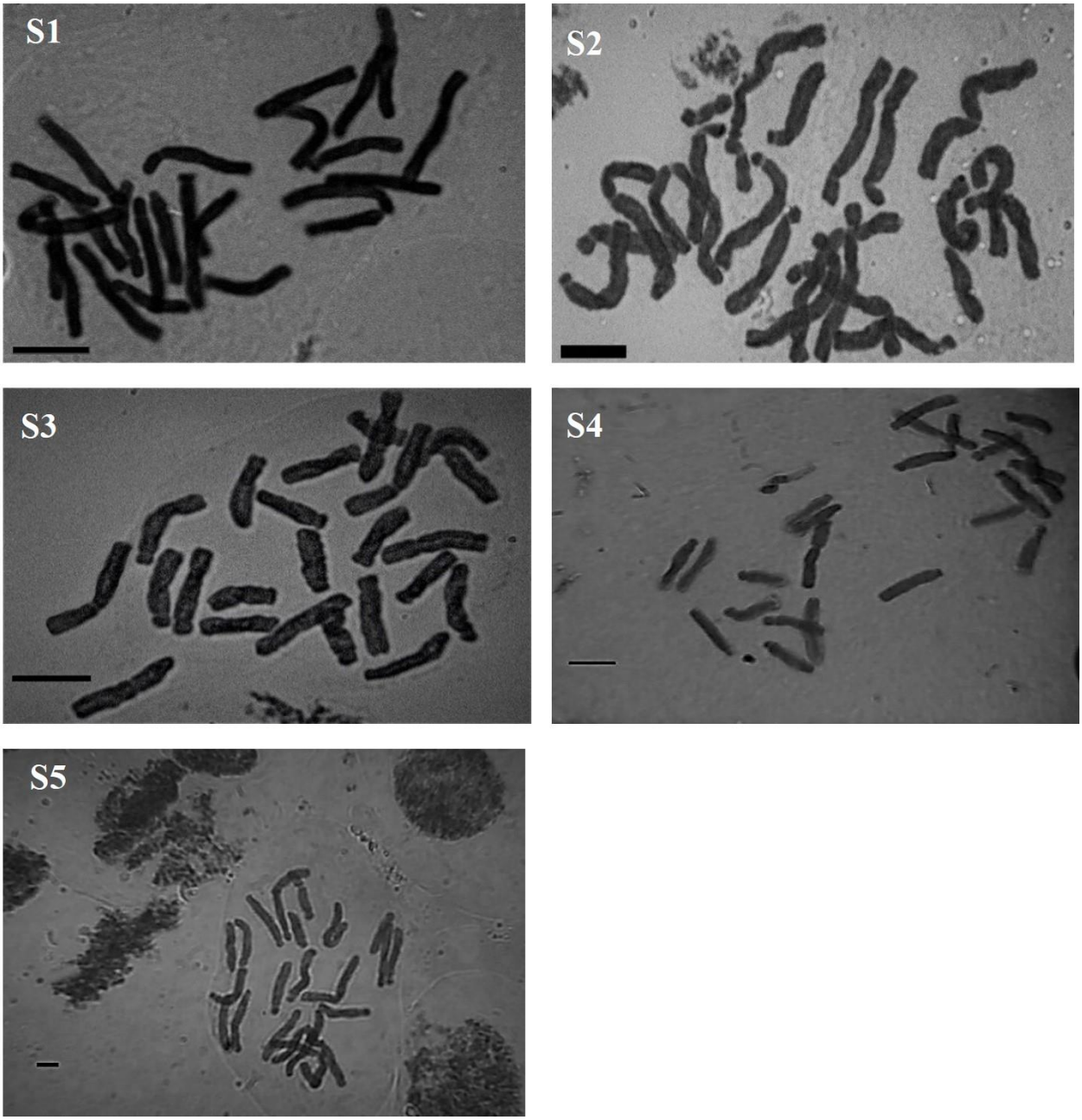

Fig.1. Somatic chromosomes in *Fritillaria*: S1, *F. avromanica*; S2, *F. assyriaca*; S3, *F. chlorantha*; S4, *persica*; S5, *F. raddeana*. Scale bar 10 µm.

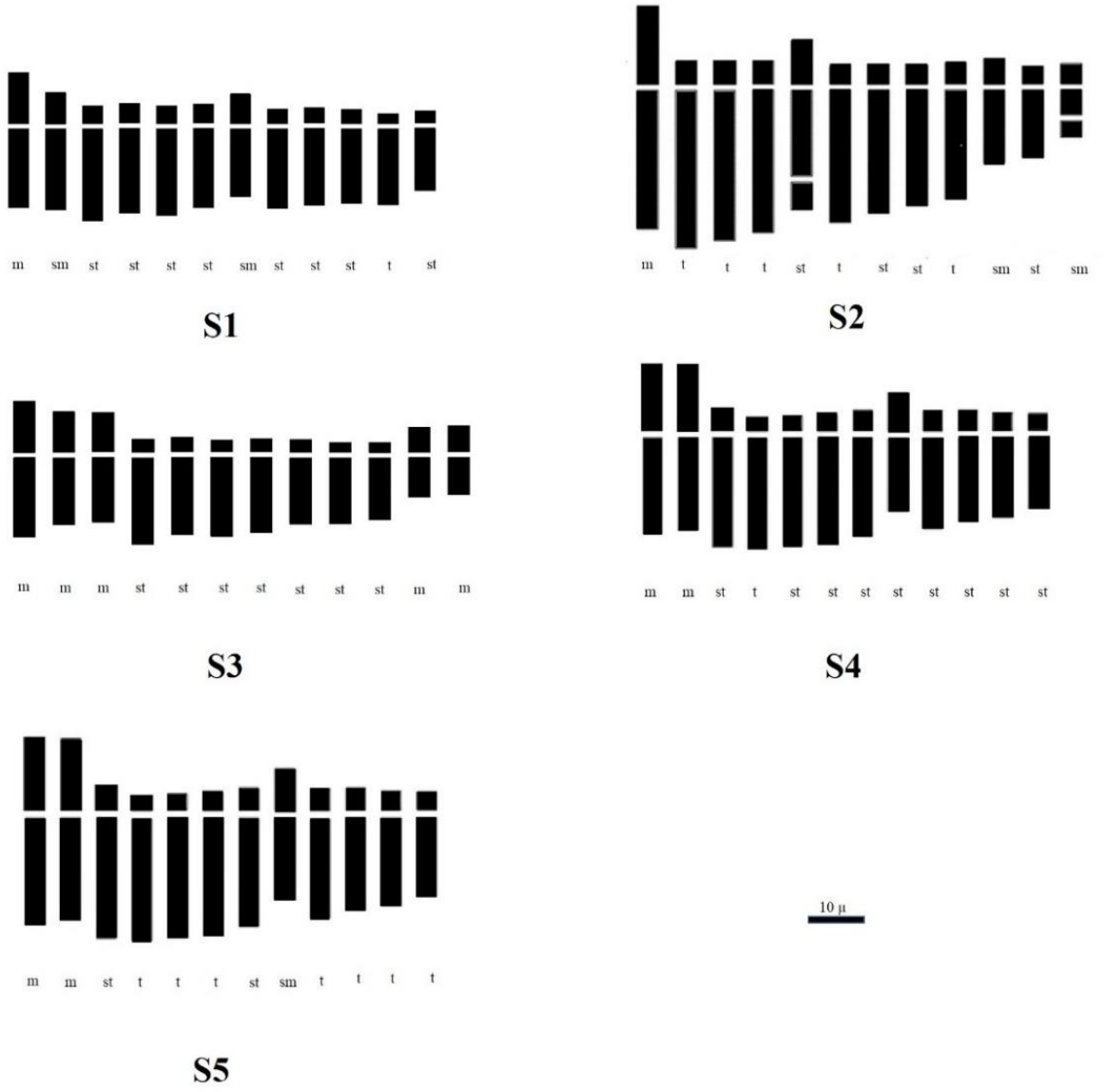

Fig. 2. Idiograms of five studied species of *Fritillaria* (2*n*=2*x*=24). S1, *F. avromanica*; S2, *F. assyriaca*; S3, *F. chlorantha*; S4, *F. persica*; S5, *F. raddeana*. Scale bar 10 µm.

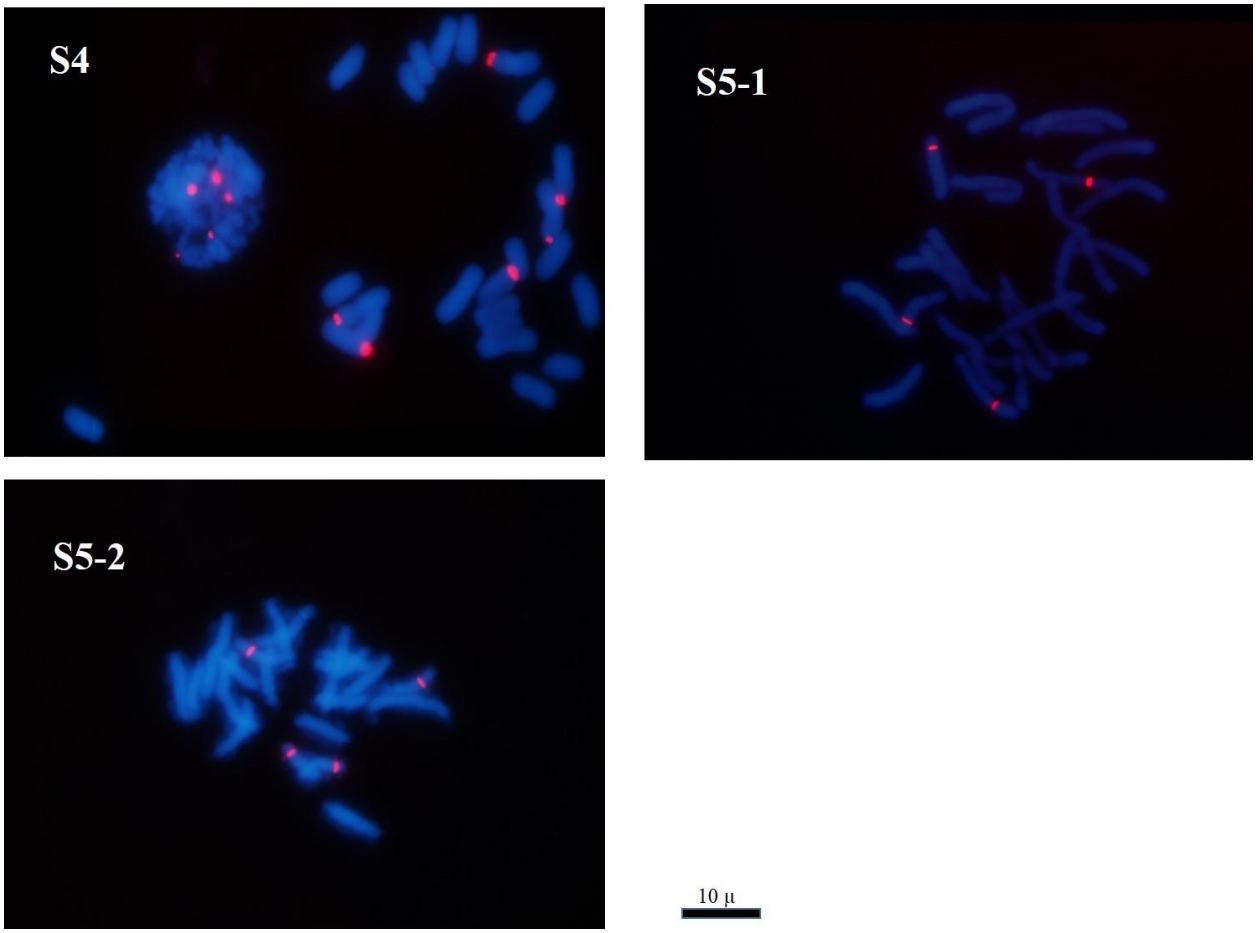

Fig. 3. FISH localization of the 35S rDNA in 2 species of *Fritillaria*. S4, *F. persica*; S5-1 and S5-2, *F. raddeana*. Scale bar=10µ.

REFERENCES

Advay, M., Teksen, M., & Maroofi, H. 2015: *Fritillaria avromanica* sp. nov. (Liliaeceae) from Iran and notes on F. melananthera in Turkey. – Nord. J. Bot. 33 (5): 526-531.

Ahmadi-Roshan, M., Karimzadeh, G., Babaei, A., & Jafari, H. 2016: Karyological studies of *Fritillaria* (Liliaceae) species from Iran. – Cytologia. 81 (2): 133-141.

Bennett, M. D., & Smith, J. 1976: Nuclear DNA amounts in angiosperms. *Philosophical Transactions of the Royal Society of London. B*. – Biol Sci. 274 (933): 227-274.

Day, P. D., Berger, M., Hill, L., Fay, M. F., Leitch, A. R., Leitch, I. J., & al. 2014: Evolutionary relationships in the medicinally important genus *Fritillaria* L. (Liliaceae). – Mol. Phylogenet. Evol. 80: 11-19.

Fedorov, A. 1969: *Chromosome numbers of flowering plants Acad. Sci USSR*: Leningrad (now St Petersburg).

Feulgen, R., & Rossenbeck, H. 1924: Mikroskopisch-chemischer Nachweis einer Nucleinsäure vom Typus der Thymonucleinsäure und die-darauf beruhende elektive Färbung von Zellkernen in mikroskopischen Präparaten. – Hoppe-Seyler s Zeitschrift für Physiologische Chemie. 135 (5-6): 203-248.

Gerlach, W., & Bedbrook, J. 1979: Cloning and characterization of ribosomal RNA genes from wheat and barley. – Nucleic. Acids. Res. 7 (7): 1869-1885.

Jafari, H., Babaei, A., Karimzadeh, G., & Ahmadi-Roshan, M. 2014: Cytogenetic study on some Fritillaria species of Iran. – Plant Syst. Evol. 300 (6): 1373-1383.

Khaniki, G. 2002a: Chromosome number of all Iranian species of Fritillaria *caucasica* group (Liliaceae). – Nucleus. 45: 6-11.

Khaniki, G. 2002b: Chromosome number of *Fritillaria* subgenera *Petilium* and *Theresia* (Liliaceae). Nucleus. 45 (1-2): 6-11.

Khaniki, G. 2005: Giemsa C-banding studies on interphase nuclei of Iranian species of *Fritillaria* and *Rhinopetalum* (Liliaceae). – Proc. Natl. Acad. Sci. India B. *75* (4): 294.

Lacour, L. F. 1978: The constitutive heterochromatin in chromosomes of Fritillaria sp., as revealed by Giemsa banding. Philosophical Transactions of the Royal Society of London. B. – Biol Sci. 285 (1004): 61-71.

Levan, A., Fredga, K., & Sandberg, A. A. 1964: Nomenclature for centromeric position on chromosomes. Hereditas. 52 (2): 201-220.

Metin, Ö. K., Türktaş, M., Aslay, M., & Kaya, E. 2013: Evaluation of the genetic relationship between Fritillaria species from Turkey's flora using fluorescent-based AFLP. – Turk. J. Biol. 37 (3): 273-279.

Mirzaghaderi, G., Abdolmalaki, Z., Zohouri, M., Moradi, Z., & Mason, A.S. 2017: Dynamic nucleolar activity in wheat— Aegilops hybrids: evidence of C-genome dominance. – Plant. Cell. Rep. 36 (8): 1277-1285.

Peruzzi, L., Leitch, I., & Caparelli, K. 2008: Chromosome diversity and evolution in Liliaceae. – Ann. Bot. 103 (3): 459-475.

Rechinger, K. 1990: *Fritillaria* L. – Flora Iranica. 165: 61-76.

Rix, E. 2001: *Fritillaria*. A Revised Classification. The *Fritillaria* Group of the Alpine Garden Society. United Kingdom.

Rix, E. M. 1997: *Fritillaria* L. (Liliaceae) in Iran. – Iran J Bot. 1:75-95.

Samaropoulou, S., Bareka, P., & Kamari, G. 2016: Karyomorphometric analysis of Fritillaria montana group in Greece. – Comparative cytogenetics. 10 (4): 679-695.

Sharifi-Tehrani, M., & Advay, M. 2015: Assessment of relationships between Iranian *Fritillaria* (Liliaceae) species using chloroplast trnH-psbA sequences and morphological characters. – J Genet Resour. 1 (2): 89-100.

Stebbins, G. L. 1971: *Chromosomal evolution in higher plants*: Edward Arnold Publisher, London.