Maejo Int. J. Sci. Technol. **2022**, *16*(03), 223-237

Maejo International Journal of Science and Technology

ISSN 1905-7873
Available online at www.mijst.mju.ac.th

Full Paper

Chromosome karyotype analysis of some *Fritillaria* species

Yongming Fan [1, 2], Lihong Hao[3], Wei Zhu[2, 4], Jaime A. Teixeira da Silva[5] and Xiaonan Yu[2, 4, *]

[1] College of Architecture, North China University of Water Resources and Electric Power, Zhengzhou, 450045, China
[2] College of Landscape Architecture, Beijing Forestry University, Beijing, China
[3] College of Horticulture, Hebei Agricultural University, Baoding, 071001, China
[4] Beijing Laboratory of Urban and Rural Ecological Environment, Beijing, 100083, China
[5] Ikenobe 3011-2, Kagawa-ken, 761-0799, Japan
* Corresponding author, e-mail: yuxiaonan626@126.com

Received: 21 August 2022 / Accepted: 17 November 2022 / Published: 23 November 2022

Abstract: *Fritillaria* (Liliaceae) are a genus of perennial bulbous plants with medicinal and ornamental importance. They are widely distributed in temperate regions of the northern hemisphere and are considered as novelty flowers on the ornamental market. In this study a genetic analysis of chromosome karyotypes of nine *Fritillaria* spp. in three subgenera was carried out using root tip squashes. Except for *F. uva-vulpis*, which is triploid ($2n = 3x = 33$), the other eight *Fritillaria* species are diploid ($2n = 2x = 24$). The karyotypes of *F. pallidiflora*, *F. tortifolia* and *F. verticillata* var. *albiflora* are 2B, while *F. imperialis*, *F. uva-vulpis*, *F. verticillate*, *F. yunminensis*, *F. anhuiensis* and *F. karelinii* are 3B. In addition, results of a clustering analysis using the karyotype near-resemblance coefficient conducted among the eight diploid species support the current classification of the *Fritillaria* L. subgenera. This study provides cytological evidence for phylogenetics and crossbreeding.

Keywords: *Fritillaria* L., chromosome, karyotype analysis, phylogeny

INTRODUCTION

There are more than 130 species of *Fritillaria* spp. (Liliaceae), which are perennial bulbous plants [1]. Fritillaries are found widely throughout temperate regions of the northern hemisphere, with a rich abundance in countries surrounding the Mediterranean [2, 3]. Rix [4] believes that *Fritillaria* is represented worldwide by eight subgenera, two sections and 165 taxa (139 species, 17 subspecies and nine varieties). However, the latest statistics at the end of December

Maejo Int. J. Sci. Technol. **2022**, *16*(03), 223-237

2010 available on the Plant List, which is compiled by the Royal Botanical Garden and Missouri Botanical Garden, showed that there were 171 taxa comprising 30 subspecies and variants [5]. Fritillaries are used mostly as ornamental bulbs and are one of the largest genera in the Liliaceae. The 2000 version of the Flora of China registers 24 species and four varieties, including 15 species that are endemic to China [6]. In China, apart from their use as garden plants, the bulbs of many species have been used in traditional Chinese medicine for over 2000 years [2, 7, 8].

There is constant demand for novelty in the ornamental plant market, and this is possible in *Fritillaria* species, which have a broad range of variation in morphological features that adapt to changes in the environment. The species developed and marketed only account for a small fraction of the 171 taxa. In China local or endemic ornamental cultivars have not yet been developed, and several ornamental cultivars (*F. imperialis* 'Maxima Lutea', *F. imperialis* 'Maxima Rubra' and *F. persica* 'Ivory Bell', among others) currently used for gardening and landscaping are imported primarily from the US or the Netherlands. Therefore, novel *Fritillaria* resources are urgently needed to improve horticultural traits such as flower colour or pattern, architecture and growth type, and resistance to abiotic and biotic stresses, via conventional hybridisation or molecular breeding in order to develop novel cultivars. However, even basic information related to production is lacking, impeding these advances [9].

The number of chromosomes and their morphological characteristics (such as length of arms, centromere position, number and length of satellites and belt type) in an organism are relatively fixed. According to Stebbins [10], species with karyotype symmetry often have original traits while those with karyotype asymmetry have more evolutionarily advanced characteristics. It is generally believed that the basic trend of chromosome karyotype evolution is from symmetry to asymmetry. Thus, evolutionarily ancient or original plants have mostly a more symmetric karyotype, while asymmetric karyotypes often occur in plants with higher degrees of evolution [11]. Two indices, namely asymmetry coefficient of karyotypes (ASK) and karyotype (KT), are often used for assessing the evolutionary relationship between species [12-14]. A high value of ASK indicates a higher degree of evolution [15, 16]. Consequently, chromosome karyotype is used as an important index of plant taxonomy and genetic research to elaborate the phylogeny of plant groups, analyse the genetic relationship between species, and reveal the process and mechanism of genetic evolution [17]. More importantly, the ploidy level can be ascertained via karyotype research and can serve as a reference for crossbreeding.

Changes in multiplicity of chromosomes, which are the main carriers of plant genes, can lead to genetic variation in plants [18] while karyotype information can provide a cytological basis for germplasm improvement. The chromosome number of more than 50 species of *Fritillaria* is currently known [19]. The earliest findings on two species (*F. amabilis* and *F. japonica*) showed a chromosome number of $2n = 22$ [20, 21], which was also true for *F. ussuriensis* [22]. The karyotypes of some other species have also been reported, most of which are diploid ($2n = 2x = 24$) and have a basic chromosome number of $x = 12$ [23-28]. Based on chromosomal positions of 5S and 45S rDNA, some Iranian *Fritillaria* species were shown to be diploid with 2n = 2x = 24 chromosomes [29]. On occasion, aneuploidy was discovered in *F. pallidiflora* ($2n = 20$) and *F. walujewii* ($2n = 30$) [30]. In addition, the karyotypes of *F. glauca* and *F. pudica* [31], as well as *F. pinetorum* [32], all of which belong to subgenus *Liliorhiza* in North America, were reported as $2n = 26$, while *F. meleagroides*, *F. montana* and *F. ruthenica*, which belong to subgenus *Fritillaria*, were reported as $2n = 18$ [33]. Triploid plants have been found in several species [34, 35], for example *F. lanceolata* [36] and *F. ussuriensis* [37]. The same species showed karyotypic variation among

studies. For example, the karyotype formula of *F. persica* was $2n = 2x = 24 = 4m + 12st + 8t$ [23], although five karyotype formulas ($2n = 2x = 24 = 4m + 16st + 4T$, $2n = 2m + 2sm + 20st$, $2n = 2m + 2sm + 18st + 2T$, $2n = 2m + 2sm + 14st + 6T$, and $2n = 4m + 2sm + 18st$) were reported by another group [26], apparently because materials were collected from different geographic locations or environments. Moreover, even the karyotypes of the same species among different cultivars or different populations are not the same, as was shown for *F. thunbergii* [38] and *F. anhuiensis* [39]. These studies indicated that karyotype polymorphism exists in *Fritillaria* species. A study of seven *Fritillaria* spp. in Anhui province in China [40], combined with the studies of Xu et al. [41], Yang and Zhu [42] and Zhang et al. [43], showed that the similarities or differences in karyotype formulas of the same or different species were not associated with the locations of the study material [40].

Zaharof [44] conducted an analysis of 12 *Fritillaria* species (*F. epirotica*, *F. tuntasia*, *F. obliqua*, *F. erhartii*, *F. conica*, *F. pontica*, *F. gussichiae*, *F. drenovskii*, *F. montana*, *F. messanensis*, *F. bithynica* and *F. carica*) of Greek origin and compared differences between their karyotypes. The karyotypes of 11 out of 12 species showed general uniformity, consisting of 4 V-type chromosomes, while 20 I (rod)-type recognisable differences were noticed in a few marker chromosomes [44]. Samaropouloua et al. [45] analysed the hybridisation and karyotype variability of three endemic *Fritillaria* species (*F. graeca*, *F. rhodocanakis* and *F. spetsiotica*) from the Argolis Peninsula in Greece. The cytotaxonomy of 11 Turkish *Fritillaria* taxa was analysed by Kocyigit et al. [46]. Their results showed that *F. uva-vulpis* was the only triploid species while other species were diploid with some B chromosomes [46]. The chromosome diversity and evolution of some Chinese species in the tribe Lilieae (Liliaceae) were studied by Gao et al [47]. They found that the karyotypic evolution included three major periods: (1) additional DNA were added or transferred from larger chromosomes to smaller chromosomes' long arms, which caused an increase in the relative variation in centromeric index and a decrease in the relative variation in chromosome length at the same time; (2) both the relative variation in centromeric index and the chromosome length decreased as a result of additional DNA or the translocation of chromosomes being added onto the short arms of smaller chromosomes; and (3) additional DNA or translocation of chromosomes were added onto the long arms of longer chromosomes. These studies indicate that phylogenetic studies of *Fritillaria* are actively being pursued. Karyotype analysis has made it possible to elucidate the phylogeny of different plant groups in *Fritillaria* and to analyse the genetic relationships between different species.

In this paper chromosomal and karyotypic parameters of nine *Fritillaria* species belonging to three subgenera (*Petillium*, *Fritillaria* and *Rhinopetalum*) were analysed. Among them, the karyotype parameters of *F. tortifolia*, *F. verticillata* var. *albiflora* and *F. karelinii* have not been previously reported. The purpose of this study is to provide a cytological basis for plant classification, phylogeny and crossbreeding of *Fritillaria* L.

MATERIALS AND METHODS

Plant Materials

For this study, the bulbs of nine *Fritillaria* species were purchased from Netherlands (via Hezhong Co., China), USA (supplied by Guangpei Lu), and obtained from Yumin experimental station in Tacheng, Xinjiang and Jinzhai counties of Liuan city, Anhui province, China in 2014 (Table 1, Figure 1). Bulbs were potted in a substrate containing peat, perlite and vermiculite (1:2:1,

Maejo Int. J. Sci. Technol. **2022**, *16*(03), 223-237

v/v) under natural light conditions at 15-18°C in September. In October when the roots had grown, fresh root tips 1-2 cm long were cut and used for analysis of somatic chromosomes.

Table 1. Basic information of nine *Fritillaria* species used in this study

Code	Subgenus name	Species name	Traits	Origin
I	*Petillium*	*F. imperialis*	Bell-shaped flowers, orange or yellow [48]	Netherlands
II	*Fritillaria*	*F. uva-vulpis*	Brown egg-shaped flowers, with a distinct yellow tip [49]	USA
III		*F. pallidiflora*	Large green-yellow flower occasionally speckled with red [49]	Xinjiang, China
IV		*F. tortifolia*	Large white, slightly tessellated, broadly-bell-shaped flowers with lots of pink spots and staining inside [49]	Xinjiang, China
V		*F. verticillata*	Large white broadly campanulate flowers with little or no pink spots inside [49]	Xinjiang, China
VI		*F. verticillata* var. *albiflora*	Large white broadly campanulate flowers with pink or green spots inside [49]	Xinjiang, China
VII		*F. yuminensis*	Curious bluish-white or slightly pink in some forms, unmarked flowers [49]	Xinjiang, China
VIII		*F. anhuiensis*	Quite large, nodding tubular-campanulate flowers, yellowish white or yellowish green and tessellated with purple spots [49]	Anhui, China
IX	*Rhinopetalum*	*F. karelinii*	Solitary, wide-open stars of rose pink with some deeper spots and veining [48]	Xinjiang, China

Figure 1. Morphological characteristics of nine *Fritillaria* species: (I) *F. imperialis*, (II) *F. uva-vulpis*, (III) *F. pallidiflora*, (IV) *F. tortifolia*, (V) *F. verticillata*, (VI) *F. verticillata* var. *albiflora*, (VII) *F. yuminensis*, (VIII) *F. anhuiensis*, (IX) *F. karelinii* (photos supplied by Xianzhen Duan)

Chromosome Preparation

Root tips were pretreated with 0.7mM cycloheximide solution in the dark at room temperature (about 25°C) for 6-8 hr. They were then transferred to Carnoy's fixative (glacial acetic acid : ethanol = 1:3 v/v) and fixed for 24 hr at 4°C. Fixed root tips were washed thoroughly with distilled water and kept in 70% ethanol stored at 4°C. The fixed root tips were hydrolysed in 1M HCl at 55°C for 3-5 min., stained with carbol fuchsin solution for 10 min. at room temperature, covered with coverslips and squashed by gently tapping on the coverslips padded by a layer of filter paper with the round end of an anatomical needle. All chemicals (cycloheximide, ethanol, glacial

acetic acid, HCl and carbol fuchsin) were purchased from Lanyi Co. (China). Chromosomes were examined under a microscope (ZEISS Scope A1, Jena, Germany) and metaphase images were collected.

Data Collection and Karyotype Analysis

At least 30 metaphases were examined in counting the chromosome number and at least 5 well-spread metaphase plates from each individual were used. Images were acquired by a chromosomal karyotype analysis system Karyo 3.1 (VideoTesT, NatureGene Corp., Hong Kong, China). Metaphases of chromosomes were collected by Leica Application Suite version 3 (Leica Microsystems, Germany) and then enhanced by Adobe Photoshop CS5 (Adobe Systems, USA). The analysis of chromosome pairs was carried out by Imag-Pro Plus 6.0 software (Media Cybernetics, SA). The values of long-arm length (L), short-arm length (S), total chromosome length (TL = L + S), relative chromosome length (RL), relative length of long arm (RLL), relative length of short arm (RLS), arm ratio (AR=L/S), r value (1/AR), longest chromosome length/shortest chromosome length (Lt/St), ASK and centromere index (CI) were measured and calculated by Adobe Photoshop CS5 and Excel 2007 (Microsoft, USA). ANOVA was performed and significant differences between means within species of several indices (L, S, TL, AR, r, RL and CI) were tested with Duncan's multiple range test in SPSS (version 20.0, IBM, USA). The karyotype pattern was drawn by inserting a 2-D bar chart in Excel 2007 (Microsoft, USA) and modified in Adobe Photoshop CS5.

The classification of relative length index was based on Li and Chen [50], karyotype classification criteria were according to Stebbins [10], and chromosome morphology was explained using nomenclature proposed by Levan et al. [51]. Nomenclature, number of chromosomes, karyotype, karyotype formula and chromosome relative length composition were also described. Gametic chromosome number, RL, CI and AR were used as cluster parameters. The karyotype near-resemblance coefficient (KRC) and genetic distance of the studied species were calculated by cluster analysis software (Visual Basic) of KRC [52] based on Tan et al. [53] and Wu [54].

RESULTS AND DISCUSSION

Except for *F. uva-vulpis*, which is triploid ($2n = 3x = 33$), the other eight *Fritillaria* species are all diploid ($2n = 2x = 24$). The relative length of each pair of chromosomes from nine *Fritillaria* species are shown in Table 2 and the related karyotype parameters of these species are shown in Table 3. The karyotypes of the somatic complement and the idiograms of the haploid complement of the studied *Fritillaria* species are shown in Figures 2 and 3 respectively. The results of ANOVA of interspecific differences of several indices are shown in Table 4. There are significant differences ($p<0.01$) in L, S, TL, AR, 1/AR and CI, but not in RL. The variation in RL, AR and CI of the nine *Fritillaria* species are shown in Table 5.

Maejo Int. J. Sci. Technol. **2022**, *16*(03), 223-237

Table 2. Relative length of each pair of chromosomes of nine *Fritillaria* species

No	*F. imperialis*			*F. uva-vulpis*			*F. pallidiflora*			*F. tortifolia*			*F. verticillata*			*F. verticillata* var. *albiflora*			*F. yuminensis*			*F. anhuiensis*			*F. karelinii*		
	RL	r	N	RL	r	N	RL	r	N	RL	r	N	RL	r	N	RL	r	N	RL	r	N	RL	r	N	RL	r	N
1	11.65	0.45	sm	12.40	0.61	m	11.85	0.80	m	13.70	0.74	m	10.97	0.38	sm	13.75	0.57	sm	11.71	0.27	st	12.31	0.20	st	12.42	0.14	t
2	10.30	0.14	st	11.34	0.16	st	10.38	0.70	m	10.92	0.27	st	10.21	0.25	st	10.52	0.66	m	10.87	0.26	st	11.22	0.27	st	11.74	0.13	t
3	9.76	0.14	t	10.95	0.40	sm	10.06	0.84	m	9.44	0.24	st	9.97	0.28	st	9.78	0.69	m	10.02	0.41	sm	9.68	0.32	st	9.91	0.09	t
4	9.35	0.14	t	10.23	0.72	m	9.53	0.94	m	8.97	0.26	st	9.71	0.33	st	9.27	0.65	m	9.84	0.25	st	8.84	0.31	st	8.82	0.40	sm
5	9.10	0.15	st	9.56	0.35	sm	9.11	0.49	sm	8.82	0.12	t	9.45	0.42	sm	8.79	0.77	m	9.57	0.30	st	8.47	0.23	st	8.50	0.11	t
6	8.53	0.13	t	9.09	0.23	st	8.55	0.46	sm	8.13	0.34	sm	8.97	0.23	st	8.27	0.68	m	8.57	0.69	m	7.89	0.23	st	8.05	0.17	st
7	7.88	0.18	st	8.47	0.51	sm	7.77	0.54	sm	7.67	0.91	m	8.06	0.83	m	7.96	0.67	m	7.64	0.30	st	7.80	0.37	sm	7.70	0.13	t
8	7.71	0.53	sm	7.96	0.28	st	7.30	0.86	m	7.18	0.94	m	7.65	0.59	m	7.34	0.79	m	7.07	0.51	sm	7.59	0.36	sm	7.35	0.74	m
9	6.98	0.18	st	7.50	0.83	m	6.77	0.67	m	6.89	0.62	m	7.12	0.65	m	7.08	0.67	m	6.54	0.41	sm	7.28	0.78	m	7.08	0.78	m
10	6.83	0.20	st	6.68	0.25	st	6.60	0.34	sm	6.65	0.89	m	6.83	0.43	sm	6.20	0.28	st	6.28	0.47	sm	7.07	0.81	m	6.53	0.20	st
11	6.48	0.26	st	5.82	0.20	st	6.25	0.42	sm	6.47	0.73	m	5.97	0.23	st	5.74	0.51	sm	6.15	0.48	sm	6.18	0.24	st	6.17	0.69	m
12	5.43	0.21	st				5.84	0.36	sm	5.17	0.78	m	5.09	0.61	m	5.28	0.31	st	5.73	0.38	sm	5.68	0.24	st	5.73	0.24	st

Notes: RL = relative length of chromosome; r = length of short arm/ length of long arm (1/AR); N = nomenclature
'm'- AR value 1.01-1.70; 'sm'- AR value 1.71-3.00; 'st'- AR value 3.01-7.00; 't'- AR value >7.01

Table 3. Mean chromosomal and karyotypic parameters of nine *Fritillaria* species

Parameter	Species code									Mean	Range	Species range	
	I	II	III	IV	V	VI	VII	VIII	IX			Min	Max
L (μm)	15.7	16.56	11.43	13.01	13.69	9.46	15.41	12.70	14.02	13.55	9.46-16.56	VI	II
S (μm)	3.38	6.50	7.11	6.32	5.49	5.74	5.69	4.18	3.51	5.32	3.38-7.11	I	III
TL (μm)	19.08	23.06	18.54	19.33	19.18	15.20	21.09	16.88	17.53	18.88	15.20-23.06	VI	II
RLL	6.86	6.53	5.14	5.61	5.95	5.19	6.09	6.27	6.67	6.03	5.14-6.86	III	I
RSL	1.47	2.56	3.20	2.72	2.38	3.15	2.25	2.06	1.67	2.38	1.47-3.20	I	III
RL	8.33	9.09	8.33	8.33	8.33	8.33	8.33	8.33	8.33	8.41	8.33-9.09	I, III-IX	II
AR	5.42	3.14	1.81	2.66	2.71	1.84	2.78	3.33	5.40	3.23	1.81-5.42	III	I
r	0.23	0.41	0.62	0.57	0.44	0.60	0.39	0.36	0.32	0.44	0.23-0.62	I	III
Lt /St	2.15	2.13	2.03	2.65	2.16	2.60	2.04	2.17	2.17	2.23	2.03-2.65	III	IV
ASK	82.30	71.82	61.65	67.31	71.39	62.22	73.04	75.26	79.99	71.66	61.65-82.30	III	I
CI	17.63	27.69	37.27	33.92	29.33	36.96	27.73	25.37	21.61	28.61	17.63-37.27	I	III

	Species code								
	I	II	III	IV	V	VI	VII	VIII	IX
NC	24	33	24	24	24	24	24	24	24
KT	3B	3B	2B	2B	3B	2B	3B	3B	3B
KF	$2n=2x=24$=4s m + 14st + 6t	$2n=3x=33$=9m + 9sm + 15st	$2n=2x=24$=12m + 12sm	$2n=2x=24$=14m + 2sm + 6st + 2t	$2n=2x=24$=8m + 6sm + 10st	$2n=2x=24$=16m + 4sm + 4st	$2n=2x=24$=2m + 12sm + 10st	$2n=2x=24$=4m + 4sm + 16st	$2n=2x=24$=6m + 2sm + 6st + 10t
CRLC	$2n$=2L + $10M_2$ + $10M_1$ + 2S	$2n$=3L + $12M_2$ + $12M_1$ + 6S	$2n$=2L + $10M_2$ + $8M_1$ + 4S	$2n$=4L + $6M_2$ + $12M_1$ + 2S	$2n$=2L + $10M_2$ + $8M_1$ + 4S	$2n$=4L + $6M_2$ + $8M_1$ + 6S	$2n$=4L + $8M_2$ + $6M_1$ + 6S	$2n$=4L + $6M_2$ + $10M_1$ + 4S	$2n$=4L + $6M_2$ + $10M_1$ + 4S

Notes: L = length of long arm; S = length of short arm; TL = total chromosome length (L+S); RLL = relative length of long arm; RSL = relative length of short arm; RL = relative length of chromosome; AR = arm ratio (L/S); r = 1/AR; Lt /St = longest chromosome length/shortest chromosome length; ASK = asymmetry coefficient of karyotype; CI = centromere index; NC = number of chromosomes; KT = karyotype; KF = karyotype formula; CRLC = chromosome relative length composition. I, *F. imperialis*; II, *F. uva-vulpis*; III, *F. pallidiflora*; IV, *F. tortifolia*; V, *F. verticillata*; VI, *F. verticillata* var. *albiflora*; VII, *F. yuminensis*; VIII, *F. anhuiensis*; IX, *F. karelinii*

Maejo Int. J. Sci. Technol. **2022**, *16*(03), 223-237

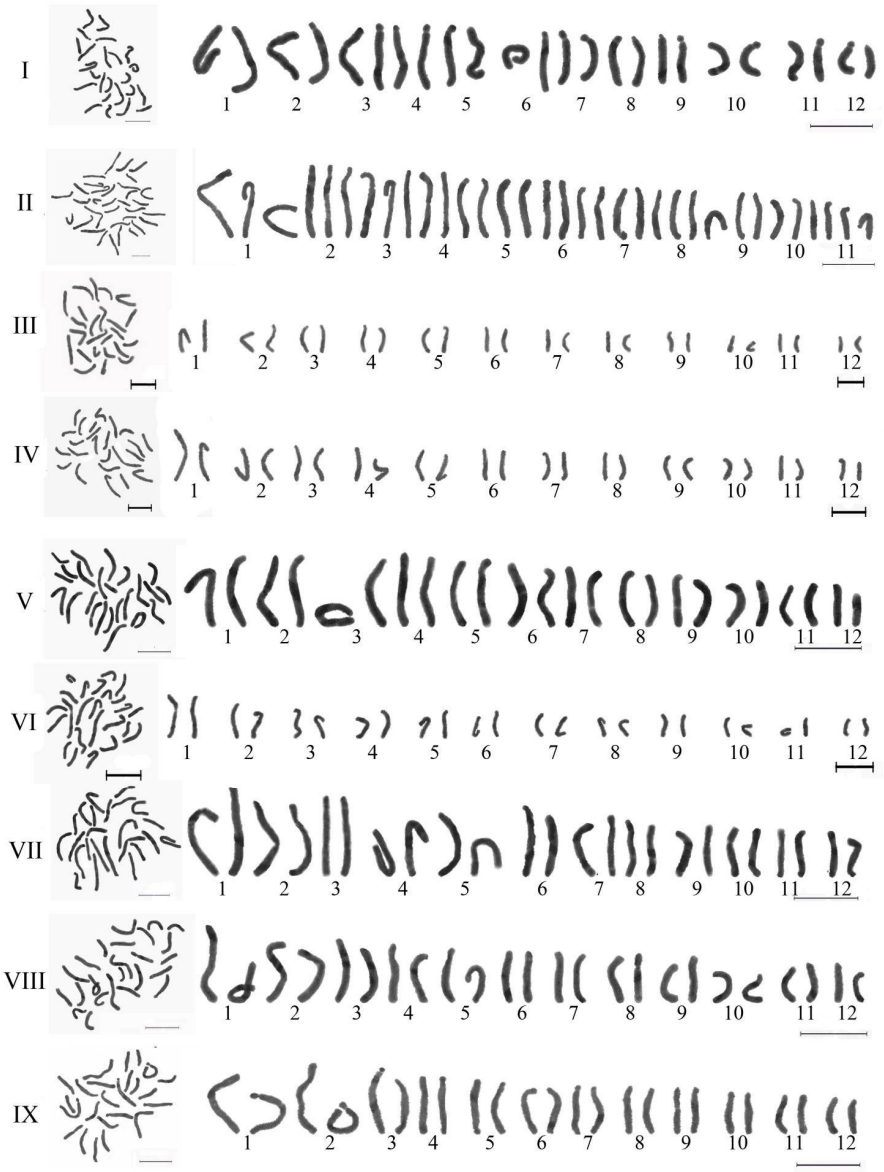

Figure 2. Somatic chromosomes of nine *Fritillaria* species. Bars = 20 μm. I, *F. imperialis*; II, *F. uva-vulpis*; III, *F. pallidiflora*; IV, *F. tortifolia*; V, *F. verticillata*; VI, *F. verticillata* var. *albiflora*; VII, *F. yuminensis*; VIII, *F. anhuiensis*; IX, *F. karelinii*

Maejo Int. J. Sci. Technol. **2022**, *16*(03), 223-237

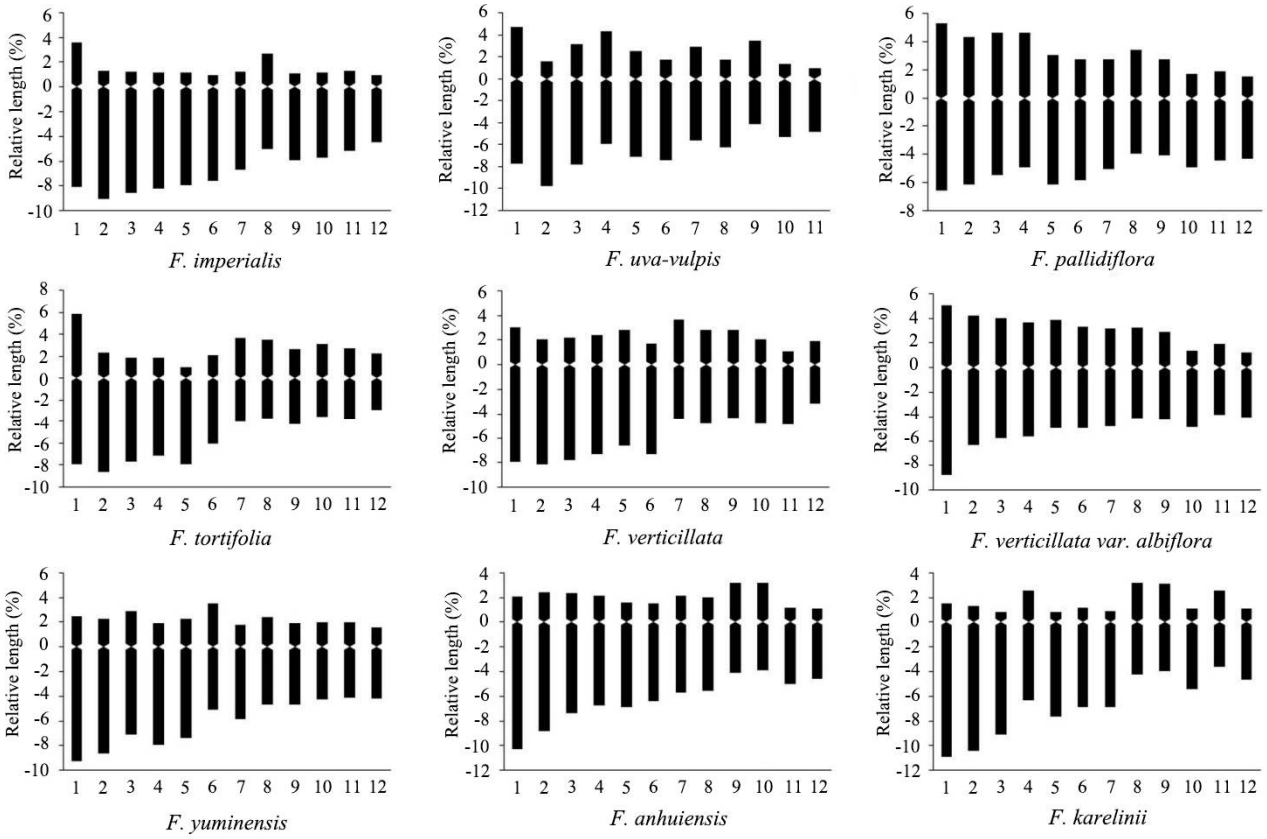

Figure 3. Idiograms of nine *Fritillaria* species

Table 4. ANOVA for chromosomal parameters of nine *Fritillaria* species

		Mean square						
	Df	S	L	TL	AR	r	RL	CI
Species	8	21.23**	58.27*	61.00*	21.41**	0.22**	0.71^{ns}	532.32**

Note: ns = non-significant difference ($p>0.05$); * significant at $p<0.01$; ** significant at $p<0.001$

Table 5. Range of relative length of chromosome, arm ratio and centromere index of nine *Fritillaria* species

Code	Relative length of chromosome (RL)	Arm ratio (AR)	Centromere index (CI)
I	5.43-11.65	1.88-7.93	11.20-34.77
II	5.82-12.40	1.21-6.12	14.04-45.21
III	5.84-11.85	1.06-2.91	25.56-48.46
IV	5.17-13.70	1.07-8.29	10.77-48.35
V	5.09-10.97	1.20-4.36	18.67-45.49
VI	5.28-13.75	1.26-3.56	21.91-44.18
VII	5.73-11.71	1.45-4.08	19.70-40.84
VIII	5.68-12.31	1.23-4.95	16.81-44.80
IX	5.73-12.42	1.28-10.91	8.39-43.93

Notes: I, *F. imperialis*; II, *F. uva-vulpis*; III, *F. pallidiflora*; IV, *F. tortifolia*; V, *F. verticillata*; VI, *F. verticillata* var. *albiflora*; VII, *F. yuminensis*; VIII, *F. anhuiensis*; IX, *F. karelinii*

Maejo Int. J. Sci. Technol. **2022**, *16*(03), 223-237

The mean values of TL show that *F. verticillata* var. *albiflora* has the shortest chromosome (TL = 15.20 μm) while *F. uva-vulpis*, a triploid species, has the longest chromosome (TL = 23.06 μm). Among the diploid species, *F. yuminensis* has the longest chromosome (TL = 21.09 μm). The mean AR values vary from 1.81 in *F. pallidiflora* to 5.42 in *F. imperialis*. The mean CI is 28.61%, ranging from 17.63% in *F. imperialis* to 37.27% in *F. pallidiflora*. In addition, *F. imperialis* has the maximum ASK value (82.3%) while *F. pallidiflora* has the minimum value (61.65%). Based on the chromosome nomenclature described by Levan et al. [51], four chromosome types are found in these nine *Fritillaria* species: m (centromere at median region), sm (centromere at sub-medium region), st (centromere at sub-terminal region) and t (centromere at terminal region), but chromosome type 'T' (centromere at terminal point), which was reported by Jafari et al [26], was not found in our study. The types of chromosome pairs for each species are shown in Table 2, and the KF and CRLC are represented in Table 3. The karyotype of *F. pallidiflora*, *F. tortifolia* and *F. verticillata* var. *albiflora* is 2B, which that of other species is 3B [10].

Four karyotype parameters, namely gametic chromosome number (x = 12), RL, CI and AR were used to calculate the KRC and genetic distance of all studied species except for *F. uva-vulpis*, which has a basic chromosome number of x = 11. A cluster analysis of the shortest distance was conducted [52] and the results are shown in Figure 4. Within eight species, the KRC between *F. pallidiflora* and *F. verticillata* var. *albiflora* is highest (0.9900), suggesting that they have a close genetic relationship. In contrast, the KRC between *F. imperialis* and *F. pallidiflora* is lowest (0.6919), suggesting a distant genetic relationship between them. The eight clustered *Fritillaria* species can be divided into three groups: *F. imperialis* belongs to subgenus *Petillium*, *F. karelinii* belongs to subgenus *Rhinopetalum*, and the other species are classified into subgenus *Fritillaria*. This result supports the division of subgenera suggested by Rix [4]. In addition, we found that the six species in the group of subgenus *Fritillaria* are divided into two smaller clusters: *F. pallidiflora*, *F. verticillata* var. *albiflora* and *F. tortifolia* are clustered together, as are *F. verticillata*, *F. yuminensis* and *F. anhuiensis*.

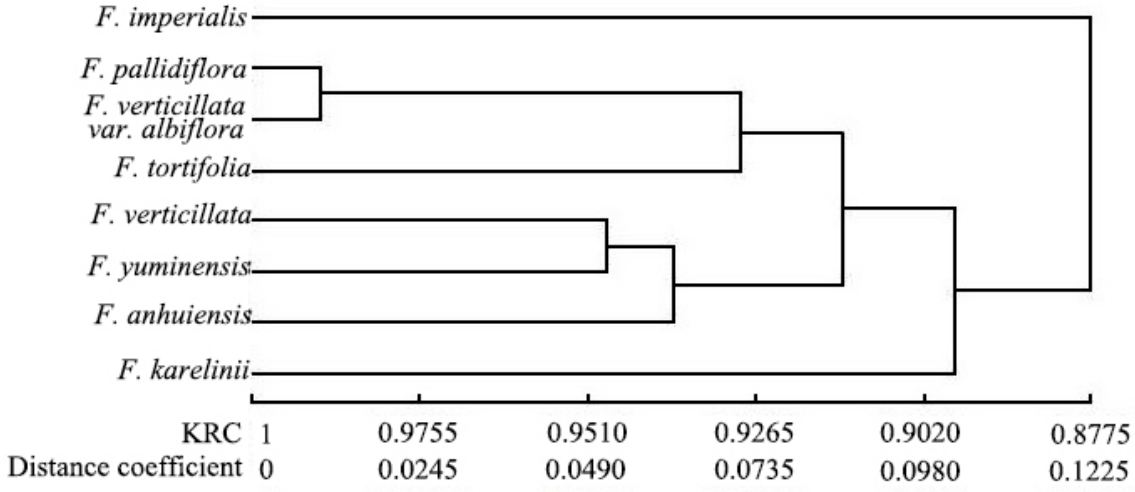

Figure 4. Cluster analysis of KRC among 8 *Fritillaria* species

The nine *Fritillaria* species studied in this research, except for *F. uva-vulpis* ($2n = 3x = 33$), are diploid ($2n = 2x = 24$). This is in agreement with previous studies [23-28, 55]. The chromosome karyotype of *F. uva-vulpis* should be considered as triploid with the basic chromosome number of x

Maejo Int. J. Sci. Technol. **2022**, *16*(03), 223-237

= 11, similar to *F. amabilis* and *F. japonica* (2*n* = 22), as reported by Noda [20, 21]. However, the result is not consistent with the findings of Khaniki [23], who determined the chromosome karyotype of *F. uva-vulpis* as 2*n* = 2*x* = 24 = 4m + 16st + 4t. This phenomenon, in which two different ploidies occur in a single species, has been reported in the chromosome research of *F. lanceolata* [36], which showed 2*n* = 3*x* = 36 and 2*n* = 2*x* = 24, and of *F. ussuriensis* [37], which showed 2*n* = 3*x* = 33 and 2*n* = 2*x* = 22. One possibility is that *F. uva-vulpis* may also have two different ploidy karyotypes.

Within the karyotype formulae of the nine *Fritillaria* species studied in this paper, four types of chromosomes are observed: 'm', 'sm', 'st' and 't'. This result corresponds with other studies on *Fritillaria* species. However, 'T' was observed in *F. persica* and *F. crassifolia* by Jafari et al. [26] for the first time and the authors considered that the existence of the 'T' chromosome type probably indicated a more evolved state compared to other *Fritillaria* species. We compared the chromosome types of the three subgenera in our study and found that subgenus *Petillium* was composed of 'sm', 'st' and 't', and subgenus *Rhinopetalum* was composed of 'm', 'sm', 'st' and 't', while most of subgenus *Fritillaria* was composed of 'm', 'sm' and 'st' except for *F. tortifolia* (2*n* = 14m + 2sm + 6st + 2t) and *F. pallidiflora* (2*n* = 12m + 12sm). In addition, we found that 'sm' was included in all studied species and located in different pairs of chromosomes (Table 2). However, we did not note a regular pattern among the six subgenera in the index of chromosome types. Jian et al. [18] argued that the difference in chromosome karyotypes indicated genetic diversity in *Fritillaria* spp. germplasm, speculating that the difference in karyotypes within a species might be related to its rich morphological variation, but this issue was not studied further.

In our study the ASK ranges from 61.65% to 82.3% with *F. pallidiflora* having the minimum value and *F. imperialis* having the maximum value. Thus, *F. imperialis* may be more evolutionarily advanced. In addition, the karyotype is usually used to assess the level of karyotype asymmetry based on qualitative and quantitative indices. Based on the Stebbins classification [10], two karyotypes, 2B and 3B, were discovered in the nine *Fritillaria* species. However, 3A, 3C and 4B karyotypes were occasionally found in a few species. Karyotype 3A was reported in *F. mellea* (classified as *F. sichuanica*) [56], *F. cirrhosa* [57, 58], *F. ussuriensis* [30], *F. pallidiflora* [30, 37], *F. persica*, *F. imperialis* and *F. straussii* [26], *F. dajinensis*, *F. delavayi*, *F. unibracteata*, *F. hupehesis* and *F. wabuensis* [58]. Karyotype 3C was found in *F. xiaobeimu* (classified as *F. thunbergii*) [40] and karyotype 4B was present in *F. kotschiana* [26], whereas 2B and 3A represent relatively symmetrical karyotypes, indicting original species, 3B, 3C and 4B represent asymmetric karyotypes, suggesting that these species are more evolutionarily advanced. In our study *F. pallidiflora*, *F. tortifolia* and *F. verticillata* var. *albiflora* probably belong to the original group in the genus *Fritillaria* L. This result is consistent with the ASK analysis.

Cluster analysis of KRC can indicate genetic relationships between species. This method has been used to study chrysanthemum [38] and *Forsythia* [59] karyotypes to analyse their genetic relationships. In our study cluster analysis of KRC shows that the nine *Fritillaria* species are divided into three groups (subgenera *Petillium*, *Fritillaria* and *Rhinopetalum*) with a value of 0.8975. This result supports the *Fritillaria* L. subgenus classification of Rix [4] and Liang [1]. However, there are still some differences in the phylogenetic relationship and morphological classification of six species in the subgenus *Fritillaria*. *F. verticillata* var. *albiflora* as a variant of *F. verticillata* [60] should be most closely related to its original species, *F. verticillata*, but the result of KRC clustering analysis indicates that it is most closely related to *F. pallidiflora*, followed by *F. tortifolia*. In contrast, *F. verticillata* has the closest relationship with *F. yuminensis*. This

result is consistent with the study of Leon et al. [61], who described *F. yuminensis* and revealed its close relationship with *F. verticillata* by DNA studies. However, Wang et al. [60] found that *F. verticillata* var. *albiflora*, *F. verticillata* and *F. tortifolia* had a close genetic relationship, while *F. pallidiflora* and *F. yuminensis* had a close relationship, using a DNA-based molecular marker, inter-simple sequence repeat.

Different results were observed by Wu et al. [62], who analysed the relationship of 22 *Fritillaria* species, also by inter-simple sequence repeat. The difference was the closest relationship between *F. stenanthera* (in fact *F. yuminensis* in their study) and *F. albidoflora*, which was classified as *F. verticillata* by Luo and Chen [63]. Moreover, these species were relatively distantly related to *F. pallidiflora.* As *Fritillaria* spp. are widely distributed and have rich natural variation, there may still be some limitations in analysing their genetic relationship by relying exclusively on conventional karyotype analysis. In order to explore the phylogenetic relationships and diversity in genetic variation of *Fritillaria* spp., a comprehensive analysis of various research results such as morphology, palynology, cytology and molecular markers is needed. In a study of genetic diversity of *Lilium* native to north-east China, the above four methods were adopted to reveal the genetic relationships among genera [64].

It is generally believed that a distant relationship between plants produces serious barriers for hybridisation and is not conducive to seed formation [65]. In order to successfully complete the process of fertilisation, breeders will prioritise genetic relationships between species for hybrid breeding. In previous studies Rønsted et al. [66] and Day et al. [67] examined the phylogenetic and evolutionary relationships respectively of the genus *Fritillaria* L. Their results consistently showed that species in one subgenus were closely related. In our study the same result was obtained in the KRC cluster analysis. At the same time, the ploidy level of these species was also recognised.

CONCLUSIONS

This study supports the current classification of the *Fritillaria* L. subgenera and provides cytological evidence for phylogenetics and crossbreeding as well as a reference for hybridisation.

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (grant no. 32071817) and the Introduced Talents Project of Hebei Agricultural University (grant no. ZD201729).

Y. F. and L. H. have contributed equally to this work.

REFERENCES

1. S. Y. Liang, “Chorology of Liliaceae (S. Str.) and its bearing on the Chinese flora”, *Acta Phytotaxon. Sin.*, **1995**, *33*, 27-51 (in Chinese).
2. S. L. Li, G. Lin, S. W. Chan and P. Li, “Determination of the major isosteroidal alkaloids in bulbs of *Fritillaria* by high performance liquid chromatography coupled with evaporative light scattering detection”, *J. Chromatogr. A*, **2001**, *909*, 207-214.
3. R. Hua, S. Q. Sun, Q. Zhou, I. Noda and B. Q. Wang, “Discrimination of fritillary according to geographical origin with Fourier transform infrared spectroscopy and two-dimensional correlation IR spectroscopy”, *J. Pharm. Biomed. Anal.*, **2003**, *33*, 199-209.
4. E. M. Rix, “*Fritillaria*: A Revised Classification”, Timber Press, Edinburgh, **2001**, pp. 56-72.

5. Royal Botanical Garden and Missouri Botanical Garden, "The plant list (2013)", **2013**, http://www.theplantlist.org/ (Accessed: November 2022).
6. Z. Y. Wu and P. H. Raven (Ed.), "Flora of China, Vol. 24", Science Press, Beijing, **2000**, pp. 127-133.
7. S. L. Li, S. W. Chan, P. Li, G Lin, G. H. Zhou, Y. J. Ren and F. C. Chiu, "Pre-column derivatization and gas chromatographic determination of alkaloids in bulbs of *Fritillaria*", *J. Chromatogr. A*, **1999**, *859*, 183-192.
8. S. L. Li, P. Li, G. Lin, S. W. Chan and Y. P. Ho, "Simultaneous determination of seven major isosteroidal alkaloids in bulbs of *Fritillaria* by gas chromatography", *J. Chromatogr. A*, **2000**, *873*, 221-228.
9. B. Vedatulug, A. B. Korkut and E. E. Sisman, "Research on propagation methods of Persian lily bulbs [*Fritillaria persica* Linn.] with various vegetative techniques", *Pak. J. Bot.*, **2010**, *42*, 2785-2792.
10. G. L. Stebbins, "Chromosomal Evolution in Higher Plants", Edward Amold, London, **1971**, pp. 87-93.
11. E. Medeiros-Neto, F. Nollet, A. P. Moraes and L. P. Felix, "Intrachromosomal karyotype asymmetry in Orchidaceae", *Genet. Mol. Biol*., **2017**, *40*, 610-619.
12. H. Więcław, A. Kalinka and J. Koopman, "Chromosome numbers of *Carex* (Cyperaceae) and their taxonomic implications", *PLoS One*, **2020**, *15*, Art. no. e0228353.
13. P. T. N. Hoang, J. Fuchs, V. Schubert, T. B. N. Tran and I. Schubert, "Chromosome numbers and genome sizes of all 36 duckweed species (Lemnaceae)", *Plants* (*Basel*), **2022**, *11*, Art. no. 2674.
14. F. P. Maragheh, D. Janus, M. Senderowicz, K. Haliloglu and B. Kolano, "Karyotype analysis of eight cultivated *Allium* species", *J. Appl. Genet*., **2019**, *60*, 1-11.
15. H. Arano, "Cytological studies in subfamily Carduoideae (Compositae) of Japan XIII", *Bot. Mag. Tokyo*, **1963**, *76*, 419-427.
16. V. Prajitha and J. E. Thoppil, "Cytogenetic characterization of *Amaranthus caudatus* L. and *Amaranthus hybridus* subsp. *cruentus* (L.) Thell", *Cytotechnol.*, **2018**, *70*, 95-101.
17. N. Liu, "Study on morphology and chromosome Karyotype of distant hybridization progenies and their parents of tree peony", *MSc Thesis*, **2014**, Beijing Forestry University, China (in Chinese).
18. H. Y. Jian, H. Zhang, T. Zhang, S. F. Li, Q. G. Wang, X. Q. Qiu and H. J. Yan, "Karyotype analysis of different varieties on *Rosa odorata* sweet", *J. Plant Genet. Resour.*, **2010**, *11*, 457-461 (in Chinese).
19. L. H. Hao, "Resources collection and evaluation of Xinjiang *Fritillaria* L. and research of flower bud differentiation of *F. pallidiflora* Schrenk", *PhD. Thesis*, **2017**, Beijing Forestry University, China (in Chinese).
20. S. Noda, "Cytology in the genus *Fritillaria*. I. Variations in karyotypes and B-chromosomes in *F. amabilis*", *Bull. Osaka Gakuin Univ.*, **1964**, *2*, 125-132.
21. S. Noda, "Cytology in the genus *Fritillaria*. II. Variation in karyotype and B-chromosomes in *F. japonica var. japonica* and *var. koidzumiana*", *Bull. Osaka Gakuin Univ.*, **1964**, *10*, 127-141.
22. R. J. Li and Z. Y. Shang, "The chromosome observation on five species of rare plants of China", *J. Wuhan Bot. Res.*, **1989**, *7*, 217-220 (in Chinese).

23. G. B. Khaniki, "Chromosome number of *Fritillaria* subgenera *Petilium* and *Theresia* (Liliaceae)", *Nucleus*, **2002**, *45*, 6-11.
24. G. B. Khaniki, "Chromosome number of all Iranian species of *Fritillaria caucasica* group (Liliaceae)", *Nucleus*, **2002**, *45*, 103-108.
25. G. B. Khaniki, "Giemsa C-banding studies on interphase nuclei of Iranian species of *Fritillaria* and *Rhinopetalum* (Liliaceae)", *Proc. Natl. Acad. Sci. India Sec. B*, **2005**, *75*, Art.no.294.
26. H. Jafari, A. Babaei, G. Karimzadeh and M. Ahmadi-Roshan, "Cytogenetic study on some *Fritillaria* species of Iran", *Plant Syst. Evol.*, **2014**, *300*, 1373-1383.
27. M. Ahmadi-Roshan, G. Karimzadeh, A. Babaei and H. Jafari, "Karyological studies of *Fritillaria* (Liliaceae) species from Iran", *Cytologia*, **2016**, *81*, 133-141.
28. F. Hazbavi, S. Hosseini, G. Mirzaghaderi and M. Advay, "Karyotypic variation in five species of the genus *Fritillaria* (Liliaceae)", *Iran. J. Bot.*, **2019**, *25*, 127-134.
29. N. Seifoori, G. Mirzaghaderi and S. Hosseini, "Chromosomal positions of 5S and 45S rDNA in some Iranian *Fritillaria* (Liliaceae) species", *Res. Square*, **2022**, doi: 10.21203/rs.3.rs-1939980/v1 (preprint).
30. S. L. Gao, A. P. Zhao and D. R. Xu, "Technique of chromosome microscopic determination in cultured material of *Fitillaria thunbergia* Miq", *J. China Pharm. Univ*., **1990**, *21*, 345-348 (in Chinese).
31. L. F. LaCour, "The constitutive heterochromatin in chromosomes of *Fritillaria* sp. as revealed by Giemsa banding", *Philos. Trans. R. Soc. Lond. B Biol. Sci.*, **1978**, *285*, 61-71.
32. M. S. Cave, "Chromosomes of the California Liliaceae", University of California Press, Berkeley, **1970**.
33. L. Hill, "A taxonomic history of Japanese endemic *Fritillaria* (Liliaceae)", *Kew Bull.*, **2011**, *66*, 227-240.
34. F. W. Sansome, "Recent advances in cytology", *Nature*, **1937**, *140*, 1033-1034.
35. S. Samaropoulou P. Bareka, and G. Kamari, "Karyomorphometric analysis of *Fritillaria montana* group in Greece", *Comp. Cytogenet.*, **2016**, *10*, 679-695.
36. C. J. Marchant and R. M. Macfarlane, "Chromosome polymorphism in triploid populations of *Fritillaria lanceolata* Pursh (Liliaceae) in California", *Bot. J. Linn. Soc*., **1980**, *81*, 135-154.
37. T. Chen, L. Zhang, X. Zhou, G. Shu and Y. Zhou, "Preliminary study of fertilizer effect on yield and quality of *Fritillaria ussuriensis*", *China J. Chin. Mater. Med.*, **2009**, *34*, 544-546 (in Chinese).
38. Y. Zhang, M. L. Zhu and S. L. Dai, "Analysis of karyotype diversity of 40 Chinese chrysanthemum cultivars", *J. Syst. Evol.*, **2013**, *51*, 335-352.
39. D. C. Zhang, J. Z. Shao, Y. S. Li and C. H. Yao, "Studies on the karyotype of three different populations of *Fritillaria anhuiensis*", *J. Anhui Norm. Univ.* (*Nat. Sci.*), **1993**, *16*, 40-44 (in Chinese).
40. Y. Wang, S. B. Zhou, X. F. Chen, J. R. Hu and Q. Luo, "A karyotypical study on *Fritillaria* L. from Anhui province", *Plant Sci. J.*, **2006**, *24*, 303-309 (in Chinese).
41. J. L. Xu, Y. Z. Li and L. W. Zhang, "Comparative study on the karyotypes of three *Fritillaries*", *J. Univ. Chin. Acad. Sci.*, **1987**, *25*, 77-80 (in Chinese).
42. D. Q. Yang and X. F. Zhu, "Studies on karyotypes of *F.huangshanensis* and *F. monantha*", *J. Wuhan Bot. Res.*, **1989**, *7*, 211-215 (in Chinese).

43. J. X. Zhang, A. N. Lao, H. Z. Huang, G. E. Ma and R. S. Xu, "Studies on the chemical constituents of *Fritillaria thunbergia* Miq. III . Isolation and identification of zhebeinone", *Acta Pharm. Sin.*, **1992**, *27*, 472-475 (in Chinese).
44. E. Zaharof, "Karyological studies of twelve *Fritillaria* species from Greece", *Caryologia*, **1989**, *42*, 91-102.
45. S. Samaropoulou, P. Bareka and G. Kamari, "Hybridization and karyotype variability of three endemic *Fritillaria* L. (Liliaceae) in Argolis Peninsula (Greece)", *Plant Biosyst.*, **2020**, *154*, 348-360.
46. M. Koçyiğit, U. Rastgeldi and E. Kaya, "Cytotaxonomical analysis of eleven Turkish *Fritillaria* L. (Liliaceae) taxa", *IUFS J. Biol.*, **2016**, *75*, 19-28.
47. Y. D. Gao, S. D. Zhou, X. J. He and J. Wan, "Chromosome diversity and evolution in tribe Lilieae (Liliaceae) with emphasis on Chinese species", *J. Plant Res.*, **2012**, *125*, 55-69.
48. K. Pratt and M. Jefferson-Brown, "The Gardener's Guide to Growing Fritillaries", Timber Press, New York, **1997**, pp. 37-42.
49. The *Fritillaria* Group, "Fritillaria image galleries", **1997**, http://www.fritillaria.org.uk/images.html (Accessed: November 2022).
50. M. X. Li and R. Y. Chen, "A suggestion on the standardization of karyotype analysis in plants", *Plant Sci. J.*, **1985**, *3*, 297-302 (in Chinese).
51. A. Levan, K. Fredga and A. A. Sandberg, "Nomenclature for centromeric position on chromosomes", *Hereditas*, **1964**, *52*, 201-220.
52. F. Li and S. Y. Pan, "A software programming for cluster analysis of the karyotype resemblance near coefficients", *J. Xuzhou Norm. Univ.* (*Nat. Sci. Ed.*), **2005**, *23*, 64-67 (in Chinese).
53. Y. D. Tan and C. M. Wu, "Cluster analysis method of karyotype resemblance-near coefficient", *Acta Genet. Sin.*, **1993**, *20*, 305-311 (in Chinese).
54. C. M. Wu, "Estimation of karyotype resemblance-near coefficient and evolutionary distance", *Acta Zootaxon. Sin.*, **1996**, *21*, 338-344 (in Chinese).
55. M. Tekşen and Z. Aytaç, "*Fritillaria mughlae* (Liliaceae), a new species from Turkey", *Ann. Bot. Fennici.*, **2008**, *45*, 141-147.
56. C. X. Li, S. F. Wang and H. L. Lei, "Report on the karyotypes of 4 species of the *Fritillaria*", *J. Agric. Univ. Hebei*, **1996**, *19*, 53-58 (in Chinese).
57. Y. D. Gao, S. D. Zhou and X. J. He, "Karyotypes of four genera in Liliaceae (S.Str.) from Hengduan Mountains of southwestern China", *Acta. Bot. Yunnanica*, **2010**, *31*, 399-405 (in Chinese).
58. K. Q. Li and W. Wu, "A karyological study of *Fritillaria* from Sichuan province", *Hubei Agric. Sci.* **2016**, *55*, 4224-4229 (in Chinese).
59. J. S. Shen, Y. J. Ye, H. T. Pan and Q. X. Zhang, "Karyotype analysis of twelve *Forsythia* species and cultivars", *J. Plant Genet. Resour.*, **2015**, *16*, 178-184 (in Chinese).
60. G. P. Wang, C. Z. Fan, X. J. Li and J. Zhu, "Genetic diversity analysis on plants in *Fritillaria* L. from Xinjiang based on ISSR", *Chin. Trad. Herb. Drugs*, **2013**, *44*, 887-890 (in Chinese).
61. C. Leon, M. F. Fay and M. Rix, "*Fritillaria yuminensis*", *Curtis's Bot. Mag.*, **2009**, *26*, 21-32.
62. X. Q. Wu, H. Z. Cheng, Y. Z. Zhou, X. M. Lou, W. J. Zhang and G. G. Tang, "Analysis of genetic relationship ornamental *Fritillaria* germplasm resources by ISSR markers", *North Hortic.*, **2010**, *18*, 157-160 (in Chinese).

Maejo Int. J. Sci. Technol. **2022**, *16*(03), 223-237

63. Y. B. Luo and S. C. Chen, "Critical notes on the genus *Fritillaria* L. (Liliaceae) of Xinjiang, China", *Acta Phytotaxon. Sin.*, **1996**, *34*, 77-85 (in Chinese).
64. L. P. Rong, "Study on germplasm resources and genetic diversity of *Lilium* native to northeast China", *PhD Thesis*, **2010**, Shenyang Agricultural University, China (in Chinese).
65. J. Li, J. Zhou, Y. Zhang, Y. Yang, Q. Pu and D. Tao, "New insights into the nature of interspecific hybrid sterility in rice", *Front. Plant Sci.*, **2020**, *11*, Art.no.555572.
66. N. Ronsted, S. Law, H. Thornton, M. F. Fay and M. W. Chase, "Molecular phylogenetic evidence for the monophyly of *Fritillaria* and *Lilium* (Liliaceae; Liliales) and the infrageneric classification of *Fritillaria*", *Mol. Phylogenet. Evol.*, **2005**, *35*, 509-527.
67. P. D. Day, M. Berger, L. Hill, M. F. Fay, A. R. Leitch, I. J. Leitch and L. J. Kelly, "Evolutionary relationships in the medicinally important genus *Fritillaria* L. (Liliaceae)", *Mol. Phylogenet. Evol.*, **2014**, *80*, 11-19.