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Karyological Study on Bellevalia and Muscari (Liliaceae) Species of Iran

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Abstract: An investigation of karyotype and chromosome numbers was carried out in different populations of *Bellevalia* Lapeyr. and *Muscari* Mill. species from Iran. In this research, six species of *Bellevalia* and six species of *Muscari* were studied. Different levels of ploidy were found in them. In *Bellevalia* with x = 4, levels of ploidy were diploid, autopentaploid and hexaploid and in *Muscari* with x = 9, it was diploid, autotriploid, tetraploid and autopentaploid. In the present research, for first time, the karyotype of *B. olivieri* was prepared and a population of *B. longistyla* with autopenploid was observed. The variation of B chromosome number in *Bellevalia* was considerable.

Key words: B chromosome, *Bellevalia*, *Botryanthus*, karyotype, *Leopoldia*, *Muscari*, *Nutans*, *Patens*

INTRODUCTION

Bellevalia Lapeyr. and *Muscari* Mill. are genera of bulbous plants belonging to Liliaceae, subfamily Lilioideae and tribe Scilleae (Engler, 1887). The both genera have a wide spread distribution. They are present in the whole Mediterranean basin as far as Caucasus, temperate Europe, North of Africa, South west of Asia (Losinskaya, 1935; Feinbrun, 1938-1940; Parsa, 1950; Bentzer, 1972; Garbari, 1973; Davis, 1984; Townsend and Guest, 1985; Feinbrun, 1986; Assadi, 1988; Rechinger, 1990; Wendelbo, 1990).

Bellevalia and *Muscari* are comprising 18 and nine species in Iran, respectively (Parsa, 1950; Wendelbo, 1967, 1980; Assadi, 1988; Rechinger, 1990). Taxonomically *Bellevalia* closely is related to *Muscari* and *Hyacinthella*. However cytologically, the larger chromosome of *Bellevalia* make it easily recognizable from the two other ones. The basic chromosome number is x = 4 in *Bellevalia* (Johnson, 2003) and x = 9 in *Muscari* (Speta, 1998).

Previous karyological studies on *Bellevalia* and *Muscari* showed in Table 1. The length of chromosome of *Bellevalia* and *Muscari* species were variable between (6-10, 18-20) and (3-8) micron, respectively (Feinbrun, 1938-1940; Bentzer, 1972).

The aim of this research was to study the karyotype of the mentioned species and to test if the relationship based on morphological characters are in accordance with cytological data. So we prepared karyogram of somatic number of chromosome from *B. fominii*, *B. tabriziana*, *B. glauca*, *B. longistyla*, *B. olivieri*, *M. comosum*, *M. caucasicum*, *M. tenuiflorum*, *M. longipes*, *M. armeniacum* var. *szovitzianum* and *M. neglectum.*

Table 1: Previous Karyological studies on studied *Bellevalia*

Species	2n	References
B. fominii	8	Delaunay (1926), Federov (1969), Bothmer and Wendelbo (1972, 1981), Johnson and Brandham (1996)
B. tabriziana	8	Persson and Wendelbo (1979)
B. glauca	8, 16, 32, 24, 24+B, 4+5B 8+3B, 17, 20, 5+2B, 3+7B	Federov (1969), Zakhariyeva and Makushenko (1969), Al-Mudarris, (1973), Podlech and Bader (1974), Aryavand (1975), Bothmer and Wendelbo (1981), Johnson and Brandham (1996)
B. longistyla	31, 35	Federov (1969), Persson and Wendelbo, (1979), Johnson and Brandham (1996),
B. saviczii	20, 24, 24+B, 3+7B, 8, 16, 5+2B, 12	Federov (1969), Zakhariyeva and Makushenko (1969), Podlech and Bader (1974), Aryavand (1975), Bothmer and Wendelbo (1981), Johnson and Brandham (1996)
M. comosum	18, 27, 18+B	Polya (1950), Damato (1950, 1952), Larsen (1956, 1960), Gadella *et al.* (1966), Stuart (1966), Garbari (1968), Bentzer (1969, 1972), Federov (1969), Goldblatt (1974), Bentzer and Ellmer (1975), Bentzer and Landstrom (1975), Love (1976), Murin and Majovsky (1987), Dalgic (1991)
M. caucasicum	18.27	Stuart (1966), Federov (1969)
M. longipes	18	Federov (1969).
M. armeniacum	18, 36, 44, 45, 54, 70, 72	Stuart (1966), Love (1973, 1974, 1976), Federov (1969), Moore (1982), Karlen (1984), Dalgic (1991)
M. neglectum	18, 36, 54, 72	Stuart (1966), Love (1973, 1974, 1976), Federov (1969), Moore (1982), Karlen (1984), Dalgic (1991)

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Table 2: Taxa, localities of *Bellevalia* and *Muscari*

Taxa	Localities
Sect. *Nutans* *B. fominii*	Azarbaijan, Ardebil, Meshkinshahr, 15 km Lahroud to Ghoutorsoee, 2345m. Jafari Emani, 45.
Sect. *Patens* *B. tabriziana*	Azarbaijan, 20 km Tabriz to Ahar, Kahlic Balaghi, 1510-1530 m, Jafari, 49.
Sect. *Bellevalia* *B. glauca*	Fars, 30 km Shiraz to Dashte-Arjan, Hossein abad station, 1970 m, Jafari, Hatami, 39.
B. longistyla	Azarbaijan, Ahar to Tabriz, Ghuijebel valley, 1830 m, Jafari Imani, 51.
B. saviczii	Fars, Shiraz, Sarvestan, Fassa, Mian Jangal station. 1775 m, Jafari, Hatami 33.
B. olivieri	Azarbaijan, 30 km Khouy to Salmas, 1503 m, Maassoumi, Safavi, 82576.
Subgen. *Leopoldia* *M. comosum* (1)	Zanjan, Zajan to Tabriz road, 15 km Ghare-Chaman, Jafari, 45.
M. comosum (2)	Hamedan, Faghireh to KhaKou, Ghesmeh-Lale Ghasem, 1940 m, Jafari, Najafi, 15.
M. caucasicum (1)	Khorassan. Mashad, Kardeh village, Jafari 42.
M. caucasicum (2)	Azarbaijan, North to Ahar, Sambouran, 1840 m, Jafari, Imani, 54.
M. tenuiflorum (1)	Kurdistan, Sanandaj, After Tazeh-abad, Chehel-Gazy, Jafari, Sheikhi, 19.
M. temuiflorum (2)	Lorestan, Doroud, Oshtoran kuh, Gheshme-Darreh, 2250 m, Jafari, Karimi, 6.
M. longipes	Zanjan, Zanjan to Tabriz, between Tazehabad and Ghule-Ghosseh, 2220 m, Mousavi, 61.
M. neglectum	Kurdistan, Tazeh abad, 65 km Sanandaj to Divan darreh. Jafari, Sheiki, 39.
M. armeniacum var. *szovitzianum*	Azarbaijan, Ardebil, Meshkin shahr, 15 km Lahroud to Ghoutorsoee, 2345 m, Jafari, Imani, 47.

MATERIALS AND METHODS

The materials were collected from the east, center and west of Iran in February until June 2003, 2004 (Table 2). Voucher specimen are deposited in herbarium of Tehran sciences and researches campus For karyotype analysis, a pretreatment at room temperature for three hours was usually applied before fixation of the root tips of six species of *Bellevalia* and *Muscari* either in 0.002 M 8-Hydroxyquinoline. After fixation in a cold mixture of ethanol and acetic acid (3:1), the following procedure involved the maceration in 1 N HCl at 60 for 5-8 min, washing in water, cutting off the meristems and squashing them in a drop of 45% acetic acid (Krahulcova, 2003). Chromosomes were described according to Levans terminology (Levan *et al*., 1964). Karyotypes were compared using total form percentage (Forni-Martin *et al*., 1994) and calculated the ratio of the longest to the shortest chromosome (Verma, 1980). Symmetry karyotypes were determined using Stebbins two way system (Stebbins, 1971).

RESULTS

The somatic chromosome number and details of karyotype of *Bellevalia* and *Muscari* studied species were shown in Table 3 and 4. *B. fominii* from sect. *Nutans* (Fig. 1a, 2a) and *B. tabrizina* from sect. *Patens* (Fig. 1b, 2b) were diploid (2X = 2n = 8) with karyotype formula (2m+1sm+1st) and (1m+2sm+1st), respectively. The rest of species belonged to sect. *Bellevalia*. *B. glauca* was diploid (2X = 2n = 8) with karyotype formula (3m+1sm) and the satellite was observed above the short arm of first pair chromosome (Fig. 1c, 2c). *B. longistyla* was autopentaploid (5X = 2n = 20+1B) with sm B-chromosome and karyotype formula (1m+5sm+4st+1B) (Fig. 1d, 2d). *B. saviczii* was hexaploid (6X = 2n = 24+1B) with sm B-chromosome and karyotype formula (7m+2sm+3st+1B) (Fig. 1e, 2e). *B. olivieri* was hexaploid (6X = 2n = 24+5B) with five sm, st and t B-chromosome and karyotype formula (7m+1sm+3st+1t+ 5B) (Fig. 1f, 2f).

Also *M. comosoum* (1) was diploid (2X = 2n = 18) with (1M+2m+2sm+4st) (Fig. 1g, 2g.), but in *M. comosum* (2) was diploid (2X = 2n = 17) with karyotype formula (2m+4sm+3st) (Fig. 1h, 2h). *M. caucasicum* (1), (2) were

Table 3: Somatic chromosome no., karyotype formula and symetoy of *Bellevalia* and *Muscari* taxa

Taxa	2n	NO. Sat	Karyotype formula	Stebbins formula	B chromosome
B. fominii	8	NO	2m+1sm+1st	2c	NO
B. tabriziana	8	1	1m+2sm+1st	3c	NO
B. glauca	8	1	3m+1sm	1c	NO
B. longistyla	16	NO	4m+3sm+1st+1B	2c	1
B. saviczii	24	NO	7m+2sm+3st+1B	2c	1
B. olivieri	24	NO	7m+1sm+3st+1t+5B	2c	5
M. comosum (1)	17	NO	1M+2m+2sm+4st	3c	NO
M. comosum (2)	18	NO	2m+4sm+3st	3c	NO
M. caucasicum (1)	18	NO	1M+3m+4sm+1st	2c	NO
M. caucasicum (2)	27	NO	7m+4sm+2st	2c	NO
M. tenuiflorum (1)	18	NO	5m+3sm+1st	2c	NO
M. tenuiflorum (2)	27	NO	1m+5sm+6st+1t	2c	NO
M. longipes	18	NO	2m+4sm+3st	3c	NO
M. neglectum	45	NO	7m+12sm+3st	3c	NO
M. armeniacum var. *szovitzianum*	36	NO	2M+10m+4sm+2st	2c	NO

NO = Not Observed M = Median point, m = Median region, sm = Submedian, st = Subterminal t = Terminal region

Table 4: Karyotypic details of the species studies of *Bellevalua* and *Muscari*

Taxa	TL (μm)	L (μm)	S (μm)	L/S	TF (%)	TV $(\mu m)^3$
B. fominii	92.50	16.00	9.62	1.66	0.349	236.66
B. tabriziana	34.24	5.44	2.88	1.88	0.264	53.23
B. glauca	88.60	19.20	5.12	3.75	0.384	514.51
B. longistyla	166.06	15.36	7.04	2.18	0.329	665.90
B. saviczii	236.12	19.20	5.12	3.75	0.336	1360.80
B. olivieri	187.34	12.80	4.48	2.85	0.304	1083.62
M. comosum (1)	56.30	10.24	1.28	8.00	0.270	182.08
M. comosum (2)	72.19	8.32	3.20	2.60	0.272	218.40
M. caucasicum (1)	59.20	6.08	1.60	3.80	0.329	152.02
M. caucasicum (2)	66.24	8.26	0.76	10.86	0.312	206.81
M. tenuiflorum (1)	189.44	17.28	4.48	3.85	0.330	492.27
M. tenuiflorum (2)	138.19	9.08	2.26	4.01	0.223	399.16
M. longipes	54.29	5.54	1.71	3.23	0.282	190.53
M. neglectum	180.26	7.04	1.92	3.66	0.306	112.15
M. armeniacum var. *szovitzianum*	178.86	10.20	1.92	5.13	0.264	70.58

TL = Total chromatin length, S = Shortest chromosome, L = Longest chromosome, L/S = Longest/Shortest, TF % = Total from percentage, TV = Total Volume

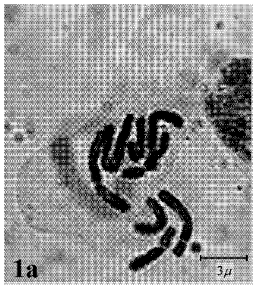

Fig. 1a: Somatic chromosomes of *B. fominii*

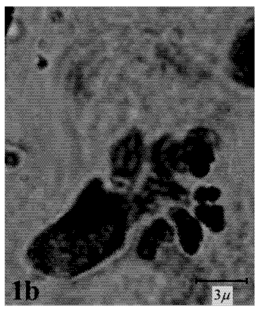

Fig. 1b: Somatic chromosomes of *B. tabriziana*

Fig. 1c: Somatic chromosomes of *B. glauca* The arrow showing satellite

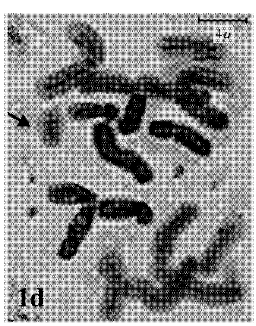

Fig. 1d: Somatic chromosomes of *B. longistyla.* The arrow showing B chromosome

Fig. 1e: Somatic chromosomes of *B. saviczii.* The arrow showing B chromosome

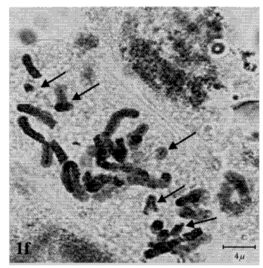

Fig. 1f: Somatic chromosomes of *B. olivieri.* The arrows showing B chromosomes

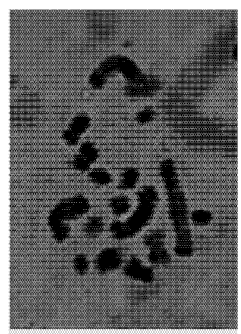

Fig. 1g: Somatic chromosomes of *M. comosum* (1)

Fig. 1h: Somatic chromosomes of *M. comosum* (2)

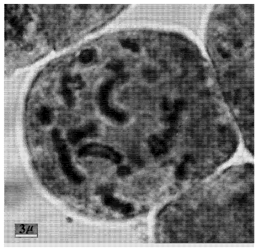

Fig. 1i: Somatic chromosomes of *M. caucasicum* (1)

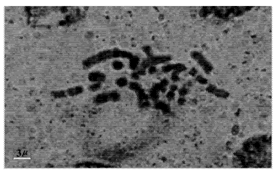

Fig. 1j: Somatic chromosomes of *M. caucasicum* (2)

Fig. 1k: Somatic chromosomes of *M. tenuiflorum* (1)

Fig. 1l: Somatic chromosomes of *M. tenuiflorum* (2)

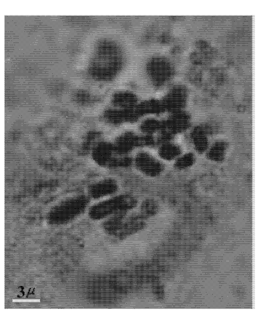

Fig. 1m: Somatic chromosomes of *M. longipes*

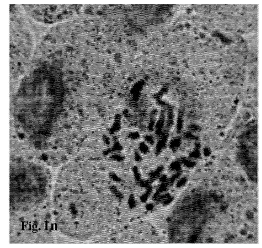

Fig. 1n: Somatic chromosomes of *M. neglectum*

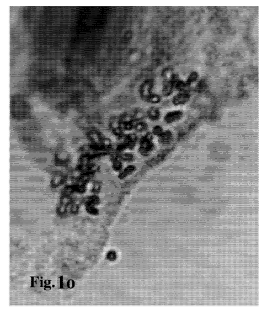

Fig. 1o: Somatic chromosomes of *M. armeniacum* var. *szovitzianum*

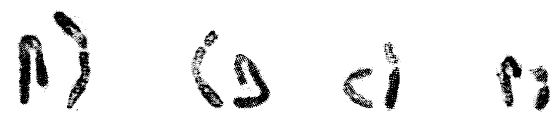

Fig. 2a: Karyotype of *B. fominii*

Fig. 2b: Karyotype of *B. tabriziana*

Fig. 2c: Karyotype of *B. glauca*

Fig. 2d: Karyotype of *B. longistyla.* The arrow showing B chromosome

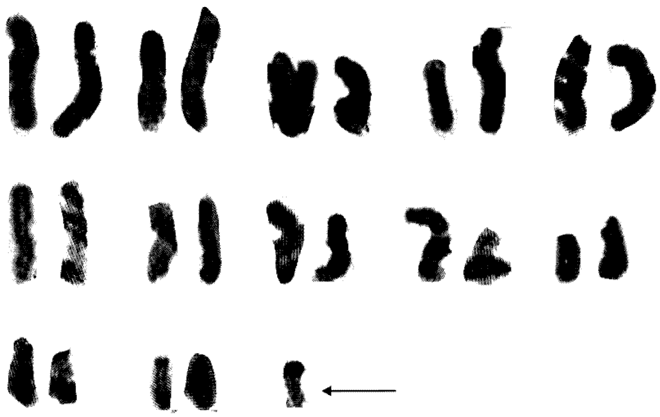

Fig. 2e: Karyotype of *B. saviczii.* The arrow showing B chromosome

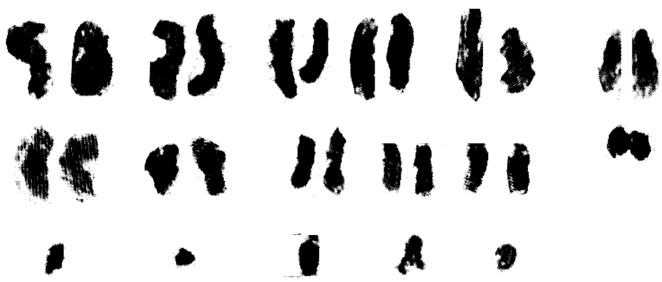

Fig. 2f: Karyotype of *B. olivieri*

Fig. 2g: Karyotype of *M. comosum* (1)

Fig. 2h: Karyotype of *M. comosum* (2)

Fig. 2i: Karyotype of *M. caucasicum* (1)

Fig. 2j: Karyotype of *M. caucasicum* (2)

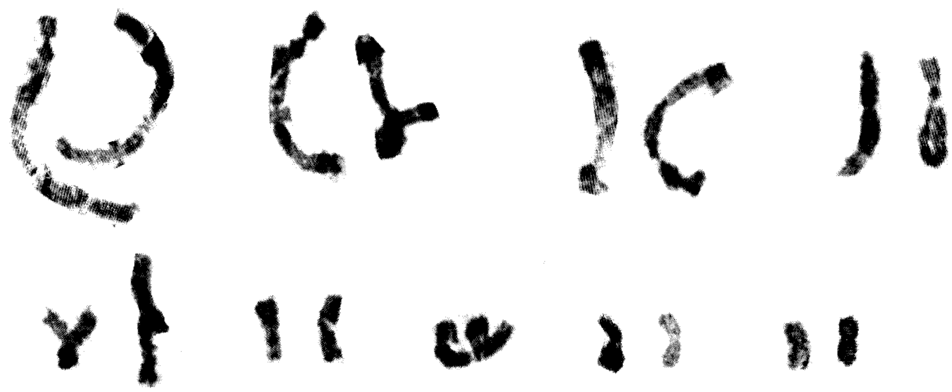

Fig. 2k: Karyotype of *M. tenuiflorum* (1)

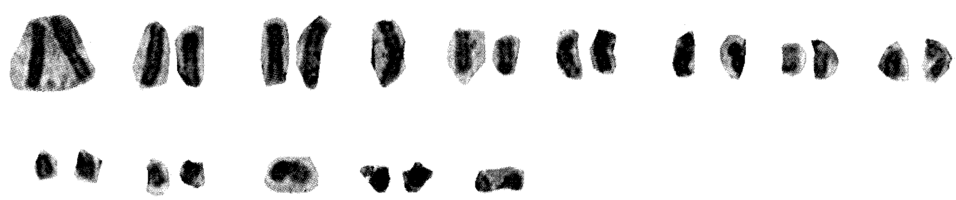

Fig. 2l: Karyotype of *M. tenuiflorum* (2)

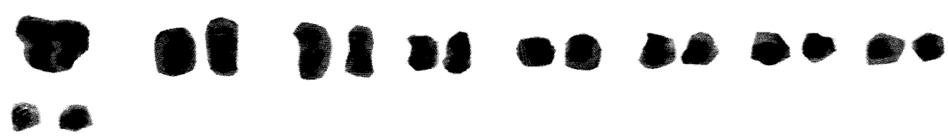

Fig. 2m: Karyotype of *M. longipes*

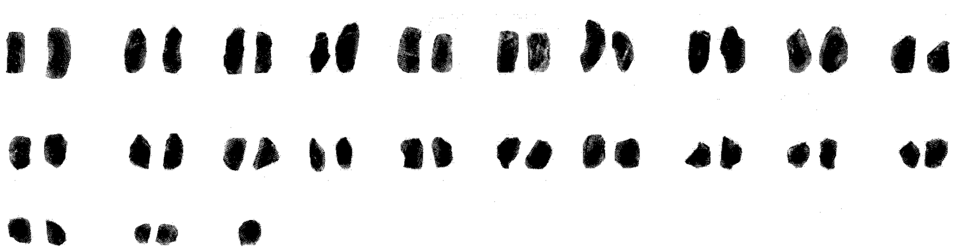

Fig. 2n: Karyotype of *M. neglectum*

Fig. 2o: Karyotype of *M. armeniacum* var. *szovitzianum*

(2X, 3X = 2n = 18, 27) with karyotype formula (1M+3m+4sm+1st) and (7m+4sm+2st), respectively (Fig. 1i, j, 2i, j). *M. tenuiflorum* (1), (2) were diploid and autotriploid (2X, 3X = 2n = 18, 27) with karyotype formula (5m+3sm+1st) and (1m+5sm+6st+1t) and some secondary constrictions (Fig. 1k, l, 2k, l). *M. longipes* was diploid (2x = 2n = 18) with karyotype formula (2m+4sm+3st) (Fig. 1m, 2m). *M. neglectum* was autopentaploid (5X = 2n = 45) with karyotype formula (7m+12sm+3st) (Fig. 1n, 2n) and *M. armeniacum* var. *szovitzianum* was tetraploid (4x = 2n = 36) with karyotype formula (2M+10m+4sm+2st) (Fig. 1o, 2o).

DISCUSSION

The basic chromosome number of *Bellevalia* is X = 4. This genus often are diploid with 2n = 8, but there is a polyploidy series of 2n = 16, 24, 32. Aneuploidy occurs only at the octaploid level (Ozhatay and Johnson, 1996). They have metacentric, acrocentric and telocentric B-chromosome (Johnson, 2003).

B. fominii was falling to class 2C. This species had the longest chromosome among the studied species which didn't have any chromosome polymorphism. Also some weak constriction were above the long arms. In Bothmers report, karyotype formulae is the same as the present research. Bothmer reported one chromosome in pair No. 4 have a longer short arm than usual which indicate a pericentric inversion (Bothmer and Wendelbo, 1981). *B. tabriziana* had the shortest and thickest chromosome among the studied species. This species was falling to class 3C. Its chromosomes were large M and short st while Persson and Wendelbo reported its chromosome have been large M, sm and st (Persson and Wendelbo, 1979). The ploidy level of *B. tabriziana* in this research paper was similar to Perssons report. *B. glauca* was falling to class 1C. The satellite was observed above the short arm of first pair chromosome. The chromosome polymorphism was occurred on first pair chromosome. The long arms of first pair were not equal. It is related to pericentric inversion. This species had the biggest and the thickest chromosomes among the studied sect. *Bellevalia*. *B. longistyla* was falling to class 2C. The autopentaploid population for this species is recorded for the first time. No obvious chromosome polymorphism was observed. *B. saviczii* with one B chromosome was falling to class 2C. Its chromosomes were smaller and thinner than the other studied species of sect. *Bellevalia* in this research. In present paper, allocyclic event and secondary constriction in long arm were observed. Only one sm B-chromosome also, was found while in Bothmers report its number was 1, 3, 7. Getner also reported 1-8 B-chromosome which the most and the least of them were m and M, t (Getner, 2005). The chromosome polymorphism was occurred in pairs No. 2, 3, 4. Getner also confirmed pericentric inversion. He believed the acrocentric was changed to metacentric. In its report aneuploidy, pentasomic (2n-1 = 23), heptasomic (2n+1 = 25) and octasomic (2n+2 = 23) were found in *B. saviczii* which collected from Shiraz (South of Iran) (Getner, 2005). Zakharieva reported pentaploid population of *B. saviczii* (Zakhariyeva and Makushenko, 1969). *B. olivieri* was falling to class 2C. The detail of karyotype is presented for first time. It has five B-chromosomes which were variable as st, sm', m. Asymmetric karyotype was bimodal. Pericentric inversion and to minor extent translocations seems to be the main background for the chromosome polymorphism (Bothmer and Wendelbo, 1981) while Feinbrun believed allopolyploidy occurred in *Bellevalia* (Feinbrun, 1938-1940). In regarding to *Muscari*, *M. comosum* (1), (2) were diploid and falling to class 3C but first population showed decreasing aneuploidy. Also one of the small chromosomes didn't have homologous. One of the first pair chromosome was longer than the other long st chromosome. Asymmetric karyotype was trimodal. In Bentzers report, *M. comosum* was 2n = 18, 27 (Bentzer, 1972). Both of the population of *M. caucasicum* were diploid and autotriploid and falling to class 2C. Asymmetric karyotype was trimodal. Both of the population of *M. tenuiflorum* were diploid and autotriploid that falling to class 2C and 3C. Some constrictions were recognized. They had trimodal asymmetric karyotype. In this specimen allocyclic event existed.

- *M. longipes* was diploid and falling to class 3C.
- *M. neglectum* and *M. armeniacum* var. *szovitzianum* were autopentaploid and tetraploid that falling to class 2C

Most of reports of polyploidy level in *Bellevalia* showed diploid and tetraploid population. Few reports exists about triploid (Musano and Maggini, 1976) and octaploid populations (Zakhariyeva and Makushenko, 1969; Bothmer and Wendelbo, 1981; Pogosjan and Torosyan, 1983). Variation of ploidy and similarity of morphological characters have been found among the species. In terms of morphological characters, Morphologically, *Bellevalia* sect. *Bellevalia* is closer to *Muscari* subgen. *Leopoldia*. Also subgen. *Botryanthus* is more advanced than subgen. *Leopoldia*. The morphology of chromosomes confirmed taxonomic position. Most of studied *Bellevalia* species were placed in class 2C of Stebbins system except *B. glauca* and *B. tabriziana*.. It is representing more primitive karyotypes

The karyotypes of both genus are markedly asymmetrical because polymorphism as confirmed by Garbari (1969, 1973), Bentzer and Ellmer (1975), Bentzer and Landstorm (1975), Dalgic (1991), Corsi *et al.* (1996) and Bareka and Kamari (2001).

Also, data regarding the total chromatin length and size of the longest and shortest chromosomes were shown in Table 4. In *Bellevalia* studied species, *B. saviczii* had the highest amount of total chromatin length (236.12 μM) and *B. tabrizana* had the least (32.24 μM). Also in *Muscari*, *M. tenuiflorum* (1) had the highest amount of total chromatin length (189.44) and *M. longipes* had the least (54.29). Also, *M. comosum* (2) with 2n = 18 had 72.19 μM total chromatin length while *M. caucasicum* (2) with 2n = 27 had 66.24 μM.

M. tenuiflorum (1) with 2n = 18 had 189.44 total chromatin length and *M. tenuiflorum* (2) with 2n = 27 had 138.19. Here *M. tenuiflorum* (1) with 2n = 18 had 189.44 total chromatin length, *M. neglectum* with 2n = 36 had 180.26 and *M. armeniacum* var. *szovitzianum* with 2n = 45 had 178.86. All of the above cases, are indicating that polyploidy has been accompanied with chromatin loss. Mean while Stebbins class about karyotype was shown in Table 3. Most of studied *Bellevalia* species were placed in class 2c of Stebbins system except *B. glauca* and *B. tabriziana*. It is representing more primitive karyotypes. Most of studied *Muscari* species were placed in class 2c. Except the both of *M. comosum* and *M. tenuiflorum* (2) populations which were placed in 3C because they have trimodal asymmetric karyotype with 3 sizes of chromosomes.

The results were obtained from the present study can be summarized as follows:

- Polyploidy variation in *Bellevalia* was more than *Muscari*.
- Ploidy increasing didn't have corresponding to total chromatin length increasing.
- Chromosome morphology of *Muscari* subgen. *Leopoldia* is larger and similar to *Bellevalia*. This subgenus is more primitive than subgen. *Botryanthus* and closer to *Bellevallia* (primitive genus).
- In *B. longistyla* and *B. olivieri* autopentaploidy and hexaploidy were observed.
- The results of karyological study proved taxonomical position of studied species of sect. *Bellevalia*.
- Presence of M chromosome in *M. armeniacum* var. *szovitzianum* and *M. caucasicum* (1) probably due to pericentric inversion.

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