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CHROMOSOME COUNTS FOR SIX SPECIES OF ALLIUM (AMARYLLIDACEAE) FROM IRAN

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Mitotic chromosome numbers are reported for ten accessions belonging to six *Allium* species. Basic chromosome number of all species was x = 8. *Allium lenkoranicum* Miscz. ex Grossh., *A. rubellum* M. Bieb., *A. scabriscapum* Boiss., *A. stipitatum* Regel. as well as *A. viride* Grossh. were diploids and *A. atroviolaceum* Boiss. was tetraploid. Chromosome count of *A. lenkoranicum* (2n = 2x = 16) is reported here for the first time.

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Key words: *Allium*; chromosome number; karyotype; ploidy

تعداد کروموزوم‌های شش گونه Allium (Amaryllidaceae) از ایران

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شمارش کروموزومی میتوزی ۱۰ جمعیت متعلق به شش گونه ازسرده پیاز گزارش می‌گردد. تعداد کروموزوم پایه همه گونه‌ها x = 8 بود. گونه‌های A. stipitatum Regel. ،A. scabriscapum Boiss. ،A. rubellum M. Bieb. Allium lenkoranicum Miscz. ex Gross. و همچنین A. viride Grossh. دیپلوئید و A. atroviolaceum Boiss. تتراپلوئید بودند. شمارش کروموزومی A. lenkoranicum (2n = 2x = 16) نخستین گزارش برای این گونه است.

INTRODUCTION

Allium L. is one of the largest genera of the Amaryllidaceae family, with more than 920 species including many economically important species used as vegetables, spices, medicines, or ornamental plants (Govaerts & al. 2020; Herden & al. 2016). The genus *Allium* is widely distributed over the Holarctic region from the dry subtropics to the boreal zones. A region of especially high species diversity stretches from the Mediterranean basin to Central Asia and Pakistan (Fritsch and Friesen 2002). This genus is one of the ten large and rich genera of the vascular flora of Iran in terms of the number of endemic species (Miri and Roughani 2019; Noroozi & al. 2019).

Most species of *Allium* have a basic chromosome number of x = 8, but x = 7 and x = 10 were found in sections *Bromatorrhiza* and *Decipientia*, respectively (Fritsch and Abbasi 2013; Fritsch and Friesen 2002). Polyploidy has also been reported in both sections (Fritsch and Friesen 2002).

Several studies were performed on chromosome number and karyotype analysis of Iranian *Allium* species and accessions (Akhavan & al. 2015; Dolatyari & al. 2018; Hosseini 2018; Mastali & al. 2018; Oroji Salmasi & al. 2019). However, due to highly heterozygous nature of *Allium* species (Jayaswall & al. 2019), it is necessary to evaluate the karyotypes of the accessions of one species to fill gaps of our knowledge in the genus *Allium*. Therefore, the objectives of this study were to determine chromosome number, ploidy

level and karyotype characteristics of six species of the genus *Allium*, of which *A. lenkoranicum* is the first report.

MATERIALS AND METHODS

The bulbs of ten *Allium* accessions belonging to six species were provided from Iranian Biological Resource Center (IBRC), Tehran, Iran, details of which are shown in table 1.

Karyological observation was made on mitotic metaphase cells of root tips according to Ao (2008) and Dolatyari & al. (2018), with some modifications. Healthy bulbs were germinated in petri dishes on moist filter paper in a growth chamber under dark conditions, and 1–1.5 cm long fresh root tips were removed for the analysis of somatic chromosomes. For arresting the meristematic cells at metaphase, two different root tip pretreatments were tested including: aqueous solution of 2 mM 8-hydroxyquineline for 3-5 h at room temperature and 0.01-0.5% colchicine solution for 30-135 min at room temperature. Root tips pretreated with colchicine 0.5% for 135 min were washed in distilled water for 5 min and fixed in freshly Carnoy's fixative (glacial acetic acid: 96% (v/v) ethanol, 3:1) for 3-5 h at room temperature. The roots were hydrolyzed in 1 N HCl for 30 min at room temperature and stained in 1% aqueous aceto-orcein for 2-10 min. The stained root tips were then squashed in a drop of 45% (v/v) acetic acid and glycerol. At least five well-spread metaphase plates were photographed, using a digital camera attached to a CX52 Olympus microscope.

Chromosomal parameters were measured as long arm length (LA), short arm length (SA), chromosome length (CL), arm ratio (AR) and centromeric index (CI). The determination of positions of centromeres as well as the measurements of S and L was performed using MicroMeasure 3.3 software. Karyotypes were prepared and chromosome pairs were classified according to Levan & al. (1964) and Stebbins (1971) and idiograms were designed for each accession using Excel software. The karyotype asymmetry parameters including total form percentage (TF%) (Huziwara, 1962), difference of relative length (DRL), asymmetry index (AsK%), (Arano 1963) and symmetry index (%S) were evaluated.

RESULTS AND DISCUSSION

The basic chromosome number of x = 8 was found in all studied accessions (table 1). The results showed that, four species were diploid (*A. lenkoranicum* Miscz. ex Grossh., *A. rubellum* M. Bieb., *A. scabriscapum* Boiss. *A. stipitatum* Regel. and *A. viride* Grossh.) and *A. atroviolaceum* Boiss. was tetraploid. Two studied accessions of *A. atroviolaceum* showed 2n = 4x = 32, as previously reported by D'Emerico and Pignone (1998) and Friesen & al. (2006). On the contrary, Miryeganeh and Movafeghi (2011) has observed the chromosome numbers of 2n = 16 for this species. Karyotypic variation in the genus *Allium* is not rare, and different karyotypes for one species or species belonging to one section could be found in accidentally occurring chromosome rearrangements (Miryeganeh and Movafeghi 2011). The somatic chromosome number and karyotypes of *A. lenkoranicum* is reported for the first time. Chromosome number of the other studied accessions were consistent with previous counts (Friesen & al. 2006; Fritsch and Abbasi 2013; Garbari & Crisman 1988; Hosseini and Go 2010; Oroji Salmasi & al. 2019).

The mean of chromosome length (CL) ranged from 14.0 µm (*A. atroviolaceum* (P1008913) to 25.4 µm (*A. viride*). The latter species had the longest LA (14.1 µm) and SA (11.3 µm). The shortest LA (7.8 µm) and SA (5.9 µm) belonged to *A. rubellum* (P1008882) and *A. atroviolaceum* (P1008913), respectively. In fact, the largest and smallest mean chromosome length was from the section *Allium*. Photographs of the mitotic metaphases and their related ideogram of the accessions are presented in figs. 1 & 2. No significant correlation was observed between the mean chromosome length and ploidy level of accessions (data not shown). *Allium lenkoranicum* (P1008868) and *A. rubellum* (P1008882) had the lowest AR (1.17) and the highest CI (0.46), while the highest and lowest of these two parameters (1.49 and 0.40, respectively) were identified in *A. lenkoranicum* (P1008822), (table 2). Although *A. viride* is considered as a synonym or variant of *A. dictyoprasum* C.A. Meyer ex Kunth., the karyotypes of these entities differ as regards to symmetry, number and position of satellited pairs (Fritsch and Abbasi 2009; Kamari & al. 1999). Dolatyari & al. (2018) reported that the total chromosome length of four *A. dictyoprasum* populations ranged from 78.4 µm to 99.5 µm and that the chromosomes were 'm' and 'sm' types and the satellite observed. While the karyotype results of *A. viride* in our study showed that despite the similarity in the number of chromosomes and karyotype formula, the length of the chromosomes was about 2.2 times longer and no satellite was observed.

Table 1. Geographical information of the studied *Allium* species.

Species	Section	Location	Latitude	Longitude	Altitude (m)	Herbarium code
A. atroviolaceum Boiss.	*Allium*	Razavi Khorasan-Quchan	N 37º 19' 16"	E 58º 41' 27"	1840	P1008913
A. atroviolaceum Boiss.	*Allium*	Semnan-Shahmirzad	N 35º 52' 05"	E 53º 12' 14"	1853	P1008773
A. lenkoranicum Miscz. ex Grossh.	*Codonprasum*	Alborz-Haljerd	N 35º 54' 18"	E 50º 53' 05"	1468	P1006717
A. lenkoranicum Miscz. ex Grossh.	*Codonprasum*	Semnan-Shahrood	N 36º 54' 55"	E 55º 20' 06"	2004	P1008822
A. lenkoranicum Miscz. ex Grossh.	*Codonprasum*	North Khorasan-Bojnurd	N 37º 49' 55"	E 55º 48' 06"	731	P1008868
A. rubellum M.Bieb.	*Avulsea*	Semnan-Shahrood	N 36º 49' 55"	E 55º 20' 06"	2004	P1008823
A. rubellum M.Bieb.	*Avulsea*	North Khorasan-Golestan National Park	N 37º 22' 06"	E 56º 14' 32"	1364	P1008882
A. scabriscapum Boiss.	*Scabriscapa*	Alborz-Haljerd	N 35º 54' 18"	E 50º 53' 05"	1468	P1006762
A. stipitatum Regel.	*Allium*	Lorestan-Aligudarz	N 33º 11' 32"	E 49º 29' 59"	2418	P1008650
A. viride Grossh.	*Allium*	Alborz-Haljerd	N 35º 54' 18"	E 50º 53' 12"	1468	P1006716

To evaluate the karyotype asymmetry, different indices were considered (table 3). Based on TF% and AsK% parameters, *A. lenkoranicum* (P1008822) was the most asymmetric species. As shown in table 3, the highest value of TF% (46.4 and 46.2) and the lowest value of AsK% (53.5 and 53.6) were recorded for *A. lenkoranicum* (P1008868) and *A. rubellum* (P1008882) (the most symmetric). The highest and the lowest values of DRL were identified in *A. scabriscapum* (6.3) and *A. rubellum* (P1008882) (2.4), respectively. High DRL value leads to more changes in the construction of chromosomes (Afshari & al. 2013). The mean of DRL values in diploid accessions were higher than the tetraploid ones (3.3 and 2.5, respectively), (table 3). This parameter is reliable only in populations with the same ploidy level (Moradi & al. 2013). The highest and the lowest values of S% were distinguished in *A. rubellum* (P1008882) (67.7) and *A. scabriscapum* (40.3), respectively. Value of this index close to 100%, indicates that the chromosomes are of similar length while some differences may exist in other karyotype features (Arabbeigi & al. 2011).

Table 2. Karyotypic parameters of the studied *Allium* species. 2n: somatic chromosome number; CL: chromosome length; LA: long arm; SA: short arm; AR: arm ratio; CI: centromeric index.

Species (Herbarium code)	2n	CL (µm)	LA (µm)	SA (µm)	AR	CI
A. atroviolaceum (P1008913)	2n=4x=32	14.0	8.0	5.9	1.40	0.42
A. atroviolaceum (P1008773)	2n=4x=32	18.1	10.4	7.6	1.42	0.42
A. lenkoranicum (P1006717)	2n=2x=16	19.3	10.7	8.6	1.29	0.44
A. lenkoranicum (P1008822)	2n=2x=16	15.1	8.9	6.2	1.49	0.40
A. lenkoranicum (P1008868)	2n=2x=16	19.9	10.6	9.2	1.17	0.46
A. rubellum (P1008823)	2n=2x=16	17.1	9.7	7.3	1.36	0.42
A. rubellum (P1008882)	2n=2x=16	14.5	7.8	6.7	1.17	0.46
A. scabriscapum	2n=2x=16	18.2	10.6	7.6	1.40	0.42
A. stipitatum	2n=2x=16	25.4	14.1	11.3	1.40	0.42
A. viride	2n=2x=16	25.4	14.1	11.3	1.36	0.44

Table 3. Asymmetrical parameters of the studied *Allium* species. SC: symmetry classes of Stebbins; TF%: total form percentage; DRL: difference of relative length; AsK%: Arano index of karyotype asymmetry; S%: symmetry index; KF: karyotype formula.

Species (Herbarium code)	SC	TF%	DRL	AsK%	S%	KF
A. atroviolaceum (P1008913)	1A	42.5	2.5	57.4	47.1	13m+2m^{sat}+1sm
A. atroviolaceum (P1008773)	1B	42.4	2.6	57.5	42.3	9m+4m^{sat}+3sm
A. lenkoranicum (P1006717)	1A	41.2	3.0	55.5	63.0	7m+1m^{sat}
A. lenkoranicum (P1008822)	1A	40.8	2.6	59.0	66.1	6m+1m^{sat}+1sm
A. lenkoranicum (P1008868)	1A	46.4	2.8	53.5	62.9	7m+1m^{sat}
A. rubellum (P1008823)	1A	42.9	4.7	57.0	63.3	6m+1m^{sat}+1sm
A. rubellum (P1008882)	1A	46.2	2.4	53.6	67.7	7m+1m^{sat}
A. scabriscapum	1B	41.9	6.3	58.0	40.3	8m
A. stipitatum	2A	43.0	4.2	57.0	48.8	7m+1sm
A. viride	1A	44.5	3.7	55.5	54.7	7m+1sm

Based on the chromosome terminology of Levan & al. (1964), two chromosome types of 'm' (metacentric) and 'sm' (submetacentric) were observed (table 3). Metacentric chromosomes were dominant in all accessions. Out of eight diploid accessions, four accessions had 7m+1sm karyotype formula (regardless of the presence of satellites) and the other were 8m. These results are in line with previous reports, stating that the common karyological feature of *Allium* consists of 5-8 metacentric and 0-4 submetacentric chromosomes (Dolatyari & al. 2018; Hosseini 2018; Mastali & al. 2018; Oroji Salmasi & al. 2019). Although, 1-2 pairs of subtelocentric chromosomes have been reported in some species (Genç & al. 2013; Hosseini and Go 2010; Li & al. 2018; Maragheh & al. 2019). We observed satellite chromosome pairs in *A. atroviolaceum*, *A. lenkoranicum* and *A. rubellum*. The presence of satellites may not be consistent in species of *Allium* and not be used to distinguish species (Oroji Salmasi & al. 2019). Our observations indicated the presence of two or four pairs of satellited chromosomes in accessions of *A. atroviolaceum*. D'Emerico and Pignone (1998) reported the presence of four satellited chromosomes, while Miryeganeh and Movafeghi (2011) only found one satellited pair. Also, two studied accessions of *A. rubellum* showed the presence of one pair of satellited chromosomes (like all accessions of *A. lenkoranicum*), conflicting with those of Hosseini and Go (2010) who reported the presence of two satellited pairs.

Karyotypes of all ten accessions were classified in the 1A (*A. atroviolaceum* (P1008913), *A. lenkoranicum*, *A. rubellum* and *A. viride*), 2A (*A. stipitatum*) and 1B (*A. atroviolaceum* (P1008773) and *A. scabriscapum*) of Stebbins categories (table 3), implying they are symmetric.

In conclusions, results of the present study showed that there is natural variation in ten accessions of six species of *Allium* that can contribute to conservation and breeding programs. The chromosome number and karyotype of *A. lenkoranicum* is reported for the first time. Karyotype analysis indicated that *Allium* species studied here have metacentric and submetacentric chromosomes and symmetric karyotypes.

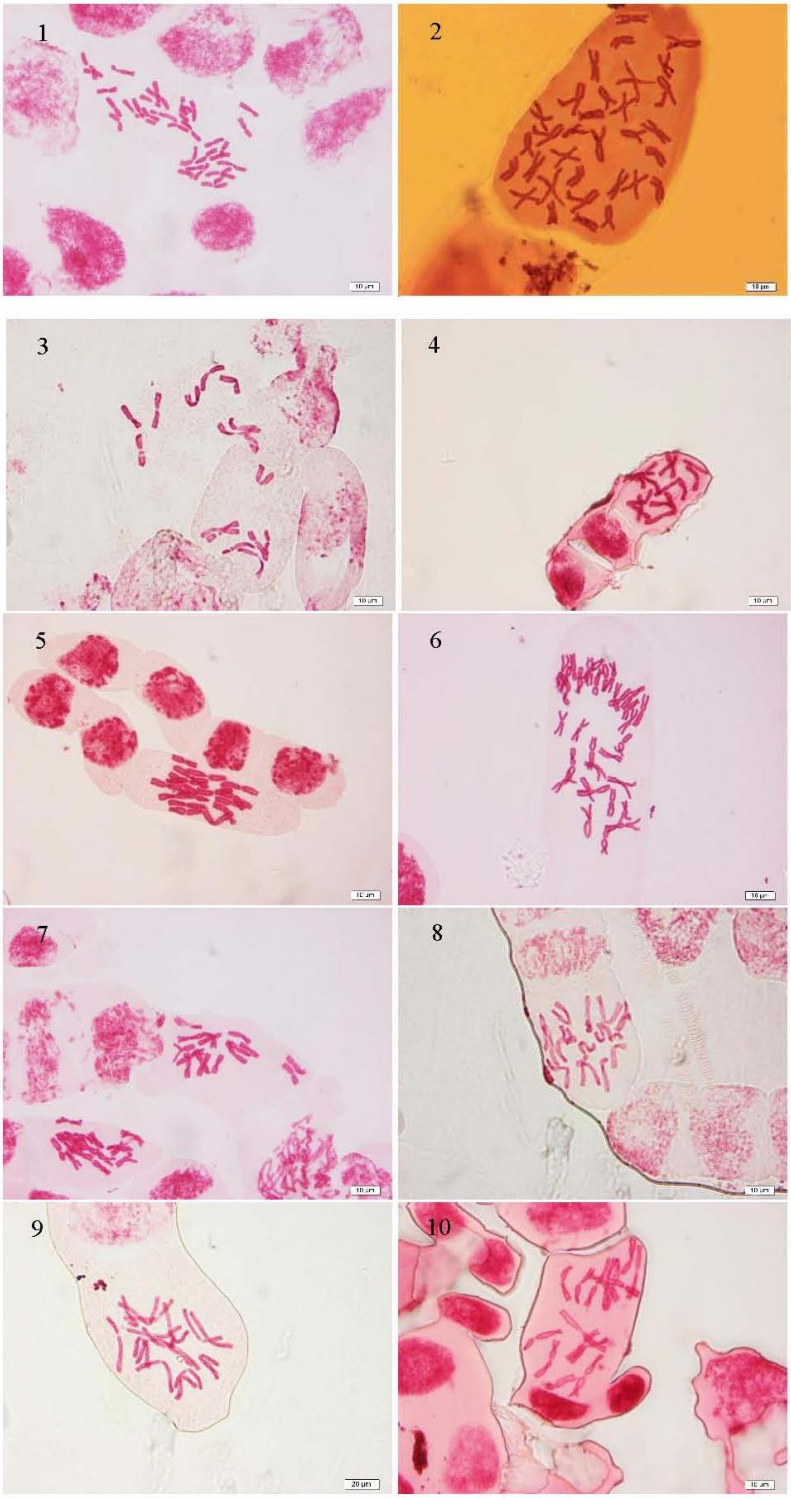

Fig.1. Somatic chromosomes of ten studied accessions of *Allium* spp. 1, *A. atroviolaceum* (P1008913); 2, *A. atroviolaceum* (P1008773); 3, *A. lenkoranicum* (P1006717); 4, *A. lenkoranicum* (P1008822); 5, *A. lenkoranicum* (P1008868); 6, *A. rubellum* (P1008823); 7, *A. rubellum* (P1008882); 8, *A. scabriscapum*; 9, *A. stipitatum*; 10, *A. viride*.

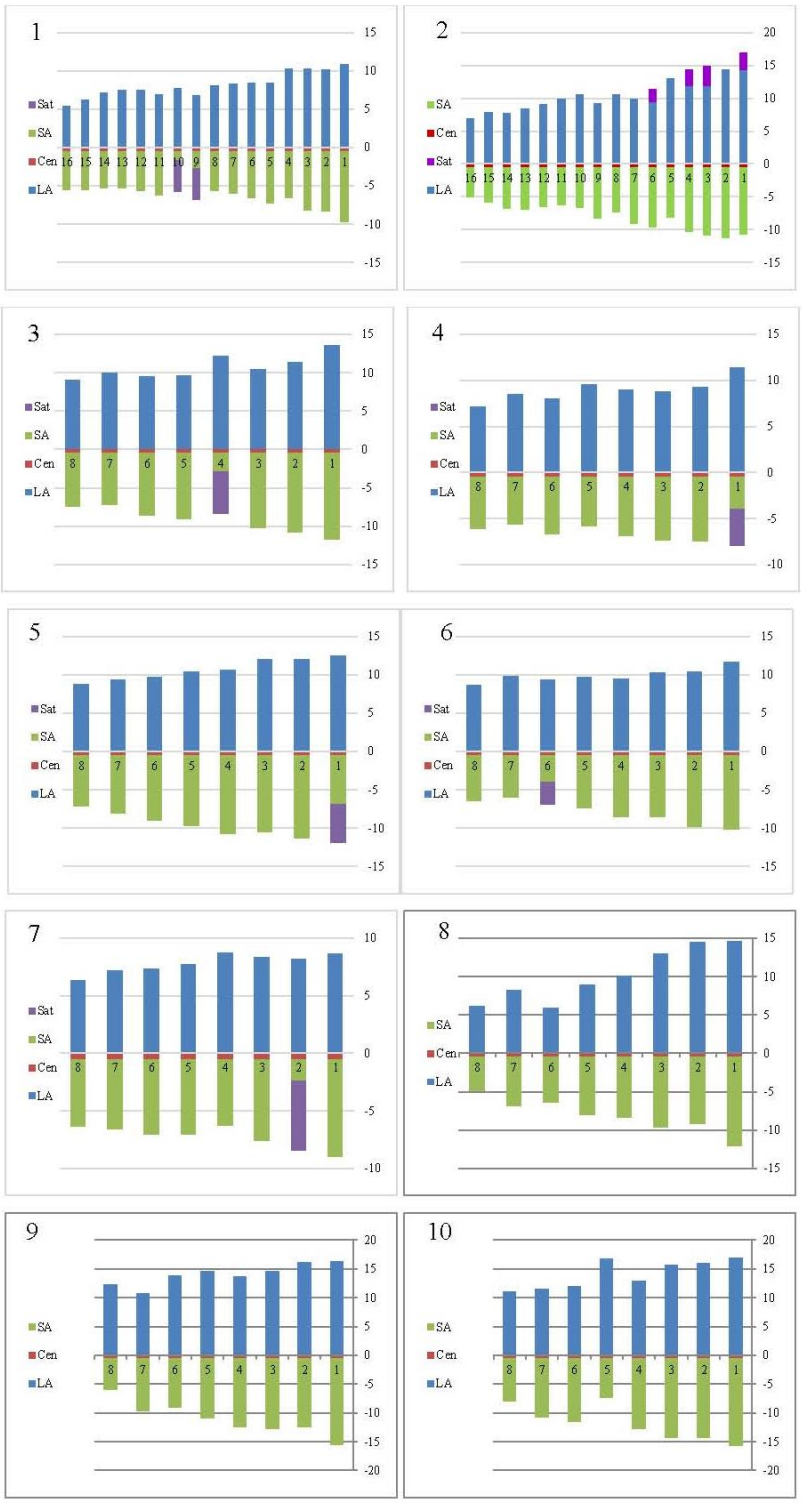

Fig. 2. Ideograms representing the mean karyotypes of ten studied accessions of *Allium* spp. 1, *A. atroviolaceum* (P1008913); 2, *A. atroviolaceum* (P1008773); 3, *A. lenkoranicum* (P1006717); 4, *A. lenkoranicum* (P1008822); 5, *A. lenkoranicum* (P1008868); 6, *A. rubellum* (P1008823); 7, *A. rubellum* (P1008882); 8, *A. scabriscapum*; 9, *A. stipitatum*; 10. *A. viride*

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