

Karyotype analysis of some *Allium* species in Iran

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Abstract

The karyotypes of 10 species (15 accessions) of *Allium* from Iran were investigated using the squash technique and 1% (w/v) aceto-iron-hematoxylin stain. The basic chromosome number was $x = 8$, and only in *A. giganteum* (1) $x = 7$. Karyotypes of 14 taxa of *Allium* were diploid with $2n = 16$; only *A. macrochaetum* was tetraploid with $2n = 32$. Satellite chromosomes were seen in *A. asarense*. All karyotypes were symmetrical, consisting of metacentric and submetacentric chromosome pairs. Only *A. caspium* and *A. stipitatum* (1) had subtelocentric chromosomes. Karyotype analysis according to Stebbins categories placed the studied taxa in symmetric classes of 1A and 2A, indicating a symmetric karyotype. The results of the analysis of variance showed significant differences for total chromosome length (TCL), mean chromosome length (CL), long arm length (LA), short arm length (SA) and intrachromosomal asymmetry index (A1). The longest chromosome length was detected on *A. asarense*, *A. elburzense*, *A. giganteum* (3), *A. rotundum* and *A. stipitatum* (3) (17.9-19.7 μm), while *A. ampeloprasum* demonstrated the shortest value (8.2 μm). Results of cluster analysis based on chromosomal parameters classified the taxa into four groups using the unweighted pair group method with arithmetic mean (UPGMA). Using principal component analysis, the first three components determined 97.3% of the total variation. The grouping of the taxa based on the 2-D scatter plot using the first two principal components, corresponding to the results of the karyotypic characteristics.

Keywords: Chromosome; Cluster analysis; Cytogenetic; Karyology; Principal component analysis.

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Introduction

The genus *Allium* is one of the largest genera of monocots comprising currently more than 900 species widely distributed on the northern hemisphere from the dry subtropics to the boreal zone. Especially, the region from the Mediterranean area to southwest and central Asia is characterized by high species diversity (Fritsch and Friesen 2002; Fritsch *et al.* 2010; Fritsch and Abbasi 2013). About 121 *Allium* species, which belong to seven subgenera and 30 sections, are grown in Iran (Fritsch and Maroofi 2010; Miri and Roughani 2019). Therefore, Iran is regarded as an important section of the Asian center of diversity

for *Allium* species (Fritsch and Friesen 2002).

Generally, all plant parts of alliums may be consumed by humans as spices, vegetables, medicinal plants and ornamentals. Many wild species are also exploited by the local inhabitants as a valuable part of the daily human diet and as part of the fodder for livestock (Fritsch and Abbasi 2013; Miri and Roughani 2018). Various *Allium* species such as *A. ascalonicum*, *A. ampeloprasum* and *A. hirtifolium* have received attention for their antimicrobial (Kyung 2012; Miri and Roughani 2018) or anticancer compounds (Ghodrati Azadi *et al.* 2008; Miri and Roughani 2018).

Allium species are adapted to diverse habitats

and exhibit a remarkable polymorphism, which is the main reason for widespread problems in their taxonomy (Fritsch and Friesen 2002). Therefore, chromosomes should be studied in detail to determine whether they can play a role in solving classification problems (Dolatyari *et al.* 2018). It has been known that chromosome number and karyotype analysis provides valuable information in identifying species and some closely related taxa in *Allium* L. (Choi and Oh 2011). Most *Allium* species are diploid. Polyploidy is less common but occurs among botanical varieties of the cultivated forms, as well as in wild species (Havey 2002). The base number of chromosomes $x = 8$ dominates (Fritsch and Astanova 1998; Fritsch and Abbasi 2013; Akhavan *et al.* 2015), but other numbers ($x = 7, 9, 10, 11$) have also been reported (Fritsch and Astanova 1998; Fritsch and Abbasi 2013). Some karyotype studies were performed on several Iranian species of *Allium* (Hosseini and Go 2010; Akhavan *et al.* 2015; Dolatyari *et al.* 2018).

Despite many studies on the karyology of *Allium* species and accessions, some differences among reports are observed. Thus, the comparative karyotype study of some species as well as additional Iranian accessions of the genus *Allium* will be essential to improve our knowledge about the variation of their karyological patterns. Hence in this article, the details of chromosome morphology, karyotype formulae and symmetry index of 10 species of *Allium* from Iran were investigated.

Materials and Methods

Plant material

The seeds of 15 accessions, representing 10 *Allium*

species were collected from Research Institute of Forests and Rangelands (RIFR), and Iranian Biological Research Center (IBRC), details of which are shown in Table 1.

Cytological studies

The seeds were hand scarified, disinfected by 0.2% (w/v) carboxin thiram (*Vitavax thiram*[®]) for 2 min and placed in Petri dishes on moist filter paper at 4 °C for a month, then they were germinated at 25 °C in a growth chamber under dark conditions. For the cytological study, 1.5-2 cm long root tips were pretreated in 1% α -bromonaphtalin for 6 h at 4 °C and washed in distilled water for 5 min. The root samples were fixed in fresh Levitsky solution, a mixture of 1:1 (v/v) chromic acid 1%: formaldehyde 10%, for 24 h at 4 °C followed by hydrolyzing in 1 N HCl for 6 min at 60 °C, and staining using 1% aqueous aceto-iron-hematoxylin for 3 h at room temperature. The stained roots were squashed in a drop of 45% (v/v) acetic acid and glycerol. At least 10 mitotic metaphases were photographed for each taxon using an Olympus (BX41) microscope equipped with Canon camera, selecting the five cells for measurements.

Chromosomal parameters were measured as long arm length (LA), short arm length (SA), mean chromosome length (CL), total chromosome length (TCL), arm ratio (AR) and centromeric index (CI). The positions of centromeres and value of SA and LA were determined using MicroMeasure 3.3 software. Karyotypes were prepared and chromosome pairs were classified according to Levan *et al.* (1964). The karyotype asymmetry parameters were determined using the total form (TF%), the difference between minimum and

Table 1. *Allium* accessions with their species and geographical description.

Species	Section	Location	Latitude	Longitude	Altitude (m)	Herbarium number
<i>A. ampeloprasum</i>	<i>Allium</i>	Ardabil, Meshkinshahr	N 38° 21' 12" E 48° 14' 28"		1260	30576
<i>A. asarense</i>	<i>Cepa</i>	Alborz	N 36° 02' 66" E 51° 12' 12"		1909	P1009482
<i>A. ascalonicum</i>	<i>Allium</i>	Chaharmahal and Bakhtiari, Shahrekord	N 32° 30' 47" E 50° 28' 23"		2712	34219
<i>A. caspium</i>	<i>Kaloprason</i>	North of Khorasan	N 37° 15' 00" E 58° 56' 86"		1454	P1008918
<i>A. elburzense</i>	<i>Asteroprason</i>	Qazvin	N 36° 28' 32" E 50° 27' 50"		2097	P1009734
<i>A. giganteum</i> (1)	<i>Compactoprason</i>	North Khorasan, Bojnord	N 37° 35' 06" E 57° 14' 37"		1658	40733
<i>A. giganteum</i> (2)	<i>Compactoprason</i>	North Khorasan, Bojnord	N 37° 35' 06" E 57° 14' 32"		1756	40776
<i>A. giganteum</i> (3)	<i>Compactoprason</i>	North Khorasan, Shirvan	N 37° 60' 15" E 57° 96' 56"		2220	30822
<i>A. hirtifolium</i> (1)	<i>Megaloprason</i>	Lorestan, Zaghe	N 33° 24' 00" E 48° 39' 00"		1840	30371
<i>A. hirtifolium</i> (2)	<i>Megaloprason</i>	Qazvin	N 35° 30' 13" E 49° 12' 78"		2097	40399
<i>A. macrochaetum</i>	<i>Allium</i>	Eeat Azarbaijan	N 37° 20' 00" E 47° 00' 00"		1350	P1009936
<i>A. rotundum</i>	<i>Allium</i>	West Azarbaijan, Urmia	N 37° 18' 51" E 45° 06' 53"		1500	36853
<i>A. stipitatum</i> (1)	<i>Megaloprason</i>	Lorestan, Rimaleh	- ^a	-	-	P1003138
<i>A. stipitatum</i> (2)	<i>Megaloprason</i>	Lorestan, Rimaleh	-	-	-	P1003140
<i>A. stipitatum</i> (3)	<i>Megaloprason</i>	Lorestan, Javanmard	N 33° 58' 00" E 48° 40' 00"		1147	P1003141

a: unknown

maximum relative length of chromosomes (DRL), intrachromosomal asymmetry index (A1), interchromosomal asymmetry index (A2) and the categories of Stebbins (1971).

Statistical analyses

After initially testing the homogeneity of variances by Bartlett's test, the karyotype data were subjected to one-way analysis of variance, based on the completely randomized design using the SAS software. Differences between each pair of means were tested by Duncan's multiple range test. Multivariate statistical analyses were carried out by the JMP software package. Cluster analysis of the 15 *Allium* taxa, based on the karyotypic characteristics, was performed by the unweighted pair group method using arithmetic mean (UPGMA) and squared Euclidean distances. Principal component analysis (PCA) was also carried out to evaluate relations among the *Allium*

taxa based on karyotype parameters by drawing a 2-D scatter plot using the two first principal components.

Results

The studied taxa had two basic chromosome numbers. The most frequent basic chromosome numbers were $x = 8$, and only *A. giganteum* (1) had $x = 7$ (Table 2). Different ploidy levels (diploidy and tetraploidy) were observed. One species (*A. macrochaetum*) was tetraploid ($2n=4x=32$), and the others were diploid ($2n = 2x = 14, 16$) (Table 2). Satellites were present on only *A. asarense*. Figure 1 illustrates the karyotypes and karyograms obtained for the taxa studied.

Types of chromosomes were metacentric, submetacentric and subtelocentric. The most common karyotype formula was obtained as $7m + 1sm$ [*A. ascalonicum*, *A. giganteum* (3), *A. stipitatum* (2), *A. stipitatum* (3)] and $5m + 3sm$ [*A.*

Table 2. Haploid karyotype parameters and formulae of the studied *Allium* taxa.

Species	2n	SC	A1	A2	TF%	DRL	KF	CI	TCL (μm)	CL (μm)	LA (μm)	SA (μm)	AR
<i>A. ampeloperasum</i>	2n=2x=16	2A	0.35a*	0.18	38.91	5.37	5m+3sm	0.38	64.94e**	8.21d**	5.01d**	3.19d**	1.70
<i>A. asarense</i>	2n=2x=16	1A	0.27ab	0.11	41.72	3.92	5m+1m ^{sat} +2sm	0.42	157.79ab	19.72a	11.51a	8.21a	1.46
<i>A. ascalonicum</i>	2n=2x=16	2A	0.29ab	0.19	41.00	7.89	7m+1sm	0.41	138.88b	17.36ab	10.22ab	7.13a	1.58
<i>A. caspium</i>	2n=2x=16	2A	0.31ab	0.15	40.28	5.38	5m+2sm+1st	0.40	139.39b	17.43ab	10.42ab	7.01ab	1.68
<i>A. elburzense</i>	2n=2x=16	1A	0.28ab	0.16	41.48	5.65	6m+2sm	0.41	143.39ab	17.92a	10.47ab	7.45a	1.48
<i>A. giganteum</i> (1)	2n=2x=14	2A	0.28ab	0.18	41.43	6.45	5m+2sm	0.41	74.05d	11.31cd	6.64cd	4.67cd	1.72
<i>A. giganteum</i> (2)	2n=2x=16	1A	0.27ab	0.15	42.16	4.93	5m+3sm	0.41	81.63d	10.42cd	6.02cd	4.40cd	1.47
<i>A. giganteum</i> (3)	2n=2x=16	2A	0.24ab	0.15	42.83	5.61	7m+1sm	0.42	150.01ab	18.75a	10.70ab	8.05a	1.43
<i>A. hirtifolium</i> (1)	2n=2x=16	2A	0.38a	0.17	37.91	6.28	3m+5sm	0.37	106.06c	13.26bc	8.24bc	5.02b-d	1.75
<i>A. hirtifolium</i> (2)	2n=2x=16	1A	0.31ab	0.18	40.37	6.26	5m+3sm	0.40	125.33b	15.66ab	9.36ab	6.30a-c	1.54
<i>A. macrochaetum</i>	2n=4x=32	1A	0.27ab	0.17	41.71	4.52	10m+6sm	0.42	168.58a	10.55cd	6.15cd	4.40cd	1.49
<i>A. rotundum</i>	2n=2x=16	1A	0.16b	0.17	45.16	7.21	8m	0.46	146.00ab	18.25a	10.00ab	8.25a	1.21
<i>A. stipitatum</i> (1)	2n=2x=16	2A	0.34a	0.16	39.28	6.10	5m+2sm+1st	0.39	138.38b	17.30ab	10.50ab	6.79ab	1.80
<i>A. stipitatum</i> (2)	2n=2x=16	1A	0.28ab	0.12	41.60	4.78	7m+1sm	0.42	134.77b	16.84ab	9.82ab	7.02ab	1.44
<i>A. stipitatum</i> (3)	2n=2x=16	2A	0.30ab	0.14	40.90	5.44	7m+1sm	0.41	145.81ab	18.23a	10.77ab	7.45a	1.52

2n: somatic chromosome number; SC: symmetry classes of Stebbins; A₁: intrachromosomal index; A₂: interchromosomal index; TF%: total form percentage; DRL: difference of relative length; KF: karyotype formula; CI: centromeric index; TCL: total chromosome length; CL: chromosome length; LA: long arm; SA: short arm; AR: arm ratio; *,**: means within a column followed by the same letter are not significantly different according to the Duncan's multiple range test at $p \leq 0.05$ and 0.01 , respectively.

ampeloperasum, *A. giganteum* (2), *A. hirtifolium* (2)], followed by 6m + 2sm [*A. asarense* and *A. elburzense*] and 5m + 2sm + 1st [*A. caspium*, *A. stipitatum* (1)].

Analysis of variance showed significant differences among taxa for TCL, CL, LA, SA and A₁. The mean length of chromosome long arm (LA) varied from 5.01 μm in *A. ampeloperasum* to 11.51 μm in *A. asarense*. The average length of chromosome short arm (SA) ranged from 3.19 μm in *A. ampeloperasum* to 8.24 μm in *A. rotundum*. The value of CL and TCL varied from 8.21 and 64.94 μm (*A. ampeloperasum*) to 19.72 μm (*A. asarense*) and 168.58 μm (*A. macrochaetum*), respectively. The mean value of the chromosome arm ratio (AR) changed from 1.21 in *A. rotundum* to 1.80 in *A. stipitatum* (1). At the intraspecific level, LA, SA, CL and TCL significantly differentiated *A. giganteum* (3) from the other two accessions; however, no significant differences were observed between *A. hirtifolium* and *A.*

stipitatum accessions (Table 2).

Karyotypes of all taxa were classified in the 1A and 2A Stebbins classes and TF values (%) were about 37.9-45.1 (Table 2), which indicates a symmetrical karyotype. However, they showed different symmetrical groups based on various karyotypic symmetrical indices. The highest value of TF% (45.1%), CI (0.46) and the lowest value of AR (1.2) were detected in *A. rotundum* (the most symmetric) and the lowest value of TF% (37.9%), CI (0.37) and the highest value of AR (1.7) were observed in *A. hirtifolium* (1) (the most asymmetric). Also, the highest and the lowest values of DRL and A₁ were determined on *A. rotundum*.

Although the lowest chromosome length was related to the northernmost species (*A. ampeloprasum*), no significant Pearson correlation coefficients were found between chromosome length and altitude ($r = 0.38$) and geographic coordinates ($r = -0.34$).



Figure 1. Karyotype and karyogram of somatic metaphases of studied *Allium* taxa.

The UPGMA phenogram assigned the accessions into four groups at the Euclidean distance of 4.12 (Figure 2). The first group comprised of six taxa [*A. hirtifolium* (1), *A. hirtifolium* (2), *A. ampeloprasum*, *A. ascalonicum*, *A. stipitatum* (1), *A. caspium*]; the second consisted of three taxa [*A. giganteum* (1), *A. giganteum* (2), *A. macrochaetum*]; the third included five taxa [*A. giganteum* (3), *A. stipitatum* (2), *A. stipitatum* (3), *A. elburzense*, *A. asarense*], while *A. rotundum* was separated in the fourth group.

The first three principal components justified 97.37% of the total variance (Table 3). The first component (59.3%) emphasized AR, CI, TF% and A_1 , while the second component (23.0%)

accentuated variation in LA, SA, CL and A_2 . The two-dimensional distribution of the accessions based on two first components (Figure 3) was almost similar to the results obtained through the UPGMA cluster analysis. The first principal component was highly related to the symmetry values and the second was strongly related to the length of complements. According to 2-D scatter plot, *A. rotundum* and *A. hirtifolium* had the highest and lowest values based on the first component, respectively. *A. ampeloprasum*, *A. giganteum* (1), *A. giganteum* (2) and *A. macrochaetum* also had the lowest values based on the second component, which corresponds to the results of the karyotype parameters.

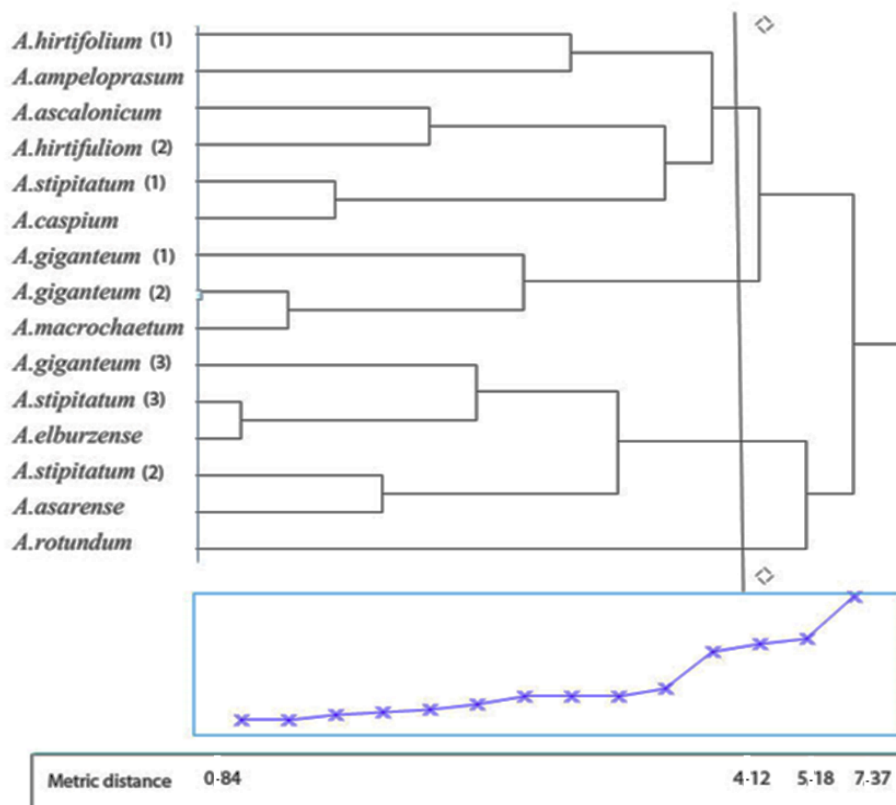


Figure 2. Dendrogram showing the phenetic relationships among the 15 accessions of *Allium* under study, constructed by the unweighted pair group method with arithmetic mean (UPGMA) using the matrix of karyotype distances.

Table 3. Loadings, eigenvalues and cumulative variances for the three principal components (PCs) of the 15 *Allium* taxa.

Parameters	PC1	PC2	PC3
CL ^a	0.229	<u>0.491</u>	0.163
LA	0.188	<u>0.533</u>	0.166
SA	0.277	<u>0.424</u>	0.156
AR	<u>-0.352^b</u>	0.111	0.115
CI	<u>-0.369</u>	-0.174	0.016
TF%	<u>-0.373</u>	-0.183	-0.013
A1	<u>-0.373</u>	0.181	-0.027
A2	-0.138	<u>-0.336</u>	<u>0.573</u>
DRL	0.009	-0.072	<u>0.761</u>
Eigen value	6.528	2.538	1.645
Variance (%)	59.35	23.07	14.95
Cumulative (%)	59.35	82.42	97.37

^asymbols as in Table 2.

^bthe parameters with the loadings of higher than 0.3 were regarded as the important constituents of each PC.

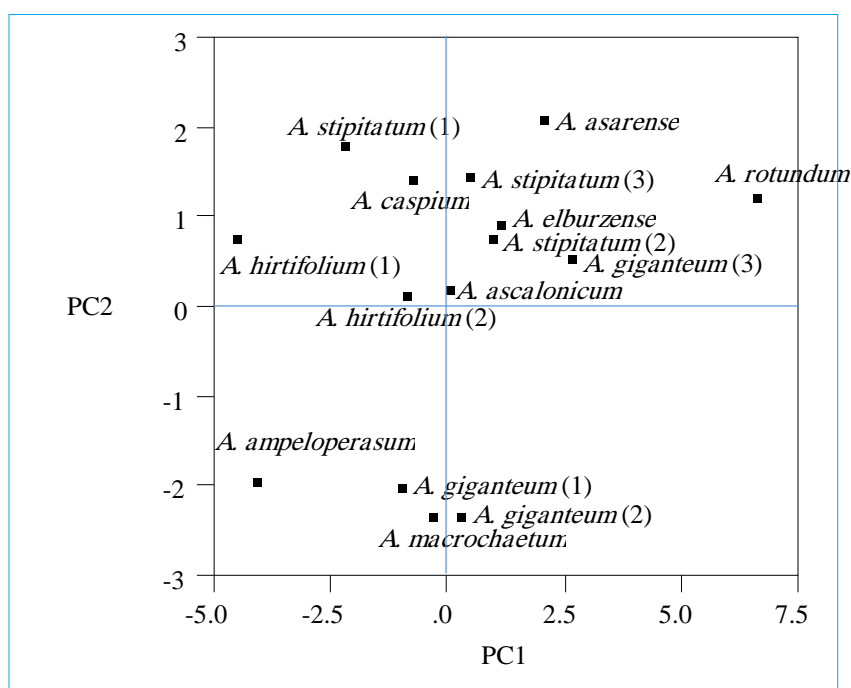


Figure 3. The 2-D scatter diagram resulted from the principal component analysis of the studied taxa.

Discussion

We studied 10 *Allium* species (15 accessions) from Iran in terms of the number of chromosomes, ploidy level and characteristics of the karyotype. The results offer a detailed picture of karyotypic variation among *Allium* species. Like most species of *Allium*, the basic chromosome number of the

most analyzed taxa was $x = 8$. Only in the *A. giganteum* accessions, two different basic chromosome numbers were characterized ($2n = 2x = 14, 16$). Also, all species were diploid except *A. macrochaetum* which was tetraploid. Intra- and interspecific karyotype diversity and multiple basic chromosome numbers among different populations

of one species have been previously reported (Özhatay and Johnson 1996; de Sarker *et al.* 1997; Ao 2008; Fritsch and Abbasi 2013). Ao (2008) and Akhavan *et al.* (2015) determined basic chromosome number of $x = 8$ in *A. przewalskianum* and 10 species of *Allium* (sect. *Acanthoprason*), respectively, which agree with most taxa of *Allium* in this study. Populations of *A. sativum* (Konvicka and Levan 1972), *A. cepa* (Paknia and Karimzadeh 2011), *A. roseum* (Guetat *et al.* 2015) and seven species of *Allium* (subgenus *Allium* and *Melanocrommyum*) (Hosseini and Go 2010) were also identified as the same basic chromosome number. On the other hand, karyotype analysis of eight species of *Allium* (sect. *Allium*) revealed that the basic chromosome number of two species is $x = 7$ (Miryeganeh and Movafeghi 2011).

Contrary to our results, previous data on *A. ampeloperasum* indicate higher levels of polyploidy, with $2n = 4x = 32$ (Von Bothmer 1975; De Sarker *et al.* 1997; Mousavi *et al.* 2006) and $2n = 6x = 48$ (Olah and DeFilippis 1969; Özhatay and Johnson 1996). Han *et al.* (2020) also presented chromosome numbers of $2n = 16, 24, 32, 40$ and 48 in different subspecies of *A. ampeloperasum*. The chromosome number of *A. giganteum* ($2n = 16$) confirmed those obtained by Gurushidze *et al.* (2012), Fritsch and Abbasi (2013) and Han *et al.* (2020); however, $2n = 14$ was also identified for the first time in this study. A higher ploidy level was exceptionally reported as $2n = 32, 48$ for *A. giganteum* (Fritsch and Abbasi 2013; Fritsch 2018). According to Özhatay and Johnson (1996), *A. macrochaetum* was diploid ($2n = 2x = 16$), while in our study it was tetraploid ($2n = 4x = 32$). The chromosome numbers and ploidy level of the other

studied species (*A. asarense*, *A. ascalonicum*, *A. elburzense*, *A. hirtifolium*, *A. rotundum*, *A. stipitatum*) agreed with those published previously (Darlington and Haque 1955; Battaglia 1957; Love 1976; Kuzmanov 1993; Özhatay and Johnson 1996; Fritsch *et al.* 2001; Ghaffari 2006; Panahandeh and Mahna 2011; Gurushidze *et al.* 2012; Fritsch and Abbasi 2013; Singh *et al.* 2016; Hosseini 2018). These chromosomal variations could be due to the accidental chromosome rearrangements, possibly for adaptation to the various environmental conditions (Fritsch and Astanova 1998), but using other techniques such as Giemsa C-banding is necessary to confirm this hypothesis.

The present study reveals that it is difficult to distinguish the studied species according to karyotype formula since accessions of one species have more than one karyotype formula, except those that correspond to *A. stipitatum* (2) and *A. stipitatum* (3). Of course, *A. asarense* can be distinguished by the presence of satellites on the chromosome pair No. 5. However, it has been previously mentioned that the presence of satellites maybe not consistent in the species of *Allium* (Miryeganeh and Movafeghi 2011). Nevertheless, quantitative and qualitative data of karyotype analyses differentiated some of the species studied. On the other hand, the results of the karyotype of *A. asarense*, a species close to the *A. cepa*, showed similarities with the findings of the karyotype of common onion populations by Paknia and Karimzadeh (2010); the karyotype formula in our study was $5m + 1m^{sat} + 2sm$, while they reported the karyotype formula of $8m, 7m + 1sm$ and $6m + 2sm$. Also, chromosome number, ploidy level, CI

and Stebbins classification were similar but mean chromosome length was longer in *A. asarense*.

There were intraspecific differences in terms of TCL, CL, LA and SA. The highest CL diversity was also observed in *A. giganteum* accessions, ranging from 10.42 to 18.75 μm . Paknia and Karimzadeh (2010) and Guetat *et al.* (2015) indicated that different populations of *A. cepa* and *A. roseum* differ in their value of total haploid chromosome length, varies from 67.79 to 96.65 μm and 106 to 172 μm , respectively. Morphological characteristics (Aryakia *et al.* 2016) and RAPD molecular markers (Ebrahimi *et al.* 2009) also supported the intraspecific diversity in some *Allium* species. Intraspecific genome size variations possibly indicate that the speciation is in progress, which could be taxonomically significant (Murray 2005).

In relation to the genome size variation, the maximum ratio between the length of the longest and the shortest complements of *Allium* diploid species was 2.43, while that calculated value from the data supplied by Hosseini and Go (2010) for diploid and tetraploid species were 1.32 and 1.55, respectively. Gurushidze *et al.* (2012) reported that the 2C genome size variation in 70 species of *Allium* varies from 26.2 to 78.7 pg. These differences support the hypothesis that variation in genome size has evolutionary implications that occurred during the divergence and evolution of the chromosome complements of this genus (Ricroch *et al.* 2005).

The studied *Allium* species were characterized by symmetrical karyotypes and classified as 1A and 2A of Stebbins classification, with a predominance of m and sm chromosomes. Ao

(2008) observed Stebbins classification 2A for *A. przewalskianum*, as well as Paknia and Karimzadeh (2010) and Akhavan *et al.* (2015) who reported 1A and 2A category for *A. cepa* populations and 10 species of *Allium* section *Acanthoprason*, respectively, which are in agreement with our findings. Paknia and Karimzadeh (2010), Miryeganeh and Movafeghi (2011) and Akhavan *et al.* (2015) found that the karyotypes of several species of *Allium* are quite symmetrical composing commonly m and sm chromosomes. In other studies, Hosseini and Go (2010) and Ao (2008), working on several species of *Allium*, reported m, sm and st chromosomes. Since the Stebbins' classification is very broad to separate the different types of karyotype asymmetry (Paszko 2006), other methods are used for the elucidation of phylogenetic relationships. According to TF%, CI, AR and A_1 values, *A. hirtifolium* (1) had the highest asymmetric karyotype and *A. rotundum* indicate a high degree of uniformity and symmetry of the karyotype; the latter is consistent with the report of Hosseini (2018). It should be noted that a symmetric karyotype does not necessarily imply "primitivity" (Peruzzi and Eroğlu 2013). Differences in karyotype formulae and asymmetry indices among species indicate that structural changes may have contributed to the diversification of the genus. These chromosome rearrangements may include small or cryptic changes or changes that did not modify the karyotype morphology, such as paracentric inversions or reciprocal translocations with segments of equal size (Seijo and Fernández 2003).

Based on the results of cluster analysis, the accessions with the maximum metric distance might lead us to induce large genetic variation by crossing different accessions. The highest distance was observed between *A. hirtifolium* (1) and *A. giganteum* (3). The accessions belonging to each individual species (except *A. hirtifolium*) were scattered across the clusters in the dendrogram. The two-dimensional plot of the accessions based on the two first components determined by PCA corresponded with the results of the cluster analysis.

Conclusion

The results revealed the natural karyological variation in 15 accessions of 10 species of *Allium*. All of the taxa had the same chromosome number with the exception of the *A. giganteum* (1) accession. Besides the earlier chromosomal counts, new chromosome numbers were also observed in *A. giganteum* and *A. macrochaetum*. Karyotype analysis indicated that *Allium* taxa studied here generally have metacentric and submetacentric chromosomes and symmetric karyotypes. Our results contribute to a better knowledge of some taxa of the genus *Allium* in Iran that may provide useful information for *Allium* evolutionary, genetic and breeding studies.

References

- Akhavan A, Saeidi H, Zarr Sh and Rahiminejad MR, 2015. Chromosome numbers and karyotype features of selected species of *Allium* L. (Amaryllidaceae) sect. *Acanthoprason* in Iran. *The Iranian Journal of Botany* 21(2): 158-164.
- Ao C, 2008. Chromosome numbers and karyotypes of *Allium przewalskianum* populations. *Acta Biologica Cracoviensia Series Botanica* 50(1): 43-49.
- Aryakia E, Karimi HR, Naghavi MR and Shahzadeh Fazeli SA, 2016. Morphological characterization of intra- and interspecific diversity in some Iranian wild *Allium* species. *Euphytica* 211(2): 185-200.
- Battaglia E, 1957. *Allium ascalonicum* L., *A. fistulosum* L., *A. cepa* L.: Analisi Cariotipica. *Caryologia* 10(1): 1-28.
- Choi HJ and Oh BU, 2011. A partial revision of *Allium* (Amaryllidaceae) in Korea and north-eastern China. *Botanical Journal of the Linnean Society* 167: 153-211.
- Darlington CD and Haque A, 1955. The timing of mitosis and meiosis in *Allium ascalonicum*: a problem of differentiation. *Heredity* 9: 117-127.
- De Sarker D, Johnson MAT, Reynolds A and Brandham PE, 1997. Cytology of the highly polyploid disjunct species, *Allium dregeanum* (Alliaceae), and of some Eurasian relatives. *Botanical Journal of the Linnean Society* 124(4): 361-373.
- Dolatyari A, Saeidi Mehrvarz Sh, Shahzadeh Fazeli SA, Naghavi MR and Fritsch RM, 2018. Karyological studies of Iranian *Allium* L. (Amaryllidaceae) species with focus on sect. *Acanthoprason*. 1. Mitotic chromosomes. *Plant Systematics and Evolution* 304: 583-606.
- Ebrahimi R, Zamani Z and Kashi A, 2009. Genetic diversity evaluation of wild Persian shallot (*Allium hirtifolium* Boiss.) using morphological and RAPD markers. *Scientia Horticulturae* 119(4): 345-351.
- Fritsch RM, 2018. *Allium monophyllum* (Amaryllidaceae) is a diploid species. *Phytotaxa* 333(2): 298-300.
- Fritsch RM and Abbasi M, 2013. A Taxonomic Review of *Allium* subg. *Melanocrommyum* in Iran. IPK Gatersleben, Germany.
- Fritsch RM and Astanova SB, 1998. Uniform karyotypes in different sections of *Allium* L. subgen. *Melanocrommyum* (Webb & Berth.) Rouy from Central Asia. *Feddes Repertorium* 109(7-8): 539-549.
- Fritsch RM, Blattner FR and Gurushidze M, 2010. New classification of *Allium* L. subg. *Melanocrommyum* (Webb & Berth) Rouy (Alliaceae) based on molecular and morphological characters. *Phyton* 49: 145-220.
- Fritsch RM and Friesen N, 2002. Evolution, domestication and taxonomy. In: Rabinowitch HD and Currah L (eds.). *Allium Crop Science*. Pp. 5-30. CABI Publishing, Wallingford, UK.

- Fritsch RM and Maroofi H, 2010. New species and new records of *Allium* L. (*Alliaceae*) from Iran. *Phyton* 50(1): 1-26.
- Fritsch RM, Matin F and Klaas M, 2001. *Allium vavilovii* M. Popov et Vved. and a new Iranian species are the closest among the known relatives of the common onion *A. cepa* L. (*Alliaceae*). *Genetic Resources and Crop Evolution* 48: 401-408.
- Ghaffari SM, 2006. New or rare chromosome counts of some angiosperm species from Iran. *The Iranian Journal of Botany* 11(2): 185-192.
- Ghodrati Azadi H, Ghaffari SM, Riazi GH, Ahmadian Sh and Vahedi F, 2008. Antiproliferative activity of chloroformic extract of Persian shallot, *Allium hirtifolium*, on tumor cell lines. *Cytotechnology* 56(3): 179-185.
- Guetat A, Rosato M, Rossello JA and Boussaid M, 2015. Karyotype analysis in *Allium roseum* L. (*Alliaceae*) using fluorescent in situ hybridization of rDNA sites and conventional stainings. *Turkish Journal of Botany* 39(5): 796-807.
- Gurushidze M, Fuchs J and Blattner FR, 2012. The evolution of genome size variation in drumstick onions (*Allium* subgenus *Melanocrommyum*). *Systematic Botany* 37(1): 96-104.
- Han T-S, Zheng Q-J, Onstein RE, Rojas-Andrés BM, Hauenschild F, Muellner-Riehl AN and Xing Y-W, 2020. Polyploidy promotes species diversification of *Allium* through ecological shifts. *New Phytologist* 225(1): 571-583.
- Havey RM, 2002. Genome organization in *Allium*. In Rabinowitch HD and Currah L (eds.). *Allium Crop Science: Recent Advances*. Pp. 5-30. CABI Publ., UK.
- Hosseini Sh, 2018. Karyological studies of some *Allium* L. (*Amaryllidaceae*) species in Iran. *The Iranian Journal of Botany* 24(1): 65-71.
- Hosseini Sh and Go R, 2010. Cytogenetic study of some *Allium* species (subgenus *Allium* and *Melanocrommyum*) in Iran. *Cytologia* 75(1): 99-108.
- Khorasani M, Saeidi Mehrvarz Sh and Zarre Sh, 2018. The genus *Allium* (*Amaryllidaceae*) in Iran: on the status of *Allium ampeloprasum* L. and its relatives. *Nova Biologica Reperta* 5(3): 299-306.
- Konvicka O and Levan A, 1972. Chromosome studies in *Allium sativum*. *Hereditas* 72: 129-148.
- Kuzmanov B, 1993. Chromosome numbers of Bulgarian angiosperms: an introduction to a chromosome atlas of the Bulgarian flora. *Flora Mediterranea* 3: 19-163.
- Kyung KH, 2012. Antimicrobial properties of *Allium* species. *Current Opinion in Biotechnology* 23(2): 142-147.
- Levan A, Fredga K and Sandberg AA, 1964. Nomenclature for centromeric position on chromosomes. *Hereditas* 52(2): 201-220.
- Love A, 1976. IOPB chromosome number reports LII. *Taxon* 25(2-3): 341-346.
- Miri SM and Roughani A, 2018. *Allium* species growing in Iran: chemical compositions and pharmacological activity. Proceedings of the First National Congress and International Fair of Medicinal Plants and Strategies for Persian Medicine that Affect Diabetes, October 9-11, Mashhad, Iran.
- Miri SM and Roughani A, 2019. *Allium* species growing in Iran: botany and distribution. Proceedings of the 2nd International Conference on Medicinal Plants, Organic Farming, Natural and Pharmaceutical Ingredients, February 13-14, Mashhad, Iran.
- Miryeganeh M and Movafeghi A, 2011. Karyotype analysis in some species of *Allium* section *Allium* (*Alliaceae*). *Romanian Journal of Biology - Plant Biology* 56(1): 17-27.
- Mousavi A, Kashi A, Davoodi D and Sanei Shariatpanahi M, 2006. Characterization of an *Allium* cultivated in Iran: the Persian leek. *Belgian Journal of Botany* 139(1): 115-123.
- Murray BG, 2005. When does intraspecific C-value variation become taxonomically significant? *Annals of Botany* 95: 119-125.
- Olla LV and DeFilipps RA, 1969. A study of the great-headed garlic in Illinois. *Illinois State Academy of Science Transactions* 62(4): 402-409.
- Özhatay N and Johnson MAT, 1996. Some karyological remarks on Turkish *Allium* sect. *Allium*, *Bellevalia*, *Muscari*, and *Ornithogalum* subg. *Ornithogalum*. *Bocconea* 5: 239-249.
- Paknia R and Karimzadeh G, 2011. Karyotypic study and chromosome evolution in some Iranian local onion populations. *Journal of Plant Physiology and Breeding*. 1(1): 49-62.
- Panahandeh J and Mahna N, 2011. The karyomorphology of *Allium hirtifolium* Bioss., a less known edible species from Iran. *Journal of Plant Physiology and Breeding* 1(2): 53-57.

- Paszko B, 2006. A critical review and a new proposal of karyotype asymmetry indices. *Plant Systematics and Evolution* 258: 39-48.
- Peruzzi L and Eroğlu HE, 2013. Karyotype asymmetry: again, how to measure and what to measure? *Comparative Cytogenetics* 7(1): 1-9.
- Ricroch A, Yockteng R, Brown SC and Nadot S, 2005. Evolution of genome size across some cultivated *Allium* species. *Genome* 48: 511-520.
- Seijo JG and Fernández A 2003. Karyotype analysis and chromosome evolution in South American species of *Lathyrus* (Leguminosae). *American Journal of Botany* 90(7): 980-987.
- Singh DC, Sharma R and Laishram JM, 2016. The karyotypic studies of three local shallot of Manipur, India. *International Journal of Humanities and Social Sciences* 5(2): 17-22.
- Stebbins GL, 1971. *Chromosomal Evolution in Higher Plants*. Addison-Wesley, USA.
- Von Bothmer R, 1975. The *Allium ampeloprasum* complex on Crete. *Mitteilungen der Botanischen Staatssammlung München* 12: 267-288.

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چکیده

کاریوتیپ‌های ۱۰ گونه (۱۵ جمعیت) آلیوم از ایران با استفاده از تکنیک اسکواش و رنگ آمیزی استو آهن همتوکسیلین یک درصد مورد بررسی قرار گرفت. تعداد کروموزوم پایه $x = 8$ و فقط در *A. giganteum* (1) $x = 7$ بود. کاریوتیپ‌های ۱۴ جمعیت آلیوم دیپلوئید ($2n = 16$) و *A. macrochaetum* تتراپلوئید ($2n = 32$) بود. در *A. asarense* کروموزوم‌های ماهواره‌دار مشاهده شد. کلیه کاریوتیپ‌ها متقارن بوده و از جفت کروموزوم‌های متاسنتریک و ساب متاسنتریک تشکیل شدند و فقط *A. caspium* (1) و *A. stipitatum* (1) کروموزوم‌های ساب تلوسنتریک داشتند. تجزیه کاریوتیپ مطابق دسته بندی استبینز، گونه‌های مورد مطالعه را در کلاس‌های تقارن 1A و 2A قرار داد که نشانگر کاریوتیپ متقارن است. نتایج تجزیه واریانس از نظر صفات مجموع طول کروموزوم‌ها، طول کروموزوم، طول بازوی بلند، طول بازوی کوتاه و شاخص عدم تقارن درون کروموزومی اختلاف معنی‌داری نشان داد. بیشترین طول کروموزوم در *A. asarense*، کمترین مقدار ($8/2$ میکرومتر) را نشان داد. نتایج حاصل از تجزیه خوشه‌ای بر اساس پارامترهای کروموزومی با استفاده از روش UPGMA، جمعیت‌ها را در چهار گروه طبقه بندی کرد. با استفاده از تجزیه به مولفه‌های اصلی، سه مؤلفه اول $97/3$ درصد از کل تنوع را تبیین کردند. پلات پراکندگی دو بعدی بر اساس گروه بندی دو مؤلفه اصلی اول مطابق با نتایج ویژگی‌های کاریوتیپی بود.

واژه‌های کلیدی: تجزیه به مولفه‌های اصلی؛ تجزیه خوشه‌ای؛ سیتوژنتیک؛ کاریولوژی؛ کروموزوم.