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* \(x = 7\). 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* 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.


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 and Abbasi 2013; 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 ºC for a month, then they were germinated at 25 ºC in a growth chamber under dark conditions. For the cytological study, 1.5-2 cm long root tips were pretreated in 1% \( \alpha \)-bromonaphtalin for 6 h at 4 ºC 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 ºC followed by hydrolyzing in 1 N HCl for 6 min at 60 ºC, 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
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.
ampeloperasum, A. giganteum (2), A. hirtifolium (2), followed by 6m + 2sm [A. asarensae 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. asarensae. 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. asarensae) 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. ampeloperasum), no significant Pearson correlation coefficients were found between chromosome length and altitude (r = 0.38) and geographic coordinates (r = -0.34).
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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. ampoloperasum, 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. asareneze], 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 A1, while the second component (23.0%) accentuated variation in LA, SA, CL and A2. 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. ampoloperasum, 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.

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

*a* symbols as in Table 2.

*b* the 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. ampelooperasum indicate higher levels of ploidy, with 2n = 4x = 32 (Von Bothmer 1975; De Sarker et al. 1997; Mousavi et al. 2006) and 2n = 6x = 48 (Olah and DeFilipps 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. ampelooperasum. 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. asarens, 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. asarens 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. asarens, 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+ + 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 A₁ 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 chromosomes 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**


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چکیده
کاریوتیپ‌های ۱۰ گونه (۱۵ جمعیت) آلیوم از ایران با استفاده از تکنیک اسکواش و رنگ آمیزی استو آهن هماتوکسیلین یک درصد مورد بررسی قرار گرفت. تعداد کروموزوم پایه \(x = 8\) و فقط در \(A. giganteum\) و \(A. macrochaetum\) \(x = 7\) بود. کاریوتیپ‌های ۱۵ جمعیت آلیوم دیپلوئید \((2n = 16)\) و ۲ تتراپلوئید \((2n = 32)\) بود. در \(A. asarensae\) کروموزوم‌های ساب متاسنتریک و ساب متاسنتریک منشأه شد. نتایج تجزیه استاندارد و سیتوبومی دسته نشان دهنده کاریوتیپ متقارن است. نتایج تجزیه واریانس از نظر صفات مجموع طول کروموزوم‌ها، طول کروموزوم‌ها، طول بازوی بلند، طول بازوی کوتاه و شاخص عدم تناسب سر در کروموزوم‌های مختلف اندازه‌داری نشان داد. بیشترین طول کروموزوم در \(A. stipitatum\) و \(A. asarensae\) و \(A. rotundum\) و \(A. ampeloperasum\) مشاهده شد، در حالی که در \(A. giganteum\) و \(A. macrochaetum\) کمترین مقدار (۲/۸ میکرونتر) را نشان داد. نتایج حاصل از تجزیه خوشه‌ای بر اساس پارامترهای کروموزوم‌ی با استفاده از روش UPGMA که در چهار مولکول اصلی رابطه ویژگی‌های کاریوتپی بود. کاریوتیپ‌های باغبانی، دانشگاه آزاد اسلامی - واحد کرج، کرج مسئول مکاتبه؛ Email: smmiri@kiau.ac.ir

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کلمات کلیدی: تجزیه به موی‌نده‌های اصلی; تجزیه خوشه‌ای; سیتوبومی‌کرک؛ کروموزووم.