CHROMOSOME NUMBERS AND KARYOTYPE FEATURES OF SELECTED SPECIES OF ALLIUM L. (AMARYLLIDACEAE) SECT. ACANTHOPRASON IN IRAN

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Chromosome numbers and karyotypes of 10 species of *Allium* section *Acanthoprason* collected from different localities in Iran are presented. Seven counts represent new reports. Chromosomal characteristics were determined using photographs complemented by statistical analyses. Our results show that the members of this section are diploid with homogeneous karyotypes characterized by the basic chromosome number of $x = 8$. The karyotypes are ±symmetrical composing mainly of metacentric and submetacentric chromosomes.

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اعداد کروموزومی و ویژگی‌های کاریوتیپی گونه‌های انتخابی از سرده پیاز (تیره نرگسیان) بخشه والک‌ها از ایران

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اعداد کروموزومی و کاریوتایپ ۱۰ گونه از سرده پیاز بخشه والک‌ها جمع‌آوری شده از ایستگاه‌های مختلفی در ایران ارائه می‌گردد. عدد کروموزومی هفت گونه برای اولین بار گزارش می‌شود. ویژگی کروموزومی با استفاده از عکس‌های تهیه شده تعیین و با معیارهای معمول آماری تکمیل شد. نتایج نشان می‌دهد که اعضای بخشه کاریوتایپ همگنی دارند که با عدد پایه کروموزومی x=۸ مشخص می‌شود. کاریوتایپ‌ها متقارن بوده و اغلب شامل کروموزوم‌های متاسانتریک و ساب‌متاسانتریک هستند.

INTRODUCTION

It has been known that somatic chromosome number provides valuable characters in delimiting species and in distinguishing some closely related taxa in *Allium* L. (Choi & Oh 2011).

Allium is the largest and cytologically most diverse genus in Amaryllidaceae (APG III 2009), comprising more than 900 species worldwide (World Checklist 2014). The most common basic chromosome number in *Allium* is x = 8, but other numbers (x=7, 9, 10, 11) and various ploidy levels (2n=14–68) have also been reported (Levan 1931, 1932; Traub 1968; Huang et al. 1995; Fritsch & Astanova 1998; Xu et al. 1998; Zhou et al. 2007). It has been shown that almost all North American species of *Allium* have a basic chromosome number of x = 7 whereas the majority of the Old World species have the basic chromosome number of x = 8 (McNeal 1992; McNeal & Jacobsen 2002; Choi & Cota-Sanchez 2010). A cytological characteristic of *Allium* is the absence of *Arabidopsis-type* telomere (TTTAGGG)n, which was first observed in *Allium.* It was suggested that the chromosomes of *Allium* may be

terminated by satellite repeats, ribosomal DNA (rDNA) repeats or mobile elements (Fuchs et al. 1995; Pich et al. 1996; Pich & Schubert 1998).

According to Fritsch & Maroofi (2010), *Allium* is represented in Iran by 121 species. Among the Iranian species of *Allium*, 76 species and subspecies are assigned to subgen. *Melanocrommyum* (Webb. & Berthel.) Rouy (Fritsch & Abbasi 2013) that is very diverse in Southwest Asia, particularly in Iran and Turkey. This subgenus is the second largest subgenus of *Allium*, comprising about 170 species worldwide classified into 20 sections, mostly diploid (x=8) with nearly uniform karyotypes (Fritsch et al. 2010; Fritsch 2012). In a karyological study on 23 species of this subgenus (Fritsch & Astanova 1998), all species were reported as diploid with 2n=16. Ghahremaninejad et al. (2013) in a cytological study of 11 Iranian *Allium* species showed that they are diploid 2n=16 or tetraploid 2n=32. Ghaffari (2006) also showed that *A. iranicum* is tetraploid (2n=32).

Allium section *Acanthoprason* Wendelbo is one of the most complicated taxonomic groups in *A.* subgen. *Melanocrommyum* Rouy in Rouy & Foucaud (Wendelbo 1971). Many species of this section are endemic to Iran. Taxonomy of this section was the subject of a long controversy. Sectional delimitation has been changed several times and there is no consensus about the number of species within the section among taxonomists as the sectional circumscription is almost completely changed since the description of the section (Wendelbo 1971; Fritsch & Abbasi 2009; Fritsch et al. 2010; Fritsch 2012).

Information on the karyology of *A.* sect. *Acanthoprason* is meager and ploidy level of the species is not well documented. According to the IPCN (Index to Plant Chromosome Numbers, www.tropicos.org/Project/IPCN), chromosome numbers for most species of this section are not reported yet.

This study was aimed to determine chromosome number and ploidy level, and to provide general information on karyotype characteristics of selected species of *A.* sect. *Acanthoprason* occur in Iran.

MATERIALS AND METHODS

The bulbs of 12 accessions representing 10 species, namely: *A. akaka* S.G.Gmel. ex Schult. & Schult.f., *A. egorovae* M.V.Agab. & Ogan., *A. austroiranicum* R.M.Fritsch (3 accessions), *A. breviscapum* Stapf, *A. chlorotepalum* R.M.Fritsch, *A. graveolens* (R.M.Fritsch) R.M.Fritsch, *A. hamedanense* R.M.Fritsch, *A. kurdistanicum* Maroofi & R.M.Fritsch, *A. materculae* Bordz. and *A. subakaka* Razyfard & Zarre were collected from natural habitats during 2010 and 2012. Details regarding the studied materials are presented in table 1.

Table 1. Geographical information of the accessions of *Allium* species investigated. All samples were collected by H. Saeidi and A. Akhavan. Herbarium vouchers are deposited in the Herbarium of the University of Isfahan.

Species	Location	Coordinates	Altitude (m)	Herbarium number
A. akaka	Ardabil, Khalkhal, Cheshmeh Aznav	N 37° 34.945' E 48° 34.363	1900	19449
A. austroiranicum	Isfahan, Fereydun Shahr, Vahdatabad	N 32° 53.486' E 50° 09.560	2750	19451
A. austroiranicum	Isfahan, Khansar, Kouhe Sangandaz	N 33° 11.414' E 50° 17.142	3000	19452
A. austroiranicum	Yazd, Barf khane, Deh Bala	N 31° 34.977' E 54° 05.414	2800	19453
A. breviscapum	Hamadan, Ganjnameh	N 34° 45.562' E 48° 26.011	2450	19454
A. chlorotepalum	Isfahan, Khansar, Kouhe Sangandaz	N 33° 12.541' E 50° 15.340	2900	19455
A. egorovae	East Azarbayjan, Marand, Zonuz, Kuhkamar	N 38° 38.238' E 45° 54.820	2400	19456
A. graveolens	Markazi, Arak, Vismeh	N 34° 15.731' E 49° 45.525	1680	19457
A. hamedanense	Hamadan, Ecbatan dam	N 34° 45.562' E 48° 26.011	2450	19458
A. kurdistanicum	Kurdistan, Saghez	N 36° 03.290' E 45° 59.127	2251	19459
A. materculae	Marand, Payam	N 38° 19.864' E 45° 48.513	2000	19460
A. subakaka	Urumieh, Sulak	N 37° 30.169' E 44° 45.433	2050	19461

The bulbs were planted in ordinary pots. The chromosome slides were prepared from the root tips according to Agayev (1998) with minor modifications. Briefly, roots were pretreated in a- monobromo naphthalene for 6 hours at 4°C. Then they were fixed in Chromic Acid/Formaldehyde (1/1) for 36 hours at 4°C, washed under tap water for 3 hours, hydrolyzed in 1N NaOH at 60 °C for 10 min. Fixed roots were stained using aceto-iron-hematoxylin at 30°C for 24 hours, washed in distilled water for at least 30 minutes and incubated for 10-15 minutes in cellulase-pectinase enzyme solution at 37°C. The stained roots were squashed in 45% acetic acid under a stereo microscope and 5-7 of the best metaphase plates from different plants of each accession were photographed under an Olympus (BX40) microscope.

Karyotypes were prepared and chromosome pairs were classified according to Levan et al. (1964) and Stebbins (1971). The chromosomes were arranged according to their length. The long arm (LA), short arm (SA) and total chromosome length (CL) were measured. Idiograms were drawn for each species. The karyotype asymmetry parameters including total form percentage (TF%) (Huziwara 1962), percentage karyotype asymmetry index (As K%) (Arano 1963), index of chromosome size resemblance (Rec), index of karyotype symmetry (Syi) (Greilhuber & Speta 1976), karyotype dispersion index (DI) (Lavania & Srivastava 1992), degree of karyotype asymmetry (A) (Watanabe et al. 1999) and intrachromosomic asymmetry index (A_1), interchromosomic asymmetry index (A_2) (Romero Zarco 1986) were evaluated.

RESULTS

Representative somatic metaphase plates are shown in figs. 1-2 and some details related to the karyotype of the studied species are presented in table 2. Only one pair satellites was found on largest chromosome in all species. Satellites were spherical or oval and connected to the short chromosome arms. No B-chromosome was observed among the materials studied.

The mean length of chromosome long arm (LA) varied from 9.41 µm in *A. akaka* to 15.07 µm in *A. hamedanense*. Averages length of chromosome short arm (SA) ranged from 5.92 µm in *A. akaka* to 9.46 µm in *A. hamedanense*. Total haploid chromosome lengths (CL) varied from 122.64 µm in *A. akaka* to 196.32 µm in *A. hamedanense*, and the mean values of chromosome arm ratios (AR) ranged from 1.35 µm in *A. austroiranicum* to 1. 61 µm in *A. hamedanense*.

Asymmetry indices including A1, A2, Rec, Syi, CVCL, CVCI, DI and AI show the degree of asymmetry related to the variation in chromosome lengths, and TF%, As K% and the A index describe the variation in centromere position.

Table 2. Chromosome statistics for Allium species studied.

Species	CV_{CL}	CV_{CI}	AI
A. akaka	14.28	7.89	1.126
A. austroiranicum	17.38	2.38	0.41
A. breviscapum	14.13	7.5	1.05
A. chlorotepalum	13.72	9.75	1.337
A. egorovae	11.36	17.94	2.03
A. graveolens	16.35	9.52	1.55
A. hamedanense	10.14	15.78	1.6
A. kurdistanicum	13.52	7.5	1.01
A. materculae	10.02	7.5	0.75
A. subakaka	12.92	12.5	1.61

CVCL, coefficient of variation of chromosome length; CVCI, coefficient of variation of centromeric index; AI, karyotype asymmetry index.

According to our results, A_1 varied from 0.25 to 0.39 and A_2 ranged from 0.1 to 0.17. The A1 and A2 indices have been used to identify the more asymmetric karyotypes among species with similar Stebbins's classes of symmetry (Genç et al. 2013).

In class 1A *A. materculae* with the highest A_1 (0.39) and the lowest A_2 (0.1) values showed the most asymmetric karyotype. On the other hand, *A. graveolens* and *A. austroiranicum* have the lowest A_1 (0.25 and 0.26, respectively) and the highest A_2 (0.16 and 0.17, respectively) values and have the most symmetric karyotypes. Within class 2A, *A. hamedanense* and *A. akaka* have the highest A_1 (0.37) value and the most asymmetric karyotypes within this class. The lowest A_1 value (0.3) was observed in *A. subakaka*; hence it represents the most symmetric karyotype within class 2A. Based on a combination of the A_1, A_2 and %TF, *A. materculae* had the most asymmetric and *A. graveolens* and *A. austroiranicum* had the most symmetrical karyotypes. TF% has a perfect negative correlation with A, A1 and As K%, and a perfect positive correlation with the Syi index. The Syi index has shown a perfect negative correlation with As K% and A . Paszko (2006) showed that among all karyotype asymmetry indices, AI, CVCL and CVCI have more precision and sensitivity to assess karyotype asymmetry compared with the other indices. Based on AI index the most asymmetric karyotype was found in *A. egorovae*, while *A. austroiranicum* showed the most symmetrical one .

For each species a detailed discussion on karyotype features is given below.

A. akaka

Pedersen & Wendelbo (1966) reported this species as diploid with 2n=16. Gurushidze et al. (2012) reported diploid and tetraploid (2n=4x=32) cytotypes in this species. The examined populations in this study, collected from the northwest of Iran (table 1), were diploid. We also figured out that six chromosome pairs were metacentric and two pairs were submetacentric (figs. 1a, 2a).

A. austroiranicum

The results showed that this species is also diploid with chromosome number of 2n=16 and symmetric karyotype with metacentric chromosomes. *Allium austroiranicum* has the highest A_2 (0.17) and DI (7.31) and the lowest AR (1.35) and A (0.15) values (figs. 1b, 2b). The chromosome number and karyotype of this species are presented here for the first time.

A. breviscapum

Chromosome number in this species was 2n=16 that is concordant with Pogosian (1983). The karyotype was formed of one pair submetacentric and 7 pairs metacentric chromosomes (figs. 1c, 2c).

A. chlorotepalum

Karyological analysis of specimens collected near the type locality showed the chromosome number of 2n=16 with six metacentric and two submetacentric chromosomes (figs. 1d, 2d). This is the first report on chromosome number of this species.

A. egorovae

The studied specimens showed a diploid chromosome number of 2n=16. The chromosome number of this species is reported here for the first time. Five chromosome pairs were metacentric and three pairs were submetacentric (figs. 1e, 2e).

A. graveolens

Chromosome number is 2n = 16 with seven metacentric and one submetacentric chromosome pairs. The chromosome number of this species is presented here for the first time. TF% (43%) and Syi (0.74) values in this species were the highest among studied species and As K% (57%) and A_1 (0.25) values were the lowest among studied species (figs. 1f, 2f).

A. hamedanense

It has an asymmetric karyotype with chromosome number of 2n=16. *Allium hamedanense* has the highest As K% (0.62), AR (1.61) and A (0.28) and the lowest S% (48%), Rec (0.81), A_2 (0.1) and DI (3.4) values among species studied (figs. 1g, 2g).

A. kurdistanicum

The diploid chromosome number of 2n=16 was counted in this species. Six chromosome pairs are metacentric and two are submetacentric (figs. 1h, 2h). This is the first report on karyology in this species.

A. materculae

All specimens studied here were collected from NW Iran (table 1). The diploid chromosome number of *A. materculae* was 2n= 1 6 that was in accordance with Pogosian (1983). Six chromosome pairs were metacentric and 2 pairs were submetacentric. TF% (36%), Syi (0.61) and A_2 (0.1) values in this species were the lowest and A_1 (0.39) and A (0.28) values were the highest among the studied species (figs. 1i, 2i).

A. subakaka

The examined specimens were collected from the type locality in Northwest Iran (table 1). Chromosome number is 2n=16 with 6 metacentric and 2 submetacentric chromosome pairs (figs. 1j, 2j). Here we recorded for the first time the karyological features of this recently described species.

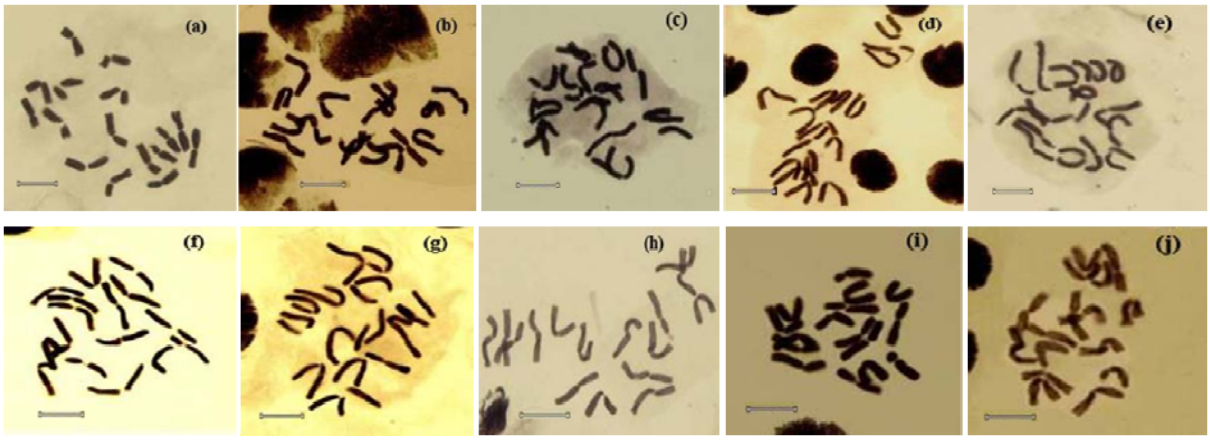

Fig. 1. Somatic chromosomes in *Allium*. a, *A. akaka*; b, *A. austroiranicum*; c, *A. breviscapum*; d, *A. chlorotepallum*; e, *A. egorovae*; f, *A. graveolens*; g, *A. hamedanense*; h, *A. kurdistanicum*; I, *A. materculae*; j, *A. subakaka*. Scale bars: 20 μm.

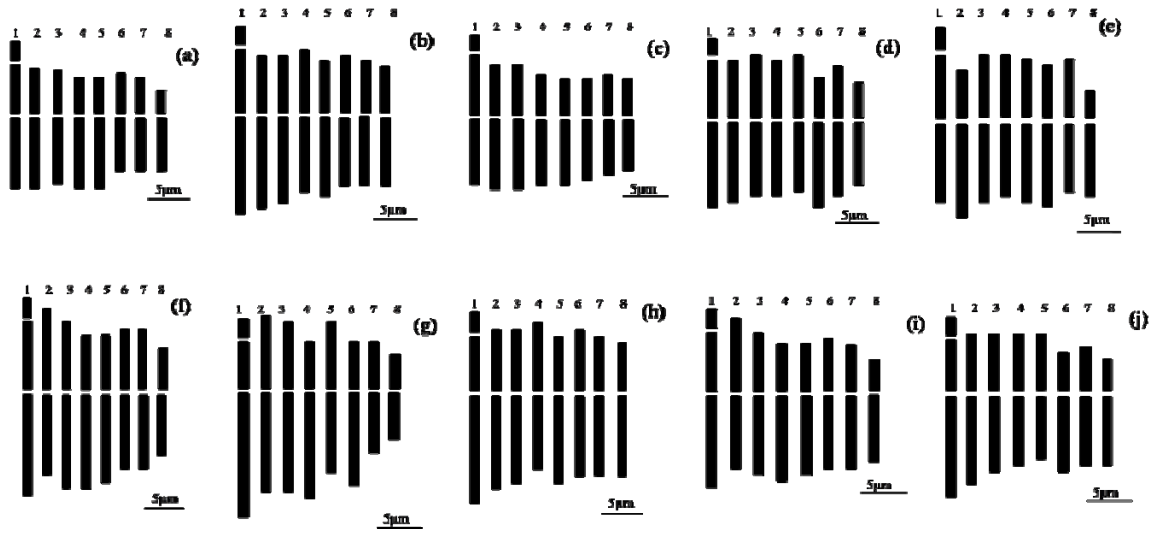

Fig. 2. Idiograms representing the mean karyotypes of the investigated species. A, *A. akaka*; b, *A. austroiranicum*; c, *A. breviscapum*; d, *A. chlorotepalum*; e, *A. egorovae*; f, *A. graveolens*; g, *A. hamedanense*; h, *A. kurdistanicum*; I, *A. materculae*; j, *A. subakaka*.

DISCUSSION

The karyotype features for 10 species of *Allium* section *Acanthoprason* as well as chromosome counts for seven species of this section are provided here for the first time. Most of the data obtained confirm the close relationship among species. The results of this study and previously published data (Genç et al. 2013; Fritsch & Astanova 1998) indicate the symmetric karyotype comprising of 5-8 metacentric and 0-3 submetacentric chromosomes as a common karyological feature of *A.* subgen. *Melanocrommyum*.

Chromosome numbers of *A. akaka*, *A. breviscapum* and *A. materculae* were already reported by Gurushidze et al. (2012) and our findings were congruent with their reports. Analyses of somatic metaphase spreads in 10 studied species showed that all of them are diploid with 2n=2x=16 and basic chromosome number of x=8 which is similar to most other species of *A.* subgen. *Melanocrommyum* (Fritsch & Astanova 1998).

In general, *Allium* sect. *Acanthoprason* represents a strongly supported monophyletic group in *A.* subgen. *Melanocromyum* (Friesen et al. 2006). Members of the section are also well characterized morphologically. Short peduncles and spine-like tepals at maturation are some important morphological synapomorphies of this section. Karyotype parameters indicate that all studied species have 5-7 metacentric and 1-3 submetacentric chromosomes except for *A. austroiranicum* with 8 uniform metacentric chromosome pairs. Considering the theory that more symmetric karyotypes are more primitive (Sharma 1990), *A. austroiranicum* might represent the most primitive karyotype among specie studied. Morphologically, *A. hamedanense* is different from other investigated species that is in accordance with karyological analysis in this study. The variations observed in karyology of the studied species were mostly in TF%, As K%, A1, A2, but these differences were not significant enough to provide further characters useful in species discrimination. The karyotype homogeneity and similar chromosome numbers are in contrary with Choi and Oh (2011) that indicated chromosome number as valuable characters in distinguishing some closely related taxa in the genus *Allium.* The results corroborate the results of Fritsch & Astanova (1998), which established no species-specific karyotype in *A.* subgen. *Melanocrommyum.* Because no species from other sections were included in this study, we were unable to have a judgment about sectional specificity of the karyotype. Our data do not automatically mean that further karyological investigations in *A.* subgen. *Melanocrommyum* could not result in taxonomically relevant data because karyotype data for many species of this section are still missing. Also our data does not reflect the possible karyological diversity within species. Based on the results of this study and published data (Fritsch & Astanova 1998) the ancestral species in this section probably have symmetric karyotype, meta- to submetacentric chromosomes and basic chromosome number of x=8.

Regarding the high morphological and karylogical similarities between species, it can be concluded that this section is diversified recently and speciation is not accompanied with notable changes in chromosome number and structure. The speciation was perhaps connected with changes at DNA level that are not reflected in chromosome structures. Analysis of DNA based molecular markers can provide useful information to resolve taxonomic and phylogenetic problems within the group.

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