

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

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Received 2015. 08.19; accepted for publication 2015. 10. 19

Akhavan, A., Saeidi, H., Zarre, Sh. & Rahiminejad, M. R. 2015. 12. 31: Chromosome numbers and karyotype features of selected species of *Allium* L. (Amaryllidaceae) sect. *Acanthoprason* in Iran. - *Iran. J. Bot.* 21 (2): 158-164. Tehran.

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  $\pm$ -symmetrical composing mainly of metacentric and submetacentric chromosomes.

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**Key words:** *Allium* subgenus *Melanocrommyum*; karyology; cytotaxonomy; phylogeny; Flora of Iran

اعداد کروموزومی و ویژگی‌های کاریوتیپی گونه‌های انتخابی از سرده پیاز (تیره نرگسیان) بخشه والک‌ها از ایران آزاده اخوان، فارغ‌التحصیل دکتری گروه زیست‌شناسی، دانشکده علوم، دانشگاه اصفهان، اصفهان، 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  $A=X$  مشخص می‌شود. کاریوتایپ‌ها متقارن بوده و اغلب شامل کروموزوم‌های متاساتریک و ساب‌متاساتریک 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)<sub>n</sub>, 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
<i>A. akaka</i>	Ardabil, Khalkhal, Cheshmeh Aznav	N 37° 34.945' E 48° 34.363	1900	19449
<i>A. austroiranicum</i>	Isfahan, Fereydun Shahr, Vahdatatabad	N 32° 53.486' E 50° 09.560	2750	19451
<i>A. austroiranicum</i>	Isfahan, Khansar, Kouhe Sangandaz	N 33° 11.414' E 50° 17.142	3000	19452
<i>A. austroiranicum</i>	Yazd, Barf khane, Deh Bala	N 31° 34.977' E 54° 05.414	2800	19453
<i>A. breviscapum</i>	Hamadan, Ganjnameh	N 34° 45.562' E 48° 26.011	2450	19454
<i>A. chlorotepalum</i>	Isfahan, Khansar, Kouhe Sangandaz	N 33° 12.541' E 50° 15.340	2900	19455
<i>A. egorovae</i>	East Azarbayjan, Marand, Zonuz, Kuhkamar	N 38° 38.238' E 45° 54.820	2400	19456
<i>A. graveolens</i>	Markazi, Arak, Vismeh	N 34° 15.731' E 49° 45.525	1680	19457
<i>A. hamedanense</i>	Hamadan, Ecbatan dam	N 34° 45.562' E 48° 26.011	2450	19458
<i>A. kurdistanicum</i>	Kurdistan, Saghez	N 36° 03.290' E 45° 59.127	2251	19459
<i>A. materculae</i>	Marand, Payam	N 38° 19.864' E 45° 48.513	2000	19460
<i>A. subakaka</i>	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  $\alpha$ -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) (Lavana & Srivastava 1992), degree of karyotype asymmetry (A) (Watanabe et al. 1999) and intrachromosomal asymmetry index ( $A_1$ ), interchromosomal 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  $\mu\text{m}$  in *A. akaka* to 15.07  $\mu\text{m}$  in *A. hamedanense*. Averages length of chromosome short arm (SA) ranged from 5.92  $\mu\text{m}$  in *A. akaka* to 9.46  $\mu\text{m}$  in *A. hamedanense*. Total haploid chromosome lengths (CL) varied from 122.64  $\mu\text{m}$  in *A. akaka* to 196.32  $\mu\text{m}$  in *A. hamedanense*, and the mean values of chromosome arm ratios (AR) ranged from 1.35  $\mu\text{m}$  in *A. austroiranicum* to 1.61  $\mu\text{m}$  in *A. hamedanense*.

Asymmetry indices including  $A_1$ ,  $A_2$ , 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 <sub>CL</sub>	CV <sub>CI</sub>	AI
<i>A. akaka</i>	14.28	7.89	1.126
<i>A. austroiranicum</i>	17.38	2.38	0.41
<i>A. breviscapum</i>	14.13	7.5	1.05
<i>A. chlorotepalum</i>	13.72	9.75	1.337
<i>A. egorovae</i>	11.36	17.94	2.03
<i>A. graveolens</i>	16.35	9.52	1.55
<i>A. hamedanense</i>	10.14	15.78	1.6
<i>A. kurdistanicum</i>	13.52	7.5	1.01
<i>A. materculae</i>	10.02	7.5	0.75
<i>A. subakaka</i>	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  $A_1$  and  $A_2$  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  $A$ ,  $A_1$  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  $2n=16$  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.

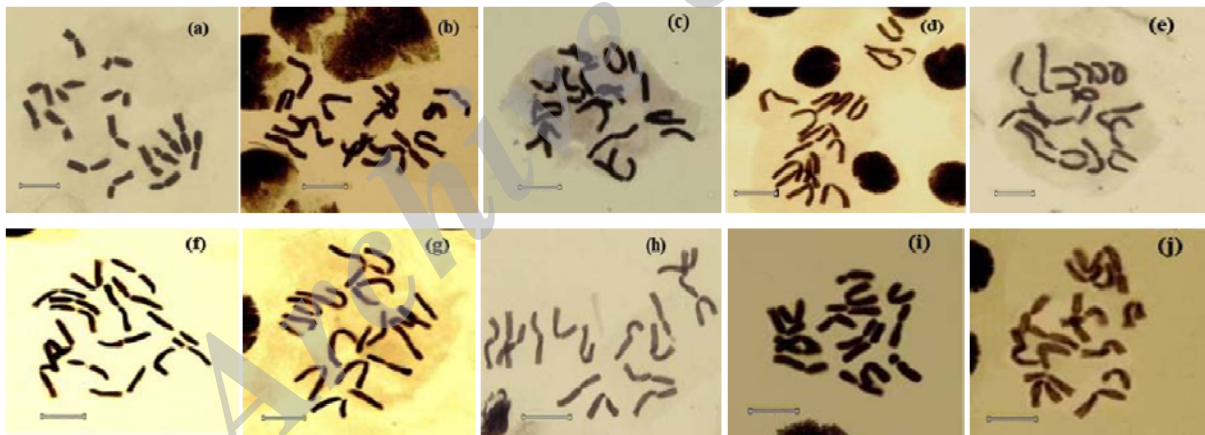


Fig. 1. Somatic chromosomes in *Allium*. a, *A. akaka*; b, *A. austroiranicum*; c, *A. breviscapum*; d, *A. chlorotepallum*; e, *A. egorovae*; f, *A. graveolens*; g, *A. hamedanense*; h, *A. kurdistanicum*; i, *A. materculae*; j, *A. subakaka*. Scale bars: 20  $\mu$ m.

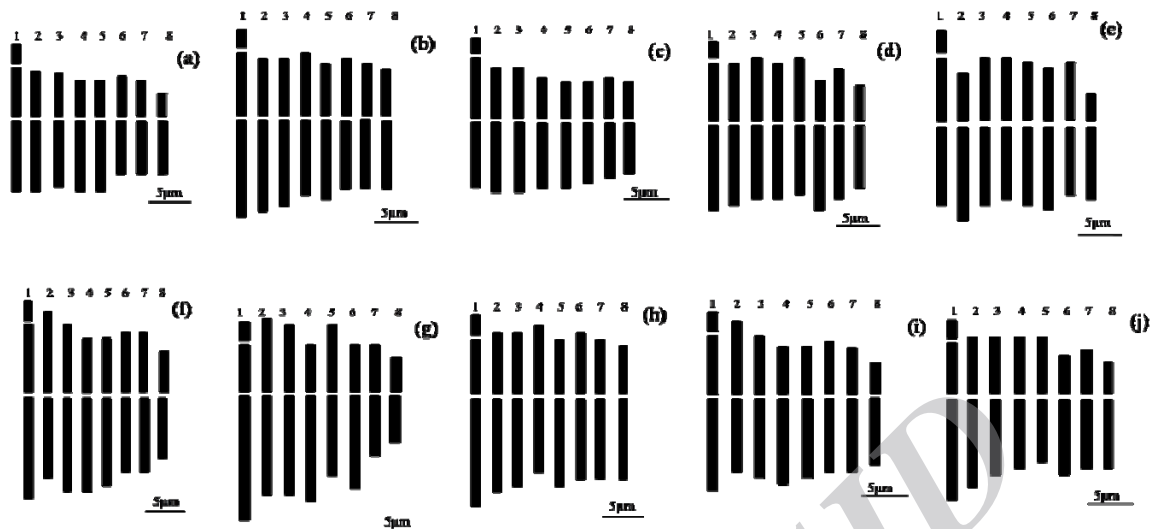


Fig. 2. Idiograms representing the mean karyotypes of the investigated species. A, *A. akaka*; b, *A. austroiranicum*; c, *A. breviscapum*; d, *A. chlorotepalum*; e, *A. egorovae*; f, *A. graveolens*; g, *A. hamedanense*; h, *A. kurdistanicum*; i, *A. materculae*; j, *A. subakaka*.

## 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. *Melanocrommyum* (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 species 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 karyological 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.

### ACKNOWLEDGEMENTS

The authors are grateful to the University of Isfahan for supporting this research.

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