BIODIVERSITAS
Volume 19, Number 2, March 2018
Pages: 720-725

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d190246

Karyomorphological variations in some populations of *Allium* subgenus *Melanocrommyum* section *Acanthoprason* in Iran

HASSAN MASTALI, MAHBOOBEH ZARE-MEHRJERDI♥, MARYAM NOROUZI, JALAL REZAEI
Department of Horticulture, College of Aburaihan, University of Tehran. Tehran, Iran. Tel./Fax. +98-21-36041089, ♥email: mzarem@ut.ac.ir

Manuscript received: 15 January 2018. Revision accepted: 28 March 2018.

Abstract. *Mastali H, Zare-Mehrjerdi M, Norouzi M, Rezaei J. 2018. Karyomorphological variations in some populations of* Allium *subgenus* Melanocrommyum *section* Acanthoprason *in Iran. Biodiversitas 19: 720-725. Allium* is the largest genus of Amaryllidaceae comprising more than 900 species belonging to 15 subgenera. Iran is reported to be a center of diversity for subgenus *Melanocrommyum*. *Acanthoprason* is a section in this subgenus. In this paper, karyomorphological variations of nine populations of four species including *Allium derderianum*, *A. kurdistanicum*, *A. minutiflorum* and *A. subakaka* of the section *Acanthoprason* growing in Iran were undertaken using squash technique and 2% (w/v) aceto-orcein stain. All of the populations have the same chromosome numbers 2n = 2x = 16 with the exception in Kochka population of *A. derderianum* 2n = 18. In the present study, the chromosome number of *A. minutiflorum* and new chromosome number of *A. derderianum* were revealed for the first time. Chromosomal characteristics were determined using photographs complemented by cluster analysis. According to dendrogram generated by the unweighted pair-group method with arithmetic average (UPGMA) cluster analysis, nine populations studied were separated into five groups at a cut off value of 5. Karyotype analysis indicated that *Allium* species studied here generally have metacentric and submetacentric chromosomes and symmetric karyotypes. Results of the present study revealed the natural variation in nine populations of four species of *Acantoprason* section which can further serve conservation and breeding planning.

Keywords: *Acanthoprason*, chromosome number, karyotypes, new count, population

INTRODUCTION

Allium is the largest genus of Amaryllidaceae comprising more than 900 species belonging to 15 subgenera. There is a main center of diversity in the eastern Mediterranean area, Southwest and Central Asia (Fritsch and Abbasi 2013). The chromosome numbers of more than 600 *Allium* species are known according to the Index to Plant Chromosome Numbers (http://www.tropicos.org/Project/IPCN). The most common basic chromosome number in *Allium* is x = 8, but other numbers (x = 7, 9, 10, 11) and various ploidy levels from diploid to hexaploid (2n = 14-68) have also been reported (Li et al. 2010).

Melanocrommyum is the second largest subgenus, comprising about 169 accepted species and subspecies grouped into 20 sections and 22 subsections (Fritsch et al. 2010; Fritsch 2012). Species of subgenus *Melanocrommyum* are naturally found in Middle East, especially in Iran and Turkey (Behcet and Rustemoglu 2012; Fritsch and Abbasi 2013; Ozhatay and Genc 2013). There are 10 sections and 82 species belonging to the subgenus *Melanocrommyum* in Iran. The sections of the subgenus in Iran are as follows: sect. *Melanocrommyum*, sect. *Acanthoprason*, sect. *Asteroprason*, sect. *Compactoprason*, sect. *Decipientia*, sect. *Kaloprason*, sect. *Megaloprason*, sect. *Procerallium*, sect. *Pseudoprason* and sect. *Regeloprason* (Fritsch and Abbasi 2013).

In this research, the ploidy levels, morphology of chromosomes, karyotype formula and symmetry indexes of nine populations related to four species including *A. derderianum*, *A. kurdistanicum*, *A. minutiflorum* and *A. subakaka* of the section *Acanthoprason* have been studied.

MATERIALS AND METHODS

The examined plant materials are presented in Table 1. Plant materials were collected from different regions of Iran (Figure 1). Ten randomly selected individuals per population were used for karyological studies.

Karyological observation was made on mitotic metaphase cells of root tips obtained from planted bulbs which were collected in natural habitats from Iran according to Ao (2008) with some modifications. Briefly, root tips were pretreated in 8-hydroxyquinoline 0.002 M for 3-5 hours in room temperature, washed with distilled water and fixed in Carnoy's solution (3:1, absolute ethanol: glacial acetic acid) for 24 hours and finally kept in 70% ethanol at 4°C. The root tips were rinsed with distilled water for 15 min before being hydrolyzed for 6 minutes in 1 N HCl at 60°C and stained with acetic-orcein 2% and squashed. A minimum of 10 slides possessing well-spread chromosomes was used for each population. The observations of the best metaphase plates were made using an Olympus BX-40 (light microscope Olympus, Tokyo, Japan) equipped with a 100×/1.3 NA oil objective and amounted DP12 digital camera (Olympus). Chromosome characteristics were measured from 10 enlarged well-spread metaphase cells of each population, using Ideokar ver.1.2 software.

Table 1. Geographical information of studied *Allium* species

Pop. no.	Species	Location (Province)	Latitude (N)	Longitude (E)	Altitude (m asl.)
1	*A. derderianum* Regel	Dareh Oson (Tehran)	35º 51’ 248’’	51º 25’ 786’’	2645
2	*A. derderianum* Regel	Kochka (Mazandaran)	36º 18’ 232’’	51 º 04’ 53’’	2248
3	*A. derderianum* Regel	Vali Abad (Mazandaran)	36 º 18’ 856’’	51º 11’ 1’’	2421
4	*A. derderianum* Regel	Vandarin (Mazandaran)	36º 22’ 55’’	51 º 1’ 41’’	2926
5	*A. kurdistanicum* Maroofi & R.M. Fritsch	Taze Abad Oryeh (Kurdistan)	35º 7’ 42’’	47º 40’ 309’’	2332
6	*A. minutiflorum* Regel	Dehdasht (Kohgiluyeh and Boyer-Ahmad)	30º 50’ 315’’	50º 33’ 067’’	1920
7	*A. subakaka* Razyfard & Zarre	Ghalelan (Kurdistan)	35º 4’ 965’’	47º 39’ 245’’	2618
8	*A. subakaka* Razyfard & Zarre	Jame Shoran (Kurdistan)	35º 5’ 733’’	47º 39’ 175’’	2318
9	*A. subakaka* Razyfard & Zarre	Pir Baba Ali (Kurdistan)	35º 6’ 17’’	47º 39’ 26’’	2351

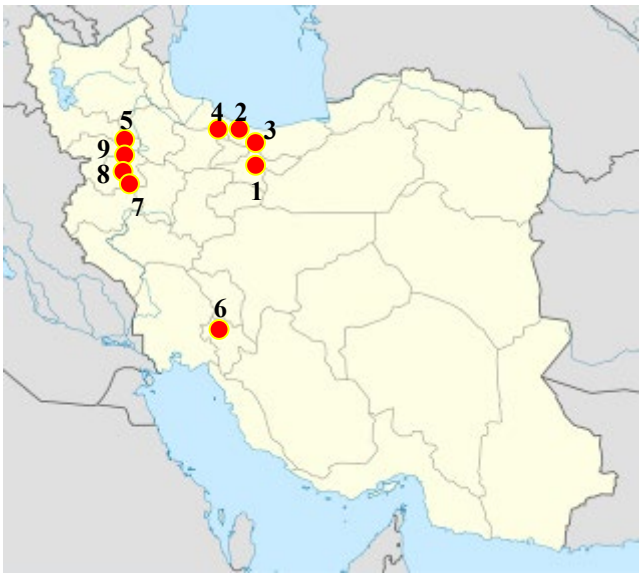

Figure 1. The nine collection sites of the genus *Allium* subgenus *Melanocrommyum* section *Acanthoprason* in Iran (the numbers indicate name of the species as represented in Table 1).

The measured variables were chromosome number, long arm length (L), short arm length (S), chromosome length (CL), arm ratio (AR), r-value, relative length of chromosome (RL%), form percentage of chromosome (F%), centromeric index (CI), total chromosome length of the haploid complement (HCL), total form percentage (TF%), Arano index of karyotype asymmetry (AsK%), symmetry index (S%), mean centromeric index (X_{CI}), degree of karyotype asymmetry (A), mean centromeric asymmetry (X_{CA}), coefficient of variation of chromosome length (CV_{CL}), coefficient of variation of centromeric Index (CV_{CI}), asymmetry index (AI), intrachromosomal asymmetry index (A_1) and interchromosomal asymmetry index (A_2) according to Mirzaghaderi and Marzangi (2015). The designation of the centromeric position as median (m) or submedian (sm) followed Levan et al. (1964). The karyotype asymmetry was classified according to Stebbins (1971). For grouping, the populations, unweighted pair-group method with arithmetic averaging (UPGMA) based cluster analysis was performed using SPSS (version 16; SPSS Inc Chicago, IL, USA) software.

RESULTS AND DISCUSSION

The chromosome numbers of nine populations belonging to four species of *Allium* subg. *Melanocrommyum* sec. *Acanthoprason* were determined in this study. All the populations collected from different locations of Iran were diploid and possessed the same somatic chromosome number (2n = 16) with the exception in Kochka population of *A. derderianum* (2n=18) (Figure 2 and 3). In the present study, the chromosome number of *A. minutiflorum* and new chromosome number of *A. derderianum* were revealed for the first time. The chromosome numbers of *A. kurdistanicum*, *A. subakaka* and three populations of *A. derderianum* were in agreement with the previous study (Pedersen and Wendelbo 1966; Akhavan et al. 2015). The intraspecies variation in chromosome number which was seen in *A. derderianum* populations was reported in other *Alliums* such as *A. sativum* (Sharma and Bal 1959; Yaghoobi and Malekzadeh Shafaroudi 2013).

The karyomorphological parameters and karyotype characteristics of each investigated population are shown in Table S1 and Table 2, respectively. The average length of chromosomes varied from 23.26 µm in *A. kurdistanicum* (Taze Abad Oryeh population) to 11.36 µm in one population of *A. derderianum* (Vandarin). In *A. derderianum* populations, the longest length of chromosomes belonged to Vali Abad (17.44 µm). The greatest and the shortest average length of chromosomes in *A. subakaka* populations were related to Ghalelan (16 µm) and Jame Shoran (12.14 µm), respectively. *A. kurdistanicum* and Vandarin population of *A. derderianum* had the longest and smallest average of long and short arms, respectively. In *A. derderianum* populations, the highest long and short arms were 9.87 µm (in Kochka population) and 7.58 µm (in Vali Abad population). Ghalelan and Jame Shoran populations of *A. subakak* had the greatest and the shortest long and short arms, respectively. Inter and intra specific variation in chromosome length may be because of developmental process which affected the chromosomes size.

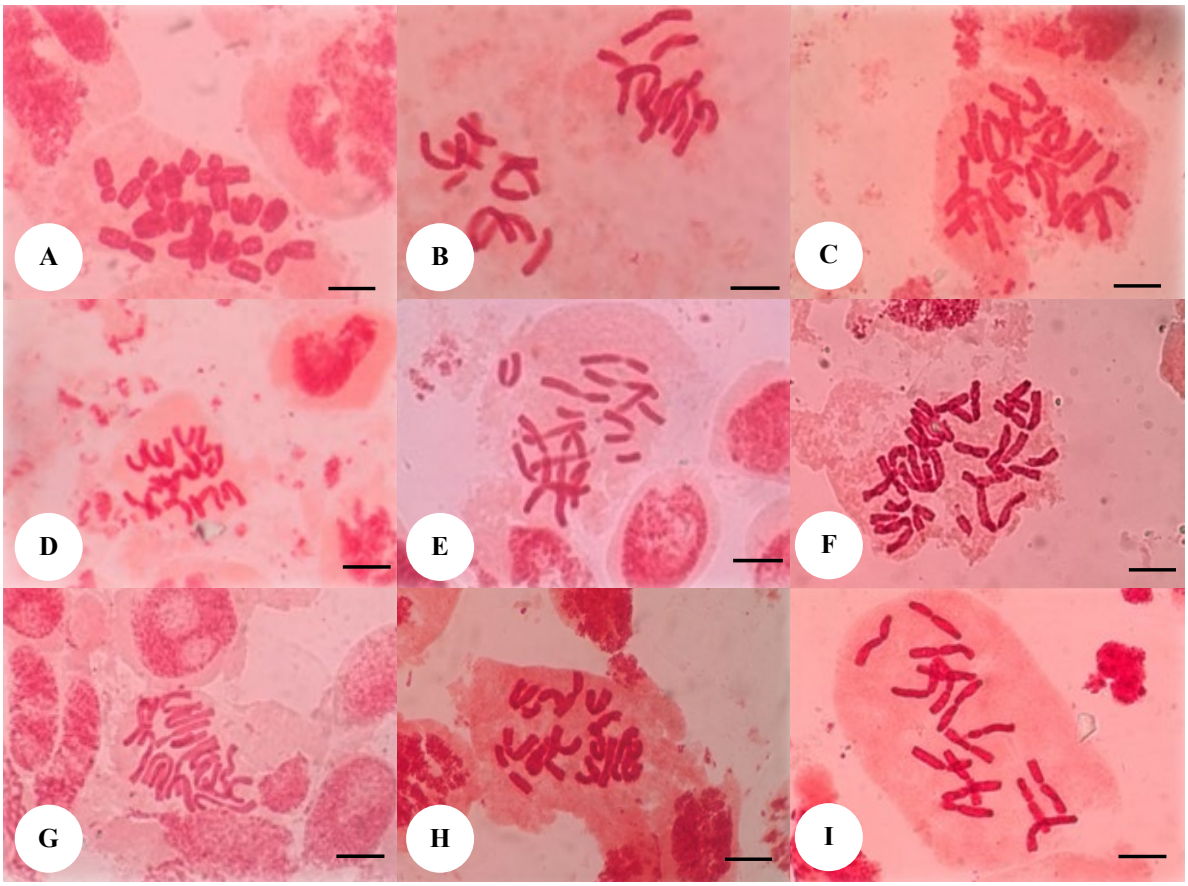

Figure 2. Somatic chromosomes of four populations of *A. derderianum* (A. DarehOson; B. Kochka; C. Vali Abad; D. Vandarin), one population of *A. kurdistanicum* (E. Taze Abad Oryeh), one population of *A. minutiflorum* (F. Dehdasht) and three populations of *A. subakaka* (G. Ghalelan; H. Jame Shoran; I. Pir Baba Ali). Scale bar = 10º µm

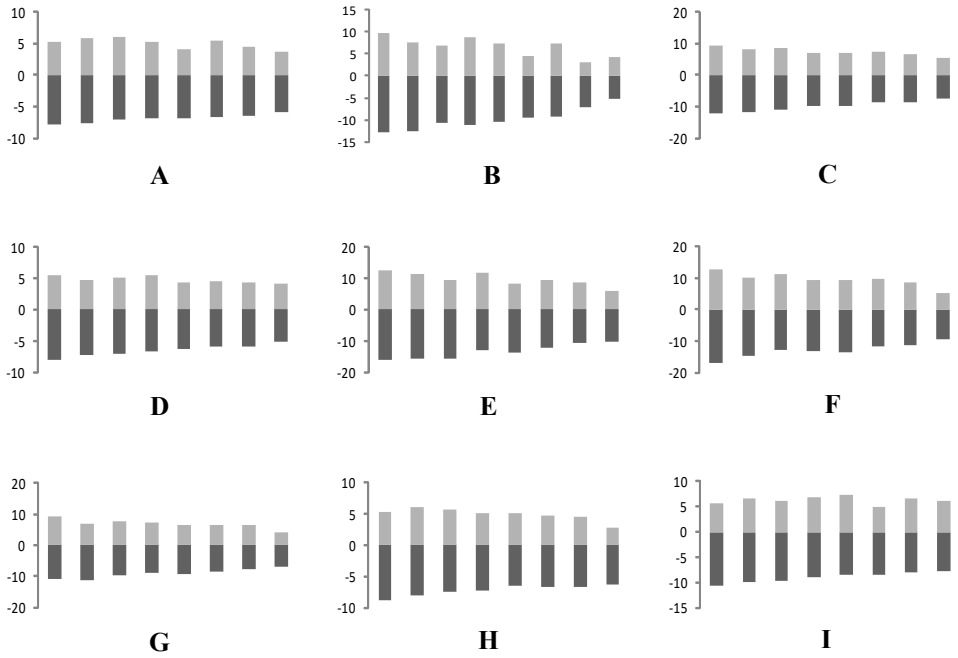

Figure 3. Ideograms of four populations of *A. derderianum* (A. DarehOson; B. Kochka; C. Vali Abad; D. Vandarin), one population of *A. kurdistanicum* (E. Taze Abad Oryeh), one population of *A. minutiflorum* (F. Dehdasht) and three populations of *A. subakaka* (G. Ghalelan; H. Jame Shoran; I. Pir Baba Ali)

Table 2. Karyotype characteristics of four populations of *A. derderianum* (1. DarehOson; 2. Kochka; 3. Vali Abad; 4. Vandarin), one population of *A. kurdistanicum* (5. Taze Abad Oryeh), one population of *A. minutiflorum* (6. Dehdasht) and three populations of *A. subakaka* (7. Ghalelan; 8. Jame Shoran; 9. Pir Baba Ali)

Pop. no.	L (µm)	S (µm)	CL (µm)	AR	r-value	CI	Type
1	6.94±0.66	5.06±0.86	12.00±1.40	1.40±0.18	0.73±0.09	0.42±0.03	8m
2	9.87±2.44	6.66±2.16	16.53±4.39	1.56±0.38	0.67±0.14	0.38±0.06	7m + 2sm
3	9.86±1.66	7.58±1.20	17.44±2.79	1.30±0.09	0.77±0.06	0.43±0.02	8m
4	6.59±0.90	4.77±0.50	11.36±1.32	1.38±0.12	0.73±0.06	0.42±0.02	8m
5	13.48±2.33	9.78±2.13	23.26±4.16	1.41±0.22	0.73±0.11	0.42±0.04	8m
6	12.95±2.31	9.65±2.17	22.61±4.31	1.37±0.19	0.74±0.10	0.43±0.03	7m + 1sm
7	9.19±1.51	6.82±1.43	16.00±2.78	1.37±0.20	0.74±0.10	0.42±0.03	7m + 1sm
8	7.20±0.84	4.95±1.01	12.14±1.67	1.50±0.33	0.69±0.11	0.41±0.05	7m + 1sm
9	8.99±1.00	6.29±0.73	15.28±1.17	1.45±0.25	0.71±0.12	0.41±0.04	6m + 2sm

Note: L= Long arm length, S= Short arm length, CL= Chromosome length, AR= Arm ratio, CI= Centromeric index

Table 3. Karyotypic parameters and asymmetry indices of four populations of *A. derderianum* (1. DarehOson; 2. Kochka; 3. Vali Abad; 4. Vandarin), one population of *A. kurdistanicum* (5. Taze Abad Oryeh), one population of *A. minutiflorum* (6. Dehdasht) and three populations of *A. subakaka* (7. Ghalelan; 8. Jame Shoran; 9. Pir Baba Ali)

Pop. no.	HCL	TF	AsK%	S%	X_{CI}	A	X_{CA}	CV_{CL}	CV_{CI}	AI	Stebbins	A_1	A_2
1	95.97	42.18	57.82	69.56	0.42	0.16	16.05	11.67	7.28	160.23	1A	0.27	0.12
2	148.74	40.26	59.74	42.67	0.39	0.20	20.32	26.60	16.46	161.61	2B	0.33	0.27
3	139.53	43.45	56.55	60.19	0.43	0.13	13.01	15.98	4.68	341.60	1A	0.23	0.16
4	90.86	41.97	58.03	70.18	0.42	0.16	15.88	11.66	5.33	218.86	1A	0.27	0.12
5	186.08	42.06	57.94	56.53	0.42	0.16	16.23	17.89	9.52	187.89	1A	0.27	0.18
6	180.85	42.70	57.30	49.47	0.43	0.15	15.14	19.08	7.34	259.93	1B	0.26	0.19
7	128.02	42.60	57.40	54.84	0.42	0.15	15.21	17.36	7.65	226.88	1A	0.26	0.17
8	97.16	40.76	59.24	64.41	0.41	0.19	19.12	13.77	11.00	125.17	2A	0.31	0.14
9	122.23	41.15	58.85	81.10	0.41	0.18	17.63	7.64	10.10	75.66	1A	0.29	0.08

Note: HCL= Total chromosome length of the haploid complement, TF= Total form percentage, AsK= Arano index of karyotype asymmetry, S= Symmetry index, X_{CI}= Mean centromeric index, A= Degree of karyotype asymmetry, X_{CA}= Mean centromeric asymmetry, CV_{CL}= Coefficient of variation of chromosome length, CV_{CI}= Coefficient of variation of centromeric Index, AI= Asymmetry index, A_1= Intrachromosomal asymmetry index, A_2= Interchromosomal asymmetry index

The highest values of r-value and CI averages and the lowest of AR among all populations belonged to Vali Abad population (*A. derderianum*) and Kochka was in the opposite. In *A. subakak*, Ghalelan had the highest values of r-value and CI averages and the lowest of AR and Jame Shoran was in contrast. All chromosomes of three populations of *A. derderianum* and *A. kurdistanicum* were metacentric (2n = 2x = 8m). Kochka population of *A. derderianum* was 2n = 2x = 7m + 2sm (chromosome pairs 6 and 8 had centromeres in the submedian position). In first report of *A. kurdistanicum* chromosome number for a population of Saghez (Kurdistan Province of Iran), six metacentric and two submetacentric chromosome pairs was observed and the largest chromosome of karyotype had one pair satellites (Akhavan et al. 2015).

The karyotypic formula of *A. minutiflorum* and two populations of *A. subakaka* were 2n = 2x = 7m + 1sm (chromosome pair 8 was submetacentric). Pir Baba Ali, another population of *A. subakaka* had 2n = 2x = 6m + 2sm (chromosome pairs 1 and 6 had centromeres in the sub median position). In Akhavan et al. (2015) work, where recorded the *A. subakaka* karyological features for the first time, a population from Sulak (Urumieh) had 6 metacentric and 2 submetacentric (chromosome pairs 6 and 8) chromosome pairs with one pair satellites on the largest chromosome. In all of studied populations in this work, no satellite was observed.

Total chromosome length of the haploid complement varied from 90.86 to 148.47 µm in *A. derderianum* populations and 97.16 to 122.23 µm in *A. subakaka* (Table 3). One studied population of *A. kurdistanicum* and *A. minutiflorum* has total chromosome length of the haploid complement with 186.08 and 180.85 µm, respectively. Asymmetry indices of populations were shown in Table 3. In *A. derderianum* populations, the highest values of AsK%, A, X_{CA}, CV_{CL}, CV_{CI}, A_1 and A_2 and the lowest of TF, S%, and X_{CI} were found in Kochka population and the smallest values of AI and A_2 were found in DarehOson. Vali Abad population has the greatest TF, X_{CI}, and AI and lowest AsK%, A, X_{CA}, CV_{CI} and A_1 and Vandarin was highest in S% and smallest in CV_{CL} and A_2 among *A. derderianum* populations. In *A. subakaka* populations, the greatest values of TF, X_{CI}, CV_{CL}, AI and A_2 and the lowest of AsK%, S%, A, X_{CA}, and A_1 belonged to Ghalelan population and Jame Shoran has the highest AsK%, A, X_{CA}, CV_{CI} and A_1 and smallest TF and X_{CI} values. The greatest value of S% and lowest of X_{CI}, CV_{CL}, AI and A_2 among *A. subakaka* populations were found in Pir Baba Ali population. Karyotypic asymmetry of three populations of *A. derderianum* (DarehOson, Vali Abad, and Vandarin) and only one population of *A. kurdistanicum* (Taze Abad Oryeh) and two populations of *A. subakaka* (Ghalelan and Pir Baba Ali) were 1A while Jame Shoran population of *A. subakaka* was 2A. Dehdasht population of *A. minutiflorum*

and one population of *A. derderianum* (Kochka) were 1B and 2B, respectively. Symmetric karyotype is relevant in *Allium* genus as reported by previous studied (Genc et al. 2013; Akhavan et al. 2015). The most asymmetric karyotype belonged to Kochka population of *A. derderianum*, which had the highest basic chromosome numbers (x = 9). Inter population variation in species level can be attributed to the chromosome evolution, possibly as a prelude to speciation. It was suggested that most speciation events appear to involve dysploidy (De Storme and Mason 2014).

UPGMA cluster analysis based on cytogenetic data classified nine studied populations into five groups at a cut off value of 5 (Figure 4). Cluster I included two populations of *A. derderianum* and one population of *A. subakaka*. Other populations of *A. subakaka* were located in Cluster II. Cluster III was composed of *A. kurdistanicum* and *A. minutiflorom* populations. Vali Abad and Vandarin populations of *A. derderianum* formed the cluster IV and V, respectively. Vandarin, the only population with the basic chromosome numbers of x = 9, had the highest distance with others. Grouping of the populations was not related to species and geographical distances. □

Cluster analysis separated populations based on their difference in cytogenetic characters. Population 1 and 4 of *A. derderianum* possessed the highest similarity, while population 2 of the same species had the highest distance from other populations. This may be due to different evolutionary history of cytological and morphological characters in this species. *A. kurdistanicum* and *A. minutiflorom* were placed in the same group, indicating that they are more closely related species.

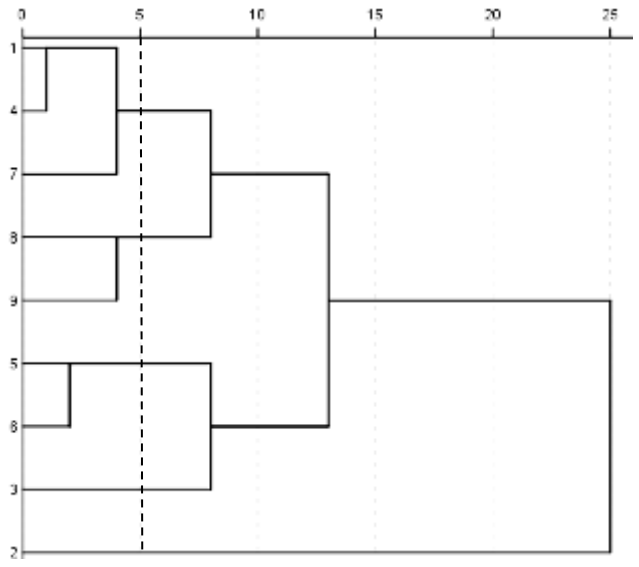

Figure 4. Dendrogram of the cluster analysis of the genus *Allium* of four populations of *A. derderianum* (1. DarehOson; 2. Kochka; 3. Vali Abad; 4. Vandarin), one population of *A. kurdistanicum* (5. Taze Abad Oryeh), one population of *A. minutiflorum* (6. Dehdasht) and three populations of *A. subakaka* (7. Ghalelan; 8. Jame Shoran; 9. Pir Baba Ali)

In conclusions, results of the present study revealed the natural variation in nine populations of four species of *Acantoprason* section from *Melanocrommyum* which can further serve conservation and breeding planning. All of the populations have the same chromosome numbers with the exception in Kochka population of *A. derderianum*. The chromosome number of *A. minutiflorum* and new chromosome number of *A. derderianum* were revealed for the first time. Karyotype analysis indicated that *Allium* species studied here generally have metacentric and submetacentric chromosomes and symmetric karyotypes. It seems that accurate taxonomic relationships and speciation in this section can be addressed by using molecular markers that can be the issue for the future studies.

ACKNOWLEDGEMENTS

This work was funded by the Iran National Science Foundation (INSF) and the author would like to thank the INSF for this support.

REFERENCES

Akhavan A, Saeidi H, Rahiminejad MR, Zare S, Blattner FR. 2015. Interspecific relationships in *Allium* subgenus *Melanocrommyum* sections *Acanthoprason* and *Asteroprason* (Amaryllidaceae) revealed using ISSR markers. Syst Bot 40: 706-715.

Ao C. 2008. Chromosome numbers and karyotypes of *Allium przewalskianum* opulations.Acta Biol Cracov Bot 50 (1): 43-49.

Behcet L, Rustemoglu M. 2012. *Allium shirnakiense*, sect. *Melanocrommyum* (Liliaceae), a new species from South-eastern Turkey. Turk J Bot 36: 450-454.

De Storme N, Mason A. 2014. Plant speciation through chromosome instability and ploidy change: cellular mechanisms, molecular factors and evolutionary relevance. Curr Plant Biol 1: 10–33.

Fritsch RM, Abbasi M. 2013. A taxonomic review of *Allium* subg. *Melanocrommyum* in Iran, Leibniz-InstitutfürPflanzengenetik und Kulturpflanzenforschung, Gatersleben (IPK). Fritsch RM, Blattner FR, Gurushidze M. 2010. New classification of *Allium* L. subg. *Melanocrommyum* (Webb &Berthel.) Rouy (Alliaceae) based on molecular and morphological characters. Phyton 49 (2): 145-220.

Fritsch RM. 2012. Illustrated key to the sections and subsections and brief general circumscription of *Allium* subg. *Melanocrommyum*. Phyton 52 (1): 1-37.

Genc I, Ozhatay N, Cevri M. 2013. A karyomorphological study of the genus *Allium* (sect.*Melanocrommyum*) from Turkey. Caryologia 66 (1): 31-40.

Levan A, Fredga K, Sandberg AA. 1964. Nomenclature for centromeric position on chromosomes. Hereditas 52 (2): 201-220.

Li QQ, Zhou SD, He XJ, Yu Y, Zhang YC, Wei XQ. 2010. Phylogeny and biogeography of *Allium* (Amaryllidaceae: Allieae) based on nuclear ribosomal internal transcribed spacer and chloroplast rps16 sequences, focusing on the inclusion of species endemic to China. Ann Bot 106 (5): 709-733.

Mirzaghaderi G, Marzangi K. 2015. Ideokar: an ideogram constructing and karyotype analyzing software. Caryologia 68 (1): 31-35.

Ozhatay FN, Genc I. 2013. *Allium cyrilli* complex (sect. *Melanocrommyum*) in Turkey. Turk J Bot 37: 39-45.

Pedersen K, Wendelbo P. 1966. Chromosome numbers of some SW Asian *Allium* species. Blyttia 24: 307-313.

Sharma AK, Bal AK. 1959. A study of spontaneous fragmentation in two varieties of *Allium sativum* and interpretation of their karyotypes. 46th session of Indian Science Congress, Dehli, India, 24 January 1959.

Stebbins GL. 1971. Chromosomal evolution in higher plants. Edward Arnold, London.

Yaghoobi E, Malekzadeh Shafaroudi S. 2013. Genetic variation of Iranian ecotypes of Garlic (*Allium* sp.) using karyotype analysis. Modern Genetics 4: 411-422. [Persian]

Table S1. Karyomorphological parameters of four populations of *A. derderianum* (1. DarehOson; 2. Kochka; 3. Vali Abad; 4. Vandarin), one population of *A. kurdistanicum* (5. Taze Abad Oryeh), one population of *A. minutiflorum* (6. Dehdasht) and three populations of *A. subakaka* (7. Ghalelan; 8. Jame Shoran; 9. Pir Baba Ali)

Pop. no.	Pair no.	L (µm)	S (µm)	CL (µm)	AR	r-value	RL%	F%	CI	Type
1	1	7.94 ± 0.27	5.27 ± 0.20	13.20 ± 0.14	1.51 ± 0.11	0.66 ± 0.05	13.76 ± 0.15	5.49 ± 0.21	0.40 ± 0.02	m
	2	7.68 ± 0.20	5.98 ± 0.29	13.66 ± 0.45	1.28 ± 0.05	0.78 ± 0.03	14.23 ± 0.46	6.23 ± 0.30	0.44 ± 0.01	m
	3	7.13 ± 0.13	6.09 ± 0.44	13.22 ± 0.55	1.17 ± 0.07	0.85 ± 0.05	13.77 ± 0.58	6.35 ± 0.46	0.46 ± 0.01	m
	4	6.85 ± 0.19	5.28 ± 0.16	12.13 ± 0.28	1.30 ± 0.05	0.77 ± 0.03	12.64 ± 0.29	5.50 ± 0.17	0.44 ± 0.01	m
	5	6.91 ± 0.29	4.16 ± 0.20	11.08 ± 0.24	1.66 ± 0.12	0.60 ± 0.05	11.54 ± 0.25	4.34 ± 0.21	0.38 ± 0.02	m
	6	6.68 ± 0.19	5.46 ± 0.08	12.14 ± 0.19	1.22 ± 0.04	0.82 ± 0.03	12.65 ± 0.19	5.69 ± 0.08	0.45 ± 0.01	m
	7	6.46 ± 0.13	4.59 ± 0.42	11.05 ± 0.52	1.41 ± 0.15	0.71 ± 0.06	11.51 ± 0.54	4.78 ± 0.44	0.41 ± 0.02	m
	8	5.85 ± 0.14	3.65 ± 0.19	9.50 ± 0.30	1.61 ± 0.06	0.62 ± 0.02	9.90 ± 0.32	3.80 ± 0.20	0.38 ± 0.01	m
2	1	12.81 ± 1.41	9.75 ± 0.99	22.56 ± 2.25	1.31 ± 0.09	0.76 ± 0.05	15.17 ± 1.51	6.55 ± 0.66	0.43 ± 0.02	m
	2	12.62 ± 1.34	7.57 ± 1.57	20.19 ± 2.87	1.66 ± 0.25	0.60 ± 0.06	13.57 ± 1.93	5.09 ± 1.05	0.35 ± 0.03	m
	3	10.69 ± 1.38	6.91 ± 1.12	17.60 ± 2.29	1.55 ± 0.48	0.65 ± 0.09	11.83 ± 1.54	4.64 ± 0.75	0.38 ± 0.04	m
	4	11.13 ± 1.31	8.79 ± 1.07	19.92 ± 2.33	1.27 ± 0.06	0.79 ± 0.04	13.39 ± 1.57	5.91 ± 0.72	0.44 ± 0.01	m
	5	10.38 ± 1.25	7.40 ± 0.89	17.78 ± 2.06	1.41 ± 0.10	0.71 ± 0.05	11.96 ± 1.38	4.97 ± 0.60	0.42 ± 0.02	m
	6	9.54 ± 1.11	4.60 ± 1.58	14.14 ± 2.64	2.08 ± 0.44	0.48 ± 0.09	9.51 ± 1.78	3.09 ± 1.06	0.27 ± 0.04	sm
	7	9.18 ± 0.86	7.32 ± 1.17	16.50 ± 2.02	1.25 ± 0.24	0.80 ± 0.08	11.09 ± 1.35	4.92 ± 0.79	0.42 ± 0.03	m
	8	7.23 ± 1.03	3.19 ± 0.49	10.43 ± 1.49	2.26 ± 0.15	0.44 ± 0.03	7.01 ± 1.00	2.15 ± 0.33	0.30 ± 0.01	sm
	9	5.26 ± 0.50	4.37 ± 0.44	9.63 ± 0.90	1.21 ± 0.07	0.83 ± 0.05	6.47 ± 0.60	2.94 ± 0.30	0.45 ± 0.01	m
3	1	12.10 ± 0.25	9.53 ± 0.86	21.64 ± 1.07	1.27 ± 0.11	0.79 ± 0.06	15.51 ± 0.77	6.83 ± 0.62	0.43 ± 0.02	m
	2	11.63 ± 0.35	8.10 ± 0.55	19.73 ± 0.74	1.44 ± 0.10	0.70 ± 0.05	14.14 ± 0.53	5.80 ± 0.39	0.41 ± 0.02	m
	3	11.05 ± 0.44	8.68 ± 0.26	19.73 ± 0.62	1.28 ± 0.05	0.78 ± 0.03	14.14 ± 0.44	6.22 ± 0.18	0.44 ± 0.01	m
	4	9.92 ± 0.16	7.18 ± 0.82	17.09 ± 0.85	1.38 ± 0.21	0.72 ± 0.08	12.25 ± 0.61	5.14 ± 0.58	0.41 ± 0.03	m
	5	9.76 ± 0.29	7.15 ± 0.34	16.91 ± 0.59	1.37 ± 0.04	0.73 ± 0.02	12.12 ± 0.43	5.12 ± 0.24	0.42 ± 0.01	m
	6	8.58 ± 0.26	7.53 ± 0.31	16.11 ± 0.45	1.14 ± 0.06	0.88 ± 0.04	11.54 ± 0.32	5.39 ± 0.22	0.47 ± 0.01	m
	7	8.46 ± 0.41	6.85 ± 0.31	15.31 ± 0.63	1.23 ± 0.06	0.81 ± 0.04	10.97 ± 0.45	4.91 ± 0.23	0.45 ± 0.01	m
	8	7.40 ± 0.36	5.62 ± 0.33	13.02 ± 0.65	1.31 ± 0.08	0.76 ± 0.03	9.33 ± 0.47	4.03 ± 0.24	0.43 ± 0.01	m
4	1	8.05 ± 0.31	5.44 ± 0.51	13.48 ± 0.69	1.48 ± 0.16	0.67 ± 0.06	14.84 ± 0.76	5.98 ± 0.56	0.40 ± 0.02	m
	2	7.33 ± 0.23	4.74 ± 0.14	12.07 ± 0.29	1.54 ± 0.06	0.65 ± 0.03	13.28 ± 0.32	5.21 ± 0.16	0.39 ± 0.01	m
	3	7.06 ± 0.19	5.05 ± 0.36	12.11 ± 0.52	1.40 ± 0.09	0.71 ± 0.04	13.33 ± 0.57	5.56 ± 0.40	0.41 ± 0.01	m
	4	6.79 ± 0.27	5.46 ± 0.26	12.25 ± 0.50	1.25 ± 0.04	0.80 ± 0.02	13.48 ± 0.55	6.01 ± 0.29	0.44 ± 0.01	m
	5	6.30 ± 0.15	4.28 ± 0.22	10.58 ± 0.34	1.47 ± 0.07	0.68 ± 0.03	11.64 ± 0.37	4.71 ± 0.24	0.40 ± 0.01	m
	6	6.01 ± 0.29	4.62 ± 0.29	10.63 ± 0.36	1.30 ± 0.16	0.77 ± 0.07	11.70 ± 0.40	5.09 ± 0.32	0.43 ± 0.02	m
	7	5.99 ± 0.27	4.29 ± 0.17	10.28 ± 0.27	1.40 ± 0.11	0.72 ± 0.05	11.31 ± 0.30	4.73 ± 0.18	0.42 ± 0.02	m
	8	5.21 ± 0.41	4.26 ± 0.30	9.46 ± 0.68	1.22 ± 0.06	0.82 ± 0.04	10.42 ± 0.75	4.69 ± 0.33	0.45 ± 0.01	m
5	1	16.29 ± 0.98	12.71 ± 0.27	29.00 ± 1.07	1.28 ± 0.08	0.78 ± 0.04	15.59 ± 0.57	6.83 ± 0.15	0.44 ± 0.01	m
	2	15.88 ± 0.37	11.51 ± 0.37	27.39 ± 0.52	1.38 ± 0.06	0.73 ± 0.03	14.72 ± 0.28	6.19 ± 0.20	0.42 ± 0.01	m
	3	15.59 ± 0.52	9.48 ± 0.41	25.07 ± 0.60	1.65 ± 0.10	0.61 ± 0.04	13.47 ± 0.32	5.09 ± 0.22	0.38 ± 0.01	m
	4	13.05 ± 0.41	11.71 ± 0.22	24.76 ± 0.55	1.11 ± 0.03	0.90 ± 0.02	13.31 ± 0.30	6.29 ± 0.12	0.47 ± 0.01	m
	5	13.82 ± 0.28	8.44 ± 0.41	22.26 ± 0.50	1.64 ± 0.09	0.61 ± 0.03	11.96 ± 0.27	4.54 ± 0.22	0.38 ± 0.01	m
	6	12.26 ± 0.73	9.66 ± 0.29	21.92 ± 0.93	1.27 ± 0.06	0.79 ± 0.04	11.78 ± 0.50	5.19 ± 0.15	0.44 ± 0.01	m
	7	10.66 ± 0.49	8.63 ± 0.90	19.29 ± 1.32	1.23 ± 0.13	0.81 ± 0.06	10.36 ± 0.71	4.64 ± 0.49	0.44 ± 0.02	m
	8	10.27 ± 0.50	6.13 ± 0.87	16.40 ± 1.30	1.68 ± 0.21	0.60 ± 0.06	8.81 ± 0.70	3.29 ± 0.47	0.36 ± 0.03	m
6	1	17.01 ± 1.84	12.83 ± 0.44	29.85 ± 2.18	1.33 ± 0.11	0.75 ± 0.05	16.50 ± 1.21	7.10 ± 0.24	0.44 ± 0.02	m
	2	14.80 ± 0.82	10.33 ± 0.20	25.13 ± 0.85	1.43 ± 0.09	0.70 ± 0.04	13.90 ± 0.47	5.71 ± 0.11	0.41 ± 0.01	m
	3	12.68 ± 0.49	11.34 ± 0.20	24.02 ± 0.65	1.12 ± 0.03	0.89 ± 0.03	13.28 ± 0.36	6.27 ± 0.11	0.47 ± 0.01	m
	4	13.23 ± 0.40	9.57 ± 0.51	22.80 ± 0.77	1.38 ± 0.06	0.72 ± 0.04	12.61 ± 0.43	5.29 ± 0.28	0.42 ± 0.01	m
	5	13.51 ± 0.80	9.26 ± 0.31	22.77 ± 0.87	1.46 ± 0.10	0.69 ± 0.04	12.59 ± 0.48	5.12 ± 0.17	0.41 ± 0.02	m
	6	11.61 ± 0.43	9.85 ± 0.33	21.46 ± 0.73	1.18 ± 0.02	0.85 ± 0.02	11.87 ± 0.40	5.45 ± 0.19	0.46 ± 0.01	m
	7	11.40 ± 0.37	8.65 ± 0.33	20.05 ± 0.60	1.32 ± 0.05	0.76 ± 0.03	11.09 ± 0.33	4.78 ± 0.18	0.43 ± 0.01	m
	8	9.39 ± 0.87	5.38 ± 0.17	14.77 ± 0.88	1.75 ± 0.17	0.57 ± 0.06	8.17 ± 0.49	2.97 ± 0.10	0.37 ± 0.02	sm
7	1	11.01 ± 0.80	9.14 ± 0.57	20.15 ± 1.31	1.20 ± 0.04	0.83 ± 0.03	15.74 ± 1.02	7.14 ± 0.44	0.45 ± 0.01	m
	2	11.36 ± 0.41	6.98 ± 0.29	18.34 ± 0.52	1.63 ± 0.09	0.61 ± 0.03	14.32 ± 0.41	5.45 ± 0.23	0.38 ± 0.01	m
	3	9.73 ± 0.59	7.63 ± 0.17	17.36 ± 0.60	1.27 ± 0.08	0.79 ± 0.04	13.56 ± 0.47	5.96 ± 0.13	0.44 ± 0.01	m
	4	9.05 ± 0.56	7.43 ± 0.29	16.47 ± 0.66	1.22 ± 0.09	0.82 ± 0.05	12.87 ± 0.52	5.80 ± 0.22	0.45 ± 0.02	m
	5	9.15 ± 0.42	6.34 ± 0.19	15.49 ± 0.52	1.44 ± 0.07	0.69 ± 0.04	12.10 ± 0.40	4.95 ± 0.15	0.41 ± 0.01	m
	6	8.54 ± 0.16	6.50 ± 0.56	15.04 ± 0.58	1.32 ± 0.15	0.76 ± 0.06	11.75 ± 0.45	5.07 ± 0.43	0.43 ± 0.02	m
	7	7.69 ± 0.37	6.42 ± 0.44	14.11 ± 0.78	1.19 ± 0.05	0.84 ± 0.03	11.02 ± 0.61	5.02 ± 0.35	0.45 ± 0.01	m
	8	6.96 ± 0.52	4.10 ± 0.22	11.05 ± 0.50	1.70 ± 0.17	0.59 ± 0.06	8.63 ± 0.39	3.20 ± 0.17	0.38 ± 0.02	sm
8	1	8.77 ± 0.92	5.41 ± 0.58	14.18 ± 1.27	1.62 ± 0.20	0.62 ± 0.08	14.60 ± 1.31	5.57 ± 0.60	0.38 ± 0.03	m
	2	7.97 ± 0.80	6.11 ± 0.56	14.08 ± 1.34	1.30 ± 0.05	0.77 ± 0.03	14.49 ± 1.38	6.29 ± 0.57	0.44 ± 0.01	m
	3	7.40 ± 0.51	5.64 ± 0.54	13.04 ± 1.01	1.31 ± 0.09	0.76 ± 0.05	13.42 ± 1.03	5.80 ± 0.56	0.43 ± 0.02	m
	4	7.29 ± 0.99	5.16 ± 0.21	12.45 ± 1.18	1.41 ± 0.13	0.71 ± 0.06	12.81 ± 1.21	5.31 ± 0.22	0.43 ± 0.02	m
	5	6.48 ± 0.54	5.20 ± 0.31	11.68 ± 0.82	1.25 ± 0.05	0.80 ± 0.03	12.02 ± 0.85	5.36 ± 0.32	0.45 ± 0.01	m
	6	6.68 ± 0.60	4.82 ± 0.49	11.50 ± 1.00	1.39 ± 0.11	0.72 ± 0.05	11.84 ± 1.03	4.97 ± 0.50	0.42 ± 0.02	m
	7	6.62 ± 0.66	4.47 ± 0.41	11.09 ± 1.04	1.47 ± 0.06	0.68 ± 0.03	11.41 ± 1.08	4.60 ± 0.42	0.40 ± 0.01	m
	8	6.35 ± 0.41	2.78 ± 0.10	9.13 ± 0.48	2.28 ± 0.11	0.44 ± 0.02	9.40 ± 0.49	2.86 ± 0.11	0.31 ± 0.01	sm
9	1	10.71 ± 0.45	5.70 ± 0.55	16.41 ± 0.71	1.88 ± 0.26	0.53 ± 0.06	13.42 ± 0.58	4.67 ± 0.45	0.34 ± 0.03	sm
	2	9.85 ± 0.35	6.59 ± 0.64	16.44 ± 0.73	1.50 ± 0.28	0.67 ± 0.06	13.45 ± 0.59	5.39 ± 0.52	0.39 ± 0.03	m
	3	9.64 ± 0.39	6.19 ± 0.16	15.83 ± 0.47	1.56 ± 0.06	0.64 ± 0.02	12.95 ± 0.39	5.06 ± 0.13	0.39 ± 0.01	m
	4	8.93 ± 0.25	6.76 ± 0.21	15.69 ± 0.35	1.32 ± 0.06	0.76 ± 0.03	12.83 ± 0.29	5.53 ± 0.17	0.43 ± 0.01	m
	5	8.62 ± 0.22	7.30 ± 0.51	15.92 ± 0.71	1.18 ± 0.06	0.85 ± 0.04	13.02 ± 0.58	5.97 ± 0.42	0.45 ± 0.01	m
	6	8.39 ± 0.26	4.94 ± 0.30	13.33 ± 0.31	1.70 ± 0.13	0.59 ± 0.05	10.91 ± 0.25	4.04 ± 0.24	0.37 ± 0.02	sm
	7	7.98 ± 0.21	6.74 ± 0.29	14.72 ± 0.47	1.18 ± 0.03	0.85 ± 0.02	12.04 ± 0.38	5.52 ± 0.23	0.46 ± 0.01	m
	8	7.82 ± 0.32	6.07 ± 0.27	13.89 ± 0.54	1.29 ± 0.05	0.78 ± 0.03	11.36 ± 0.44	4.97 ± 0.22	0.44 ± 0.01	m