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Chromosome counts and karyotype features of different ecotypes of *Allium* L. species (Amaryllidaceae – Subg. *Melanocrommyum*) in Iran

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Abstract. This study aimed to investigate the karyotypes of five *Allium* species, which belong to three sections of the subgenus *Melanocrommyum*. Bulbs from these species were collected from natural habitats in Iran, and their somatic chromosomes were analysed. The results revealed that all examined members of subg. *Melanocrommyum* had a basic chromosome number of $x=8$ and were diploid ($2n=2x=16$). Chromosomal data for *A. saralicum* and *A. shatakiense* are reported here for the first time. The karyotypes exhibited a variety of chromosome types and sizes, including variations observed among different accessions of the same species. In particular, *A. saralicum* showed satellite chromosomes ranging in size from 2.2 to 3.71 μm , located on the short arm. Seven accessions of *A. saralicum*, *A. stipitatum*, and *A. haemanthoides* demonstrated the presence of 1-3 B chromosomes with centromeres located in the median or sub-terminal position. Notably, the number of B chromosomes varied even among different accessions of the same species. Based on various indices, the karyotypes of the species were classified into symmetric and asymmetric groups. All karyotype asymmetry methods consistently identified *A. stipitatum* as the species with the most asymmetric chromosomes, while *A. ubipetrense* was recognized as the most symmetric species. This study contributes to the karyological knowledge of the genus *Allium* and provides valuable data for future taxonomic research. It emphasizes the significance of chromosomal characteristics in understanding plant evolution and species diversity within the *Allium* genus.

Keywords: *Allium*, B chromosome, ideogram, karyology, *Melanocrommyum*.

INTRODUCTION

Allium is a notable monocot genus comprising over 900 species, primarily concentrated in the eastern Mediterranean region, Southwest, and Central Asia. Within different plant communities, *Allium* species play a significant role (Fritsch and Abbasi, 2013). Iran exhibits diverse geographic and climatic conditions, allowing *Allium* species to thrive in a range of habitats. These plants typically inhabit open, sunny, and relatively dry

sites, adapting to both arid and moderately humid climates (Fritsch and Friesen, 2002). Subgenus *Melanocrommyum* of *Allium* encompasses Iranian species that occupy various ecological habitats, spanning from lowlands to highlands. Although they can be found in dry steppes, semi-deserts, and arid mountains, the majority of these species thrive in such environments (Fritsch and Abbasi, 2013). Currently, Iran is home to 148 recognized *Allium* species and subspecies, representing eight subgenera and 32 sections (Fritsch and Amini Rad, 2013). Subgenus *Melanocrommyum* is the second-largest subgenus within *Allium*, encompassing approximately 170 species worldwide, classified into 20 sections (Fritsch, 2012).

Chromosomes serve as the carriers of genetic information, and alterations in their number and structure play a crucial role in plant evolution (Escudero et al., 2014). Chromosomal characteristics, including number, size, and shape (karyotype), serve as defining features for numerous plant taxa across different taxonomic levels (Baltisberger and Hörandl, 2016). In the genus *Allium*, a basic chromosome number of $x=8$ is predominant, although a few sections exhibit $x=7$, 9, and 11 (Friesen et al., 2006). Subgenus *Melanocrommyum* typically possesses a basic chromosome number of $x=8$. However, a small number of species, such as *A. karataviense* Regel and *A. rnonophyllum* Vved. have been identified with basic chromosome numbers of $x = 9$ and $x=10$, respectively (Fritsch and Astanova, 1998). Satellite chromosomes have proven to be a valuable cytological marker in *Allium*, and different types of satellites have been studied based on the centromere position and secondary constrictions on chromosome arms (Dolatyari et al., 2018).

Several karyological investigations have been conducted on Iranian *Allium* species, revealing symmetrical karyotypes composed of metacentric and submetacentric chromosomes in the *Melanocrommyum* subgenus (Pedersen and Wendelbo, 1966; Pogolian, 1983;

Fritsch and Astanova, 1998; Gurushidze et al., 2010, 2012; Hosseini and Go, 2010; Akhavan et al., 2015; Dolatyari et al., 2018; Hosseini, 2018). Approximately 55% of Iranian *Allium* species have been karyologically characterized, providing a valuable foundation for future taxonomic research (Dolatyari et al., 2018). Nonetheless, the karyological data for many species, including endemic ones in Iran, remains completely unknown. This study aims to contribute to the karyological investigation of selected Iranian *Allium* accessions, expanding our knowledge of the chromosomes within the genus *Allium*.

MATERIALS AND METHODS

In this study, bulbs of five species (seven ecotypes) were collected from their natural habitats in 2018 (Figure 1). The species include *A. ubipetrense* R.M. Fritsch, *A. haemanthoides* Boiss. & Reut. *A. stipitatum* Regel, *A. saralicum* R.M. Fritsch, and *A. shatakiense* Rech. f. Details about the collected materials can be found in Table 1. Among the species studied, *A. ubipetrense* was determined to be endemic to Iran, while the other species were indigenous and had distributions in various regions, including Iran, Iraq, Turkey, Tajikistan, Afghanistan, Kazakhstan, Kyrgyzstan, Pakistan, Turkmenistan, and Uzbekistan.

To break the dormancy of the bulbs, they were stored at 4 °C. Subsequently, the bulbs were rooted in wet, sterile cotton gauze in a refrigerator. For somatic chromosome analysis, fresh root tips measuring 1–1.2 cm were collected from the cultivated bulbs in the early morning. The roots underwent a pre-treatment with a-bromo naphthalene for 3 hours at 4 °C, followed by three washes with distilled water (each lasting 5 minutes) at room temperature. The roots were then fixed overnight at 4 °C in Carnoy's fixative, which consists of glacial acetic acid and ethanol in a 3:1 ratio. After thor-

Table 1. Characterization and sampling location of the studied taxa.

Section	Species	Species code	Locality	Voucher specimen	Origin
sect. <i>Acanthoprason</i>	<i>A. ubipetrense</i>	S1	Kurdistan, Marivan road, kalatarzan, jannat boo village	UOK-130	Native- endemic
	<i>A. haemanthoides</i>	S5	Kurdistan, Saral Area, Zardavan	UOK-131	Native-non endemic
sect. <i>Melanocrommyum</i>	<i>A. saralicum</i>	S2-E1	Kurdistan, Saral Area, hezarkanian Village	UOK-113	Native-non endemic
	<i>A. saralicum</i>	S2-E2	Kurdistan, Saral Area, Chatan Village	UOK-114	Native-non endemic
	<i>A. shatakiense</i>	S3	Kurdistan, Saral Area, zardavan	UOK-126	Native-non endemic
sect. <i>Procerallium</i>	<i>A. stipitatum</i>	S4-E1	Kurdistan, Saral Area, Kapak Village	UOK-101	Native-non endemic
	<i>A. stipitatum</i>	S4-E2	Kurdistan, Saral Area, zardavan	UOK- 102	Native-non endemic

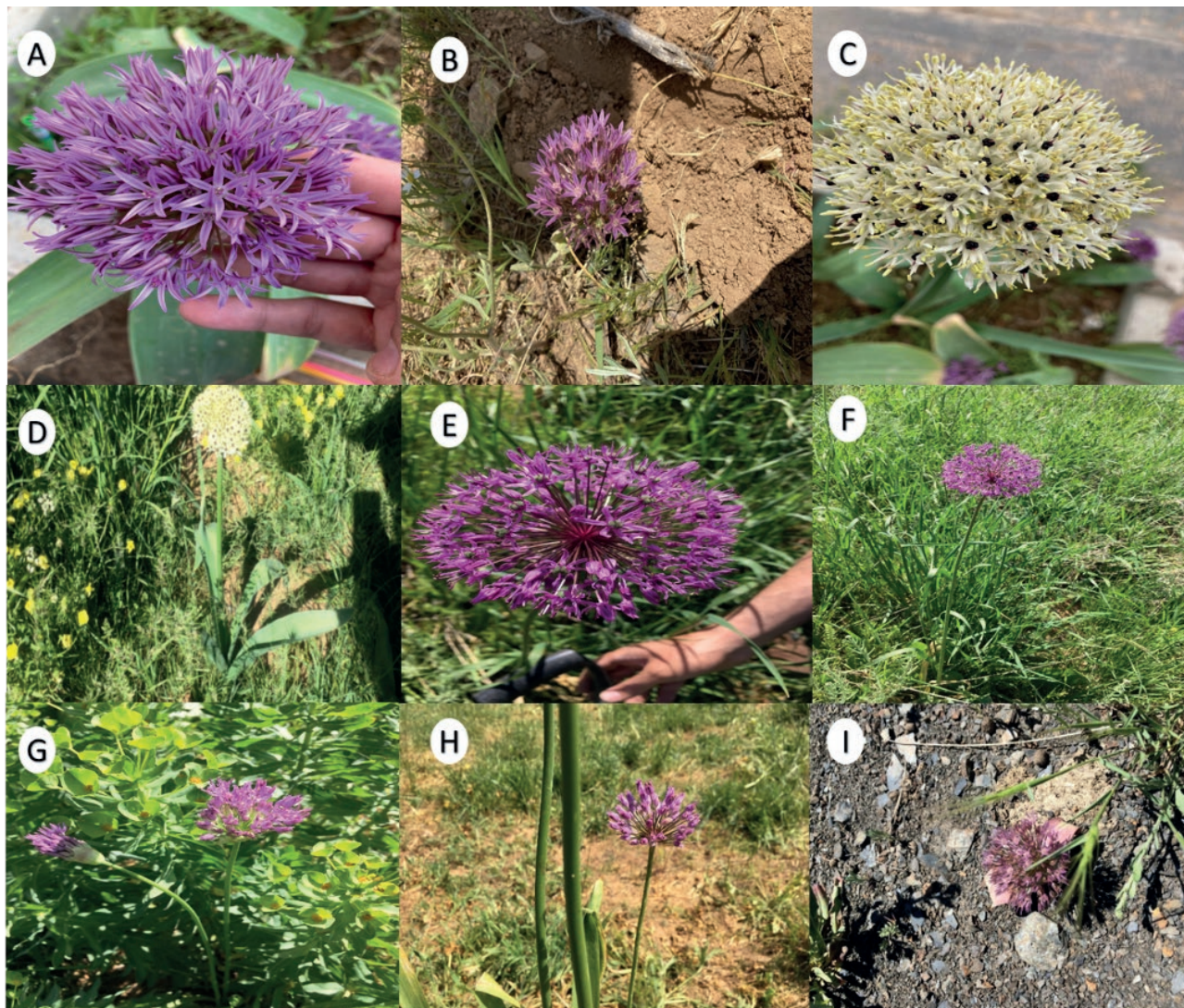


Figure 1. Studied species in their habitat in saral region in Kurdistan. A, B: *A. ubipetrense*; C, D: *A. saralicum*; E, F: *A. stipitatum*; G, H: *A. shatakiense*; I: *A. haemanthoides*.

ough washing with distilled water, the excised roots were transferred to 70% (v/v) aqueous ethanol and stored in a refrigerator until further use.

Hydrolysis was carried out by treating the root tips with 1 M HCl for 15 minutes at 60 °C. Subsequently, the root tips were stained with feulgen solution for 1 hour and then squashed in a drop of 45% (v/v) acetic acid. The best metaphase plates were photographed using a DP72 digital camera attached to the BX51 Olympus microscope. At least five metaphase plates were analyzed for each accession, and the short (S) and long arms (L) of the chromosomes were measured using IdeoKar software (<http://agri.uok.ac.ir/ideokar/index.html>) (Mirzaghaderi and Marzangi 2015). The morphology of the

chromosomes was determined based on the nomenclature proposed by Levan et al. (1964). Karyotype formulas were established using centromere indices (CI) and arm ratio (AR).

In addition to the basic karyological data such as $2n$ (chromosome number), x (basic chromosome number), and the total chromosome length of the haploid (HCL), several chromosomal parameters were analyzed. These parameters include the long arm (L), short arm (S), chromosome length (CL), arm ratio (AR), r value, relative length of chromosome (RL), chromosome form percentage (F %), and centromeric index (CI %). Furthermore, 12 karyotype parameters were calculated to quantify the asymmetry of the karyotypes. These parameters

include the mean centromeric asymmetry (MCA), coefficient of variation of chromosome length (CVCL), coefficient of variation of centromeric index (CVCI), total form percentage (TF %), mean centromeric index (XCI), asymmetry index (AI), degree of karyotype asymmetry (A), percentage of karyotype symmetry (S%), intra chromosomal asymmetry index (A1 and A2), and percentage karyotype asymmetry index (AsK %). It's worth noting that B chromosomes were not considered in the computation of these parameters due to their effects on calculating asymmetry factors.

RESULTS

The analysis focused on metaphase plates of five species belonging to the *Melanocrommyum* subgenus of the genus *Allium*. These species are diploid, and their basic chromosome number is $x=8$. The chromosomal data for *A. saralicum* and *A. shatakiense* are presented for the first time in this study. Among the three species analyzed (*A. saralicum*, *A. stipitatum*, *A. haemanthoides*), seven accessions showed the presence of 1–3 B chromosomes with centromeres located in the median or subterminal position (Figure 2). Notably, the number of B chromosomes varied even among different accessions of the same species. Karyotypes of somatic complement and the ideograms of the haploid complement of studied *Alliums* are demonstrated in Figures 3.

There were significant differences in size between the longest and shortest chromosomes in each complement. *Allium haemanthoides* had the longest chromosome (14.2 μm), while *A. stipitatum* had the shortest chromosome (5.42 μm). The mean total chromosome length (TL) ranged from 9.27 μm (*A. stipitatum*) to 11.37 μm (*A. haemanthoides*), with an overall mean value of 10.15 μm . The centromeric index (CI) of the complements varied from 37% (*A. stipitatum*) to 41% (*A. ubipetrense*). Based on the nomenclature proposed by Levan et al. (1964), two chromosome types, 'm' (centromere at the median region) and 'sm' (centromere at the submedian region), formed six different karyotype formulas (Table 2). Additionally, there were pairs of satellites with sizes ranging from 2.2 to 3.71 μm in *A. saralicum* (S2-E1 and S2-E2) located on the short arm.

The karyotypes of all five species were classified into the 1A, 1B, and 2B classes of Stebbins classification (Stebbins 1971). Various methods were used to assess karyotype asymmetry, and most methods identified different species as symmetric or asymmetric. For example, *A. ubipetrense* had the highest value of total form percentage (TF %) at 41.56 (indicating the most symmet-

ric species), while *A. stipitatum* had the lowest value at 37.52 (the most asymmetric species). The coefficient of variation (CV %) showed the highest value in *A. stipitatum* (26.88%, the most asymmetric) and the lowest value in *A. ubipetrense* (11.77%, the most symmetric). The highest XCA value (mean centromeric asymmetry) was observed in *A. stipitatum* (24.11%), while the lowest value was found in *A. shatakiense* (17.95%). The mean coefficient of variation of centromeric index (CVCI) was determined as 13.56 μm , ranging from 7.7 μm (*A. ubipetrense*) to 14.32 μm (*A. stipitatum*). *Allium stipitatum* had the highest value of asymmetry index (A) at 0.24, while *A. ubipetrense* and *A. shatakiense* showed the lowest value at 0.17. The highest and lowest values of percentage of karyotype symmetry (S %) were observed in *A. ubipetrense* (68.83) and *A. stipitatum* (43.20), respectively. *Allium stipitatum* had the highest value of percentage karyotype asymmetry index (AsK %) at 62.24 but *A. ubipetrense* showed the lowest value (54.90) (Table 2). According to the data presented in Table 2, all the karyotype asymmetry methods consistently identified *A. stipitatum* as the species with the most asymmetric chromosomes, while *A. ubipetrense* was recognized as the most symmetric species. These findings highlight the distinct karyotype characteristics and asymmetry levels among the studied *Allium* species.

DISCUSSION

The karyotypic characteristics of several *Allium* species, including *A. saralicum*, *A. shatakiense*, *A. stipitatum*, *A. ubipetrense* and *A. haemanthoides* are discussed in this study. All studied taxa were found to be diploid with a chromosome number of $2n=2x=16$. *Allium stipitatum*, on the other hand, exhibited the same diploid chromosome number of $2n=2x=16$, which is consistent with previous studies (Fritsch and Astanova, 1998; Ohri and Pistrick, 2001; Oroji Salmasi et al., 2019). However, reports also exist of *A. stipitatum* having a basic chromosome number of $2n=16$ and $2n=48$ (Pogosian 1983).

In *A. saralicum*, a pair of satellite chromosomes was observed, located on the short arm of the chromosomes. The present study identified two chromosome types, 'm', 'sm', and 'st', in five different *Allium* species. The karyotypic analysis, combined with previously published data on the *Melanocrommyum* subgenus, indicated a common symmetric karyotype with 2-8 metacentric and 0-4 submetacentric chromosomes, as well as 0-4 subtelocentric chromosome pairs.

Chromosomes in the *Acanthoprason* section (*A. ubipetrense* and *A. haemanthoides*) displayed a median or

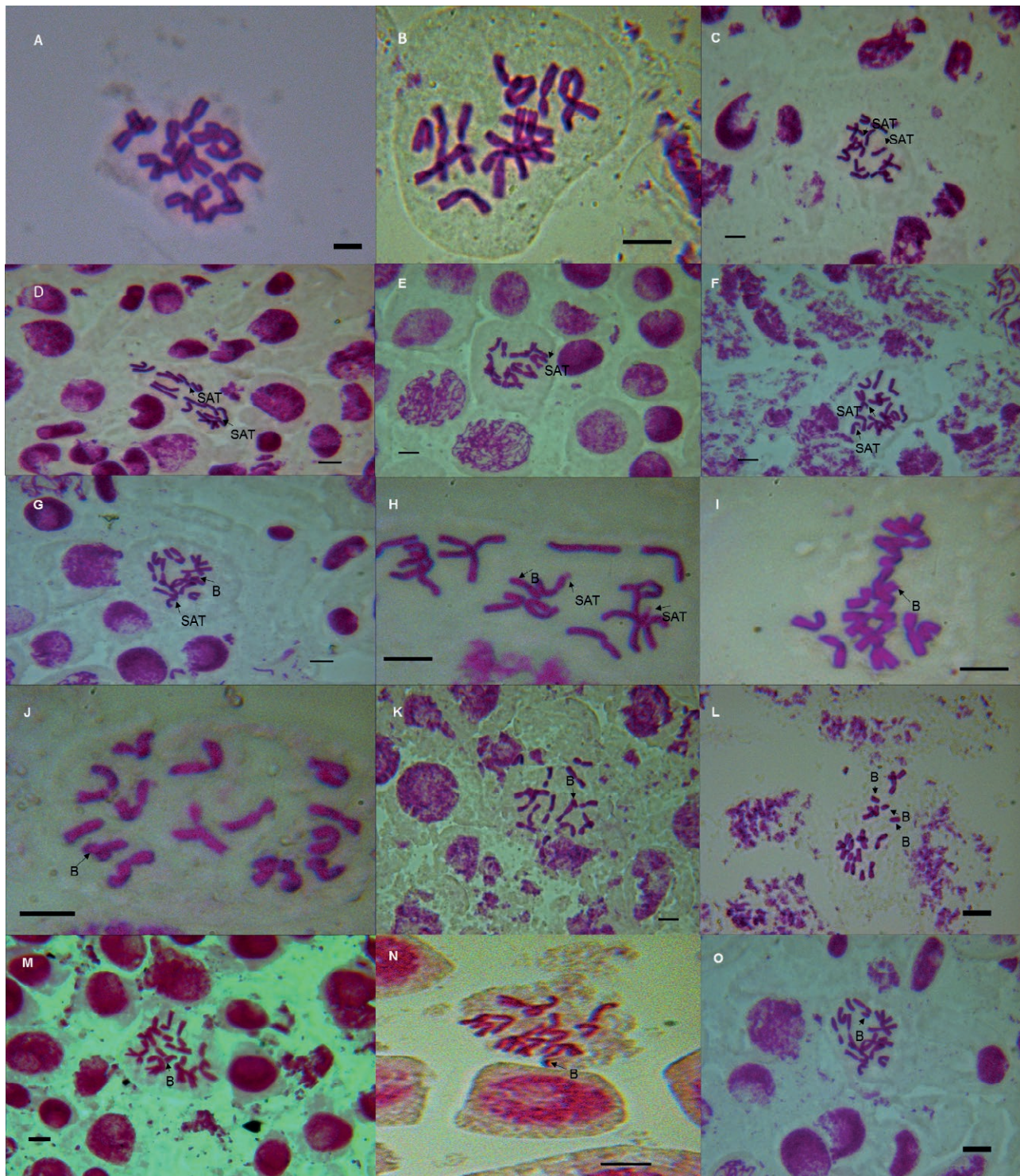


Figure 2. Mitotic metaphase plates of the investigated accessions of selected *Allium*, subg. *Melanocrommyum*: A, B: *A. ubipetrense*; C-F: *A. saralicum* (E1); G, H; *A. saralicum* (E2); I-K; *A. stipitatum* (E1); L; *A. stipitatum* (E2); M; *A. haemanthoides*; N,O; *A. shatakiense*. Scale bars = 10µm.

submedian centromere position and a gradual decline in arm lengths. *Allium ubipetrense* had chromosomes with

centromeres only in the median position, while *A. haemanthoides* exhibited submedian centromeres and one B



Figure 3. Idiograms and karyotypes of the investigated accessions of selected *Allium* subg. *Melanocrommyum*. A: *A. ubipetrense*; B: *A. saralicum*; C: *A. shatakiense*; D: *A. stipitatum*; E: *A. haemanthoides*. Scale bars = 10µm.

chromosome in addition to metacentric ones. The coefficient of variation for centromere index (CVCI) in *A. stipitatum* was more than twice (14.32) that of *A. ubipetrense* (6.35). The coefficient of variation for centromere length (CVCL) index confirmed *A. ubipetrense* as having the most symmetric chromosomes. A study by Dolatyari et al. (2018) on *A. haemanthoides* did not detect any B

chromosomes but identified two satellite (SAT) chromosomes with centromeres positioned between the median and sub median positions.

Allium ubipetrense is primarily found in the north-western parts of the Zagros mountain range in Iran. This species exhibits different morphotypes, which sometimes leads to misidentifications. *Allium haeman-*

Table 2. Mean chromosomal and karyotypic parameters of *Allium* spp. S1: *A. ubipetrense* S2: *A. saralicum*, S3: *A. shatakiense*, S4: *A. stipitatum*, S5: *A. haemanthoides*.

Parameters	Species					Mean	Range	
	S1	S2	S3	S4	S5		Min	Max
S (µm)	4.09	4.30	3.89	3.47	4.52	4.05	3.47-4.52	S4 S5
L (µm)	5.79	6.55	5.50	5.79	6.84	6.09	5.50-6.84	S3 S5
TL (µm)	9.89	10.86	9.40	9.27	11.37	10.15	9.27-11.37	S4 S5
AR	1.44	1.63	1.45	1.69	1.54	1.55	1.44-1.69	S1 S4
r value	0.69	0.64	0.70	0.60	0.66	0.65	0.60-0.70	S4 S3
HCL	79.21	89.03	75.27	75.74	93.61	82.57	75.27-93.61	S3 S5
RL%	12.49	12.24	12.49	12.25	12.14	12.32	12.14-12.49	S5 S1,S3
F %	5.15	4.86	5.13	4.60	4.83	4.91	4.60-5.15	S4 S1
CI	0.41	0.38	0.40	0.37	0.39	0.39	0.37-0.41	S4 S1
TF %	41.56	39.65	41.20	37.52	40.00	39.98	37.52-41.56	S4 S1
CVCL	11.77	19.89	20.72	26.88	18.90	19.63	11.77-26.88	S1 S4
A1	0.30	0.33	0.28	0.37	0.32	0.32	0.28-0.37	S3 S4
A2	0.11	0.19	0.20	0.26	0.18	0.18	0.11-0.26	S1 S4
AI	185.24	155.31	170.48	214.10	174.87	180	155.31-214.10	S2 S4
AsK %	54.90	60.34	58.43	62.24	58.79	58.94	54.90-62.24	S1 S4
S%	68.83	52.49	51.91	43.20	54.90	54.26	43.20-68.83	S4 S1
A	0.17	0.21	0.17	0.24	0.19	0.19	0.17-0.24	S1,S3 S4
XcA	17.95	21.72	17.53	24.11	19.74	20.21	17.53-24.11	S3 S4
XcI	0.17	0.39	0.41	0.37	0.40	0.34	0.17-0.41	S1 S3
CVCI	6.35	12.47	12.09	14.32	11.45	13.56	6.35-14.32	S1 S4

	Species						
	S1	S2-E1	S2-E2	S3	S4-E1	S4-E2	S5
ST ^a	1A	1A	1A	1A	2B	2B	1B
KF ^b	16m	10m+6sm	10m+6sm+ 1smB	12m+4sm+1smB	8m+8sm+1stB	12m+4sm+3stB	10m+6sm+1smB

^a ST Stebbin's (1971) classification; ^b KF Karyotype formula.

thoides, classified under sect. *Acanthoprason*, shares one subgroup with *A. ubipetrense* and *A. kurdistanicum* from the Kurdistan province, based on molecular markers (ITS sequences of nuclear rDNA) (Fritsch and Abbasi 2013). Chromosomal parameters suggest a high probability of differentiation between the two species, *A. haemanthoides* and *A. ubipetrense*, into different subgroups.

Regarding the *Melanocrommyum* section, *A. saralicum* and *A. shatakiense* demonstrated closely similar values for all parameters and indices. The karyotypes of both species consisted of metacentric and sub metacentric chromosomes, with one B chromosome and centromeres in the median position. This study reports the symmetric karyotype of section *Melanocrommyum* for the first time in *A. saralicum* and *A. shatakiense*. Previous studies have also shown this symmetric karyotype in the *Melanocrommyum* section (Fritsch & Astanova

1998; Hosseini and Go, 2010; Akhavan & al. 2015; Dolatyari et al., 2018; Hosseini, 2018).

Allium stipitatum, the only species analyzed karyologically in section *Procerallium*, was also diploid with $2n=16$. However, its karyotype differed from the other species in subg. *Melanocrommyum*, as it included 1-3 subtelocentric B chromosomes. Among the studied taxa, *Allium stipitatum* exhibited the highest values for all asymmetric indices, such as CVCL (26.88), CVCI (14.32), ASK% (62.24), and the lowest values for TF% (37.52), rvalue (0.6), F% (4.6), and CI (0.37).

Nevertheless, when it comes to distinguishing closely related taxa at the section level, the uniformity of karyotypes and comparable chromosome counts do not hold significant value. This research focused on a five species across three different sections, and it would be inaccurate to apply these findings universally to the entire *Allium* genus. To achieve a clearer understanding

of sectional boundaries, it would be beneficial to conduct future studies that involve a larger variety of species from diverse sections, while also examining chromosomal karyotypes in greater detail.

CONCLUSION

In conclusion, the karyological investigation of five *Allium* species belonging to the *Melanocrommyum* subgenus provides valuable insights into the chromosomal characteristics and karyotype diversity within this genus. The species studied, *A. ubipetrense*, *A. haemanthoides*, *A. stipitatum*, *A. saralicum*, and *A. shatakiense*, exhibited diploid chromosome numbers and a basic chromosome number of $x=8$. However, some variations were observed, such as the presence of 1-3 B chromosomes in certain accessions. The karyotypes of these species displayed a combination of metacentric, submetacentric, and subtelocentric chromosomes, with centromeres positioned at the median or submedian regions. Additionally, satellite chromosomes were observed in *A. saralicum*. The karyotype asymmetry analysis revealed variations among the species, with *A. stipitatum* exhibiting the most asymmetric chromosomes and *A. ubipetrense* displaying the most symmetric chromosomes.

These findings contribute to the understanding of the genetic diversity and evolutionary patterns within the *Allium* genus, particularly the *Melanocrommyum* subgenus. The data obtained from this study adds to the existing knowledge of *Allium* species in Iran and serves as a foundation for future taxonomic and evolutionary research. Further investigations of karyological characteristics and chromosomal variations in other Iranian *Allium* species would provide a more comprehensive understanding of their genetic makeup and phylogenetic relationships.

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