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Cytotaxonomy of some species of *Acanthophyllum* (Caryophyllaceae) from Iran

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Original observations on meiotic cells in 17 species of the genus *Acanthophyllum* are reported. Of these, the chromosome numbers, for 8 taxa viz, *A. laxiusculum, A. heratense, A. caespitosum, A. pachycephalum, A. khuzistanicum, A. mucronatum, A. verticillatum* and *A. crassinodum* are new observations. The basic chromosome numbers for the genus are $x = 14$ and $x = 15$. Three ploidy levels were observed for $x = 15$, diploidy ($2n = 2x = 30$), tetraploidy ($2n = 4x = 60$) and hexaploidy ($2n = 6x = 90$). Comparison of the morphological characters, chiasma average and chromosome configuration have shown some similarities between three tetraploid species of *A. microcephalum, A. mucronatum* and *A. verticillatum* and two hexaploid species of *A. crassinodum* and *A. glandulosum. A. caespitosum* is quite different from the others, especially in chromosome number ($n = 14$) and the morphology of inflorescence. Therefore, it seems that this taxon should be placed in a new section. The occurrence of polyploidy in some species indicates that this phenomenon plays an important role in the evolution of the *Acanthophyllum* genus. Chromosome configurations for polyploid species and chiasma average for all species are reported here for the first time.

Key words: Caryophyllaceae, *Acanthophyllum,* meiotic analysis, chromosome number, chiasma formation, Iran.

Introduction

Acanthophyllum C. A. Meyer is a genus with a total of c. 61 species in the world. Of these 33 occur in Iran of which 23 species are endemic. All species of *Acanthophyllum* are divided into 7 sections of which 4 sections, namely *Oligosperma*, *Macrostegia*, *Acanthophyllum,* and *Pleiosperma,* occur in Iran. According to literature (Boissier, 1867; Huber-Morath, 1967; Komarov, 1970; Ghazanfar & Nasir, 1986; Parsa, 1951; Leonard, 1986; Schiman-Czeika, 1988) the highest number of species has been recorded in east of Iran (Khorrasan province) and in the adjacent area i.e., Turkmenistan and Afghanistan. The east of Afghanistan towards China and the west of Turkey towards Syria are poor in species, where only a single species occurs in China (*A. punges*) and Syria (*A. verticillatum*). Considering the floristic regions according to Takhtajan (1986), all the species of this genus belong to Irano-Turanian region.

Chromosome studies serve as a source of data for biologists concerned with systematic and evolutionary investigations. Unlike the morphological characters of specimens that can be measured and remeasured by succeeding genera-

Table 1. The species and origin of material examined.

Taxon	Collection No.	Origin
Section *Oligosperma*		
A. caespitosum Boiss.	10666	Hamadan; Toyserkan
	9966	Ilam: Eslamabad
	10166	Bakhtaran: between Sahneh & Kangavar
A. elatius Bunge	7366	Khorrasan: betweeb Sabzevar & Shahroud
A. heratense Schim.-Czeika	7866	Khorrasan: between Mshhad & Torbat-Heydariyah
	8066	Khorrasan: between Kashmar & Totbat-Heydariyah
	11766	Khorrasan: Bojnourd
A. korshinskyi Schischk.	3165	Khorrasan: between Mshhad & Shandiz
	8366	Khorrasan: between Sabzevar & Mehr
	8266	Khorrasan: between Nayshabour & Kashmar
	7266	Semnan: Shahroud, 60 Km. Towards Sabzevar
A. laxiusculum Schim.-Czeika	8466	Khorrasan: between Sabzevar & Mehr
	9366	Ghazvin: between Boinzahra & Takestan
	7666	Khorrasan: between Mashhad & Nayshabour
	5666	Semnan: Garmsar, Behbar
	2566	Semnan: Garmsar, Behbar
	4065	Semnan: Shahroud, Mojen
	12364	Tehran: 60 Km. Towards Saveh
	6466	Tehran: Ab-Ali
	6064	Tehran: NW
A. lilacinum Schischk.	6966	Semnan: Shahroud, Cheldokhtar, Tang-e-Olang
Section *Macrostegia*		
A. bracteatum Boiss.	167	Zanjan: between Avaj & Abgarm
A. gracile Bunge ex Boiss.	5665	Khorrasan: between Mashhad & Torbat-Haydariyeh
A. khuzistanicum Schim.-Czeika	1267	Khuzestan: Dezful
	673	Khuzestan: Omidiyeh
A. pachycephalum Schim.-Czeika	2467	Tehran: towards Karj
	14164	Tehran: Karaj, 5 Km. towards Chalus
Section Acanthophyllum		
A. crassifolium Boiss.	16564	Tehran: Jajroud
	17464	Tehran: Firouzkouh
	14764	Markazi: between Arak & Qom
A. microcephalum Boiss.	15364	Tehran: Karaj, 5 Km. towards Chalus
	15064	Qom: towards Arak
	16364	Tehran: Sorkhehesar
A. mucronatum C.A. Mey.	13267	Zanjan: 2 Km. N
	10266	Hamadan: Assadabad
A. verticillatum (Willd.) Hand.-Mzt.	16664	Tehran: Jajroud
Section *Pleiosperma*		
A. crassinodum Yukhan. & Edmondson	12466	Khorrasan: between Ghoochan & Dareh-Gaz
	12066	Khorrasan: Dareh-Gaz, Tandureh park
A. glandulosum Bunge ex Boiss.	17164	Tehran: between Firouzkouh & Damavand
	5265	Khorrasan: Ghoochan
	8166	Khorrasan: Kashmar, Rivash
	4965	Gorgan: Golestan park
A. sordidum Bunge ex Boiss.	2965	Qom: 55 Km. towards Tehran
	5966	Semnan: Garmsar, Behbar
	3465	Gorgan: Golestan park

tions of botanists, the chromosomal information is ephemeral and it is not directly retrievable from dried plants (herbarium specimens). Consequently, the documentation of karyological analysis and meiotic behavior serve as a unique source of information for both current and future investigations.

Except of my previous reports (Ghaffari,

Table 2. Chromosome numbers of *Acanthophyllum* species.

Taxon	Present count (n)	Previous count (n)	Previous count (2n)	References
Section *Oligosperma*				
A. caespitosum	14	15		GHAFFARI 1988
A. elatius	15	15		GHAFFARI 1988
A. heratense	15	–		–
A. korshinskyi (syn. *A. khorasanicum*)	15	15		GHAFFARI 1988
A. laxiusculum	15 + 0-3B	–		–
A. lilacinum	15	15		GHAFFARI 1988
Section *Macrostegia*				
A. bracteatum	15	15		GHAFFARI 1986
A. gracile	15	15		GHAFFARI 1987
A. khuzistanicun	15	–		–
A. pachycephalum	15	–		–
Section *Acanthophyllum*				
A. crassifolium	30	30		GHAFFARI 1986
A. mirocephalum	30	30	60	ARYAVAND & FAVARGER 1980 GHAFFARI 1986 NUSSBAUMER 1964
A. mucronatum	30	–		–
A. verticillatum	30	–		–
Section *Pleiosperma*				
A. crassinodum	45	–		–
A. glandulosum	45	45	90	GHAFFARI 1986 NUSSBAUMER 1964
A. sordidum	30	30		GHAFFARI 1987

1986, 1987, 1988), chromosome counts on the *Acanthophyllum* are limited to 6 species (NUSSBAUMER, 1964; ARYAVAND & FAVARGER, 1980). Because of the occurrence of some indistinct species in this genus, SCHIMAN-CZEIKA (personal communications) believed that the chromosome studies are useful and that they may help to clear up the status of indistinct species.

The present study describes the meiotic chromosome numbers of 45 collections in Iran, representing 17 species. The data on mean chiasma frequency and meiotic behaviour are reported here for the first time.

Material and methods

The origin of the plant material studied here is shown in Table 1. Floral buds of plants found in nature were collected and immediately fixed in Piennr's fluid containing ethanol 96% – chloroform – propionic acid, 6:3:2 (v/v/v) for 24 hours at room temperature. Anthers dissected out from the buds were squashed and stained with 2% acetocarmine. Chromosome counts obtained from a minimum of 50 pollen mother cells within each collection (at different stages of meiosis). Because of the difficult spreading of the meiotic chromosomes, the pairing analyses were conducted on limited number of cells (Tabs 2–3).

All slides were made permanent by the Vanetian turpentine (WILSON, 1945). Photographs of chromosomes were taken by Olympus Photomicroscope at initial magnification of 330X. Voucher specimens were deposited in the Central Herbarium of Tehran University (TUH).

Results

The previous and present counts and the results of the analysis of metaphase I of meiosis in pollen mother cells are summarized in Tables 2, 3 and 4. In addition, each section is discussed bellow in details.

Section Oligosperma SCHISCHK.

Section *Oligosperma* has the highest number of species amongst other section of *Acanthophyllum* genus. This section possesses 25 species, from which nine are narrow endemic (SCHIMAN-CZEIKA, 1988; KOMAROV, 1975). Twenty-one collections representing 6 species were studied in this section. They were uniformly diploid with $n = 15$

Table 3. Types of bivalents, chiasma average per bivalent and positions in diploid taxa of *Acanthophyllum*

Taxon	No. of cells	Bivalents situation		Chiasma position		Chiasma average
		Ring	Rod	Terminal	Interstitial	
Section *Oligosperma*						
A. caespitosum	22	89	241	419	1	1.36
A. elatius	7	76	29	181	4	1.76
A. heratense	5	44	31	119	–	1.59
A. korshinskyi	8	86	34	206	25	1.92
A. laxiusculum	13	90	105	285	4	1.48
A. lilacinum	12	142	39	323	53	2.08
Section *Macrostegia*						
A. bracteatum	6	49	41	139	2	1.57
A. gracile	7	41	64	146	3	1.42
A. khuzistanicum	5	43	32	118	1	1.59
A. pachycephalum	14	99	111	309	7	1.60

Table 4. Summary of the analysis of meiotic metaphase I in tetraploid and hexaploid species of *Acanthophyllum.*

Taxon	No. of cells analyzed	Configurations						Chiasma average	Level of ploidy
		I	II	III	IV	V	VI		
A. crassifolium	6	6	127	–	10	–		1.37	4X
	%	4.19	88.81	–	6.99	–	1.43		
A. microcephalum	5	12	120	–	12	–	–	1.55	4X
	%	8.33	83.33	–	8.33	–	–		
A. mucronatum	10	24	234	–	27	–	–	1.55	4X
	%	8.42	82.10	–	9.47	–	–		
A. verticillatum	7	16	164	–	19	–	–	1.54	4X
	%	8.04	82.41	–	9.54	–	–		
A. crassinodum	6	2	183	2	17	–	17	1.62	6X
	%	.90	82.80	.90	7.69	–	7.69		
A. glandulosum	10	4	307	4	39	–	19	1.61	6X
	%	1.07	82.30	1.07	10.45	–	5.09		
A. sordidum	9	–	202	–	34	–	–	1.31	4X
	%	–	85.59	–	14.40	–	–		

(except *A. caespitosum*) (Tabs 1–2, Figs 1A–J,). Chromosome counts for *A. caespitosum* in 3 different populations were $n = 14$, which was different from my earlier report (GHAFFARI, 1988). I have reported the haploid chromosome number for this taxon to be $n = 15$. The erroneous count has been due to the false recording of two univalents at first metaphase that considered incorrectly as bivalents.

Many specimens which SCHIMAN-CZEIKA (1985, 1988) named as *A. laxiusculum* had previously been determined as *A. squarrosum* . Therefore, my earlier report (GHAFFARI, 1986) about *A. squarrosum* corresponds to *A. laxciusculum.* In the samples numbered as 5666 and 2566 (Tab. 1) in addition to 15 bivalents, 0 to 3 B-chromosomes were found in many cells at meiosis and mitosis stages. These B-chromosomes showed a tendency to lag at first anaphase. The similar phenomenon in *Centaurea kandavanensis* has been reported by GHAFFARI (1998). B-chromosomes appeared as univalents without pairing with each other. Chiasmata average in samples without B-chromosomes was 1.47 for one bivalent (see Tab. 3). The results of behaviour of B-chromosomes in this taxon will be published separately. Chromosome counts for *A. laxiusculum* and *A. heratense*, and also chiasma average and positions of bivalents for all species in this study, are reported here for the first time.

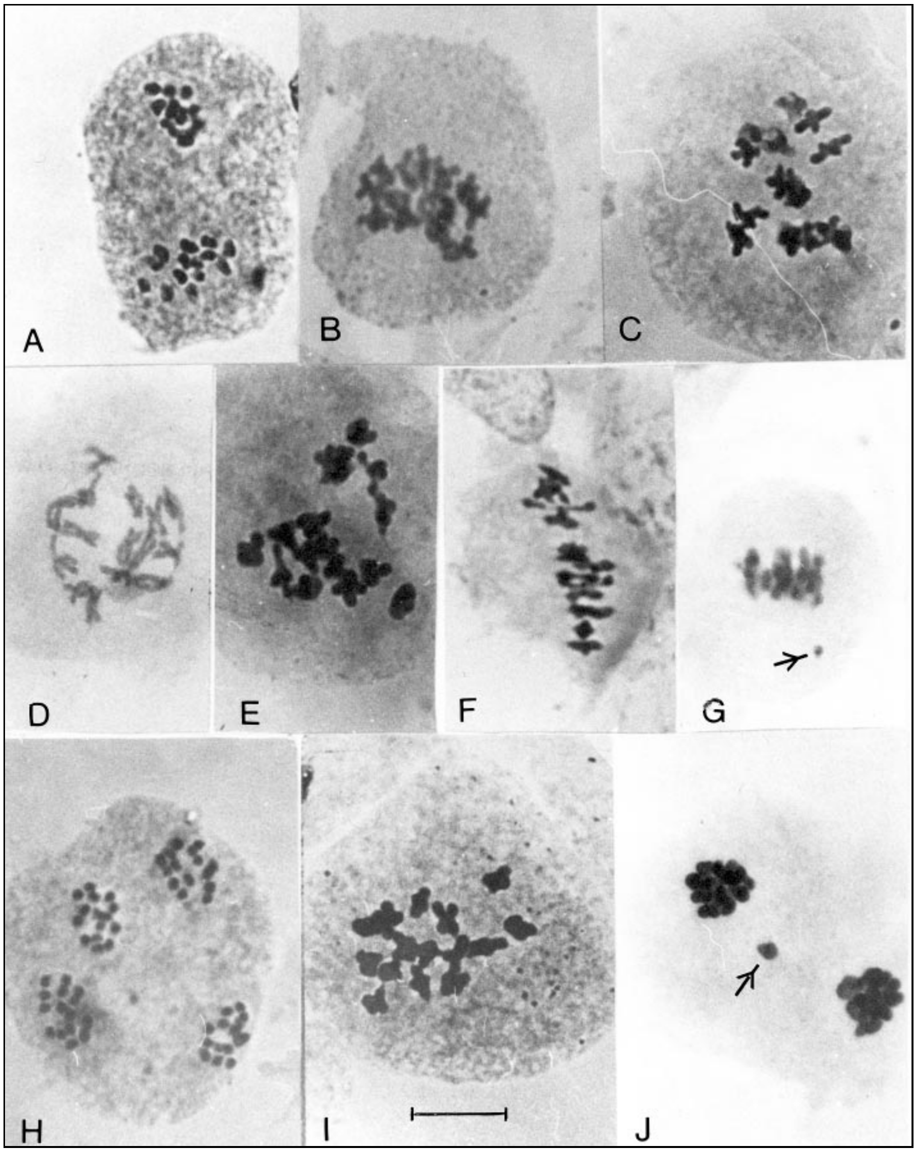

Fig. 1. Meiosis. A – *A. caespitosum*, metaphase II, n=14; B – *A. elatius*, metaphase I, $n = 15$; C – *A. heratense*, metaphase I, $n = 15$; D – *A. heratense*, diplotene, $n = 15$; E – *A. korshinski*, metaphase I, $n = 15$; F – *A. laxiusculum*, metaphase I, $n = 15$; G – *A. laxiusculu*, metaphase I, showing B-chromosome (arrow); H – *A. laxiusculum*, anaphase II; I – *A. lilacinum*, metaphase I, $n = 15$; J – *A. lilacinum*, late anaphase I, showing laggard bivalent chromosome (arrow). Scale bar 10 μm.

Section Macrostegia BOISS.

Section *Macrostegia* is characterized by board hyaline margin of bracteole, it is comprised of 9 species in the world (SCHIMAN-CZEIKA, 1988; KOMAROV, 1975). Six collections studied in this work represented 4 species that were diploid with gametic chromosome number of $n = 15$ (Tabs 1–2, Figs 2A–D). Many specimens which were named by SCHIMAN-CZEIKA (1988) *A. pachycephalum* had previously been determined as *A. bracteatum*. Therefore, my previous report (GHAFFARI, 1986) about *A. bracteatum* refers to *A. pachycephalum*.

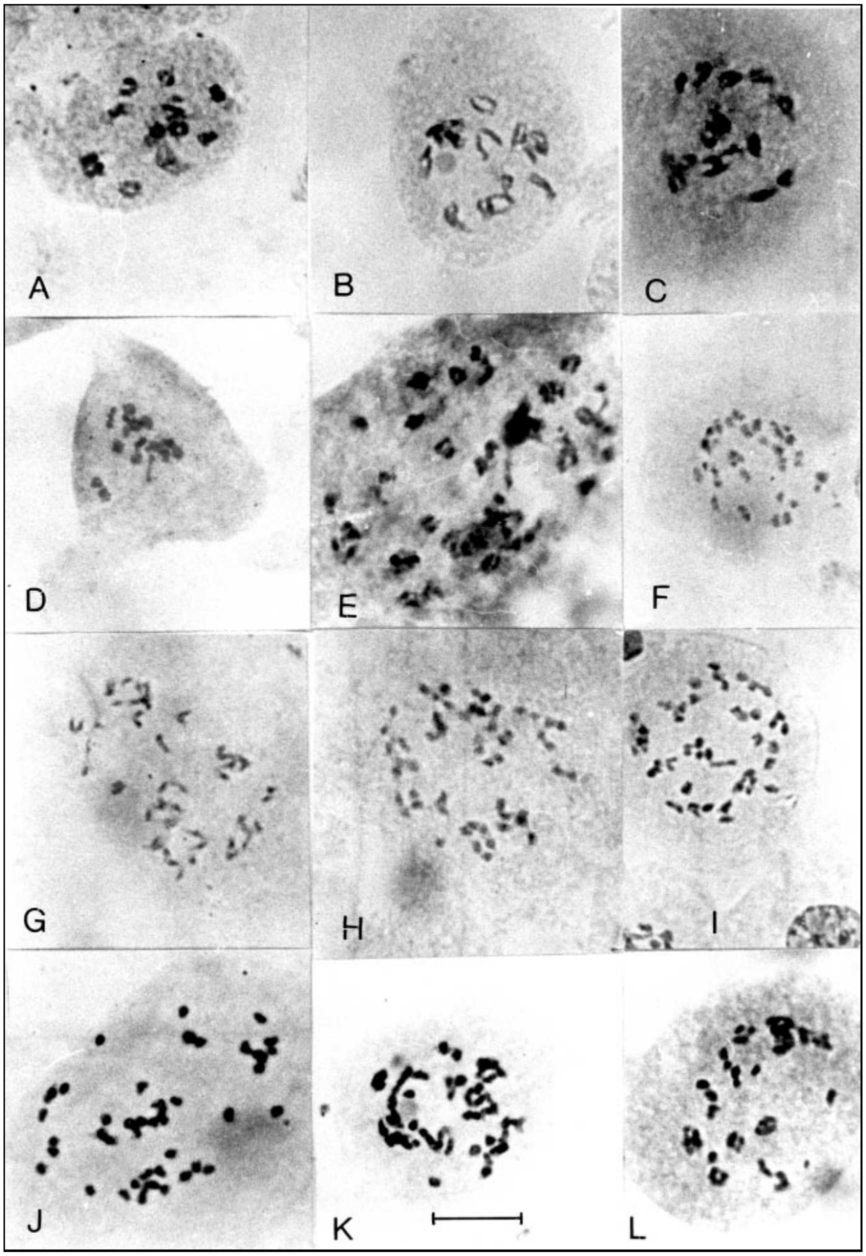

Fig. 2. Meiosis. A – *A. brcteatum*, diakinesis, $n = 15$; B – *A. gracile*, diakinesis, $n = 15$; C – *A. khuzistanicum*, diakinesis, $n = 15$; D – *A. pachycephalum*, metaphase I, $n = 15$; E – *A. crassifolium*, diakinesis, $n = 30$; F – *A. microcephalum*, metaphase I, $n = 30$; G – *A. mucronatum*, metaphase I, $n = 30$; H – *A. verticillatum*, metaphase I, $n = 30$; I – *A. crassinodum*, metaphase I, $n = 45$; J – *A. glandulosum*, metaphase I, $n = 45$; K – *A. glandulosum*, metaphase I, showing multivalents configuration; L – *A. sordidum*, metaphase I, $n = 30$. Scale bar 10 μm.

Chromosome counts for *A. pachycephalum* and *A. kuzistanicum* are also repotted here for the first time.

Section Acanthophyllum

Section *Acanthophyllum* has 7 species in the world (Schiman-Czeika, 1988). Nine collections studied here represented 4 species which were uniformly tetraploid with gametic chromosome number of $n = 30$ (Tabs 1–2, Figs 2E–H). The similarity in chiasma average and chromosome configurations in *A. microcephalum*, *A. mucronatum* and *A. verticillatum* shows an affinity between them (Tab. 4), but more cell analysis is needed. Chromosome counts for *A. mucronatum* and *A. verticillatum* are reported here for the first time too.

Section Pleiosperma BOISS.
This section has 7 species in the world, two of which are narrow endemic (SCHIMAN-CZEIKA, 1988, KOMAROV, 1975) Nine collections represented 3 species, two of them, *A. crassinodum* and *A. gladulosum*, were hexaploid with gametic chromosome number of $n = 45$ and the third one, *A. sordidum*, was tetraploid with $n = 30$ (Fig. 2I). The results of chromosome pairing and chiasma average in *A. crassinodum* and *A. glandulosum* may indicate a similarity between them (Tab. 4), but more material examined is needed. Chromosome count for *A. crassinodum* is reported here for the first time.

Discussion

The results obtained from the chromosome studies on pollen mother cells, showed an equal basic chromosome number, $x = 15$, in all species of four sections except for *A. caespitosum*. Table 2 indicates that nearly all the members of sections *Macrostegia* and *Oligosperma* are diploid with $2n = 2x = 30$ (except for diploid *A. caespitosum* with $2n = 2x = 28$), members of section *Acanthophyllum* are tetraploid with $2n = 4x = 60$, and members of section *Pleiosperma* are hexaploid with $2n = 6x = 90$ (except for *A. sordidum*). Comparison of the morphological characteristics (see SCHIMAN-CZEIKA, 1988) and the results of meiotic analysis (Tab. 3) between *A. microcephalum, A. mucronatum* and *A. verticillatum* have shown similarities between them. These similarities are especially pronounced in the mean chiasma frequency, rate of univalents, bivalents and tetravalents formation (Tab. 4). It seems that, these three species present a single species with differences in alleles which carry a few characters. For this reason, in some Floras (HUBER-MORATH, 1975; POST, 1933) these species have been mentioned as synonyms. Also in Iran, they have been grown in a single locality. Further, MOBAYEN (1979) did not find any clear differences to separate these species.

Acanthophyllum crassinodum is mophologically very close to *A. glandulosum* (SCHIMAN-CZEIKA, 1988; YUKHANANOV & EDMONDSON, 1977). As it is shown in Table 3, similarities are especially pronounced in the mean chiasma frequency, and bivalents formation. MOBAYEN (1979) believed that characters such as little branches of the stem and swollen nodes are not enough to separate them. On the other hand, we found a great morphological variation for *A. glandulosum* in nature (especially in different altitudes).

Acanthophyllum caespitosum differs from the others in morphology and in karyology as well. All species of *Acanthophyllum* follow the basic chromosome number of $x = 15$, whereas the *A. caespitosum* has a basic number of $x = 14$. SCHIMAN-CZEIKA (personal communications) believes that this taxon is quite separated from the others. In her personal letter to me she says "Although in the Flora Iranica, I compared it with *A. pulchrum*, the two are quite different, for instance *A. pulchrum* has beautiful blossoms. I do not know any species which has such inconspicuous inflorescence like *A. caespitosum*. All of the others have distinct inflorescenes separated from the leaves". Therefore it seems that this taxon must be placed in a new section.

In the *Acanthophyllum* genus, polyploidy appears to have played an important role in its evolution and speciation. Up to now in two sections, *Acanthophyllum* and *Pleiosperma,* tetraploidy and hexaploidy were recorded. Also most of the species which were found in the western and in the central parts of Irano-Turanian region (Turkey, Syria and many parts of Iran), are tetraploid and those at eastern and northern parts (north-eastern of Iran, South of Turkmenistan and Pamir) are hexaploid. This wide geographic distribution supports the previous reports of REESE (1958), STEBBINS (1972) and EHRENDORFER (1980). They have considered that the polyploids have greater ability to colonize in a new and wider geographic distributions than their diploid ancestors.

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