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Systematic study of *Astragalus chrysostachys* Boiss. (Fabaceae) in Iran, with the description of a new species

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Abstract

This paper reports the first known study of morphology, meiotic chromosome number, meiotic behavior and pollen morphology in different populations of *A. chrysostachys* Boiss. to evaluate and determine the population limits of this species in Iran. With exception of one tetraploid population, all studied taxa are diploid and possess $2n = 2x = 16$ chromosomes consistent with the proposed base number of $x = 8$ for the species from the check list of Legumes of northern Eurasia. Almost all samples displayed regular bivalent pairing and chromosome segregation at meiosis. Meiotic abnormalities observed included a variable number of sticky chromosomes with laggards and bridges in telophase I or II, asynchronous nuclei in telophase II and cytomixis. Data obtained from cytogenetic study support the phenetic groupings based on habit morphology. Results from pollen morphology showed a rather large variation in different populations. In addition, *Astragalus sekaniensis* Ranjbar, Assadi & Karamian, a new diploid species ($2n = 16$) restricted to the western Nersita mountain, NW Iran, is described.

Key Words: Flora of Iran, *Astragalus chrysostachys*, *A. sekaniensis*, meiotic behavior, chromsome number.

Introduction

Astragalus L., a genus belonging to the tribe Astragaleae of Papilionoideae in the Fabaceae, occurs in cold mountainous regions of Europe, Asia, and North America, and has its centr of diversity in Central Asia (Ranjbar & Karamian 2003a, Polhill 1981). In terms of species number, *Astragalus* may be the largest genus of vascular plants, represented by a total of ca 2500 taxa (Lock & Simpson 1991, Mabberley 1997, Maassoumi 1998, Ranjbar & Karamian 2002). It is also the most speciose genus in Iran, and *A.* sect. *Hymenostegis* with ca 44 species is one of its largest sections in Iran (Ranjbar & Karamian 2003b, Podlech & Maassoumi 2003, Ranjbar & Rahiminejad 2005, Ranjbar et al. 2005). The importance of chromosomal information in plant systematics and evolution has attracted the attention of several workers. At the generic level and below chromosome features have provided a range of possibilities for understanding the affinities of taxa. As reported by several authors (Ledingham 1957, 1960, Aryavand 1983, Maassoumi 1987, Maassoumi 1989, Bader and Sherif 2007, Al-Turki et al. 2000) variation in chromosome number in the genus *Astragalus* differentiates Old World species from those of America. Most of the cytological studies in the tribe Astragaleae have concentrated on the chromosome count (Aryavand 1983, Maassoumi 1987, 1989, Sheidai et al. 1996, 2000, 2007, Bader & Sherif 2007). The basic chromosome number ($x = 8$) and five ploidy levels ($2n = 2x = 16$, $2n = 4x = 32$, $2n = 6x = 48$, $2n = 8x = 64$ and $2n = 12x = 96$) are present in the genus. However, studies on the impact of cytogenetic data on the interspecific and phylogenetic relationships in the

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genus are still limited. Also, little is known about the nature of genetic variability in diploid species and the taxonomic relationships of the different taxa in the genus.

The study of pollen grains of the leguminous plants (e.g. CLARKE & KUPICHA 1976, FERGUSON 1990, FERGUSON & SKVARLA 1981, FERGUSON & STIRTON 1993, DIEZ & FERGUSON 1994, HUGHES 1997) has dealt mainly with the description of the pollen grains of certain genera or sometimes tribes.

Hence, investigations in different aspects can be useful to solve taxonomic problems of this problematic group. This work follows previous studies conducted on leguminous fodder species in Iran (RANJBAR et al. 2004, 2006, 2007a, 2007b, 2008, 2009, RANJBAR 2009) and aims to: increase knowledge about patterns of morphological variation, chromosome number, meiotic behavior and pollen morphology in different populations of *A. chrysostachys* of *A.* sect. *Hymenostegis* Bunge in Iran. It establishes relationships between the cytogenetic data, pollen morphology and taxonomic delimitation. During field work in May 2007 and 2008 for a project on systematics of *A. chrysostachys* BOISS. in Iran, one morphologically deviant population was collected from around Oshnavieh, south of Uroumiyeh, NW Iran. Upon closer investigation, including chromosome count, meiotic behavior and patterns of morphological variation, this population proved to be distinct and to merit consideration as a separate species.

Material and methods

Morphology

A. chrysostachys and *A. sekaniensis* were collected from the field in different regions of their natural geographical distributions during several excursions in Iran. The collected material was in vegetative or fruiting phase and deposited at BASU*, Hamedan, Iran. Also several sheets of herbarium specimens were examined for each taxon from the following herbaria: W, WU and PR. The specimens studied morphologically are listed in Table 1 and used as operational taxonomic units (OTUs). A numerical taxonomic analysis of the different individuals from these populations was carried out based on 45 quantitative/qualitative characters related to vegetative and reproductive organs. The list of morphological characters studied here is presented in Table 2. Data were entered onto a computerized spreadsheet program, Microsoft Excel version 7. The spreadsheet was later transformed into a file format suitable for phenetic analysis. Principal coordinates analysis (PCO) was carried out using MVSP software version 3.2 (KOVACH 1985-2002), with a matrix of standardized data. For PCO, an Average Distance Matrix of standardized data was obtained.

Cytogenetic study

Chromosome number and meiotic behavior were analyzed in different populations of *A. chrysostachys* and also in *A. sekaniensis*. Voucher specimens are kept at BASU (Table 1). 15 flower buds from at least 5 plants at an appropriate stage of development were fixed in 96% ethanol, chloroform and propionic acid (6:3:2) for 24 h at room tempera-

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Abbreviations for herbaria follow Index Herbariorum: http://sweetgum.nybg.org/ih/

ture and then stored in 70% ethanol at 4°C until used. Anthers were squashed and stained with 2% acetocarmine. All slides were made permanent using Venetian turpentine. Photographs of chromosomes were taken on an Olympus BX-41 photomicroscope at initial magnification of X 1000. Chromosome counts were made from well-spread metaphases in intact cells, by direct observation and from photomicrographs. All data obtained from cytogenetic study were analyzed by MVSP software and the relationships between different populations were examined.

Pollen morphology

Pollen samples were obtained from the materials collected during several excursions and prepared using the standard method described by ERDTMAN (1960). Voucher specimens are kept at BASU (Table 1). Then, the pollen grains were mounted on unstained glycerin jelly and observations were made with a Nikon Type-2 microscope. The measurements were based on 25 readings from each specimen. Equatorial diameter (E), polar axis (P), colpus length (CL), colpus width in granule site (CG), colpus width in non-granule site (CN), granule length (GL), granule diameter (GD) and the shape index (P/E) were measured. Data were analyzed by MVSP software version 3.2 and the relationships between different populations were studied. The terminology used here is according to FAEGRI (1956).

Results and discussion

Morphology

Morphological study showed a high level of intraspecific variation and resulted in five groups. CHR17, CHR13, CHR19 and CHR 20 populations were included in group 1; CHR18 in group 2; CHR12 in group 3; CHR11 in group 4 and SEK15 in group 5 (Fig. 1). It seems that among the morphological characters studied (Table 2), plant height, leaflet mucron length, calyx length, calyx teeth and tube length, played decisive roles in differentiating populations. Group 1 with 4 populations is morphologically related to *A.*

Table 1: Taxa studied and acronyms.

Abbreviation	Voucher	Altitude (m)	Locality	Species
CHR11	BASU 16811	1705	Iran: Kurdestan, Serishabad to Bijar, 30 km after Serishabad	*A. chrysostachys*
CHR12	BASU 16812	1815	Iran: Bojnurd to Quchan, 15 km after Bajgiran	*A. chrysostachys*
CHR13	BASU 16813	1520	Iran: Naqadeh to Oshnavieh, after Jaldian neck, before Piranshahr bifurcate	*A. chrysostachys*
SEK15	BASU 16815	1635	Iran: Oshnavieh to Uroumiyeh, the first neck before Sekani village	*A. sekaniensis*
CHR17	BASU 16817	1563	Iran: Qazvin to Kuheyn	*A. chrysostachys*
CHR18	BASU 16818	2150	Iran: Tehran, Abali to Mobarkabad	*A. chrysostachys*
CHR19	BASU 16819	1571	Iran: Tehran to Firuzkhuh, 5 km after Polure bifurcate	*A. chrysostachys*
CHR20	BASU 16820	2400	Iran: Firuzkuh, 5 km after Vazna village	*A. chrysostachys*

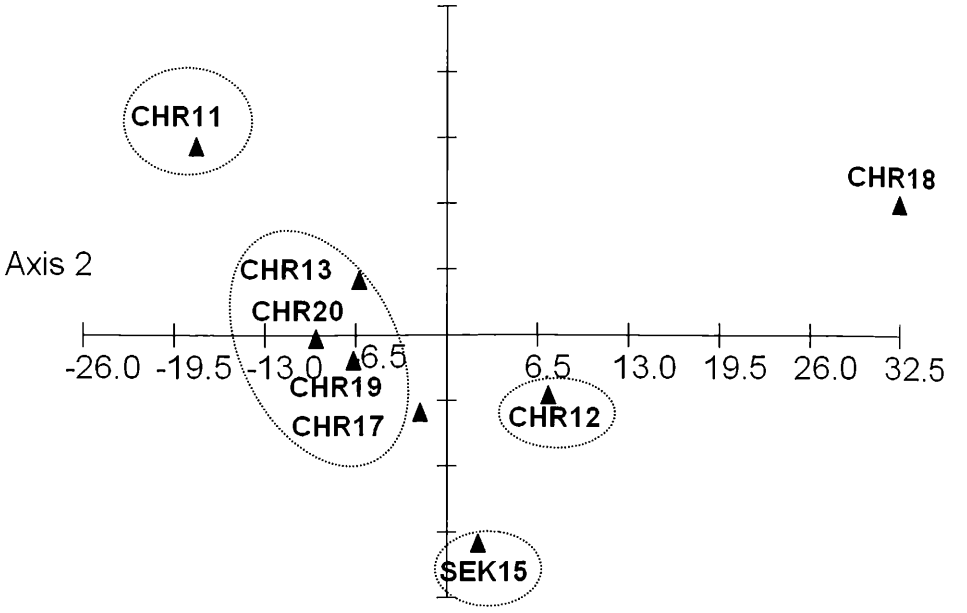

Fig. 1. PCO analysis of different population of *A. chrysostachys* and *A. sekaniensis* based on morphological characters (abbreviations are as listed in Table 1).

chrysostachys BOISS. (Type: Persia,. Ispahan Province: AUCHER ELOY 4401), therefore we recognized this group as *Astragalus chrysostachys* BOISS. Group 2, which comprises CHR18 population, is separated from the other groups by its plant height, leaf length, leaflet mucron length, petiole length, hair density at the base of the stipule and keel length. The only population CHR12 in group 3 is separated from the others by its stipule length, length of free and connated parts of the stipule, leaflet length and calyx length. We concluded that this population can be recognized as *A. chrysostachys* Boiss. var. *khorasanicus* SIRJ. & RECH.f. (Type: Persia borealis, Khorasan Province, montes Kopetdagh). The CHR11 population is differentiated from other groups by the large size of the plant, leaf length, length of stem growing in the first year, leaflet length, leaflet mucron length, calyx tube length and wing length. This group is recognized as *A. chrysostachys* ssp. *nervistipulus* (BOISS.) MAASSOUMI. SEK15 population formed a distinct group. The diagnostic morphological characters for differentiating it as the new species of *A. sekaniensis* from the populations of *A. chrysostachys* are shown in Table 3.

Astragalus sekaniensis RANJBAR, ASSADI & KARAMIAN sp.n. (Fig. 2)

Ab *Astragalo doghrunensis* Maassoumi & Podlech stipulis 13–15 mm (nec ad 18 mm) longis, bracteis ad 14 mm (nec 9–10 mm) longis, foliolis 10–12 × 3-3.5 mm (nec 5–9 × 1.5–5 mm), calyce 20–23 mm (nec 11–13 mm) longo, dense pilis patentibus albis 4–5 mm longis (nec laxe ad densiuscule pilis brevibus 0.2–0.5 mm longis et pilis ascendentibus 1–1.5 mm longis) obtectis, dentibus filiformibus, viridibus, 8–9 mm longis (nec anguste subulatis, flavis, 4–5 mm) longis, vexillo 22-23 (nec ca. 19 mm) longis et carina 20–21 mm (nec ca. 15 mm) longa differt.

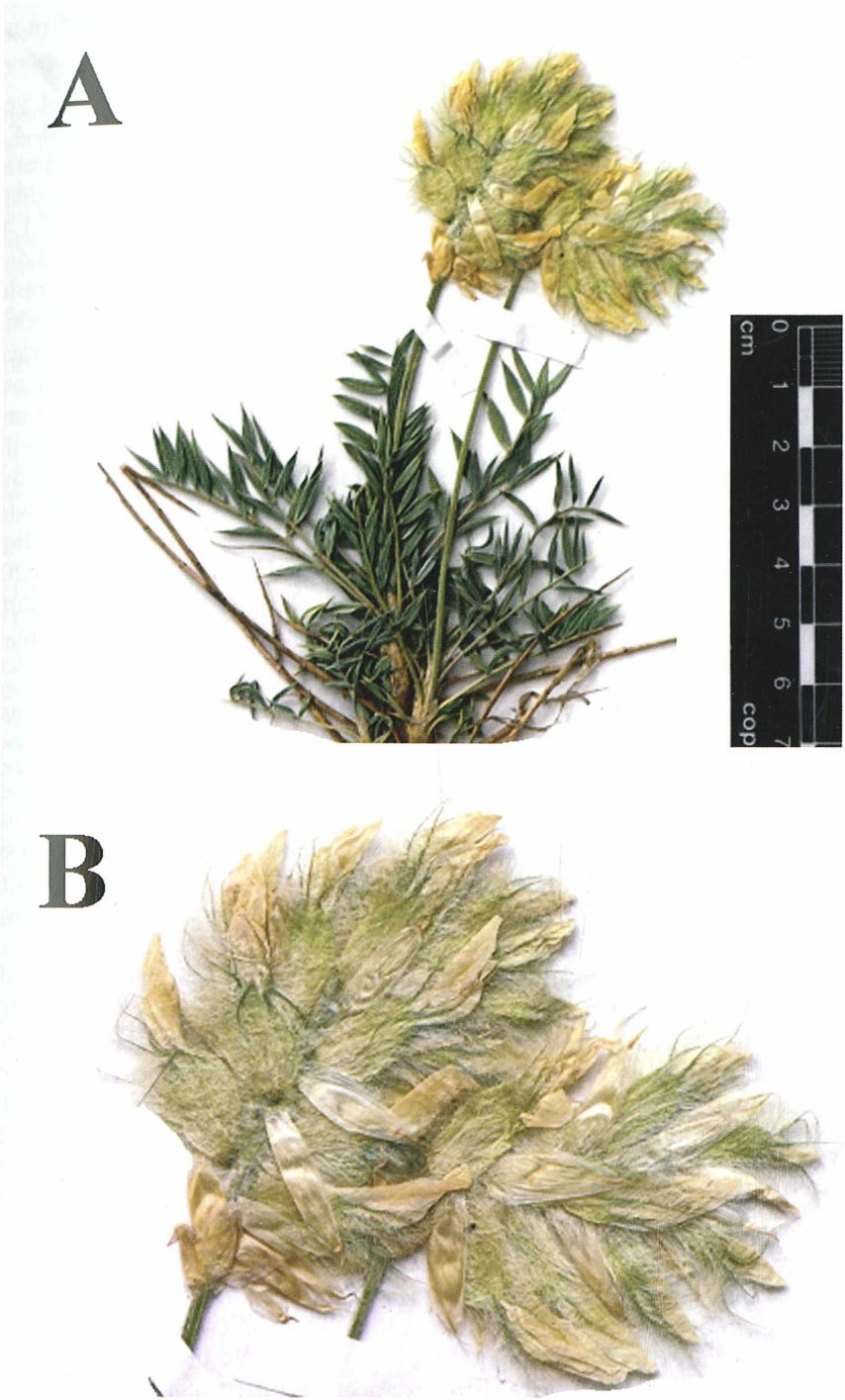

Fig. 2: *A. sekaniensis* (Ranjbar & Assadi 16815, BASU): A) Type specimen; B) Close up of inflorescence and flowers.

Type: Iran, Oshnavieh to Orumieh, the first neck before Sekani village, 1635 m a.s.l., 13.5.2008 Ranjbar & Assadi 16815 [holotype BASU!, isotypes TARI!, W!].

Perennial herbaceous plants, woody at the base and with remainders of last year's rachids, 13–15 cm tall, subacaulescent, densely covered with white hairs 0.4–4 mm long. Stipules 13–15 mm long, narrowly triangular, adnate to the petiole for ca 5 mm, at the base connate for ca 7 mm; mebranaceous, yellowish white at base, with distinct nerves, sparsely ciliate at the margin. Leaves paripinnate, 3–5 cm long, petiole 1–1.4 cm long, densely appressed-hairy. Leaflets in 5–6 pairs, oblong-elliptic, 10–12 × 3–3.5 mm, acute, with mid vein prominent on lower surface, with distinct mucro 1.5–2 mm long, both surfaces densely appressed hairy. Inflorescence dense, 15–20-flowered. Peduncle erect to ascending, 5–7 cm long, densely appressed to rarely subappressed hairy; raceme 3–4 cm long, dense ovate. Bracts papery and membranaceous at apex, narrowly ovate, 13–14 × 3–4 mm, glabrous, sparsely covered by ciliate hairs. Calyx inflated after anthesis, 20–23 mm long, ellipsoid, densely covered with villous hairs 4–5 mm long; teeth filiform, green, 8–9 mm long. Corolla yellowish, standard 22–23 mm long, limb ovate, 6–8 mm wide, sub-abruptly contracted into the long and in the upper part widely rounded, in the basal part narrow claw. Wings 16–17 mm long, limbs oblong, slightly rounded, ca 6 × ca 3.5 mm, auricle ca 0.8–1 mm long, claw 10–11 mm long; keel 20–21 × ca 3 mm, limbs 8–9 mm long, obovate to triangular, claw ca 10 mm long. Stamens 20–21 mm long, the distal 3–3.5 mm free from each other. Ovary shortly stipitate, narrowly ellipsoid, 5–6 mm long, densely appressed-hairy. Pods unknown.

Etymology: The specific epithet is in named after the type-locality, "Sekani", Azarbaijan Garbi Province, Iran.

Taxonomie remarks

A. sekaniensis is a rare and local endemic in NW Iran and only known from seven specimens collected at a single locality. It occurs in dry-steppe zone in the sub-mountainous region near the village Oshnaviyeh, south of Uroumiyeh in Azarbaijan Garbi Province (Fig. 3). It is closely related to *A. doghrunensis* by having yellow flowers and short peduncle. However, both are separated by the size of the calyx, calyx teeth, standard, wing and keel (Table 3). The type material of *A. doghrunensis* was not available to us, but the detailed original description, type photo and specimens from the type locality gave an impression of the range of variation of *A. doghrunensis*. This species occurs at elevations higher than 2000 m (up to 2800 m), in contrast to *A. sekaniensis* that occurs at elevations lower than 2000 m (up to 1700 m); otherwise the most favorable altitude for growth of *A.* sect. *Hymenostegis* is between 800-3500 m. The other species of *A.* sect. *Hymenostegis*, *A. chrysostachys*, which is closely related to *A. sekaniensis*, favors submontane regions with dry and windy conditions. The type materials of *A. chrysostachys* Boiss. was available to us at W (Fig. 4). The new species shows morphological similarities in the size of the leaflet, bract and stipule with *A. chrysostachys* (Fig. 5). However, they differ from each other mainly in the size of the peduncle, calyx, calyx teeth, standard, wing and keel, calyx indumentum, number of flowers and the shape of the inflorescence. In *A. chrysostachys*, the calyx teeth are mostly shorter than the tube, but in *A. sekaniensis* the calyx teeth are longer than the tube.

Table 2: Morphological characters and character state matrix of different populations of *A. chrysostachys* and *A. sekaniensis*.

Morphological characters	CHR20	CHR19	CHR18	CHR17	SEK15	CHR13	CHR12	CHR11
Height (cm)	27.5	27	32	25	14	36	32	40
Leaf length (cm)	4.75	5.5	7.6	5.5	4	4.5	4.5	6.5
Petiole length (mm)	13.5	13.5	22.75	16	11.5	16	13.25	14.5
Rachis length (mm)	30	20	35	20	15	15	20	40
Number of leaflet pairs	5	6	6	6	6	7	5	6
Leaflet length (mm)	11	17.33	11.35	15	11	10	13.25	26
Leaflet width (mm)	3.5	3.25	4.25	2.7	3.25	3.25	2.25	3
Leaflet shape (Oblong = 0, Elliptic = 1)	1	1	1	1	1	1	1	0
Leaflet mucro length (mm)	1	2	2	1.75	2	1	4	2
Hair density on leaflet (Loose = 0, Sparse = 1)	0	1	0	0	0	1	0	0
Hair length upper surface (mm)	0.75	0.6	0.6	0.6	0.4	0.7	0.6	0.6
Hair length lower surface (mm)	0.9	0.55	0.50	0.90	0.30	0.65	0.5	0.55
Stipule length (mm)	25	18	21.5	22	12	12	19.5	16
Stipule width (mm)	12	12	8	10	5	8	10	14
Hair density on stipule (Loose = 0, Dense =1)	0	1	0	1	0	1	1	1
Hair length at base of stipule (mm)	1.9	1.75	0.65	1.25	1	1.5	2	0.35
Free portion of stipule length (mm)	10	11	13	9	7	6	9	7
Connation part of stipule (mm)	15	12	11	10	5	6	10.5	10
Hair density on stipule margin (Glabrous = 0, Ciliate = 1)	0	0	1	0	0	0	0	0
Peduncle length (cm)	17	14.5	20	10	6.5	12	8.5	25
Inflorescence width (mm)	20	23	30	27	32	29	25	20
Inflorescence length (mm)	90	67	75	47	35	65	70	53
Bract length (mm)	15	14	13	13	13.5	10	12	15
Bract width (mm)	7	5.5	7	5	3.5	6	5.5	6
Bract indumentum (Appressed = 0, Subappressed = 1)	0	0	1	0	1	0	1	1
Hair density on bract (Sparse = 0, Loose = 1)	0	1	0	1	1	1	1	1
Hair length on bract (mm)	0.8	0.55	0.75	0.9	0.9	1	2.75	1.1
Calyx length (mm)	16.5	15.5	15.5	16.25	21.5	15.5	19	13.5
Calyx width (mm)	8.5	8	8	7	5.5	5.75	8.5	6
Calyx teeth length (mm)	6.25	4.5	5	5.5	8.5	5.5	7	5
Calyx tube length (mm)	10.5	11	9	10.5	12	9.5	11	8
Hair length on calyx (mm)	3.5	2.75	2.25	2.75	4.5	2.5	3.5	2
Standard width (mm)	19.5	18	23.25	21.5	22.5	24.5	20.5	13
Standard length (mm)	6.5	5.5	6.5	6.25	7	7	7.5	5.5
Standard claw length (mm)	10	10	9	8.5	10	11	8	7
Standard color (Green = 0, Yellow = 1, Whitish yellow = 2)	1	0	0	1	2	0	0	1
Wing length (mm)	15.5	15	16	16.5	16.5	16.5	15.5	11.5
Wing width (mm)	2.5	2.1	3	3	3.5	3	3	2.75
Wing color (Green = 0, Yellow = 1, Whitish yellow = 2)	1	0	0	1	2	0	0	1
Wing angulate length (mm)	.90	.50	.50	.60	.90	.70	.80	1
Wing claw length (mm)	10.5	10.5	11	12	10.5	11.25	9.5	7
Keel length (mm)	16.5	17.25	20.75	17	20.5	20.5	17.5	11.5
Keel width (mm)	2.5	2	3.5	3	3	2.75	2.5	2
Keel claw length (mm)	10.5	11.5	12	10.5	11	11.5	10	6
Keel color (Green = 0, Yellow = 1, Whitish yellow = 2)	1	0	0	1	2	0	0	1

Table 3: Diagnostic morphological characters of *A. doghrunensis* and *A. sekaniensis*.

Characters	*A. doghrunensis*	*A. sekaniensis*
Height (cm)	18–20	13–15
Number of leaflet pairs	5–7	5–6
Leaf length (cm)	2–6	3–5
Leaflet length (mm)	5–9	10–12
Leaflet width (mm)	1.5–3	3–3.5
Leaflet mucro length (mm)	0.5–1	ca. 2
Stipule length (mm)	up to 18	ca. 12
Bract length (mm)	9–10	13–14
Bract width (mm)	3–5	3–4
Calyx length (mm)	11–13	20–23
Calyx teeth shape	subulate	filiform
Calyx color	pale yellow	green
Calyx teeth length (mm)	4–5	8–9
Hair length on calyx (mm)	0.2–1.5	4–5
Standard length (mm)	ca. 19	22–23
Keel length (mm)	ca. 15	20–21

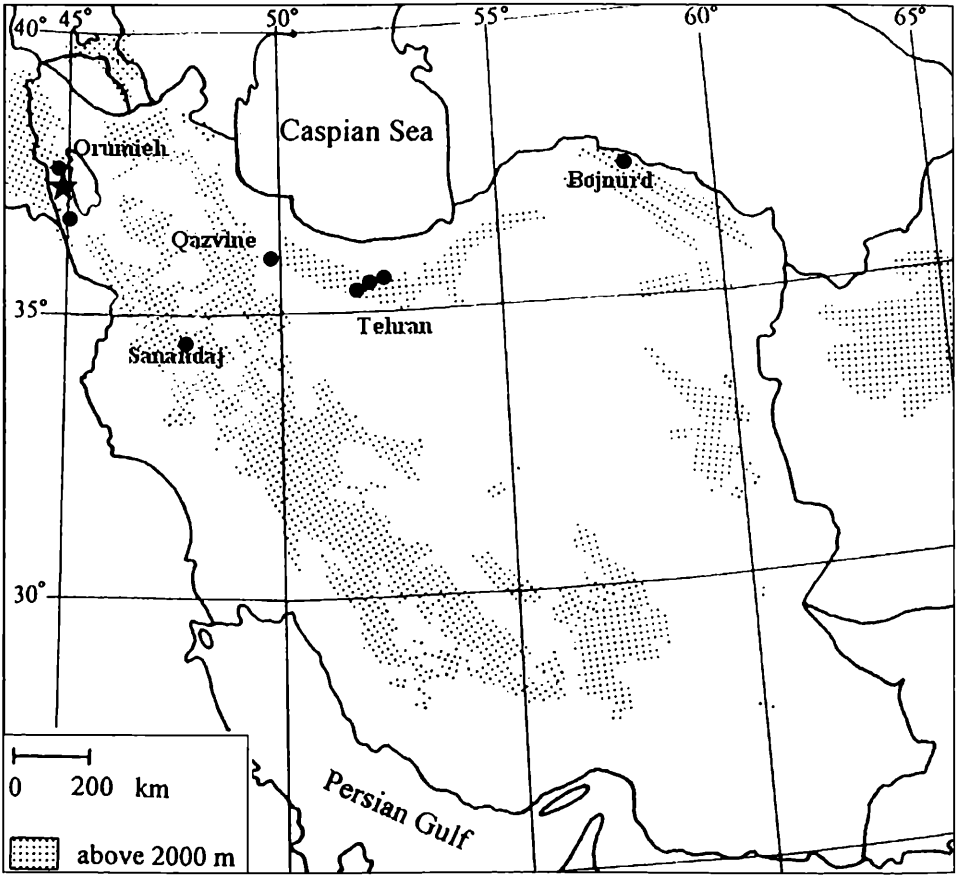

Fig. 3. Distribution of *A. chrysostachys* (●) and *A. sekaniensis* (★) in Iran.

Fig. 4. Isotype of *A. chrysostachys* Boiss. (Aucher-Eloy 440 [W]).

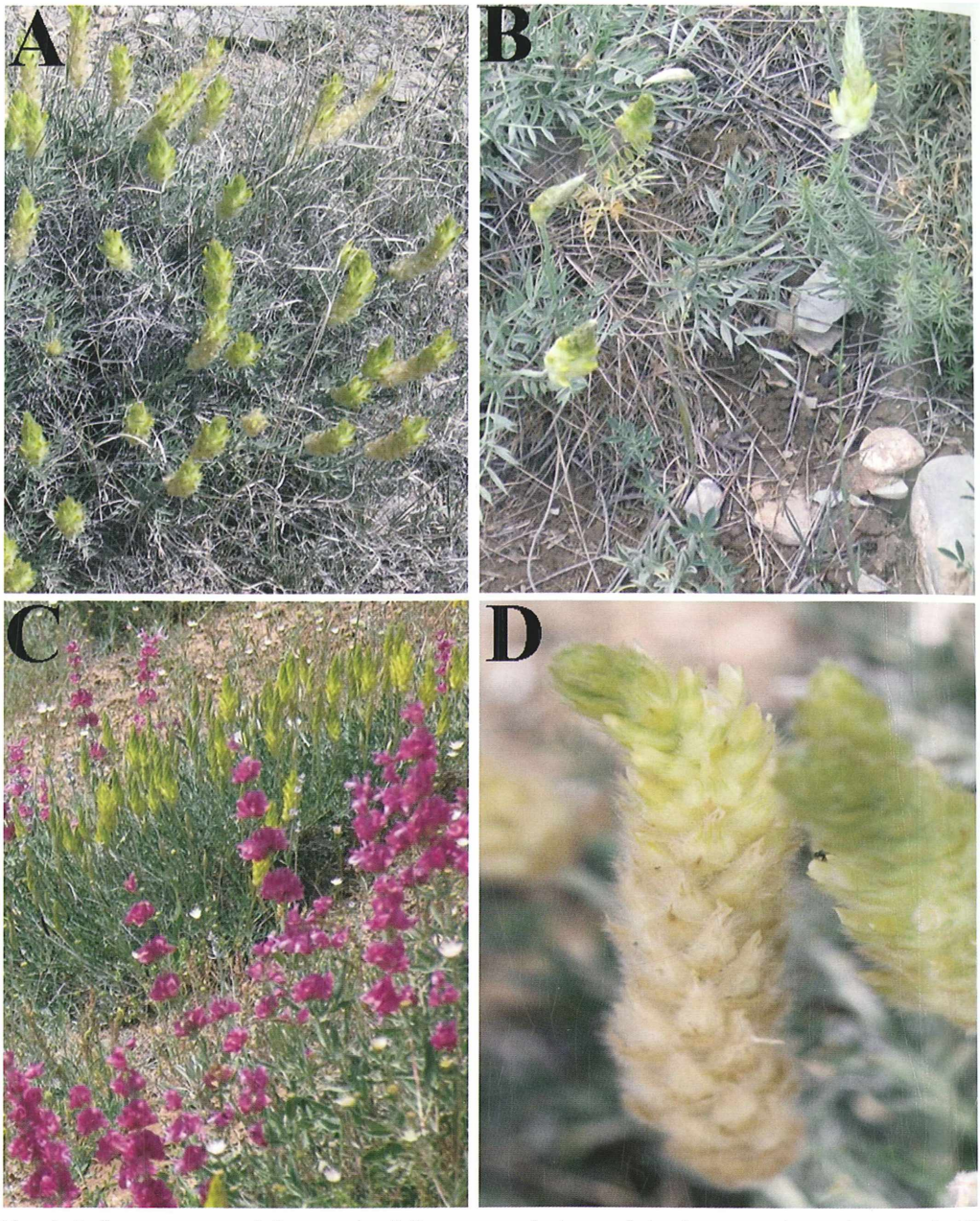

Fig. 5: Inflorescence and flowers in different populations of *A. chrysostachys*: A) CHR12; B) CHR18; C) CHR 11; D) CHR 13.

Cytogenetic study

Data with regard to meiotic chromosome number, meiotic stages, as well as abnormalities that have been observed in each stage are presented in Table 4. A total of 3840 diakinesis/metaphases I (D/MI), 3346 anaphase I/telophase I (AI/TI), 86 methaphase II

Table 4: Number of pollen mother cells (PMCs) analyzed and percentage of PMCs meiotic behavior in different populations of *A. chrysostachys* and *A. sekaniensis*.

Meiotic characters	CHR20	CHR19	CHR18	CHR17	SEK15	CHR13	CHR12	CHR11
Cell number	771	2604	2295	4728	127	1744	1617	998
D/MI	21	783	497	1650	19	518	129	223
% D/MI	27.62	30.06	21.61	34.82	3.92	29.9	7.97	22.66
% Cytomixis	0	0	0.002	0	0	0	2.32	0
% Fragmented chromosome	1.87	1.14	0	1.81	15.78	1.35	4.65	2.69
AI/TI	153	837	626	1702	57	561	1656	344
% AI/TI	19.84	32.14	27.75	35.75	44.09	44.09	40.56	34.95

Abbreviations: D/MI: Diakinesis/Metaphase I, AI/TI: Anaphase I/Telophase I, MII: Metaphase II, AII/TII: Anaphase II/Telophase II, n = Chromosome number.

(MII), and 5574 anaphase II/telophase II (AII/MII) cells were analyzed. CHR17 is the only *A. chrysostachys* population which is tetraploid and possesses 2n = 4x = 32 chromosomes. The meiotic irregularities observed in different studied taxa included chromosome stickiness resulting in bridges, the occurrence of laggard chromosomes, formation of micronuclei in tetrad cells, multipolar cells and cytomixis, discussed below.

Laggards, fragmented and sticky chromosomes

Fragmented chromosomes, being unable to orient at the metaphase plate, were observed during metaphase I or metaphase II (Figs. 7, 8). The highest frequency of fragmented chromosomes of metaphase I cells was observed in population SEK15. Laggard chromosomes were observed during anaphase I in populations CHR12 and CHR17 (Fig. 6). According to NICKLAS & WARD (1994), non-oriented bivalents may be related to impaired attachment of kinetochores to the spindle fibers. PAGLIARINI (1990) reported that laggards may result from late chiasma terminalization (SOUZA et al. 2006). These laggards might have degenerated or may have resulted in the formation of polyads particularly at the resting phase (BASI et al. 2006).

Cytomixis

The phenomenon of cytomixis consists in the migration of chromosome between meiocytes through cytoplasmic connection. Since cytomixis creates variation in the chromosome number of the gametes, it could be considered as a mechanism of evolution (GHAFFARI 2006). This phenomenon was observed in all populations at different stages and CHR12 showed the highest percent in D/MI (2.32%) and D/MII (1.73%) stages (Table 4).

Chromosome bridges

Chromosome bridges resulting from stickiness were observed in all populations at anaphase I cells with exception of populations CHR12 and CHR17 and at anaphase II cells in populations CHR17 and CHR18 (Figs. 6, 7; Table 4). The number of chromosomes involved in their formation varied among different meiocytes. Genetic as well as environmental factors have been considered as reasons for chromosome stickiness in different plant species (NIRMALA & RAO 1996).

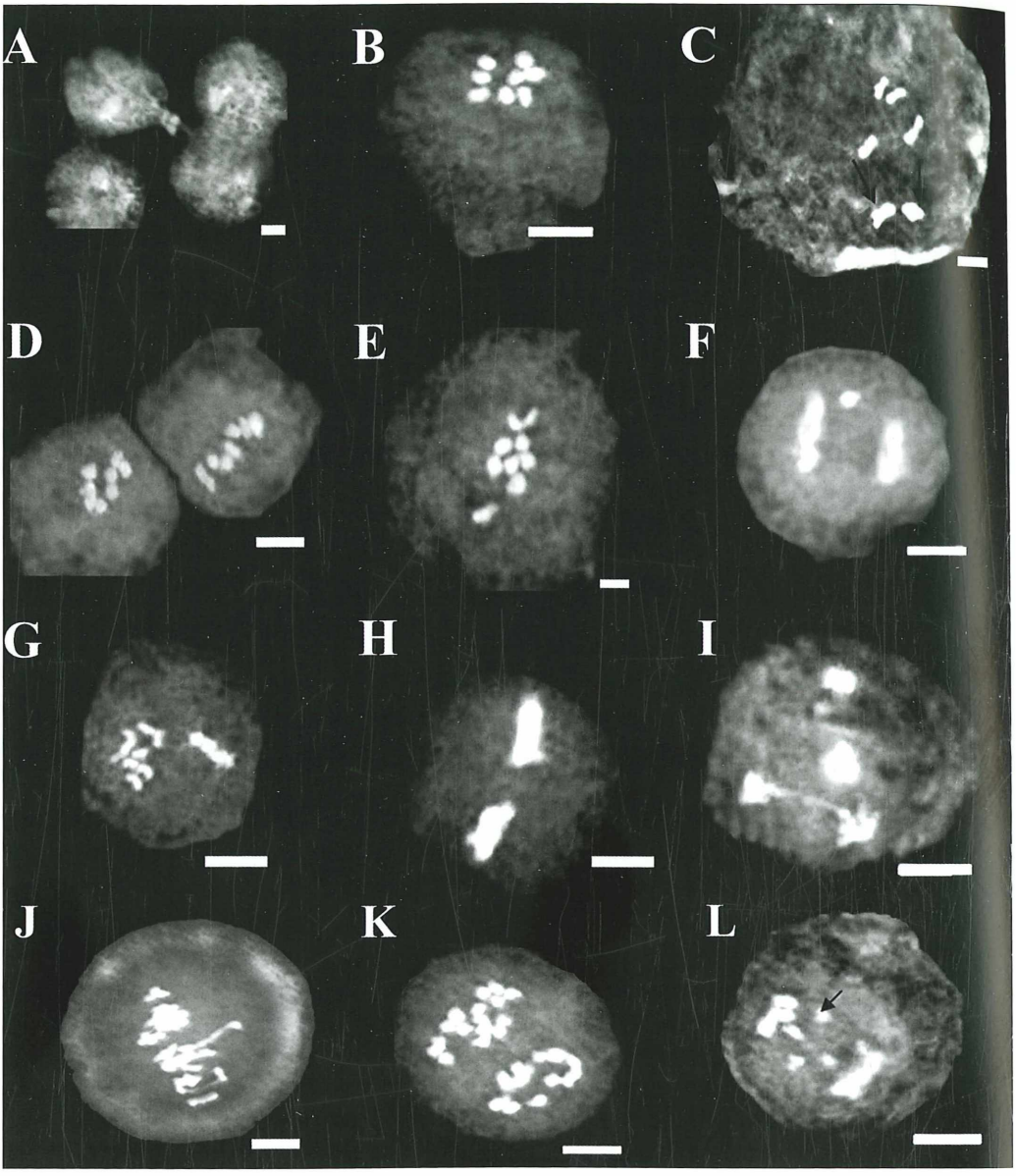

Fig. 6: Meiotic behavior in diploid populations of *A. chrysostachys:* A) Cytomixis in CHR18; B) Diakinesis with 8 bivalents in CHR13; C) Diakinesis with 4 bivalents and 2 tetrads in CHR11; D) Metaphase I in CHR11; E) Metaphase I with 8 bivalents in CHR13; F) Laggard in CHR12; G) Asynchronous nuclei in CHR19; H) Metaphase II in CHR18; I) Bridge in CHR20; J) Metaphase I in CHR17; K) Telophase I in CHR17; L) Laggards in CHR17. Scale: 6 μm.

Micronucleus

Chromosomes that produced micronuclei during meiosis were eliminated from microspores as microcytes. The micronucleus reached the microspore wall and formed a kind

Table 5: Pollen characteristics in different populations of *A. chrysostachys* and *A. sekaniensis*.

Characters / Populations	P	E	L	CG	CN	GD	GL
CHR20	25(27.3)29	25(22.35)27	21(23.65)27	14(16.95)20	9(11)12	3(3.8)5	2(3.17)4
CHR19	28(29.5)32	22(24.5)30	24(25.6)29	19(22.1)23	9(10.65)11	2(3.85)4	3(3.4)5
CHR18	27(27.8)29	21(24.8)26	25(25.5)27	18(21.1)27	11(12.5)14	3(3.43)5	2(2.3)3
CHR17	25(32.1)35	22(25.23)27	26(30.3)33	22(23.2)27	10(14.05)16	4(4.4)5	2(2.98)4
SEK15	26(30.15)30	30(29.15)31	25(28.37)31	23(25.20)28	8(9.5)11	2(3.27)4	3(5.35)7
CHR13	28(31.1)32	24(26.55)29	25(28.35)32	19(23.45)46	9(9.5)13	3(4.6)6	3(4.2)6
CHR11	29(32.1)35	22(25.35)29	29(29.3)33	16(23.2)27	10(14.5)15	4(4.4)5	2(2.98)4
CHR12	27(30.05)32	23(25.31)27	25(27.63)30	20(23.15)25	10(11.1)14	2(4.26)6	2(4.1)5

Abbreviations: E: Equatorial diameter, P: Polar axis, L: Colpus length, CG: Colpus width in granule site, CN: Colpus width in none granule site, GL: Granule length, GD: Granule diameter, P/E: Shape index.

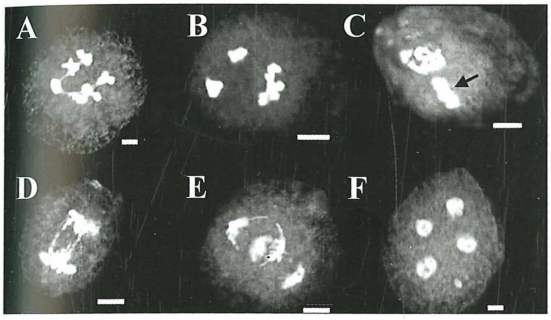

Fig. 7: Meiotic behavior in tetraploid population (CHR17) of *A. chrysostachys*; A) Chromosome stickiness; B) Metaphase I with fragmented chromosomes; C) Asynchronous nuclei; D) Bridge; E) Anaphase II; F) Micronucleus. Scale: 6 µm.

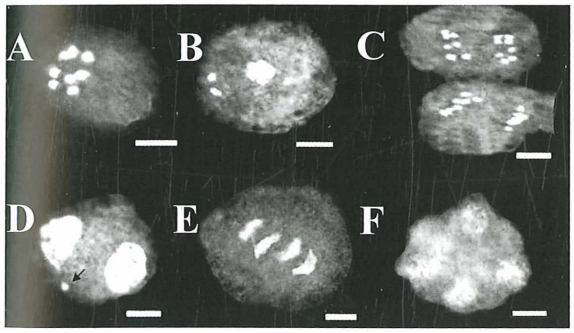

Fig. 8: Meiotic behavior in *A. sekaniensis*: A) Diakinesis; B) Metaphase I with fragmented chromosomes; C) Telophase I; D) Micronucleus; E) Anaphase II; F) Pentapolar cell. Scale: 6 µm.

of bud, separated from the microspore. The eliminated microcytes gave origin to small and sterile pollen grains (BAPTISTA-GIACOMELLI et al. 2000). Micronuclei are seen in some populations (Table 4), with the highest percentage in population SEK15 (Fig. 7).

Multipolar cells

The spindle apparatus is normally bipolar and acts as a single unit, playing a crucial role in chromosome alignment during metaphase. Any distortion or breakage in the spindle may result in random sub-grouping of the chromosomes (NIMALA & RAO 1996). Pentapolar cells were observed in population SEK15 (Fig. 8). Such cells may lead to the formation of abnormal tetrads and infertile pollen grains.

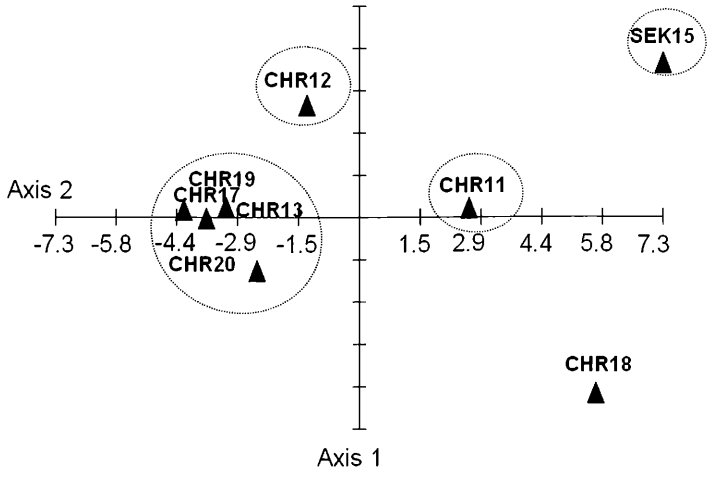

Fig. 9. PCO analysis of different populations of *A. chrysostachys* and *A. sekaniensis* based on meiotic characters (abbreviations are as listed in Table 1).

PCO analysis based on cytogenetic data showed intraspecific variation as well as morphological characters and resulted in five groups (Fig. 9). Group 1 includes populations CHR13, CHR17, CHR19 and CHR20, group 2 includes CHR11, group 3 includes CHR18, group 4 includes CHR12 and group 5 includes population SEK15. The SEK15 population represents the new species *A. sekaniensis* and showed a high degree of abnormality, for example high score in the formation of a micronucleus (3.57%), fragmented chromosomes (15.78%) and pentapolar cells (2.27%).

Pollen morphology

Pollen grains in the populations studied are large-, rarely medium-sized ranging from: P = 25– (27.3) –29 µm; E = 25– (22.35) –27 µm to P = 29– (32.1) –35 µm; E = 22– (25.35) –29 µm. The smallest pollen grains belong to population CHR20, while the largest ones belong to population CHR11 (Table 5). The pollen grains in taxa studied are prolate-subprolate to spheroidal-rhombic or subquadrate-rhombic and often protruding at the equator, tricolpate or colporate, the size is variable between populations and granules are clearly defined, or absent in a few populations. The colpi are long, extending onto the poles with tapering ends, coarsely granulated membranes and with either smooth or ornamented margins. The mean values and ranges of seven quantitative characters which were useful in separating different populations are given in Table 5.

PCO analysis based on pollen morphology resulted in 6 groups (Fig. 10). Populations CHR13 and CHR12 were included in group 1, populations CHR17 and CHR19 in group 2, population CHR18 in group 3, population CHR11 in group 4, population CHR20 in group 5 and population SEK15 in group 6. Populations CHR12 and CHR13 form a single group for their small granule width and length. Populations CHR17 and CHR19 are far from the other populations because of their spherical pollen. Population CHR18 is

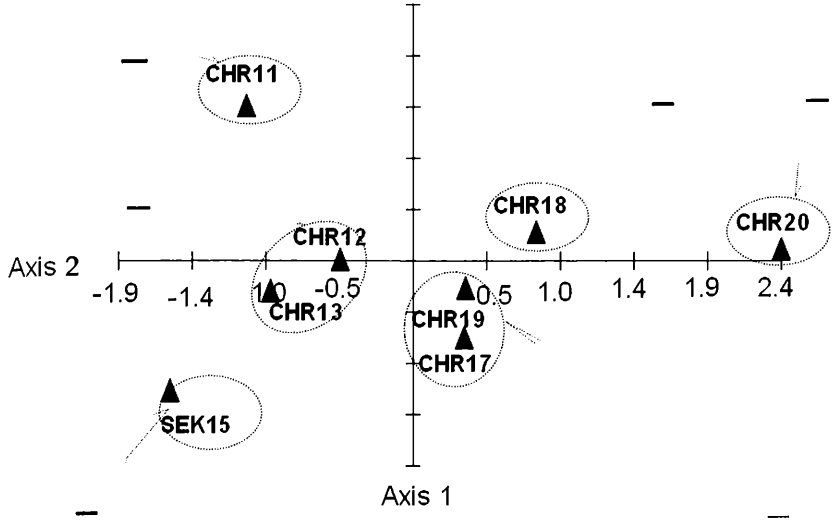

Fig. 10: PCO analysis of different populations of *A. chrysostachys* and *A. sekaniensis* based on pollen morphological characters (abbreviations are as listed in Table 1).

separated by its large granule diameter. The SEK15 population is distinguished from other populations for its short colpus length in the non-granule site, CHR20 for the smallest and CHR11 for the largest pollen grains.

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