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KARYOLOGICAL INVESTIGATIONS IN SOME SPECIES OF VICIA (FABACEAE) FROM IRAN

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Mitotic chromosomes of 13 species from four sections (*Cracca, Ervillia, Ervum* and *Variegata*) of the genus *Vicia* subgenus *Vicilla* were studied. Chromosome numbers of two species: *V. armena*, (2n=10) and *V. multijuga* (2n=10) are reported for the first time. 2n=14 for *V. ciceroidea* is a new count for this species, the previous report was 2n=28. Karyological data are presented for all the studied species. The chromosomes of all species were mainly metacentric to submetacentric.
Based on Stebbines karyotype classification, the studied *Vicia* species were placed in three classes: 2A, 3A and 1B. *V. monantha* had the most symmetrical karyotype and could be considered more primitive than other studied species. Five species namely *V. armena, V. aucheri, V. akhmaganica, V. persica* and *V. variegata* showed 2n= 10 chromosomes and also had similar karyotype characters.

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Key words: *Vicia*; mitose; chromose numbers; karyotype; Iran

مطالعات کروموزومی بر روی برخی گونه های ماشک (Vicia) در ایران

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حبیب زارع: استادیار پژوهش مرکز تحقیقات کشاورزی و منابع طبیعی مازندران

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بررسی میتوزی ۱۳ گونه از ۴ بخشه (Cracca, Ervillia, Ervum و Variegata) از جنس .Vicia L زیر جنس Viciellaمورد مطالعه قرار گرفت. عدد کروموزومی دو گونه: V. armena (2n=10), V. multijuga (2n=10) برای اولین بار در جهان گزارش می شوند. 2n=14 برای V. ciceroiseae شمارش جدیدی برای این جنس است. گزارش قبلی برای این گونه 2n=28 بود. اطلاعات کاریولوژیکی برای تمام گونه‌های مورد مطالعه در این تحقیق ارائه شده است. کروموزوم‌ها در همه گونه‌ها بیشتر متاسنتریک و ساب متاسنتریک بودند. بر اساس طبقه‌بندی کاریوتایپی استبینز گونه‌های ماشک در سه رده قرار گرفتند: 2A, 3A و1B. V. monantha متقارن ترین کاریوتایپ را داشته و می‌تواند ابتدایی‌تر از سایر گونه‌ها مورد ملاحظه قرار گیرد. پنج گونه V. armena, V. aucheri, V. akhmaganica, V. persica و V. variegata نه تنها عدد کروموزومی 2n= 10 را نشان می‌دهند بلکه ویژگی‌های کاریوتایپی مشابهی دارند.

INTRODUCTION

The genus *Vicia* L. (Fabaceae) includes over 170 species widely distributed through Europe, Asia and North America, extending to the temperate regions of south America and tropical Africa (Bisht & al. 1998). *Vicia* species are of many uses to human, as nutrition source for people and domestic animals and some are used as ornamental plants and green manure. Kupicha

(1976) divided the species into two subgenera, *Vicia* and Vicilla (Schur) Rouy and 22 sections. With a revision, Maxted (1991a) classified the species into 26 section. Most of these species are temperate annuals and perennials (Kupicha 1976). Cheretkova- Zertkova (1979) reported 46 species from Iran, Pakravan (2000) identified 40 species from 14 sections and Jalilian & al. (2014) 40 species from 15 sections.

There are different base numbers and ploidy levels reported in this genus (2n= 10, 12, 14, 24, 28; x= 5, 6, 7) (Veerasethakul & Lassetter 1981; Raina & Rees 1983; Maxted & al. 1991; Yamamoto 1973; Mettin & Hanelt 1968; Gaffarzadeh & al. 2008; Marti & al. 2012; Jalilian & Rahiminejad 2012 & 2015; Ei-Bok & al. 2014). Jalilian and Rahiminejad (2012) have used the chromosomal data for sectional divisions.

V. canescens is a very variable species and there are some different opinions on the infra species level division.

Chrtkova-Zertova (1979) considered eight species (*V. persica* Boiss., *V. armena* Boiss., *V. variegata* Willd., *V. akhmaganica* Kazar., *V. gregaria* Boiss. et Heldr., *V. aucheri* Jaub. et Spach *V. rechingeri* Chrtkova-Zertova and *V. afghanica* Chrtkova- Zertova) in the sect. *Variegata* Radzhi., while Davis & Plitmann (1970), Townsend (1974) and Pakravan (2000) accepted *V. variegata* species group as *V. canescens* Labill. treated as a polytypic species including 2 subspecies in Iranian materials. Jalilian & al. (2012) recognized *V. canescens* as its sensu lato with no infra-specific taxa. There are more or less overlapping characters (upper and lower leaflets, leaflets apex, tendril, stipule length, indumentums of legumes) used by Davis & Plitmann (1970) and Pakravan (2000) for infra species division of *V. canescens* which make it very difficult to decide about it.

The aim of this study is (i) to study the chromosomes of various species, (ii) to revise the taxonomy of some *Vicia* species and subspecies (*V. canescens*) by using cytological analysis.

MATERIALS AND METHODS

In this study, we examined 29 accessions from 13 species (table 1).

The seeds were treated with a solution of 10% sodium hydro chlorite, washed with distilled water and germinated on filter paper in Petri dishes. Root tips were pre-treated with 8-hydroxyquinoline for 3. 5 hours, fixed in a solution of glacial acetic acid and absolute ethanol (1: 3) for 24 hours and stored in 70% alcohol. Acetocarmine used for staining the chromosome. The squashed specimens were studied by an Olympus B51 microscope and photographs were taken by a digital camera (DP12). At least 10 cells of each accession were studied. The chromosomes were classified according to Levan & al. (1964). The symmetry of karyotypes were determined by TF% (Total form percentage) and the Stebbins classification.

RESULTS AND DISCUSSION

Karyotypes of 13 *Vicia* species were studied (table 1, figs. 1 & 2)

Chromosome numbers of two species *V. multijuga* (2n= 14+1B) and *V. armena* are reported for the first time in the world.

2n=14 is a new count for *V. ciceroidea*, the previous count for this species was 2n= 28 (Jalilian & Rahiminejad 2012). 2n=12 for *V. cracca* confirms the previous counts for this species (Ghaffari & al. 1987; Nishikuwa & al. 1985a, and Lou & Wang 1989). There are reports of 2n=14 (Akpinar & R. Bilaloğlu 1997, Inceer & Hayirloglu-Ayas 2005; Jalilian & Rahiminejad 2015), 2n= 24 (Lou & Wang 1989)and 2n=28 (Ryka 1954) for this species, which show different ploidy level and disploidy in *V. cracca*. 0 to 2 B-chromosomes were observed in our preparations (fig. 1. b). *V. monantha* had 2n= 14 chromosomes wich confirmed the Jalilian and Rahiminejad (2015) report. Also 2n=24 is reported for this species (El-Bok & al. 2014). Chromosome number of 2n=14 for *V. villosa, V. monantha, V. ervilia* (L.) Wild and *V. tetrasperma* (L.) Schreb are in agreement with previous counts for these species (Jalilian & Rahiminejad 2012, 2015; El-Bok & al. 2014, Youssef & al. 1966; Raina & Rees 1983; Yamamoto 1973; Rahiminejad & al. 2000; Oberprieler & Vogt 1996; Dempsey & al. 1994). Chromosome number of 2n=10 for *V. variegata, V. akhmaganica* and *V. persica* are in agreement with previous counts for these species (Rahiminejad & al. 2000; Ghaffari & al. 1987; Nazarova. 2004; Jalilian & Rahiminejad 2015). Chromosome count for *V. variabilis* (2n=14= 1B) has confirmed the previous report for this species (Jalilian & Rahiminejad 2015).

Table. 1. *Vicia* species examined in karyological study.

Species	Collector	Locality
V. akhmaganica Kaz.	Amini & Abbasi	Mazandaran: Challus, Kandovan road, 2000m; 2832 ALUH
V. akhmaganica Kaz.	Hosseinzadeh	Tehran: Darbandsar; 1833 ALUH
V. akhmaganica Kaz.	Hosseinzadeh	Tehran: Shahrestanak; 1834 ALUH
V. armena Boiss.	Hosseinzadeh	Tehran: Darbandsar; 1836 ALUH
V. armena Boiss.	Amini & Abbasi	Mazandaran: Challus, Kandovan road, 2800-3000m; 2834 ALUH
V. aucheri Jaub. & Spach	Pakravan	Tehran: 3 Km from Dizin to Shemshak; 1505-2 ALUH
V. aucheri Jaub. & Spach	Assdi & Pakravan	Tehran: Firuzkuh road, near Havir village 2700 m ;76592 TARI
V. ciceroidea Boiss.	Assdi & Pakravan	Tehran: Firuzkuh road, near Havir village 2700 m ; 76670 TARI
V. ciceroidea Boiss.	Pakravan	Tehran: Shahrestanak 2800 m; 1876 ALUH
V. cracca L.	Pakravan	Mazandaran: Kandovan road, Siahbisheh 2400m; 1839 ALUH
V. cracca L.	Pakravan	Zanjan: Yahya abbad; 1238 ALUH
V. ervilia (L.) Willd.	Pakravan	Hamedan: Tueserkan; 4-90 ALUH
V. ervilia (L.) Willd.	Pakravan	Lorestan: Kuhdasht; 44-104 ALUH
V. ervilia (L.) Willd.	Pakravan	Hamedan: Malayer; 44-201 ALUH
V. ervilia (L.) Willd.	Pakravan	Hamedan: Nahavand; 44-88 ALUH
V. monantha Retz.	Pakravan	Zanjan: Fileh khame village; 481 ALUH
V. monantha Retz.	Pakravan	Fars: Bamo national park; 3421 ALUH
V. multijuga (Boiss.) Rech.f.	Pakravan	Tehran: Kandovan rod; 1851 ALUH
V. multijuga (Boiss.) Rech.f.	Pakravan	Tehran: Firuzkuh,; 2314 ALUH
V. persica Boiss.	Pakravan	Tehran: 3 km from Dizin to Shemshak; 1505-1 ALUH
V. persica Boiss.	Hosseinzadeh	Tehran: Shemshak; 1840 ALUH
V. variabilis Freyn & Sint.	Pakravan	Chaharmahal va Bakhtiari: Babaheydar; 4804 ALUH
V. variabilis Freyn & Sint.	Pakravan	Esfahan: 14 Km w of Esfahan; 2315 ALUH
V. variegata Willd.	Amini	Mazandaran: Pole-Zanguleh to Yush, 2800 m; 1841 ALUH
V. variegata Willd.	Hosseinzadeh	Tehran: Shemshak1842 ALUH
V. tetrasperma (L.) Schreb.	Hosseinzadeh	Mazandaran: Sisangan park, 1844 ALUH
V. tetrasperma (L.)Schreb.	Pakravan	Mazandaran: 18 km to Chaboksar; 2174 ALUH
V. villosa Roth.	Hosseinzadeh	Tehran: Darband; 1843 ALUH
V. villosa Roth.	Pakravan	Markazi: Arak, Senejan village; 2175 ALHU

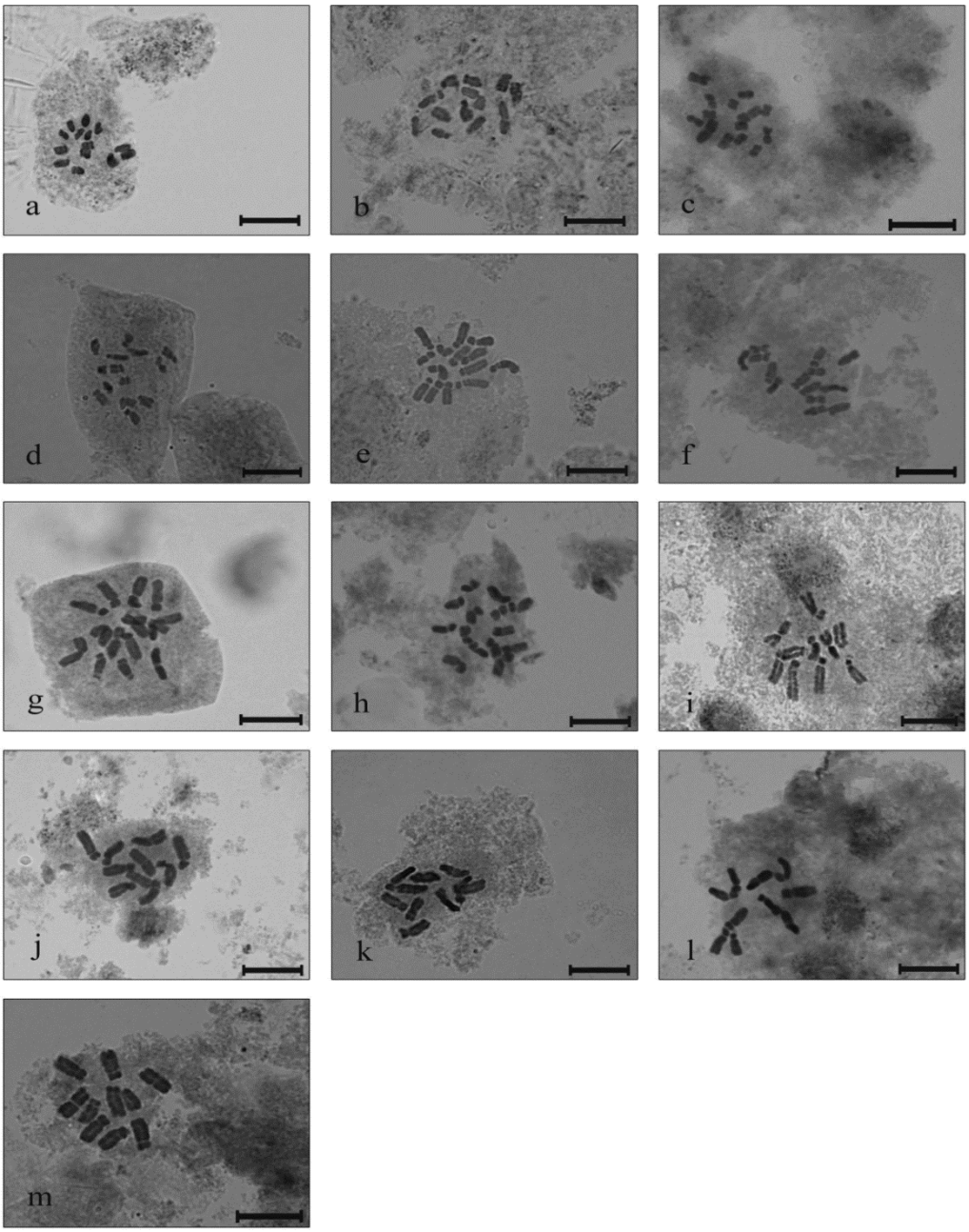

Fig. 1. Somatic chromosomes of *Vicia* species. a, *V. ciceroidea* (2n=14); b, *V. cracca* (2n=12); c, *V. monantha* (2n=14); d, *V. multijuga* (2n=14); e, *V. variabilis* (2n=14); f, *V. villosa* (2n=14); g, *V. ervilia* (2n=14); h, *V. tetrasperma* (2n=14); I, *V. akhmaganica* (2n=10); j, *V. armena* (2n=10); k, *V. aucheri* (2n=10); l, *V. persica* (2n=10); m, *V. variegata* (2n=10). The arrows indicate B-chromosomes.

Karyological characters of species studied are presented in table 2.

The chromosomes of all the species studied were mostly metacentric and submetacentric and five species had one pair of subtelocentric (table 2). Karyotype of *V. ciceroidea* include 2 metacentric (m) pairs, 3 pairs of submetacentric (sm) and 2 pairs of subtelocentric (st) chromosomes (table 2, fig. 1 & 2). *Vicia ciceroidea* was the only species with 2 pairs of subtelocentric chromosomes. (table 2, fig. 2 a).

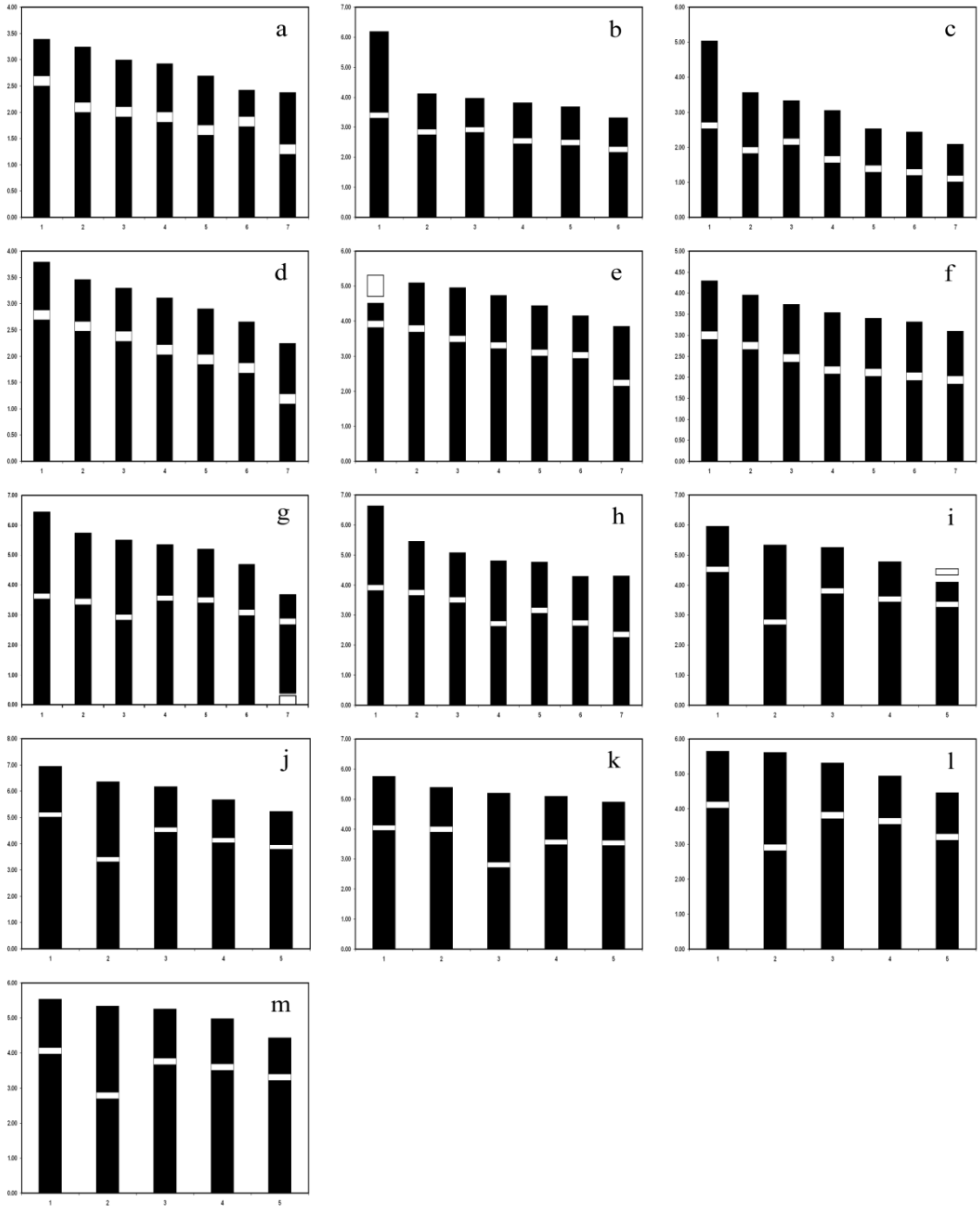

Fig. 2. Idiograms of the karyotypes of *Vicia* species. a, *V. ciceroidea* (2n=14); b, *V. cracca* (2n=12); c, *V. monantha* (2n=14); d, *V. multijuga* (2n=14); e, *V. variabilis* (2n=14); f, *V. villosa* (2n=14); g, *V. ervilia* (2n=14); h, *V. tetrasperma* (2n=14); I, *V. akhmaganica* (2n=10); j, *V. armena* (2n=10); k. *V. aucheri* (2n=10); l, *V. persica* (2n=10); m, *V. variegata* (2n=10). The satellites indicate by leaving blanks.

Table 2. The chromosome lengths, Total length and TF% of *Vicia* species. Abreviations, μm: micron; m: metacentric; Sm: s
satellite present.The numbers I-VII correspond different chromosomes measurments.

Species / **Chromosome**	Chromosome I	Chromosome II	Chromosome III	Chromosome IV	Chromosome V	Chromosome VI	Chromosome VII	To
V. ciceroidea	3.20 St	3.04 Sm	2.80 Sm	2.72 Sm	2.49 m	2.22 St	2.18 m	
V. cracca	6.01 m	3.96 Sm	3.79 Sm	3.67 Sm	3.51 Sm	3.12 Sm		
V. monantha	4.83 m	3.36 m	3.14 Sm	2.86 m	2.34 m	2.23 m	1.91 m	
V. multijuga	3.60 Sm	3.29 St	3.10 Sm	2.91 Sm	2.70 Sm	2.45 Sm	2.08 m	
V. variabilis	5.13 Sm*	4.93 St	4.75 Sm	4.56 Sm	4.26 Sm	3.96 Sm	3.66 m	
V. villosa	4.09 Sm	3.76 Sm	3.56 Sm	3.34 m	3.21 m	3.12 m	2.90 Sm	
V. ervilia	6.26 m	5.56 m	5.32 m	5.16 Sm	5.00 Sm	4.50 Sm	3.56 St*	
V. tetrasperma	6.46 m	5.26 Sm	4.87 Sm	4.61 m	4.60 Sm	4.30 Sm	4.12 m	
V. akhmaganica	5.83 St	5.16 m	5.13 Sm	4.62 Sm	4.37 Sm*			
V. armena	6.75 Sm	6.16 m	5.98 Sm	5.49 Sm	5.07 Sm			
V. aucheri	5.60 Sm	5.19 Sm	5.00 m	4.89 Sm	4.71 Sm			
V. persica	5.45 Sm	5.42 m	5.12 Sm	4.77 Sm	4.27 Sm			
V. variegata	5.38 Sm	5.17 m	5.11 Sm	4.78 Sm	4.25 St			

V. cracca has one pair of metacentric and 5 pairs of metacentric chromosomes. *V. monantha* (2n=14) had 6 pairs of metacentric and 1 pair of submetacentric chromosomes that confirms the previous report (Youssef & al. 1996). *V. ervillia* had 3 pairs of metacentric, 3 pairs of submetacentri chromosomes and pair 1 pair of subtelocentric. *V. variabilis* and *V. multijuga* had 1 pair of metacentric, 5 pairs of submetacentric and 1 pairs of subtelocentric chromosomes. *V. villosa* and *V. tetrasperma* had 3 pairs of metacentric and 4 pairs of submetacentric chromosomes.

Among the species with 2n=10 chromosomes *V. armena, V. aucheri, V. persica* and *V. variegata* had only one pair of meta and 4 pairs of submetacentric chromosomes, while *V. akhmaganica* had one pair of subtelocentric chromosomes plus one pair of meta and 3 pairs of submetacentric chromosomes.

In terms of the Stebbins system (1971) the karyotypes of our *Vicia* species are placed in 2A, 3A & 1B classes. The most symmetrical karyotype was observed in *V. monantha* which falls in Stebbins 1B category. Therfore V. monantha is considered as more primitive in this system (table 2).

The most asymmetrical karyotype was observed in the species of sect. *Variegata* (3A) (table 3). Jalilian & Rahiminejad (2012) reported the asymetrical type of chromosomes for *V. aucheri*. Except *V. monantha* other species of the sections Cracca, Ervillia (*V. ervillia*) and Ervum (*V. tetrasperma*) fall in Stebbins 2A category. Jalilian & Rahiminejad (2012) considered *V. ciceroidea* in the 2A category.

The observed basic chromosome numbers are somehow in accordance with the sectional taxonomic treatments applied by Kupicha (1976) & Pakravan (2000) and Jalilian & Rahiminejad (2012).

The five species including *V. armena, V. aucheri, V. akhmaganica, V. persica* and *V. variegata* with 2n= 10 chromosomes had also similar karyotype characters. These results confirm the Jalilian & al. (2014) and Jalilian (2011) which recognized *V. canescens* based on molecular markers as its *sensu lato* with no infra-specific taxa. The close relationships between these taxa have been confirmed by seed sculpturing study (Hosseinzadeh & al. 2008). They have mentioned similar character of seed coat for these species and considered them as a complex species. Therefore these five species have been considered as synonyme of *V. canescens.*

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