Karyotype analysis and new chromosome number reports for nine *Vicia* species in Iran

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Abstract

This study concerns diploid chromosome numbers and karyotype analysis of nine species belonging to four sections (*Vicilla*, *Cracca*, *Variegata* and *Anatropostylia*) of the subgenus *Vicilla* of the genus *Vicia* (*Fabaceae*). The chromosome numbers of *Vicia alpestris* subsp. *hypoleuca*, *V. aucheri*, *V. ciceroideae*, *V. gariensis*, *V. iranica*, *V. koeieana*, *V. rechingeri*, *V. sojakii* and *V. venulosa* are reported here for the first time. The basic chromosome number showed a range of x = 5, x = 6 and x = 7. The majority of the studied taxa were diploid with 2n = 10, 2n = 12 and 2n = 14. In a group of x = 7, three tetraploids species (2n = 28) were determined. Karyologically, the chromosomes observed were mainly from submetacentric and subtelocentric types. Based on Stebbines karyotype classification the studied *Vicia* species were placed in three classes: 2A, 3A & 4A.

Keywords: *Fabaceae*, *Vicia alpestris* subsp. *hypoleuca*, *Vicia aucheri*, *Vicia ciceroideae*, *Vicia gariensis*, *Vicia iranica*, *Vicia koeieana*, *Vicia rechingeri*, *Vicia sojakii*, *Vicia venulosa*

بررسی کاریوتیپی و گزارش جدید کروموزومی نه گونه از جنس *Vicia* در ایران*

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خلاصه

مطالعات سیتوژنتیک روی نه گونه متعلق به چهار بخش (*Vicilla, Cracca, Variegata, Anatropostylia*) از زیرجنس *Vicilla* و جنس *Vicia* از تیره بقولات صورت پذیرفت. سه عدد پایه کروموزومی متفاوت x = ۷، x = ۶ و x = ۵ مشاهده شد، به طوری که اکثر آرایه‌ها دیپلوییـد ۲n = ۱۴، ۲n = ۱۲ و ۲n = ۱۰ بودند و تنها در سه آرایه با عدد پایه کروموزومی x = ۷ تتراپلوییدی مشاهده شد. شمارش کروموزومی برای آرایه‌های *Vicia alpestris* subsp. *hypoleuca*، *V. aucheri*، *V. ciceroideae*، *V. gariensis*، *V. koeieana*، *V. iranica*، *V. rechingeri*، *V. sojakii* و *V. venulosa* برای نخستین بار گزارش می‌شود. کروموزوم‌ها غالبا از نوع ساب‌متاسنتریک و ساب‌تلوسنتریک بودند. برپایه طبقه‌بندی Stebbins، گونه‌های مورد مطالعه در کلاس‌های ۲A، ۳A و ۴A قرار گرفتند.

واژه‌های کلیدی: بقولات، *Vicia alpestris* subsp. *hypoleuca*، *Vicia aucheri*، *Vicia ciceroideae*، *Vicia gariensis*، *Vicia koeieana*، *Vicia iranica*، *Vicia rechingeri*، *Vicia sojakii*، *Vicia venulosa*

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Introduction

Vicia L. (tribe *Vicieae*, family *Fabaceae*), an important element of the Iranian flora, comprising about 150 (Kupicha 1976) to 210 (Hanelt & Mettin 1989) species widely occurring all around the country. It has been suggested that the Mediterranean area is the main centre of diversity of *Vicia* (Naranjo *et al.* 1998). The genus is characterized by having a style pubescent all around, or bearded on the adaxial side, never twisted and stem non winged. Usually the genus has been subdivided into three or four major groups recognized as different taxonomic levels. In his account of *Vicia*, Kupicha (1976) classified it into two subgenera: *Vicilla* (Schur.) Rouy (including 17 sections) and *Vicia* (including five sections); a notion accepted widely.

The genus has been treated by several authors taxonomically in Iran. Boissier (1872), in his account of *Vicia* recognized 24 Iranian species; a number that has been increased to 34 by Parsa (1948), 46 by Chrtková-Žertová (1979) and 39 by Pakravan (2000).

The basic chromosome numbers reported in *Vicia* are: x=5, 6, & 7. Most of the *Vicia* species studied are believed to be diploids with 2n=2x=10, 12, 14; however, a number of tetra- and hexaploids were also reported (Hanelt & Mettin 1989). It was suggested that, the karyotypic variability encountered in the genus is a result of the speciation and evolution process within this taxon (Raina & Rees 1983, Hanelt & Mettin 1989, Maxted *et al.* 1991). Cytologically, the Iranian materials of the genus were examined by Rahiminejad & Ehtemam (2000) and Hesamzadeh & Rasuli (2006). However, there is no cytological report regarding the Iranian *Vicia* endemic species. This study aims to study the mitosis and investigate the basic chromosome number among nine *Vicia* species in which no cytological study has been reported.

Materials and Methods

Plant and seed materials were collected from the distributional range of the species under study all around Iran (Table 1). Voucher specimens are deposited in the herbarium of the University of Isfahan (Isfahan, Iran).

Diploid chromosome numbers were counted from the seedlings' root tips which were grown in Petri dishes. Pretreatment was performed using 0.5% saturated α-Bromo naphthalene at 4° C for 4 h. Root tips were fixed in 10% formaldehyde and 1% chromic acid (1:1) for at least 16 h at room temperature, then were rinsed for 3 h in distilled water and hydrolyzed in 60° C 1 N NaOH for about 10–25 min. Staining was carried out using hematoxylin for 3–4 h. Meristematic tissues were macerated for 10–15 min in cellulose-pectinase enzyme solution at 37° C and squashed in a droplet of 45% acetic acid and lactic acid (10:1) (Wittmann 1965). At least five chromosome spreads for each accession were examined and photographed under light microscope. Chromosomal measurements were done utilizing Micro measure 3.3 software (Reeves & Tear 2000). Karyotypic descriptions were based on Levan *et al.* (1964) and their relevant indicating parameters were that of: Stebbins (1971) (karyotype symmetry); Huziwara (1962) [total form percentage (TF %)]; Romero-Zarco (1986) [value of relative chromatin (VRC), as well as A1 and A2 indices]. The chromosomes were identified according to Levan *et al.* (1964), while other karyotype parameters like, difference of relative length (DRL), of were determined.

Results and Discussion

The results of this study are summarized in Figure 1 and Table 2. As our results showed, the basic chromosome number among the materials studied was varied between x=5, 6 & 7.

Among the species associated with x=5, two diploid (2n=10) species, in the group with x=6, one diploid (2n=12) and in the group with x=7, three diploid (2n=14), three tetraploid (2n=28) exist (Table 2).

The mean value of chromosome long arm (LA) was varied from 2.16 µm in *V. gariensis* to 8.19 µm in *V. iranica.* Averages of chromosomes short arm (SA) were different from 0.95 µm in *V. gariensis* to 2.09 µm in *V. iranica*. Total haploid chromosome length (TCL) was varied from 21.76 µm in *V. gariensis* to 149.68 µm in *V. iranica* and finally the mean value of chromosome

arm ratio (AR) was changing from 2.11 µm in *V. koeieana* to 5.41 µm in *V. iranica* (Table. 2). In terms of the Stebbins system, the karyotypes of our *Vicia* species placed in 2A, 3A & 4A classes, which are considered as primitive classes in this system (Table 2). By using the Romero-Zarco asymmetry indices of A1 and A2 we determined the more asymmetric karyotype among the species which showed the similar Stebbins' classes of symmetry. For example in the species with 3A class, *V. iranica* possesses the highest A1 value (0.74), and almost the lowest DRL value (4.61), accordingly it has a more asymmetric karyotype (Table. 2). The species which are classified as 2A and 3A groups also showed the lowest value of A2 in range of 0.01–0.04 and the highest value of %TF ranged from 19.6 to 34.2 (Table 2). Total haploid chromosome length (TCL) in the studied diploid taxa with x=5 was in the range of 23.95 µm in *V. aucheri* (2n=10) to 34.19 µm in *V. rechingeri* (2n=10), and the same parameter in taxa with x=7 was in the range of 21.76 µm in *V. gariensis* (2n=14) to 39.39 µm in *V. koeieana* (2n=14) (Table 2). In the studied tetraploid taxa with x=7, TCL was in the range of 45.09 µm in *V. sojakii* (2n=28) to 149.68 µm in *V. iranica* (2n=28) (Table 2). The highest VRC (value of relative chromatin) amongst all species was obtained for *V. iranica* and the lowest was obtained for *V. gariensis*.

Vicia venulosa characterized by a pair of satellite on the short arms of chromosome 4 and *V. iranica* had two pairs of satellites which connected to the long arms of chromosomes 6 and 12 (Fig 1B & E).

Based on A1 and TF%, *V. iranica* had the most asymmetrical and evolutionary karyotype and *V. venulosa* had the most symmetrical karyotype. According to A2 and DRL, V. gariensis had the most asymmetrical karyotype (Table 2).

Based on the results of this study, it can be suggested that among the material studied, basic chromosome number approved to be a sectional associated trait as below: sect. *Anatropostylia* (a monotypic section) with 2n=14, x=7; sect. *Cracca* with five studied species and 2n=14 & 28, x=7; sect. *Variegata* with two studied species 2n=10, x=5; and sect. *Vicilla* with one species studied and 2n=12, x=6 (Fig. 1). The observed basic chromosome numbers are to some extent in accordance with the sectional taxonomic treatments applied by Kupicha (1976) and Pakravan (2000). The only exception is the common x=7 between sections *Anatropostylia* and *Cracca*. Reviewing the reported *Vicia* chromosome counts (Goldblat & Johnson 1979) showed that our species specific observations are mostly in accordance with their co-sectional species as Kupicha's classification (Kupicha 1976).

Table 1. Localities of the *Vicia* species studied

Subgenus	Section	Species	Locality	No.
Vicilla	*Vicilla*	*V. venulosa*	Tehran: Road of Firoozkuh to Semnan	7633
Vicilla	*Cracca*	*V. iranica*	Hamadan: Road of Hamadan to Malayer	7617
Vicilla	*Cracca*	*V. ciceroideae*	Tehran: Dizin	7609
Vicilla	*Cracca*	*V. sojakii*	Mazandaran: Golestanak	7630
Vicilla	*Cracca*	*V. alpestris* subsp. *hypoleuca*	Azerbayejan: Khoy, Qotur	7605
Vicilla	*Cracca*	*V. gariensis*	Hamadan: Nahavand, Garin mountain	53922
Vicilla	*Anatropostylia*	*V. koeieana*	Isfahan: Fereydoon-shahr, Posht kuh	7619
Vicilla	*Variegata*	*V. rechingeri*	Azerbayejan: Tabriz, Kandavan	7646
Vicilla	*Variegata*	*V. aucheri*	Azerbayejan: 30 km N. of Sarab, Sabal mountain	7648

Table 2. Karyotype features of the *Vicia* species studied

Species	Pl. level	2n	TCL	LA	SA	MCL =VRC	AR	ST	DRL	TF%	A_1	A_2	KF
V. venulosa	2X	12	28.22	2.94	1.74	4.7	2.13	3A	7.3	34.2	0.44	0.01	2m + 3sm + 1st
V. iranica	4X	28	149.68	8.19	2.09	10.69	5.41	3A	4.61	19.6	0.74	0.01	2m + 1sm + 10st
V. ciceroideae	4X	28	45.09	2.19	1.03	3.22	2.15	2A	4.47	32	0.51	0.03	3m +11sm
V. sojakii	4X	28	64.5	3.21	1.39	4.6	2.42	3A	3.41	30.2	0.54	0.01	3m + 8sm + 3st
V. alpestris subsp. *hypoleuca*	2x	14	22.56	2.27	0.96	3.22	2.38	3A	5.57	29.7	0.56	0.04	7sm
V. gariensis	2X	14	21.76	2.16	0.95	3.1	2.29	4A	5.41	30.4	0.56	0.09	7sm
V. koeieana	2X	14	39.39	3.65	1.85	5.63	2.11	3A	3.96	32.9	0.48	0.02	2m + 4sm + 1st
V. rechingeri	2x	10	34.19	4.79	2.05	6.84	2.45	3A	3.88	30	0.56	0.03	4sm + 1st
V. aucheri	2x	10	23.95	3.34	1.45	4.79	2.47	3A	4.69	30.2	0.54	0.03	1m + 3sm + 1st

PL: Ploidy level, 2n: Somatic chromosome number, ST: Symmetry classes of stebbines, TCL: total haploid chromosome length, LA: Mean long arm, SA: Mean short arm, MCL: Mean haploid chromosome length, VRC: Value of relative chromatin, AR: Mean arm ratio, KF: Karyotype formula (m: metacentric, st: submetacentric, st: subtelocentric), TF%: Total form percentage, DRL: Different of relative length, VRC: Value of relative chromatin, A_1: Intrachromosome asymmetry index, A_2: Interchromosome asymmetry index

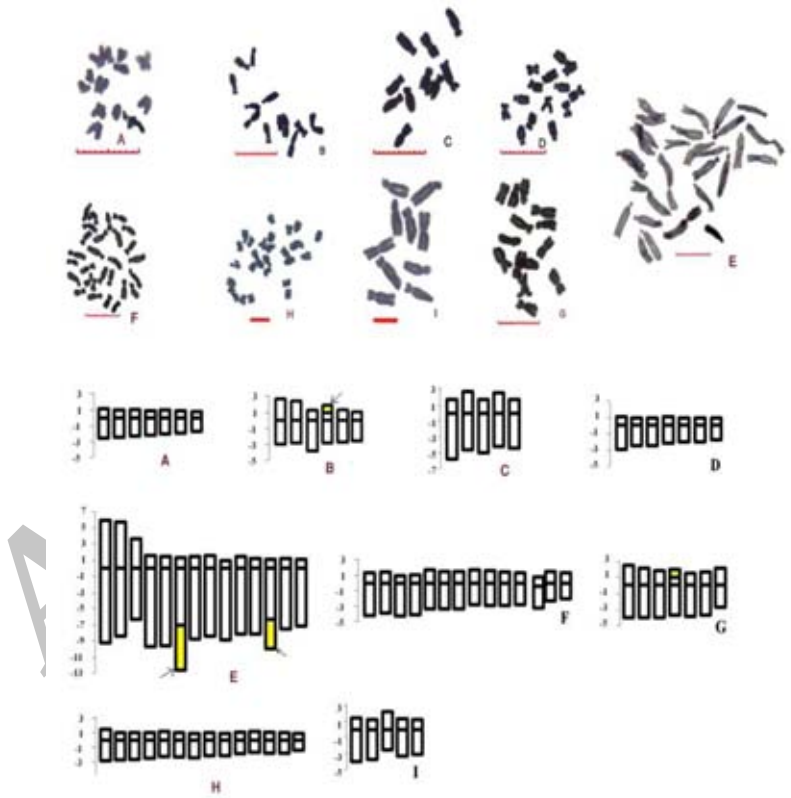

Fig. 1. Mitotic metaphase and ideograms in *Vicia* species studied: A. *V. gariensis*, B. *V. venulosa*, C. *V. rechingeri*, D. *V. alpestris* subsp. *hypoleuca*, E. *V. iranica*, F. *V. sojakii*, G. *V. koeieana*, H. *V. ciceroideae*, I. *V. aucheri* (Bar = 10 µm, arrow: Sattelite).

There are some species specific chromosomal traits for the materials studied. The results of this study showed a positive correlation between the ploidy level and TCL however, no consistency was found among the MCL and ploidy levels. In the same way no relation was observed between the AR and DRL and polidy levels. Hanelt & Mettin (1989) reported that meta- and submetacentric chromosomes are the dominant chromosomal forms in the subgenus *Cracca* (subgenus *Vicilla* in Kupica sense). There is an inconsistency between our results and this opinion. The observations of this study revealed that sm (62%) and st (22%) chromosomal shapes predominant other forms (Fig. 1 and Table 2).

This study showed that mostly in the complements examined the shortest chromosomes were the stones and the longest belonged to m types, this is in accordance with the Robertsonian translocation theory (Raina & Rees 1983). Despite the same basic chromosome number revealed in both sections *Anatropostylia* and *Cracca* however, their karyotypic formula separated them clearly, the lowest value of the ratio of the longest to the shortest arms of the chromosome was seen in *V. koeieana* (AR = 2.11). The multi-basic chromosome number is a common phenomenon in the genus *Vicia*, which was assigned as Robertsonian translocation (Raina & Rees 1983). Regarding the parameters presented in Table. 2 do not confirm this suggestion however, the number of species examined in this study is not sufficient to make a general conclusion about this opinion.

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