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## Karyotype of Several *Vicia* Species from Iran

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**Abstract:** The genus *Vicia* L. (Fabaceae) comprises approximately 190 species in the world. It includes 47 annual or perennial species in Iran. The karyotype of two species and two subspecies of the genus from Northwest Iran were investigated. Aceto-iron-hematoxilin was used to stain chromosomes. Chromosome characteristics including number, long arm, short arm and lengths, total length of chromosome set, arm ratio index and relative chromosome length were measured using the Micromeasure software. Results showed that these species were different in chromosome number, karyotype formula and karyotype characteristics such as *V. villosa* ( $2n = 2x = 14$ ), *V. hyrcanica* ( $2n = 2x = 12$ ), *V. sativa* subsp. *sativa* ( $2n = 2x = 12$ ) and *V. sativa* subsp. *nigra* ( $2n = 2x = 12$ ).

**Key words:** Aceto-Iron-hematoxilin staining, Karyotype, *Vicia*

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### INTRODUCTION

The genus *Vicia* L. (Fabaceae) comprises approximately 190 species in the world (ILDIS, 1999). The genus mostly located in Europe, Asia and North America, extending to the temperate regions of south America and tropical Africa (Maxted, 1993). Most of them are diploids with a basic number  $x = 5, 6$  or  $7$  (Maxted *et al.*, 1991; Darlington *et al.*, 1945) while only six of them are polyploids (Cremonini *et al.*, 1992). The chromosome numbers of half of the 166 species are known (Maxted, 1993; Maxted *et al.*, 1991).

Maxted *et al.* (1991) have divided the genus into two subgenera, including *Vicilla* and *Vicia*. The subgenus *Vicilla* divided into 17 sections and the subgenus *Vicia* into 9 sections. Most of these species are annual, but a few of them belonging to the section *Cracca* are perennial (Yamamoto, 1973). Cytological investigations have mainly concerned the determination of chromosome number and the examination of chromosome morphology (Maxted *et al.*, 1991; Sahin and Babag, 1990). Karyological studies have played an important role in solving taxonomic problems (Goday and Pimpinelli, 1986).

The objective of this study was construction of a detailed karyotype analysis of *Vicia villosa*, *Vicia hyrcanica*, *Vicia sativa* L. subsp. *sativa*, *Vicia sativa* L. subsp. *nigra* and *Vicia lathyroides* using aceto-iron-hematoxilin staining method.

### MATERIALS AND METHODS

Seed samples of vetches were collected from different parts of rangelands and farmlands of the Ardabil province (Northwest of Iran) in 2006. These vetches include *Vicia villosa* L., *Vicia hyrcanica* Fish and Mey., *Vicia sativa* L. subsp. *sativa* and *Vicia sativa* L. subsp. *nigra*. These vetches were identified according to Flora of Turkey (Davis, 1988). Seeds of vetches were germinated on moist blotting paper and the root tips were obtained. For karyotype analyses they were pretreated with 0.05% colchicines for 3 h at 25°C, then fixed in 1:1 (v/v) solution of formalin 10% and chromic acid 1% for 36 h at 4°C and stored in 70% ethanol at -20°C. The root tips were hydrolyzed with 1 N NaOH at 60°C for 8 min. They were stained with Aceto-iron-hematoxilin for 12 h at 25°C. After each step root tips were washed briefly in distilled water. Meristematic region with 1 mm of length excised and macerated in cytase enzyme at room for 2 h. Squash preparations on slides were made in 45% acetic acid (Asghari-Zakaria *et al.*, 2002).

Chromosomes were measured for long arm, short arm, chromosome lengths, total length of chromosome set, arm ratio index and relative chromosome length, on 10 enlarged well-spread metaphase cells, using Micromeasure software developed by the department of Colorado State University available on Internet At <http://www.colostate.edu/Depts/Biology/Micromeasure>.

Homologous chromosomes were identified based on the position of centromers. The nomenclature followed by Levan *et al.* (1964).

**RESULTS AND DISCUSSION**

***Vicia villosa*:** Mitotic chromosomes are shown in Fig. 1. Karyotypic characters of the mitotic chromosomes are shown in Table 1. The analysis of karyotype showed that this species has  $2n = 2x = 14$  chromosomes (Fig. 1). Chromosome length in *Vicia villosa* ranged from 4.53  $\mu\text{m}$  in chromosome 7 to 5.89  $\mu\text{m}$  in chromosome 1. Furthermore, arm ratio index values ranged from 1.46 in chromosome 7 to 2.52 in chromosome 5 (Table 1). The ratio between the largest and the smallest chromosome was 1.30:1. The karyotype is made of seven pairs of chromosomes (one metacentric and six sub-metacentrics).

Chromosome 7 (SAT chromosome), a secondary constriction near centromeric region. With the viewpoint of Stebbins (1971) symmetry class, *Vicia villosa* fell in A2 category and showed the most symmetric karyotype.

***Vicia sativa* L. subsp. *sativa*:** Mitotic chromosomes are shown in Fig. 2. Karyotypic characters of the mitotic chromosomes are shown in Table 2. The analysis of karyotype showed that this species has  $2n = 2x = 12$  chromosomes (Fig. 2). Chromosome length in *Vicia sativa* L. subsp. *sativa* ranged from 2.89  $\mu\text{m}$  in chromosome 6 to 5.69  $\mu\text{m}$  in chromosome 1. Furthermore, arm ratio index values ranged from 1.38 in chromosome 4 to 6.07 in chromosome 2 (Table 2). The ratio between the largest and the smallest chromosome was 1.97:1. The karyotype is made of six pairs of chromosomes (one metacentric and five sub-acrocentrics).

Navratilova *et al.* (2003) showed that the karyotype of *Vicia sativa* L. subsp. *sativa* composed of one

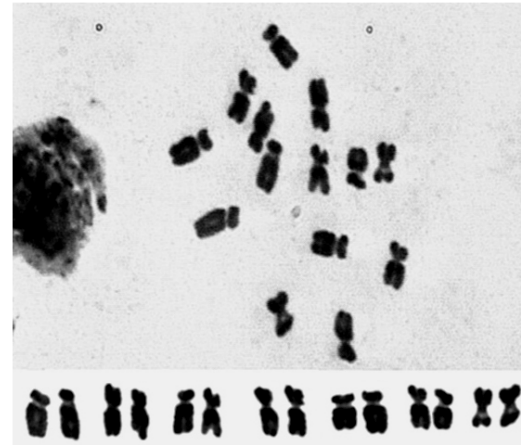


Fig. 1: Somatic metaphase chromosomes of *Vicia villosa* stained with aceto-iron-hematoxilin



Fig. 2: Somatic metaphase chromosomes of *Vicia sativa* L. subsp. *sativa* stained with aceto-iron-hematoxilin

Table 1: The mean karyotypic characters of seven mitotic chromosomes in *Vicia villosa*

Chr.	Type	Chromosome ( $\mu\text{m}$ )	Relative length (%)	Long arm ( $\mu\text{m}$ )	Short arm ( $\mu\text{m}$ )	Arm ratio
1	sm	5.89±0.28	16.42±0.49	4.21±0.22	1.68±0.07	2.52±0.09
2	sm	5.43±0.16	15.28±0.56	3.52±0.18	1.92±0.08	1.88±0.16
3	sm	5.30±0.18	14.84±0.28	3.59±0.13	1.71±0.07	2.11±0.07
4	sm	5.03±0.14	14.14±0.47	3.15±0.08	1.88±0.07	1.69±0.06
5	sm	4.95±0.22	13.82±0.39	3.55±0.19	1.40±0.05	2.52±0.09
6	sm	4.57±0.16	12.79±0.26	2.99±0.11	1.59±0.07	1.91±0.09
7*	m	4.53±0.14	12.71±0.41	2.67±0.11	1.86±0.08	1.46±0.10

\*SAT-chromosome

Table 2: The mean karyotypic characters of six mitotic chromosomes in *Vicia sativa* subsp. *sativa*

Chr.	Type	Chromosome ( $\mu\text{m}$ )	Relative length (%)	Long arm ( $\mu\text{m}$ )	Short arm ( $\mu\text{m}$ )	Arm ratio
1	sa	5.69±0.13	19.73±0.37	4.74±0.14	0.95±0.03	5.02±0.21
2*	sa	5.61±0.22	19.37±0.53	4.79±0.17	0.81±0.06	6.07±0.31
3*	sa	5.18±0.23	17.88±0.58	4.17±0.19	1.01±0.06	4.18±0.17
4	m	4.98±0.14	17.23±0.26	2.88±0.08	2.10±0.08	1.38±0.04
5	sa	4.56±0.17	15.76±0.38	3.59±0.10	0.96±0.07	3.87±0.22
6	sa	2.89±0.09	10.02±0.21	2.18±0.06	0.72±0.03	3.06±0.09

\*SAT-chromosome

Table 3: The mean karyotypic characters of six mitotic chromosomes in *Vicia sativa* subsp. *nigra*

Chr.	Type	Chromosome ( $\mu\text{m}$ )	Relative length (%)	Long arm ( $\mu\text{m}$ )	Short arm ( $\mu\text{m}$ )	Arm ratio
1	sm	8.28 $\pm$ 0.50	21.74 $\pm$ 0.54	6.13 $\pm$ 0.41	2.16 $\pm$ 0.10	2.82 $\pm$ 0.09
2	sa	7.64 $\pm$ 0.29	20.29 $\pm$ 0.41	5.99 $\pm$ 0.19	1.64 $\pm$ 0.11	3.72 $\pm$ 0.13
3	sa	7.23 $\pm$ 0.45	19.03 $\pm$ 0.59	5.99 $\pm$ 0.42	1.24 $\pm$ 0.05	4.81 $\pm$ 0.25
4	sa	5.54 $\pm$ 0.25	14.70 $\pm$ 0.33	4.37 $\pm$ 0.22	1.18 $\pm$ 0.04	3.70 $\pm$ 0.12
5*	sa	5.13 $\pm$ 0.25	13.61 $\pm$ 0.43	4.12 $\pm$ 0.22	1.00 $\pm$ 0.05	4.15 $\pm$ 0.21
6	sm	4.00 $\pm$ 0.18	10.63 $\pm$ 0.34	2.91 $\pm$ 0.16	1.09 $\pm$ 0.03	2.65 $\pm$ 0.11

\*SAT-chromosome

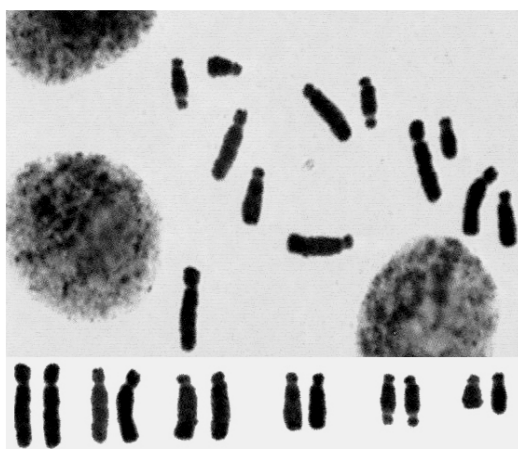


Fig. 3: Somatic metaphase chromosomes of *Vicia sativa* L. subsp. *nigra* stained with aceto-iron-hematoxylin

metacentric, four subacrocentrics and one acrocentric chromosome. It seems that they used another criterion for naming chromosomes.

Chromosome 2 and Chromosome 3 (SAT chromosomes), are distinguishable from other chromosomes through a satellite located on the telomeric region of their long arm. In the viewpoint of Stebbins (1971) symmetry class, *Vicia sativa* L. subsp. *sativa* fell in A3 category.

***Vicia sativa* L. subsp. *nigra*:** Mitotic chromosomes are shown in Fig. 3. Karyotypic characters of the mitotic chromosomes are shown in Table 3. The analysis of karyotype showed that this species has  $2n = 2x = 12$  chromosomes (Fig. 3). Chromosome length in *Vicia sativa* subsp. *nigra* ranged from 4  $\mu\text{m}$  in chromosome 6 to 8.28  $\mu\text{m}$  in chromosome 1. Furthermore, arm ratio index values ranged from 2.65 in chromosome 6 to 4.81 in chromosome 3 (Table 3). The ratio between the largest and the smallest chromosome was 2.07:1. The karyotype is made of six pairs of chromosomes (two submetacentrics and four subacrocentrics).

Weber and Schifino-Wittmann (1999) have shown that the karyotype of *Vicia sativa* subsp. *nigra* (synonym: *Vicia angustifolia* L.) composed of six acrocentric chromosomes and Chromosome 5 has a secondary



Fig. 4: Somatic metaphase chromosomes of *Vicia hyrcanica* stained with aceto-iron-hematoxylin

constriction in the long arm. The results of this study revealed that the genome of this species has six pairs of chromosomes (two submetacentrics and four subacrocentrics) and Chromosome 5 had a secondary constriction in the long arm. It seems that they used another criterion for naming chromosomes.

Schifino-Wittmann *et al.* (1994) described *V. angustifolia* with  $2n = 14$  but no such chromosome number was found in accessions included in this study.

Chromosome 5 (SAT chromosomes), are distinguishable from other chromosomes through a satellite located in the telomeric region of the long arm. In the viewpoint of Stebbins (1971) symmetry class, *Vicia sativa* subsp. *nigra* fell in B4 category. *Vicia sativa* subsp. *nigra* showed the most asymmetric karyotype.

***Vicia hyrcanica*:** Mitotic chromosomes are shown in Fig. 4. Karyotypic characters of the mitotic chromosomes are shown in Table 4. The analysis of karyotype showed that this species has  $2n = 2x = 12$  chromosomes (Fig. 4). Chromosome length in, *Vicia hyrcanica* ranged from 8.37  $\mu\text{m}$  in chromosome 6 to 11.60  $\mu\text{m}$  in chromosome 1. On the other hand, arm ratio index values ranged from 2.54 in chromosome 1 to 3.40 in chromosome 5 (Table 4). The ratio between the largest and the smallest chromosome was 1.39:1, *Vicia hyrcanica* has the largest genome

Table 4: The mean karyotypic characters of six mitotic chromosomes in *Vicia hyrcanica*

Chr.	Type	Chromosome (µm)	Relative length (%)	Long arm (µm)	Short arm (µm)	Arm ratio
1	sm	11.60±0.30	20.40±0.16	8.32±0.24	3.28±0.08	2.54±0.05
2*	sm	10.16±0.33	17.86±0.34	7.35±0.26	2.81±0.11	2.64±0.09
3	sm	9.35±0.18	16.46±0.23	6.88±0.16	2.47±0.07	2.80±0.10
4	sa	8.93±0.17	15.73±0.19	6.71±0.14	2.22±0.08	3.05±0.13
5	sa	8.47±0.30	14.86±0.32	6.53±0.23	1.93±0.08	3.40±0.09
6	sm	8.37±0.26	14.71±0.28	6.22±0.20	2.15±0.06	2.90±0.05

\*SAT-chromosome

Table 5: Karyotypic characters of four species and subspecies in *Vicia*

Species	Chromosome (No.)	Formula karyotype	SAT position	Total length of haploid chromosome	Symmetry class	Ploidy level
<i>V. villosa</i>	14	6s.m + 1m	7L	35.70	A2	Diploid
<i>V. sativa</i> ssp. <i>sativa</i>	12	5s.a + 1m	2L, 3L	28.91	A3	Diploid
<i>V. sativa</i> ssp. <i>nigra</i>	12	4s.a + 2s.m	5L	37.82	B4	Diploid
<i>V. hyrcanica</i>	12	4s.m + 2s.a	2L	56.87	A4	Diploid

among the species investigated in this study. It possesses six pairs of chromosomes (four submetacentrics and two subacrocentrics).

Chromosome 2 (SAT chromosome), had a secondary constriction near centromeric region. Yamamoto (1973) showed that in *V. hyrcanica* (2n = 12) the total chromosome length was long. One long subterminal chromosome and one middle sized submedian chromosome which had a long satellite connected to the short arm were observed. The rest of the chromosomes were all subterminal. In the viewpoint of Stebbins (1971) symmetry class, *Vicia hyrcanica* fell in A4 category (Table 5).

### CONCLUSIONS

The results of this study revealed that these species were different in chromosome number, karyotype formula and karyotype characteristics.

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