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Research Note

Giemsa C-Banded Karyotypes of *Vicia cracca* L. subsp. *cracca* and *V. bithynica* L.

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Abstract: The distribution of heterochromatin on the chromosomes of *V. cracca* L. subsp. *cracca* (2n = 14) and *V. bithynica* L. (2n = 14) was established with the aid of the C-banding technique. The chromosomes of both species have centromeric C-bands. When compared, prominent bands at the ends of the short arms on 4 chromosomes of *V. cracca* subsp. *cracca* were observed, while in *V. bithynica* only 1 chromosome had a prominent band at the end of the long arm. The C-banding patterns in the 2 species were different, and it was determined that heterochromatic regions were more dense on the chromosomes of *V. cracca* subsp. *cracca*. The present results are compared with previously published data on Vicia species.

Key Words: *Vicia*, karyotype, C-banding, heterochromatin.

Vicia cracca L. subsp. *cracca* ve *V. bithynica* L.'nın Giemsa C-Band Karyotipleri

Özet: *Vicia cracca* L. subsp. *cracca* (2n = 14) ve *V. bithynica* L. (2n = 14)'nın kromozomlarında heterokromatin dağılımı C-band tekniği ile tespit edildi. Her iki türün tüm kromozomları sentromeric C- bandlarına sahiptir. *V. bithynica*'nın sadece bir kromozomunda kalıcı bantlar varken *V. cracca* subsp. *cracca*'nın dört kromozomunda kalıcı bandlar mevcuttur. İki türdeki C-band modelleri farklıdır ve *V. cracca* subsp. *cracca*'nın kromozomlarında heterokromatin bölgelerinin daha yoğun oldunu tespit edildi. Mevcut sonuçlar daha önce yayınlanmış olan verilerle tartışıldı.

Anahtar Sözcükler: *Vicia*, karyotip, C-bandı, heterokromatin.

Introduction

The genus *Vicia* L. (*Fabaceae*) consists of 166 species distributed mainly in Europe, Asia and North America, extending to the temperate regions of South America and tropical Africa (Maxted, 1993). The genus was grouped in 4 major species clusters *Cracca* S.F.Gray, *Vicia, Ervum* (L.) S.F.Gray and *Faba* Aschers. & Graebn., by Kupicha (1976). Maxted et al. (1991) the genus divided into 2 subgenera, *Vicilla* (Schur) Rouy. and *Vicia*, with the subgenus *Vicilla* divided into 17 sections, and the subgenus *Vicia* divided into 9 sections. Most of these species are annuals, but a few of them belonging to the section *Cracca* are perennials (Yamamoto, 1973). In Turkey, 64 species, 22 subspecies and 18 varieties of this genus have been recorded (Davis & Plitmann, 1970; Vural, 2000).

There are many taxonomic problems in the genus *Vicia*, which is poorly delimited from *Lathyrus* L. and *Lens* Mill. The species in the sect. *Faba* have similarities to *Lathyrus*. *Vicia crocea* (Desf.) B.Fedtsch. is readily mistaken for *Lathyrus aureus* (Stev.) Brandza. *V. cappadocica* Boiss. & Ball. also constitutes a problem, with similarities to both *Vicia* and *Lathyrus*. Many of the species are highly variable both genetically and in response to environmental differences (Davis & Plitmann, 1970).

In terms of morphological characteristics, *V. koeiana* Rech.f. is very similar to taxa of the sect. *Cracca* and it is also similar to some species of the genera *Lathyrus* and *Lens* with regard to style characteristics (Davis & Plitmann, 1970). Later, Kupicha (1976) raised this species to the level of monotypic genus and then added it to the sect. *Anatropostylia* Plitm. of the genus *Vicia*. *Vicia noeana* Reut. ex Boiss. var. *megalondonta* Rech. is similar to *V. hyrcanica* Fisch. & C.A.Mey. but with some different charactistics in the calyx and leaflets so that sometimes they are confused with each other.

In recent years, karyological studies have played an important role in solving taxonomic problems. The karyotypes of some *Vicia* species are presented in Table 1.

The traditional way of making cytogenetic comparisons between related species is to stain chromosomes with dyes such as Feulgen, orcein or carmine. Great advances have been made in cytogenetics by the introduction of much more precise staining methods such as C-banding, silver staining and fluorescence differential staining for examining chromosome banding patterns. These techniques are particularly useful for comparing the content, location and composition of heterochromatic regions of the chromosomes belonging to closely related species (Goday & Pimpinelli, 1986).

Table 1. List of previous karyological investigations on some *Vicia* L.

Taxa	Origin of the material	Chromosome numbers (2n)	Author(s)
Vicia cracca L. subsp. *cracca*	Turkey	14	Şahin & Babaç, 1990
			Beyazoğlu & Hayırlıoğlu, 1991
			Akpınar & Bilaloğlu, 1997
			İnceer et al., 2002
V. tetrasperma (L.) Schreb. Spic.	Czechoslovakia	14	Löve, 1975
	Australia	14	Yamamoto, 1973
	Poland	14	Kuta, 1980
	Turkey	14	Şahin & Babaç, 1990
			Tabur et al., 2001
			İnceer et al., 2002
V. villosa Roth.	Iceland	14	Löve & Löve, 1956
	East Germany	14	Yamamoto, 1973
	Poland	14	Kuta, 1980
V. dasycarpa Ten.	Australia	14	Yamamoto, 1973
	Poland	14	Kuta, 1980
V. hirsuta (L.) S.F.Gray	Japan	14	Yamamoto, 1973
	Iceland	14	Löve & Löve, 1956
	Poland	14	Kuta, 1980
	Turkey	14	Beyazoğlu & Hayırlıoğlu, 1991
			Tabur et al., 2001
			İnceer et al., 2002
	Poland	14	Kuta, 1980
	Bulgaria	14	Terziiski & Dimitrov, 1983
V. sepium L.	Iceland	14	Löve & Löve, 1956
	Denmark	14	Larsen, 1954
	France	14	Larsen, 1954
	Poland	14	Kuta, 1980
	Turkey	14	Akpınar & Bilaloğlu, 1997
			İnceer et al., 2002
V. hyrcanica Fish. & Mey.	East Germany	12	Yamamoto, 1973
	Turkey	12	Akpınar & Bilaloğlu, 1997
	Turkey	12	İnceer et al., 2002
V. melanops Sibth. Sm.	East Germany	10	Yamamoto, 1973
	Turkey	10	İnceer et al., 2002
V. galilaea Plitm. & Zoh.	Israel	14	Yamamoto, 1986
	Turkey	14	Şahin & Babaç, 1995
V. bithynica L.	East Germany	14	Yamamoto, 1973
	Turkey	14	İnceer et al., 2002

Chromosome banding is a valuable tool for identifying homologous chromosomes in karyologically similar species and especially for detecting chromosomal rearrangements that contribute to karyotype evolution. A number of attempts have been made to reconstruct karyotype phylogenies of various taxa based on banding pattern analysis. Giemsa C-banding indicates the presence of constitutive heterochromatin at different locations on chromomomes.

To sum up, problems related to the genus *Vicia* in Turkey have not been solved. In an attempt to provide more cytological details on the taxa, based on karyotypic analysis of 2 *Vicia* taxa from north-east Anatolia, Giemsa C-banding patterns were studied. As a consequence, this study provides a contribution to the knowledge of the phylogeny of the genus.

Materials and Methods

All the plants used in the study were collected from natural populations. Voucher specimens are kept at the Herbarium KTUB. Localities of the examined taxa and populations are as follows:

Vicia cracca L. subsp. *cracca*, A7 Trabzon: Zigana Dağı, open slopes, roadsides, 1700 m, 14.viii.2003, *İnceer* 182.

V. bithynica L., A7 Trabzon: KTU Kanuni Kampüsü, 30 m, 15.v.2003, *İnceer* 179.

Seeds were germinated on moist filter paper, and for Feulgen staining the root tips were cut off and pre-treated with 0.05% colchicine for 3-4 h and then fixed in ethanol-acetic acid (3:1) for at least 24 h at 4 °C (İnceer et al., 2002). The root tips were hydrolysed in 1 N HCl at 60 °C for 13-15 min and then rinsed with tap water for a minimum of 5-6 min. Staining was carried out in Feulgen for 1 h and squash preparations were made.

For C-banding with Giemsa, the root tips were pre-treated with 0.05% colchicine for 4-5 h at room temperature and then fixed as above. They were rinsed in distilled water and then softened by incubation in 1% aqueous cellulase-pectinase for 20-30 min. They were subsequently dissected and macerated in 45% acetic acid. The cover slip was removed with the aid of CO_2 freezing and the slides were then immersed in absolute ethanol for 5 min, prior to transferring to acetic acid for 20 s, air-dried and stored overnight at 60 °C. The next day, the slides were transferred to 0.2 N HCl for 1 h, then to 5% $Ba(OH)_2$ for 5 min, rinsed in running tap water, treated with 2 x SSC at 60 °C for 1 h, rinsed with deionised water for 15 min, and immediately stained in a 3% Giemsa solution until well stained. After rinsing with deionised water, the slides were air-dried and a cover slip mounted using Entellan.

The best metaphase plates of each specimen, normally at least 3, were photographed and idiograms (Figure 3) prepared from enlarged prints by measuring the total length of the chromosomes and their arms and satellites. The centromeric index (r) was then calculated as the long to short arm ratio and chromosomes classified according to Levan et al. (1964).

Results and Discussion

Distinct darkly stained heterochromatin spots were observed at prophase in both *Vicia* taxa studied, indicating the likelihood that the chromosomes would exhibit C-bands. Descriptions of the banding patterns and general morphology of the chromosomes were undertaken at metaphase.

All chromosomes in both taxa had C-bands at the centromeric region. Several chromosomes also showed C-bands at the telomeric ends. These telomeric bands appeared mostly as dot-like structures at the ends of the chromosome. Interstitial bands were seen on some chromosomes.

Figure 1a shows the Feulgen stained chromosomes of *V. cracca* subsp. *cracca* and Figure 2a the Giemsa banded chromosomes. Figure 3a shows a high degree of symmetry consisting of 12 submetacentric and 2 metacentric chromosomes, of which 2 have satellites on the short arms. All the chromosomes had centromeric C-bands. Chromosomes 1, 2, 5 and 6 all have a prominent band at the ends of the short arms. Chromosome 3 has a frequent dot-like structure in the middle of the short arm and chromosome 7 has a weaker band on the tip of the short arm. The long arm of chromosome 4 clearly shows the nucleolar organising region (NOR).

Figure 1b shows the Feulgen stained chromosomes of *V. bithynica* and Figure 2b those stained with Giemsa. Figure 3b shows a high degree of asymmetry in the karyotype, which consists of 4 submetacentrics, 2 subacrocentrics, and 8 acrocentric chromosomes, and 2

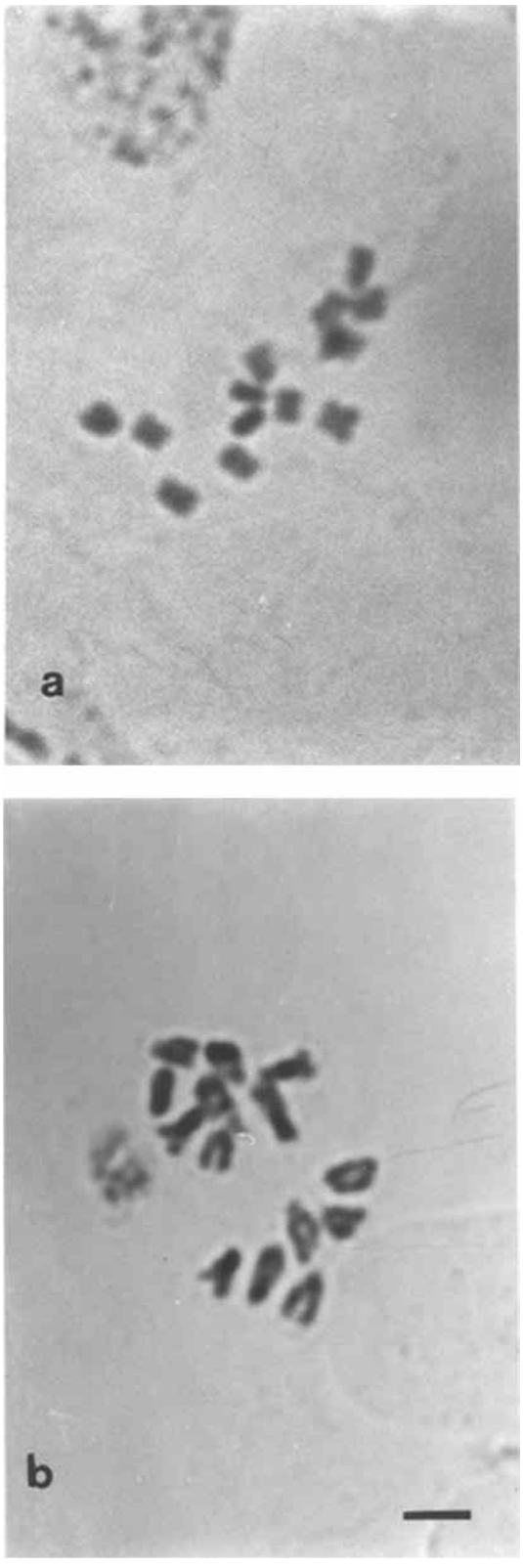

Figure 1. Feulgen-stained metaphase plates. a: *V. cracca* subsp. *cracca*, 2n = 14. b: *V. bithynica*, 2n = 14. Scale bar = 5 µm.

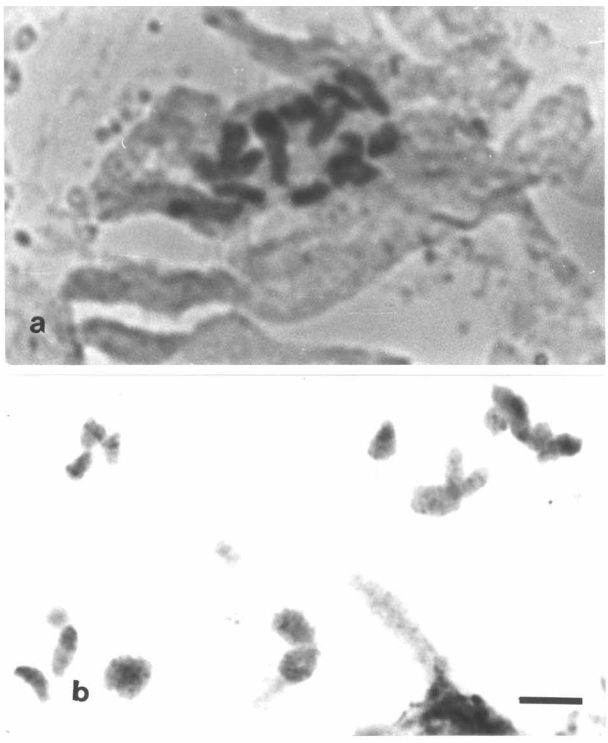

Figure 2. Giemsa-stained metaphase plates. a: *V. cracca* subsp. *cracca*, 2n = 14. b: V. bithynica, 2n = 14. Scale bar = 5 µm.

have satellites on the short arms. Again, all the chromosomes have centromeric C-bands. In the short arm of chromosome 6, there is a clear secondary constriction that is apparently an NOR. The NOR and satellites are frequently associated with the nucleolus in metaphase cells. Chromosome 1 has weaker bands on both the short arm and the long arm. Chromosome 5 has terminal dots on the short arm. Other chromosomes only have bands in the centromeric regions.

Our results show different banding patterns in the 2 species, which agrees with those obtained by Hizume et al. (1980) and Hayırlıoglu-Ayaz et al. (1999) for different *Vicia* species.

Diagnostic centromeric C-bands were identified in the *Vicia* taxa studied in the present work. With some differences between the taxa, the C-bands in *V. cracca* subsp. *cracca* stained more intensely than those in *V. bithynica*. Therefore, *V. cracca* subsp. *cracca* has more dense heterochromatic regions on its chromosomes.

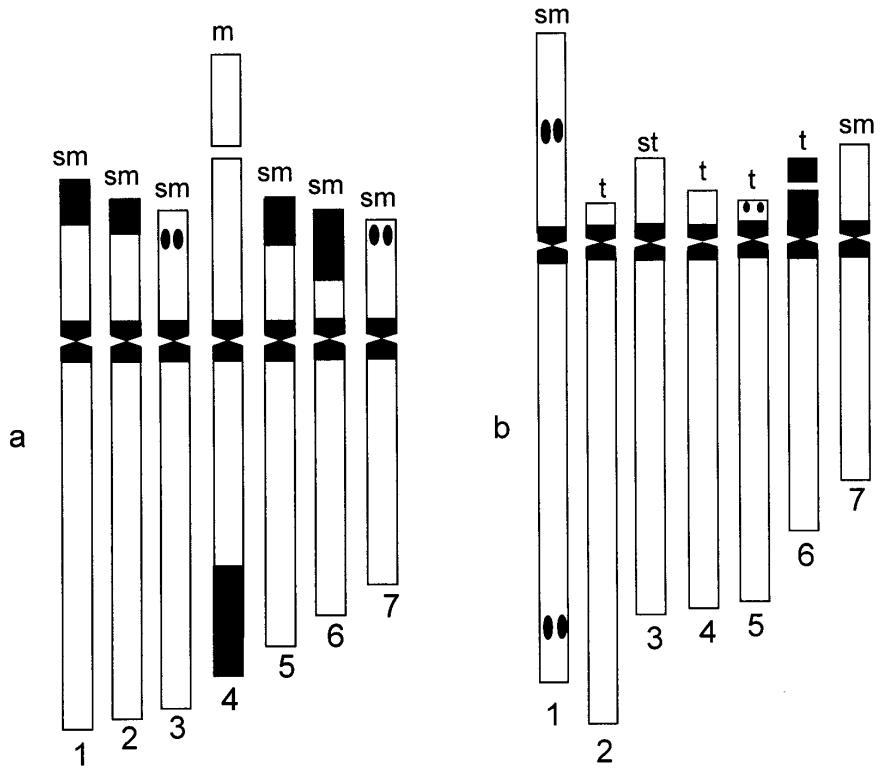

Figure 3. Haploid idiograms. a: *V. cracca* subsp. *cracca*, b: *V. bithynica*

In *Vicia*, as in many other genera of flowering plants, speciation is accompanied by large changes in chromosome size and DNA amount. The variations in total nuclear DNA amount in the chromosome complement within the genus *Vicia* were distinctively quantified by Raina & Rees (1983). Nuclear DNA content (2C) was reported for *V. hybrida* L. (13.55 pg), *V. sativa* L. (4.50 pg), *V. cracca* (10.60 pg) and *V. bithynica* (9.15 pg) (Bennett & Smith, 1976). Later, the nuclear DNA contents of *V. tetrasperma* and *V. hirsuta* were found to be 7.20 pg and 7.95 pg, respectively (Bennett & Leitch, 1995). Hayırlıoğlu-Ayaz et al. (1999) reported the most intensive C-banding patterns in *V. hybrida* with regard to the slight C-banding patterns in *V. sativa*. In the present study, we detected more intensive C-banding patterns in *V. cracca* subsp. *cracca* than in *V. bithynica*. In the light of cytogenetical data, we suggest that there is a considerable correlation between nuclear DNA content and C-banding in *Vicia* species.

It is well known that the chromosome karyotype can often contribute a great deal to the interpretation of phylogenetic relationships between different taxonomic groups (Mariani & Falistocco, 1990). The results of this study help to clarify the chromosomal differences between *V. cracca* subsp. *cracca* and *V. bithynica* with regard to the amount and location of heterochromatin. Once these banding patterns have been established, in other species a more detailed analysis of the evolutionary relationships of the species will be possible.

References

Akpınar N & Bilaloğlu R (1997). Cytological investigations of certain species of *Vicia* L. *Turk J Biol* 21: 197-207.

Beyazoğlu O & Hayırlıoğlu S (1991). Karyotype analysis of some *Vicia* species in Turkey. *La Kromosomo* II-63-64: 2143-2148.

Davis PH & Plitmann U (1970). *Vicia* L. In: Davis PH (ed.) *Flora of Turkey and the East Aegean Islands*, Vol. 3, pp. 274-325. Edinburgh: Edinburgh University Press.

Goday C & Pimpinelli S (1986). Cytological analysis of chromosomes in the two species *Parascaris univalens* and *P. equorum*. *Chromosoma* 94: 1-10.

Hayırlıoğlu-Ayaz S, Viinikka Y & Beyazoğlu O (1999). C- banded chromosomes of four *Vicia* species. *Pakistan Journal of Botany* 31: 307-313.

Hizume M, Tanaka A, Yonezawa Y & Tanaka R (1980). A technique for C-banding in *Vicia faba* chromosomes. *Japan J Genetics* 55: 301-305.

İnceer H, Hayırlıoğlu-Ayaz S & Beyazoğlu O (2002). Cytotaxonomic investigations on some taxa of the genus *Vicia* L. from north-eastern Anatolia. *Acta Botanica Gallica* 149: 125-138.

Kupicha FK (1976). The infrageneric structure of *Vicia* L. *Notes Roy Bot Garden Edinb* 34: 287-326.

Kuta E (1980). Karyological studies on the genus *Vicia* L. *Acta Biol Cracov Ser Botanica* 22: 81- 89.

Larsen K (1954). Chromosome numbers of some European flowering plants. *Bot Tidsskr* 50 (2): 163-174.

Levan A, Fredga K & Sandberg AA (1964). Nomenclature for centromeric position on chromosomes. *Hereditas* 52: 201-220.

Löve A & Löve D (1956). Cytotaxonomical conspectus of the Icelandic flora. *Acta Horti Gotob.* 20 (4): 65-291.

Löve A (1973). IOPB chromosome number reports 49. *Taxon*: 24 (4): 501-516.

Löve A (1975). IOPB chromosome number reports 49. *Taxon*: 24 (4): 501-516.

Mariani A & Falistocco E (1990). Chromosome studies in 2n = 14 and 2n = 16 types of *Medicago murex*. *Genome* 33: 159-163.

Maxted N, Callimassia MA & Bennett MD (1991). Cytotaxonomic studies of eastern Mediterranean *Vicia* species (*Leguminosae*). *Plant Syst Evol* 177: 221-234.

Maxted N (1993). A phenetic investigation of *Vicia* (*Leguminosae, Vicieae). Botanical Journal of the Linnean Society* 111: 155-182.

Raina SN & Rees H (1983). DNA variation between and within chromosome complements of *Vicia* species. *The Genetical Society of Great Britian* 51: 335-346.

Şahin A & Babaç MT (1990). Cytotaxonomic investigations on some *Vicia* L. species in east and southeast Anatolia I. *Turk J Bot* 14: 24-38.

Şahin A & Babaç MT (1995). Cytotaxonomic investigations on some *Vicia* L. species in east and southeast Anatolia II. *Turk J Bot* 19: 293-297.

Tabur S, Civelek S & Bagci E (2001). Cytotaxonomic studies on some *Vicia* L. species growing in eastern Mediterranean and southern Aegean regions I. *Acta Botanica Gallica* 148: 159-174.

Terziiskii D & Dimitrov B (1983). Karyotype analyses in *Vicia hirsuta* (L.) S.F.Gray and *V. meyeri* Boiss. *Caryologia* 36: 345-354.

Vural M (2000). *Vicia* L. In: Güner A, Özhatay N, Ekim T & Başer KHC (eds.) *Flora of Turkey and the East Aegean Islands,* Volume 11, pp. 89-92. Edinburgh: Edinburgh University Press.

Yamamoto K (1973). Karyotaxonomical studies on *Vicia* I. on the karyotype and character of some annual species of *Vicia. Japan J Genetics* 48: 315-327.

Yamamoto K (1986). Interspecific hybridization among *Vicia narbonensis* and its related species. *Biol Zentralbl* 105: 181-197.