

C-BANDED CHROMOSOMES OF FOUR *VICIA* L., SPECIES

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Abstract

The distribution of heterochromatin on the chromosomes of *Vicia hybrida* L. ($2n=12$), *V. hirsuta* (L.) S. F. Gray ($2n=14$), *V. tetrasperma* (L.) Schreb, Spic ($2n=14$) and *V. sativa* L. subsp. *nigra* (L.) Ehrh. ($2n=12$) have been established with the aid of the C-banding technique.

Introduction

Chromosome banding is valuable for identifying homologous chromosomes in karyologically differentiated species and especially for detecting chromosomal rearrangements that contributed to the 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 discrete locations in chromosomes.

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

Studies on the interspecific hybridization of *Vicia* have made it necessary to clarify the genetic relationships among the species of this important genus. The genus *Vicia* L., one of the common genus of the family Fabaceae, is comprised of more than 150-200 species and is subdivided into six sections viz., Cracca, Ervum, Euvicia, Faba, Anatrophyllia and Trigonellopsis (Smith, 1977). In Turkey, 59 species, 22 subspecies and 18 varieties of this genus have been recorded (Davis, 1970). Most of these species are annuals but a few of them belonging to the section Cracca are perennials. The basic chromosome numbers are $x=5, 6$ and 7 (Darlington & Wylie, 1955). Many of them are diploid species but some representatives of the section Cracca are polyploid. The karyotypes of *Vicia* species were reported by Sveschnikova (1940), Elçi (1965), Hanelt & Mettin (1970), Cincura (1962), Rousi (1961), Zertova (1969), Huziwara & Kondo (1963), Plitmann (1967), Yamamoto (1966; 1968; 1973), Guidetta & Caffaro (1984), Kuta (1980), Sahin & Babaç (1990), Terziiskii & Dimitrow (1983), Beyazoglu &

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Hayirlioglu (1991). The present report describes the Giemsa C-banding patterns of the four *Vicia* species distributed in Northeast Anatolia on the basis of karyotypic analysis in an attempt to provide more cytological details for the species.

Material and Methods

Seeds of *Vicia hybrida* L., *V. hirsuta* (L.) S. F. Gray, *V. tetrasperma* (L.) Schreb, Spic. and *V. sativa* L. subsp. *nigra* (L.) Ehrh. were collected from natural populations at the Campus of Karadeniz Technical University, Trabzon, Turkey. Seeds were germinated on moist filter paper, treated with 0.5% colchicine for 3.5 h. Excised root tips were fixed in 3:1 mixture of absolute ethanol and acetic acid and squashed in 45 % acetic acid. The banding technique was made according to method of Viinikka & Kallio (1993). The cover slip was removed with the aid of CO₂ freezing and the slides were then treated in absolute ethanol for 5 min., and in absolute acetic acid for 20 sec, air-dried and stored overnight at 60°C. On 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 with running tap water, treated with 2xSSC at 60°C for 1h, rinsed with deionized water for 15 min., and stained immediately with 3% Giemsa solution until properly stained. After rinsing with deionized water the slides were air-dried and a cover slip was mounted with "Entellan".

The idiograms were prepared with measurements taken on enlarged micrographs on five mitotic metaphases in each species. The classification of the chromosomes into median (m), submedian (sm) and subterminal (st) types was based on the analysis of metaphase chromosomes (Levan *et al.*, 1965).

Results and Discussion

Distinct darkly stained heterochromatin spots were observed in metaphase chromosomes of the four *Vicia* species studied, indicating the likelihood that the chromosomes would exhibit C-bands. Description of the banding pattern and general morphology of the chromosomes was more advantageously made on metaphase.

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

Vicia hybrida L. (2n=12): The C-banding pattern is exemplified by the karyotype and idiogram of metaphase chromosomes (Figs.1a,2a,3a). All chromosomes had stained centromeric C-bands. The chromosome complement consisted of five sm and one st pair (s).

Chromosomes 1, 2 and 3 all have a prominent band at the end of short arm, widest in chromosome 2. In addition, there is a weak band in the middle of long arm of these chromosomes. Chromosome 4 obviously bears the nucleolus organizing region (NOR) that separates the satellite from the rest of the chromosome. At NOR there is a rather large band. There is also a weaker band on the same arm between NOR and

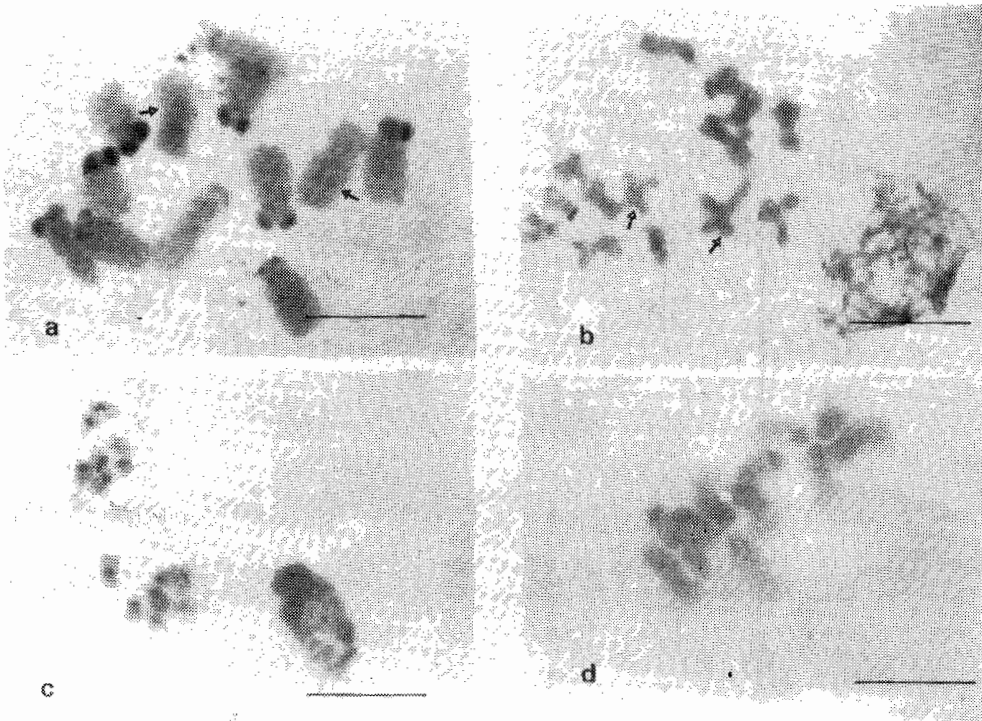


Fig.1. Mitotic metaphase chromosomes of *Vicia* species a. *V. hybrida*; b. *V. hirsuta* c. *V. tetrasperma*, d. *V. sativa* subsp. *nigra*. Arrows indicate the nucleolar organizers. Bars represent 10 μm .

centromere. Occasionally there are still weaker bands visible in the other parts of the chromosome. But due to the small number of the cells examined their location could not be confirmed. Chromosomes 5 and 6 also have a large band at the end of short arm. The location of the weaker bands could not be determined.

Vicia hirsuta (L.) S.F. Gray ($2n=14$): A karyotype and an idiogram are presented in Figs.1b,2b,3b. All chromosomes had centromeric C-bands. The chromosome complement consisted of three sm and four m pair (s). Chromosome 1 has a weaker band on the tip of short arm. Chromosome 2 has a frequent knob structure in the middle of one arm and chromosome 3 a clear band at the end of short arm. The supposed NOR band on the chromosome 6 is remarkably weak.

Vicia tetrasperma (L.) Schreb, Spic. ($2n=14$): A karyotype and idiogram are presented in Figs.1c,2c,3c. The chromosomes were classified into the following groups: six m and one sm pair (s). No other bands than the centromeric ones were visible.

Vicia sativa L., subsp. *nigra* (L.) Ehrh. ($2n=12$): A karyotype and an idiogram are presented in Figs. 1d,2d, 3d. All chromosomes had centromeric C-bands that not so intensely stained as other *Vicia* species. The chromosomes were classified into the following groups: two st and four sm pair(s). Chromosome 3 had a infrequent knob structure near the secondary constriction.

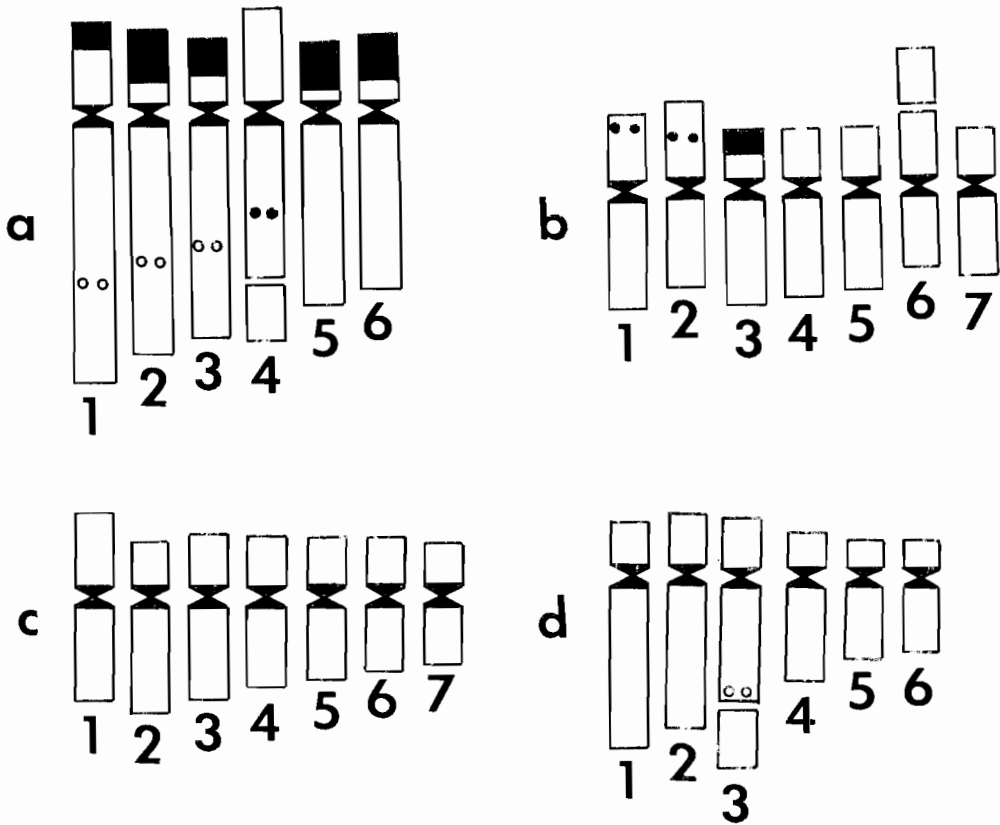


Fig. 2. Idiograms of C- banded karyotype of *Vicia* species. a. *V. hybrida*; b. *V. hirsuta*; c. *V. tetrasperma*; d. *V. sativa* subsp. *nigra*. Black segments: distinct C- bands. Solid circles: frequent knob structures. Open circles: infrequent knob structures.

Diagnostic centromeric C-bands, which were similar in size, were found for each species of studied *Vicia*. While all of *V. hybrida* chromosomes and one of *V. hirsuta* chromosomes have prominent bands at the end of short arms and NORs, the others (*V. tetrasperma* and *V. sativa* subsp. *nigra*) have not. Similar to our results, Hizume *et al.*, (1980) identified centromeric C-bands, prominent bands and NOR in *V. faba* chromosomes.

In *Vicia* as in many other genera of flowering plants the speciation was accompanied by massive changes in chromosome size and DNA amount. The variation of total nuclear DNA amount and chromosome complements within the genus of *Vicia* were distinctively quantified by Raina & Rees (1983). In the study, the highest amount of total DNA was found to be 16.46 picogram for *V. hybrida* and 8.95 picogram for *V. hirsuta*. According to our results, the most intensive C-banding patterns were detected in *V. hybrida* and *V. hirsuta*.

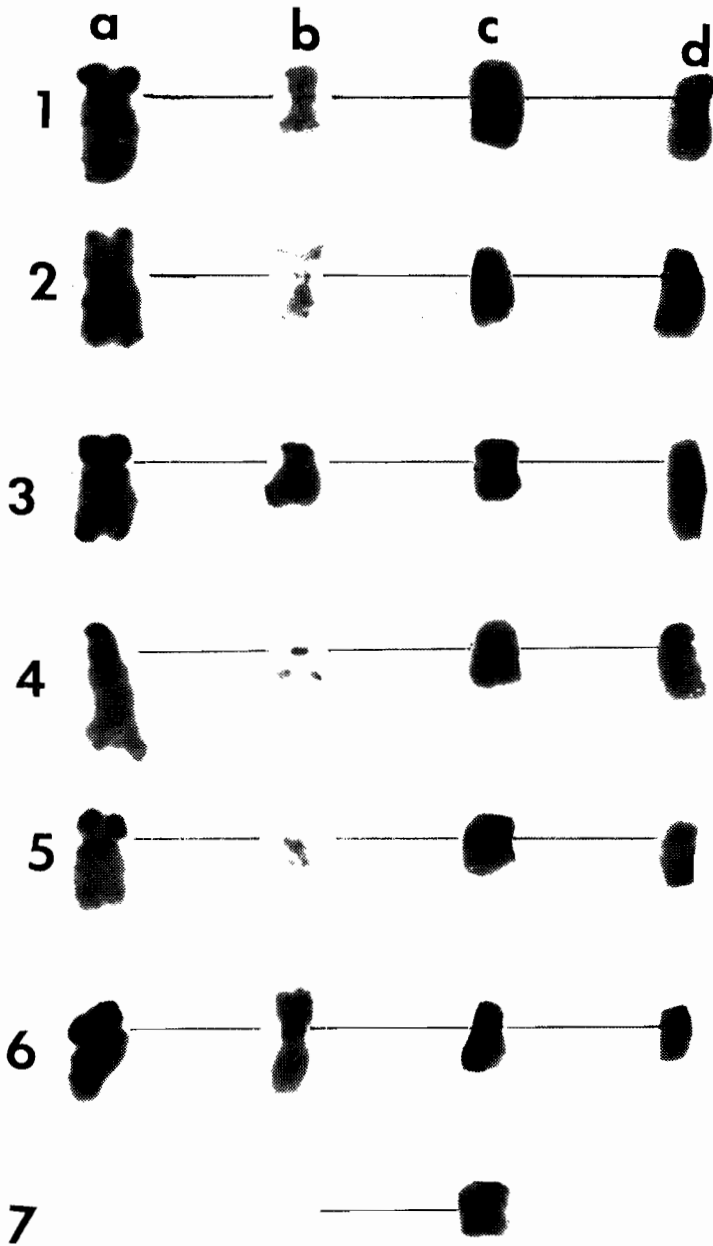


Fig.3. Karyograms of C- banded karyotype of *Vicia* species. a. *V. hybrida*; b. *V. hirsuta*; c. *V. tetrasperma*; d. *V. sativa* subsp. *nigra*.

It is well known that the somatic complement has often contributed a great deal to the interpretation of the phylogenetic relationships between different taxonomic groups (Mariani & Falistocco, 1990). The results of this study helps to clarify the chromosomal differences among *V. hybrida*, *V. hirsuta*, *V. tetrasperma* and *V. sativa* subsp. *nigra* with regard to the amount and location of heterochromatin. Once these banding patterns have been established, a more detailed analysis of the evolutionary relationships of the species will be possible.

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