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# GIEMSA C BANDING IN *VICIA* L.[1]

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## ABSTRACT

Giemsa C-banding analysis was performed on the somatic chromosomes of six diploid species of *Vicia*, namely *V. orobus* (2n=12), *V. villosa* (2n=14), *V. disperma* (2n=14), *V. lutea* (2n=14) *V. hybrida* (2n=12) and *V. faba* (2n=12). Giemsa C-banding patterns were specific of the species and the characteristic trend of distribution of heterochromatin all over the length of the chromosomes is maintained in all the species investigated. Karyotype analysis together with banding pattern show evolutionary reduction in chromosome number from 2n=14 to 2n=12 accompanying the diminution in number, size and amount of the heterochromatic segments. Banding pattern of the species within same section resembles more closely than between the sections in the genus, despite variation in chromosome number.

## INTRODUCTION

The genus *Vicia* is comprised of more than 170 species and is subdivided into four sections i.e. *Cracca, Ervum, Euvicia* and *Faba* (Ehra 1950, Plitmann 1967). Most ofthe species are annuals but a few of them belonging to section *Cracca* are parennials. Karyotype analyses of *Vicia* species have been worked out by many workers and species relationship on the basis of their data have been suggested (Plitmann 1967, Yamamoto 1973, Hollings and Stace 1974).

Various banding methods for the linear differentiation of chromosomes developed in recent years have provided valuable data in working out intra and infra specific variation and species relationship (Lavania and Sharma 1983). Correlation between the DNA base composition of the heterochromatin and the response of various staining techniques have given interesting results (Marks and Schweizer, 1974, Vosa 1975, 1977; Badr and Elkington 1977 and La Cour 1978). Though the first ever report on chromosome banding (Q—banding) comes from plants i.e. *Vicia faba* (*Caspersson et al.*, 1969) and the fundamental information on the nature and classification of various heterochromatin types (Greilhuber, 1975, Vosa 1976) has been obtained through characteristic banding patterns, the work has not been extended to the other species of this genus. It was therefore desired to work out the C-banding patterns of some species of this interesting genus with a view to know the sequence complexity of DNA with respect to heterochromation distribution and its significance in tracing the evolutionary

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relationship and the direction of change regarding the constitutive heterochromatic band endowment.

## MATERIALS AND METHODS

The name of the various species of *Vicia* investigated, their diploid chromosome number, name of the section within the genus and the source of the seed material are given in Table I.

TABLE I

| Name of the species | 2n | Section* | Source |
|---|---|---|---|
| *V. orobus* DC. | 12 | Cracca | Royal Botanic Gardens, Kew |
| *V. villosa* Roth. | 14 | | —do— |
| *V. disperma* DC. | 14 | Ervum | —do— |
| *V. lutea* L. | 14 | Vicia | —do— |
| *V. hybrida* L. | 12 | | —do— |
| *V. faba* L. | 12 | Faba | Sutton & Sons, Calcutta |

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*C-banding* :

Giemsa C-banding procedure of Vosa and Marchi (1972) for plants was followed. Pretreated fixed root tips were hydrolysed in IN.HCl for 30 seconds at 60°C and squashed in 45% acetic acid. Air dried preparations (preferably stored overnight) were treated with saturated aqueous solution of barium hydroxide for 4-8 minutes, specific to the particular species at 20-22°C followed by incubation in 2×SSC at 60°C for about an hour. Staining was performed in 5% BDH Giemsa in M/15 phosphate buffer (pH 6.8) for 10-15 minutes.

Somatic karyotypes were prepared for the various species following root tip squashing and orcein staining. In each case approximately 10-15 scattered C-banded metaphase plates were scored and C-bands were plotted on the idiograms as shown in Fig. 1.

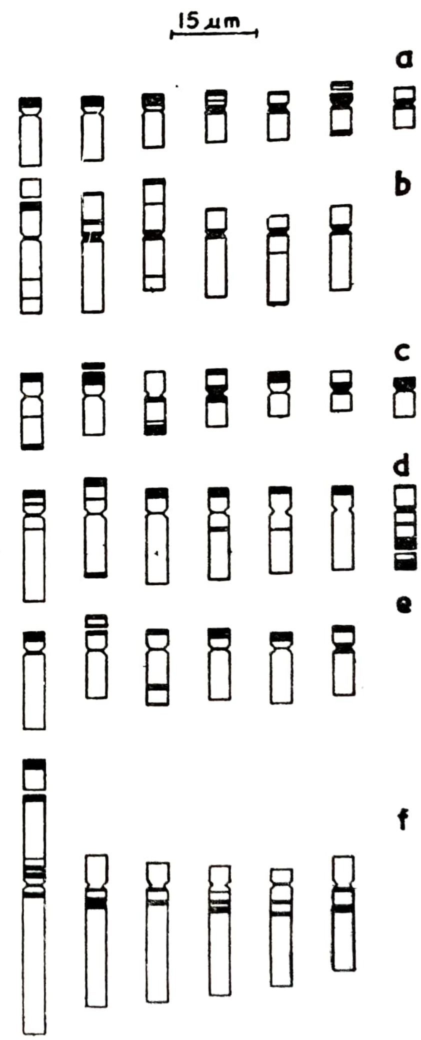


Fig. 1. C banded karyotypes in Vicia species: a. *V. villosa*, b. *V. orobus*, c. *V. disperma*, d. *V. lutea*, e. *V. hybrida* f. *V. faba*.

## RESULTS AND DISCUSSION

The C-band distribution and the diagrammatic representation in the form of C-banded karyotypes of the various species investigated are shown in Fig.

1. The species have two different chromosome numbers viz. 2n=14 and 2n=12 as depicted in Table I. In general, C-bands are observed in centromeric region, terminal end, nucleolar constriction and intercalary position on the two arms, specific for the particular species. Giemsa C-banding patterns of *Vicia faba* have been worked out by various other workers (Dobel *et al.*, 1973, Takehisa and Utsumi 1973, Klasterska and Natarajan 1975, Greilhuber 1975). The present findings corroborate the earlier observations on this species. The general trend of distribution of heterochromatic segments of *Vicia faba* is maintained also in other species studied. It is remarkable and of taxonomic significance that there is more resemblance in the banding pattern within the same section in the genus as compared to other sections despite variation in chromosome number.

A survey of chromosome numbers (Fedorov, 1969), reported in the genus *Vicia*, shows that 2n=14 is the most widespread number (as it is in several related genera of tribe *Viceae* e.g. *Lens*, *Lathyrus* and *Pisum*). Hanelt and Mettin (1966), Hollings and Stace (1974) from their karyotypic studies on several *Vicia* spp. suggested that 2n=14, 2n=12 and 2n=10 chromosomes are related to each other in reductional evolutionary sequence. On the basis of karyotype analysis, they referred that this reduction has occurred through various ways at different periods in the genus. It may involve centric fusion type of interchange or various other types of structural rearrangements. It is not unlikely that n=7 represents the ancestral basic number in the genus.

An analysis of present and previous data (Hollings and Stace 1974, Yamamoto 1973) indicate that evolution of karyotypes in the genus *Vicia* as a whole fits very well into the evolutionary trend shown by many plants, namely an increasing karyotype asymetry together with a decrease in basic number (Stebbins 1971).

Phylogenetic reduction in chromosome number is usually associated with the occurrence of unequal translocatons between nonhomologous chromosomes, followed by the loss of heterochromatic, genetically inert or non-essential centromeric regions (Stabbins 1966). This diminution in heterochromatin content reflected in the form of number and position of C-bands obtained by C-banding procedure is noted in the present case as well. Ancestral species of *Vicia* with 2n=14 chromosomes have more C-bands considering both the number and size of C-bands as compared to derived species with 2n=12 chromosomes. The unequal translocations between the chromosomes might have brought this change leading to asymmetry in advanced species with low chromosome numbers. This type of evolutionary relationship in karyotype is more closely observed within the same section of the genus as evident from earlier cytological informations and present data on C-banding analyses. That diminution in number, size and position of C-bands has accompanied the evolution of various species has also been observed in the various species of the related genus *Lathyrus* and several others (Lavania and Sharma, 1980, 1983).

It is clear, therefore, that patterns of C-banding may provide a valuable criterion for assessing the validity of taxonomic groups within the genus as well as in interpretation of relationships between and within species.

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*Note added in proof:* While this paper was in press, we have come across a relevant paper by G. Ramsay (C-banding in *vicia* species, pp. 28-39, *in* Chapman, G. P. and S. A. Tarawali (eds.): *System for Cytogenetic analysis in Vicia faba* L. 1984, Martinus Nijhoff Publ.) describing the C-banding patterns in six species of *Vicia* two of which viz. *V. faba* and *V. lutea* are common with us. The banding patteru reported by Ramsay for these two species resembles in general with that of ours but for C-banding polymorphism in nucleolar Chromosomes. Applying Ramsay's data to our studiess it substantiates our contention regarding sectional characteristics in banding pattern for *Vicia* and also corroborates our suggestion pertaining to heterochromatin diminution with the course of evolution.

## REFERENCES

BADR, A. AND T. T. ELKINGTON 1977. Variation of Giemsa C-band and fluorochrome banded karyotypes and relationships in *Allium* subgen. *Molium. Pl. Syst. Evol.* **128** : 23-35.

CASPERSSON, T., L., ZECH, E. J. MODEST, G. E., FOLEY, U. WAGH AND E. SIMONSSAN 1969. Chemical differentiation with fluorescent alkylating agents in *Vicia faba* metaphase chromosomes. *Exp. Cell Res.* **58** : 147-152.

DOBEL, P., R. REIGER AND A. MICHAELIS 1973. The Giemsa banding patterns of the standard and four reconstructed karyotypes of *Vicia faba. Chromosomc* **43** : 409-422.

EHARA, K., 1950. *Forage Crops* I. Yokendo, Tokyo 355-359.

FEDOROV, A. A. 1969. *Chromosome numbers of flowering plants*. Academy of Sciences of USSR.

GREILHUBER, J., 1975. Heterogeneity of heterochromation in plants : Comparison of Hy- and C-bands in *Vicia faba. Plant Syst. Evol.* **124** : 139-156.

HANELT, P. AND D. METTIN 1966. Cytosystematische untersuchungen in der artengruppe um *Vicia sativa* L., II. *Kulturpflanze* **14** : 137-161.

HOLLINGS, E. AND C. A. STACE 1974. Karyotype variation and evolution in *Vicia sativa* aggregate. *New Phytol.* **73** : 195-208.

KLASTERSKA, I. AND A. T. NATARAJAN 1975. Distribution of heterochromation in the chromosomes of *Nigella damascena* and *Vicia faba. Hereditas* **79** : 154-156.

LA COUR, L. F., 1978. Two types of constitutive heterochromatin in the chromosomes of some *Fritillaria* species. *Chromosoma* **67** : 67-75.

LAVANIA, U. C. AND A. K. SHARMA 1980. Giemsa C-banding in *Lathyrus. Bot. Gaz.* **141** : 199-203.

LAVANIA, U. C. AND A. K. SHARMA 1983. Chromosome banding in evoluticnary plant cytogenetics. *Proc. Indian Acad. Sci.* (Plant Sci.) **92** 51-79.

MARKS, G. E. AND D. SCHWEIZER 1974. Giemsa banding : Karyotype differences in some species of *Anemone* and in *Hepatica nobilis. Chromosoma* **44** : 405-416.

PLITMANN, U., 1967. *Biosystematical study of Vicia of the middle east*. Private publ. : 1-128.

STEBBINS, G. L., 1966. Chromosomal variation and evolution. *Science* **152** : 1463-1469.

STEBBINS, G. L., 1971. *Chromosomal evolution in higher plants*. Edward Arnold (Publ.) Ltd., London.

TAKEHISA, S. AND S. UTSUMI 1973. Visualization of metaphase heterochromatin in *Vicia faba* by the denaturation-renaturation Giemsa staining method. *Experientia* **29** : 120-121.

TUTIN, T. G., V. H. HEYWOOD, N. A. BURGES, D. M. MOORE, D. H. VALENTINE S. M. WALTERS AND D. A. WEBB (eds.) 1968. *Flora Europaea* Vol. II Cambridge at the University press.

VOSA, C. G., 1975. The use of Giemsa and other staining techniques in karyotype analysis. *Curr. Adv. Pl. Sci.* **14** : 495-510.

VOSA, C. G., 1976. Heterochromatin classification in *Vicia faba* and *Scilla sibirica. Chromosomes Today* **5** : 185-192.

VOSA, C. G., 1977. Heterochromatic patterns and species relationship. *Nucleus* **20** : 33-41.

VOSA, C. G. AND P. MARCHI 1972. Quinacrine fluorescence and Giemsa staining in Plants. *Nature* (New Biol.) **237** : 191-192.

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.