Giemsa C-banding Pattern in Some Cultivars of *Trichosanthes dioica* Roxb.

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In recent years remarkable advances have been made in techniques for the study of linear differentiation of chromosomes which have revolutionized the cytogenetic research. The extreme usefulness of the banding techniques has been proved in its application in precise identification of individual chromosomes.

Recently developed techniques for visualization of constitutive heterochromatin provide means for studying the distribution pattern of the same in plant and animal materials. There have been relatively few studies so far which have attempted to exploit Giemsa C-banding techniques to investigate the relatedness between taxa. Filion (1974) has investigated Giemsa banding patterns in some cultivars of *Tulipa*. Recently, El-gadi and Elkington (1975) have made a comparison of Giemsa C-banding patterns in some species of *Allium* and have suggested their relationship. Similar studies have been made by Thomas (1981) in *Lolium* species and by Chakraborty (1982) in several species and cultivars of *Allium*, *Aloe*, *Gasteria* and *Haworthia*. Excepting a lone report, information is entirely lacking in this direction not only in the genus *Trichosanthes* but also in the family Cucurbitaceae as a whole.

Although a few attempts including those of Chakraborty and Datta (1981) and Chakraborty (1982) have been made in some liliaceous taxa, studies on C-heterochromatin in members of Cucurbitaceae to assess the role of heterochromatin, if any, in intraspecific diversification are completely lacking. With these objects in view, a detailed study of the distribution pattern of Giemsa C-heterochromatin in some cultivars of *Trichosanthes* has been undertaken.

Materials and methods

Three cultivars of *T. dioica* were used for chromosome analysis following the principle of BSG-technique (Vosa 1976a). The taxa investigated were 'Kalyani', 'Sanjiban' and 'Kali'. The gynoeceious individuals of the cultivars were included in the present investigation. Adventitious roots were obtained from the plants propagated in the experimental garden. The apical root meristems were pretreated in saturated aqueous solution of alpha-bromo naphthalene for 5 min at $4^{\circ} \pm 1^{\circ}\text{C}$ followed by 25 min at 12° – 14°C . After thorough washing in distilled water the materials were fixed in acetic ethanol (1:3, v/v) for about 1 h to overnight. Fixed root tips following hydrolysis in 1N HCl at 60°C for 30 sec and thorough washing in distilled water were squashed. The slides with squashed materials, after detachment of cover glass, were air dried for 20 h–24 h and treated with saturated aqueous solution of barium hydroxide [$\text{Ba}(\text{OH})_2$, H_2O] for 4 min at room temperature. The preparations were then washed thoroughly in distilled water and incubated in $2 \times \text{SSC}$ (0.3 M NaCl and 0.03 M trisodium citrate) at 62 – 63°C for 20 min. After rinsing in distilled water the preparations were stained in 2% Giemsa (BDH) solution in phosphate buffer (M/15) at pH 6.9 for 15–30 min. Finally, the preparations were rinsed in distilled water, dried rapidly in air and mounted in euparal. All the observations, drawings and photomicrographs were made from the permanent Giemsa banding preparations thus obtained.

Observations

The present observations have been dealt with three gynoecious cultivars of *T. dioica* Roxb. The distribution pattern of Giemsa C-heterochromatin in the somatic metaphase chromosomes has been recorded. As the stringent treatments inherent in the BGS-technique for chromosome banding distort the chromosome morphology beyond identification, on the basis of chromosome length, number and position of bands, the chromosomes have been arranged in the following groups.

Group-I: Long chromosomes ($5.89\ \mu\text{m}$ – $3.43\ \mu\text{m}$) having telomeric bands located at one arm.

Group-II: Long chromosomes ($5.40\ \mu\text{m}$ – $3.60\ \mu\text{m}$) having telomeric bands located at both arms.

Group-III: Medium to short chromosomes ($3.27\ \mu\text{m}$ – $1.97\ \mu\text{m}$) having telomeric bands located at one arm.

Group-IV: Medium to short chromosomes ($2.94\ \mu\text{m}$ – $2.29\ \mu\text{m}$) having telomeric bands located at both arms.

Group-V: Medium to long chromosomes ($6.41\ \mu\text{m}$ – $2.49\ \mu\text{m}$) having two telomeric and one or more intercalary bands.

Group-VI: Medium to short chromosomes without band and with diffuse Giemsa staining ($2.78\ \mu\text{m}$ – $2.29\ \mu\text{m}$).

The salient features of the banded karyotypes are presented in the Table 1.

The somatic chromosomes of the investigated taxa, in general, are characterized by distinct telomeric bands (Figs. 1–3). Some members of the complement are provided with two telomeric bands while a few are characterized by the absence of bands. The characteristic variation in band position and length can be conveniently used for identification of a taxon (Figs. 4–6). One pair of chromosomes in each of the taxa investigated is characterized by diffused Giemsa staining. The highest amount of Giemsa C-heterochromatin

Figs. 1–3. Banded karyotypes of different cultivars of *Trichosanthes dioica* Roxb. 1, *T. dioica* cv 'Kali'. 2, *T. dioica* cv 'Kalyani'. 3, *T. dioica* cv 'Sanjiban'.

romatin is represented in *T. dioica* cv 'Sanjiban' followed by *T. dioica* cv 'Kali' and *T. dioica* cv 'Kalyani'. The proportion of diffuse Giemsa staining varies from species to species (Fig. 7).

The interphase nuclei of the taxa investigated reveal bands as darkly stained dots which are

Table 1. Salient Giemsa C-band karyotypic features in different gynoeious cultivars of *T. dioica*

Cultivars	Total chromosome length (TCL) (μm)	C-banded region		C-banded karyotypic formulae
		Total banded length (μm)	% TCL	
'Kali'	78.15	15.00	19.19	15+II4+III10+IV1+VI2
'Kalyani'	87.60	17.49	19.96	II2+II4+IV4+VI2
'Sanjiban'	68.55	23.43	42.93	II3+III4+IV6+V7+VI2

Figs. 4-7. 4-6, banded idiograms of different cultivars of *T. dioica*. Arranged in the same order as that of Figs. 1-3. 7, relative proportion of haploid non-band, diffuse band and band chromatin matter in different cultivars of *T. dioica*.

more or less uniformly distributed. The salient features of band karyotypes are given in the Table 1.

Discussions

Conventional karyotype analysis reveals a general homogeneity of chromosome complements of the members of *Trichosanthes* L. investigated. The striking similarity in the numerical and structural features of the karyotypes characterizes the taxa. *T. dioica* with their gynoeious cultivars are all characterized by a uniform number of 22 chromosomes in the somatic complement which confirm the previous reports (Bhaduri and Bose 1947, Sen 1976). The cultivars of *T. dioica* seem to represent a homogeneous assemblage of closely related taxa, which are apparently indistinguishable from each other on karyotypic features as revealed through usual orcein staining. Four pairs of nucleolar chromosomes are common to the taxa included for banding studies.

Karyotype analysis following Giemsa banding technique, however, reveals a number of distinguishing features between the varieties investigated. Similar differences in the heterochromatin pattern between species of *Allium* have been noted by Vosa (1976b) as well. In recent past, Chakraborty (1982) has demonstrated differential C-heterochromatin pattern in some species and cultivars of *Allium* and a few members belonging to *Aloe*, *Gasteria* and *Haworthia*. The pattern differs not only between the species but also between the varieties. The lone report of banding studies in Cucurbitaceae is that of Seikiova *et al.* (1978) who have used a modified Giemsa technique for identifying cucumber chromosomes.

T. dioica, in general, is characterized by distinct telomeric bands. The Giemsa C-banding patterns differ between the cultivars and as such can be conveniently used for distinguishing one variety from the other. A comparative analysis of the distribution pattern of C-heterochromatin clearly brings out certain significant differences between the taxa. The cultivar 'Sanjiban' stands apart from the rest in possessing a distinguishing banding pattern revealing as many as seven chromosomes with one or more intercalary bands in addition to the telomeric bands at both arms. Besides these, eight chromosomes show telomeric bands at both arms which are conspicuously thick in one chromosome. Banding heteromorphism is represented in as many as three pairs of chromosomes. The remaining cultivars investigated are conspicuous by the absence of chromosomes with intercalary band. The cultivars 'Kali' and 'Kalyani', however, can be distinguished from each other by the presence of five chromosomes with two telomeric bands in each in the former while four such chromosomes are represented in the latter. The variety 'Kali' is further characterized by the occurrence of banding heteromorphism involving a structurally heteromorphic pair of chromosomes. The additional banding parameters differentiating the gynoeious cultivars of *T. dioica* are total number of bands in the somatic complement as well as the percentage of C-banded length in respective taxa.

A striking numerical uniformity coupled with remarkable homogeneity in gross morphology of chromosomes characterizes the cutivars of *T. dioica*. The close phyletic relatedness of the taxa is strongly reflected in chromosomal features as well. The karyotype analysis following conventional staining techniques seems to be far from adequate for distinguishing between the cultivars. Application of recently developed Giemsa C-banding method, however, reveals a differential distribution of C-heterochromatin in the cultivars of *T. dioica* thus demonstrating the active involvement of heterochromatin in intraspecific diversification.

Summary

To assess the role of heterochromatin, if any, in intraspecific diversification, a detailed study of the distribution pattern of Giemsa C-heterochromatin in three gynoeceous cultivars of *Trichosanthes dioica* Roxb., namely, 'Kali', 'Kalyani' and 'Sanjiban' has been undertaken.

The somatic chromosomes are characterized by distinct telomeric bands. The differential banding pattern can be conveniently used for distinguishing between the cultivars. The cultivar 'Sanjiban' stands apart from the rest in possessing seven chromosomes with one or more intercalary bands in addition to the telomeric bands at both arms. The cultivars 'Kali' and 'Kalyani', however, can be distinguished from each other by the presence of five chromosomes with two telomeric bands in each in the former while four such chromosomes are represented in the latter.

The application of Giemsa C-banding method reveals a differential distribution of C-heterochromatin in the cultivars investigated thus demonstrating the active involvement of heterochromatin in intraspecific diversification of *T. dioica*.

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