Cytologia 56: 409–417, 1991

Chromosome Studies and Nuclear DNA in Relation to Sex Difference and Plant Habit in Two Species of Cucurbitaceae

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Accepted April 4, 1991

The species of Cucurbitaceae have exclusively unisexual flowers and populations may be monoecious or dioecious. Of the 48 genera and 135 species recorded for this family, 67 are dioecious spread over 19 genera (Chakravarty 1959, 1966, 1968, Whitaker and Davis 1962). Several genera are monotypic in nature. The importance of this family in agriculture and medicine is well established.

The dioecious members of this family provide ideal materials for study of the chromosomal basis of sex determination. The species of *Trichosanthes*, *Momordica*, *Melothira*, *Edgaria*, *Luffa* and *Coccinia* have been subjected to cytological analysis (Sinoto 1929, Nakajima 1937, Kurita 1939, Kumar and Vishveshwaraiah 1952, Patel 1952, Roy *et al.* 1966, Roy 1970, Roy and Roy 1971, Trivedi and Roy 1972, Thakur and Sinha 1972–73, Roy *et al.* 1982, Mukherjee 1974–75, Datta and Roy 1982, Pandey and Saran 1986, Sarkar *et al.* 1987, Sarkar and Datta 1988). The chromosome numbers range from n=11 to 14 for these genera. Intraspecific polyploidy in nature has so far been recorded only in *Momordica dioica* and *Trichosanthes dioica* (Trivedi and Roy 1972, Roy *et al.* 1982).

In three species of *Trichosanthes* the XY type of sex determining mechanism has been reported (Nakajima 1937, Kurita 1939). Westergaard (1958) however, emphasized the need for a more careful assessment of the chromosomal status of these species in relation to unisexuality. The convincing evidence of the presence of eex chromosome has been obtained for *Coccinia indica* (Roy and Roy 1971). The Y chromosome is considerably larger than X and heteromorphicity is evident both in mitosis and meiosis. Artificial polyploids have been raised in *C. indica* (Roy and Roy 1971) to determine the influence of sex chromosomes and autosomes in the determination of sex. A series of individuals were obtained with different sets of autosomes and different dosage of X and Y chromosomes. The plant with XXX and single Y became male, denoting the importance of Y in sex determination. In *Trichosanthes dioica*, indication of differential meiotic behaviour has been recorded for some chromosomes though clear sex chromosomes could not be demarcated (Roy *et al.* 1982).

Despite the occurrence of X and Y chromosomes in *Coccinia indica* and indication of differential staining behaviour in *Trichsanthes dioica*, no detailed analysis of chromosome structure has been done with the aid of improved techniques. Studies to a large extent have so far been concentrated principally on meiotic behaviour. The presence of sex chromosomes also adds to the heterochromatic content of the nucleus which is reflected in the staining behaviour. The heterochromatic content may also affect the amount of nucleic acid in the nucleus. No data are yet available on the estimation of nuclear DNA in both male and female plants.

In view of the above lacunae of our knowledge in relation to the factors associated with sex determination in Cucurbitaceae, this investigation was undertaken. The paper deals with critical analysis of the structure and behaviour of chromosomes in *Coccinia indica* and *Trichosanthes dioica*, where both male and female plants have been subjected to analysis. In

both species nuclear DNA has been estimated through *in situ* cytophotometric technique. The extent of relationship of these data with sex difference has been analysed.

Materials and methods

The materials for the present investigation include two unisexual dioecious species of the family Cucurbitaceae.

1) *Trichosanthes dioica* Roxb. (Male and Female plants)
2) *Coccinia indica* Wight and Arn. (Male and Female plants)

The somatic chromosomes in most cases were studied from temporary squash preparations of root-tips. Trials with various pretreatment chemicals and fixatives revealed a mixture of saturated solution of paradichlorobenzene and 0.002 M oxyquinoline (1: 1) for 2 hr in case of *Coccinia indica* and 2.30 hr at 14–16°C in case of *Trichosanthes dioica* to be the most effective pretreating agent. After pretreatment the root tips were fixed in acetic acid-ethanol mixture (1: 3) for overnight at room temperature, hydrolysed in 1N HCl at 60°C for 10–12 min. and washed in distilled water. The root-tips were then treated with 45% acetic acid for 5 min., stained in 2% acetic-orcein staining solution for overnight and finally squashed in a drop of 45% acetic acid.

For meiotic analysis, young flower buds were fixed in acetic–alcohol (1: 3). The anthers were dissected out from floral buds and smeared in 2% acetic-carmine solution.

For the estimation of nuclear DNA the root tips of the male and female plants of both the species and that of *Allium cepa* were fixed in 1: 3 acetic ethanol for overnight and were then washed in distilled water, and hydrolysed in 1N HCl at 60°C for 15 min. in case of *Trichosanthes dioica* and 20 min. in case of *Coccinia indica*. The root tips were then weshed thoroughly in distilled water, kept in 45% acetic acid for 2–3 min., stained in Schiff's reagent for 2–3 hr at 16–18°C and finally squashed in a drop of 45% acetic acid.

Cytophotometric measurements were made by using a Leitz Wetzlar Aristophot with microspectrophotometer, following the single wavelength (550nm) method (Sharma and Sharma 1980). One hundred metaphase plates were scanned in all the cases. 4C nuclear DNA values were calculated on the basis of optical density, in terms of relative arbitrary units of absorbance which were then converted to absolute units (Picogram 10^{-12}g) by using the formula:

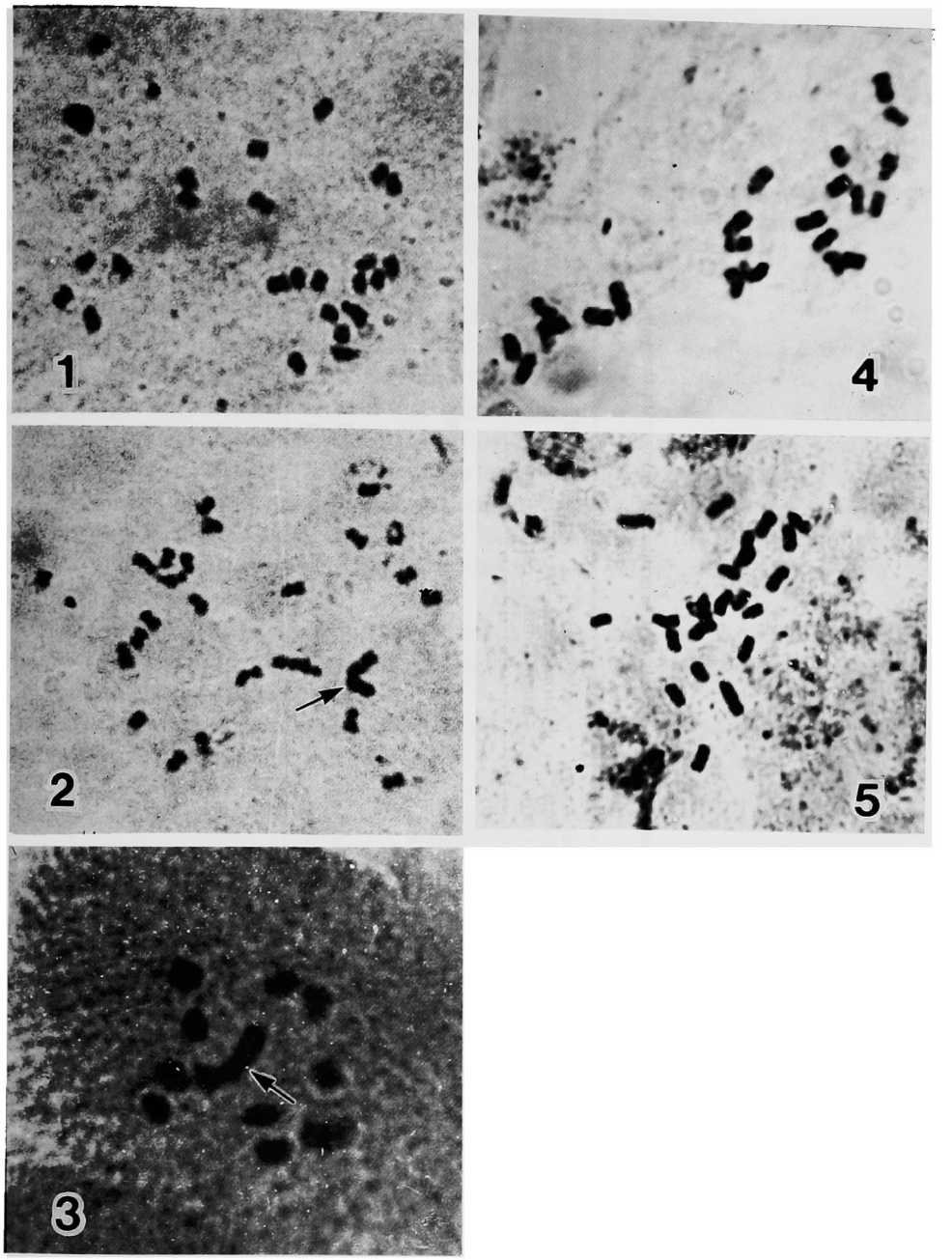
$$\text{4C nuclear DNA} = \frac{A}{B} \times C$$

whers A=4C nuclear DNA value of *Allium cepa* i. e. 67×10^{-12}g derived from the determinations of Van't Hof's (1965) 2C nuclear DNA value; B= the mean absorption figure measured in the *A. cepa* standard root tips and C= the mean of the absorption figures measured for the sample nucleus (metaphase plates) minus those of the background readings.

Results

Cytological data and DNA content in Trichosanthes dioica Roxb.

Studies on the chromosomes of both male and female plants of this species showed similarity in their number, size and morphology. Both contain 2n=22 chromosomes in their complements (Figs. 4, 5). The chromosome size ranged from 1.52 to 3.04 μm in both cases. The total chromosome lengths were 47.14 μm and 46.08 μm and the mean of TF% 36.03 and 36.04 in female and male plants respectively. The chromosomes were classified into four types namely A, B, C and D on the basis of the position of primary and secondary constrictions.



Figs. 1, 2. Somatic metaphase plates showing 2n=24 chromosomes respectively of the female and male plant (arrow indicating the large Y chromosome in fig. 2) of *Coccinia indica* Wight. and Arn. (×1720 approx.).

Fig. 3. Meiotic metaphase I plate showing 12 bivalents (arrow indicating the large sex chromosomal bivalent) of male plant of *C. indica* Wight. and Arn. (×2300 approx.).

Figs. 4, 5. Somatic metaphase plates showing 2n=22 chromosomes respectively of the female and male plant of *Trichosanthes dioica* Roxb. (×1600 approx.).

Table 1. Amount of 4C nuclear DNA in root meristems in two dioecious species of Cucurbitaceae along with the values of other cytological parameters

Name of the species	Somatic chromosome number (2n)	Karyotype formula	Total chromosome length (μm)	Average chromosome length (μm)	Amount of 4C nuclear DNA in root (pgs)$\pm$S. E.	Amount of 4C nuclear DNA per genome	Amount of 4C nuclear DNA per chromosome
Coccinia indica							
Female plant	24	2A+12C+10D	34.68	1.44	8.25$\pm$0.10	4.13	0.34
Male plant	24	2A+10C+10D+XY	36.86	1.54	10.35$\pm$0.12	5.18	0.43
Trichosanthes dioica							
Female plant	22	2A+2B+8C+10D	47.14	2.14	11.30$\pm$0.13	5.65	0.51
Male plant	22	2A+2B+8C+10D	46.08	2.09	11.66$\pm$0.11	5.83	0.53

Type A: Chromosomes with two constrictions, one nearly median, the other nearly submedian or nearly subterminal on the same arm.
Type B: Chromosomes with two constrictions, at opposite ends, both nearly submedian.
Type C: Chromosomes with nearly median constrictions.
Type D: Chromosomes with nearly submedian constrictions.

Meiotic study revealed eleven bivalents in late prophase and metaphase I. No pollen dimorphism was noticed. The amounts of 4C nuclear DNA were 11.30 picograms and 11.66 picograms in female and male respectively.

Cytological data and DNA content in Coccinia indica Wight and Arn.

A detailed karyotypic study of both male and female plants of *C. indica* showed a similarity in their chromosome morphology and numbers which were 2n=24, except one comparatively larger chromosome without any homologue present in the male plant (Figs. 1, 2). The length of the chromosomes varied from 1.04–2.07 μm in case of female plant and 0.98–3.91 μm in case of male plant. The size of the larger (Y) chromosome in male plant was 3.91 μm which consists of both primary and secondary constrictions. Total chromosome length is 34.68 μm and 36.86 μm and mean of TF% is 38.61 and 35.84 in female and male plants respectively. The chromosomes were classified into 3 types, namely A, C and D on the basis of the position of the constrictions.

Meiosis revealed 12 bivalents of which one is larger than the rest in metaphase I and diakinesis. The larger bivalent shows a very short pairing contact (Fig. 3). The larger chromosome shows a strong heteropycnosity.

The 4C DNA values for each sex are 8.25 picograms and 10.35 picograms in female and male plants respectively (Table 1).

Discussion

Previous studies on *Trichosanthes dioica* carry discrepant reports. Heteromorphism indicating an XY mechanism was noted by Patel (1952). However, later investigations (Mukherjee 1974–75, Singh and Roy 1979, Roy *et al.* 1982) show the absence of visible structural difference in any of the chromosome pairs, but heteropycnosis and precocious separation of one bivalent were clearly recorded (Roy *et al.* 1982). It has been suggested that *T. dioica* represents a stage between species without and with sex chromosome respectively. In other species of *Trichosanthes* no such incipient chromosomal differentiation could be recorded (Pandey and Saran 1986).

In the present study both male and female plants show identical chromosome morphology. There is remarkable similarity in the number of secondary constrictions, the total chromosome length and even in the average range in chromosome size. The similarity between the two sexes is also evident in their DNA value. The 4C DNA is approximately 11.66 picograms in male and 11.3 picograms in female; the average size ranging from 2.09 to 2.14 respectively. Therefore, in karyotypes, total chromosome lengths as well as the amount of nuclear DNA no marked differences could be observed. In absence of any detectable morphological difference it may be argued that the determination of sex in this species is principally controlled at the autosomal level.

Pollen dimorphism associated with sex chromosome has been reported by certain authors (Strasburger 1910, Westergaard 1958). No dimorphism in the characters of the pollen grains could be observed in *T. dioica*. In the meiotic cells differential behaviour of any pair of chromosomes was not observed. The technique adopted hereby permitted a critical analysis and identification of chromosome segments. No supernumerary constricted chromosomes were

observed as suggested by earlier authors. On the other hand, the application of banding technique reveals clearly identifiable chromosome bands. The band homology between male and female individuals has been recorded (Chattopadhyay and Sharma 1988). Simultaneously, several bands could not be fully homologized with its partner. The extent to which such differences is associated with sex dimorphism is not yet fully resolved. Dimorphism in chromosome bands may even reveal the cryptic genetic diversity existing between different individuals of a species. However, application of this improved banding technique in a large number of male and female individuals of *Trichosanthes dioica* may reval differences, if any, associated with the expression of sex.

At the present state of our knowledge and on the basis of the evidences gathered during this study the sex difference in *T. dioica* can not be correlated with any chromosome heteromorphicity.

In *Coccinia indica* XY mechanism is well established. In the induced tetraploids and their segregation individuals with variable number of sex chromosome have been obtained. The strong male determining characteristics of Y as compared to that of femaleness in X has been established (Roy and Roy 1971).

In the present investigation too, such heteromorphic pair has been clearly observed. The Y chromosome is very long as compared to the rest. The karyotypes of both male and female individuals do not show a marked difference excepting the presence of XY in male. The XX chromosome in female could not be clearly differentiated because of the absence of any detectable difference from other short chromosome pairs. The Y chromosome is provided with a secondary constriction. In addition, there is another pair of chromosomes with secondary constrictions present in both male and female plants. The chromosome with secondary constrictions belonging to A type, common to both, is fairly large as compared to the rest. The range in chromosome size between the two sexes differs markedly at the upper level because of the presence of Y chromosome. The total chromosome length too is slightly more being 36.86 μm in male as compared to 34.68 μm in female.

The similar difference between the male and the female in 4C DNA value is also observed because of the presence of long Y chromosome. It is 10.35 picograms in male as against 8.35 picograms in female. Therefore, the differences in chromosome size in males of *Coccinia indica* reflect additional amount of chromatin rather than differential condensation. The differential chemical nature is clearly indicated.

The heterochromatic nature of Y is also indicated in its staining behaviour. In the both prophase and metaphase it remains highly condensed, in the intermitotic stage as well strong heterochromatic block has been noted. The Y chromosome of *C. indica*, therefore, has a high heterochromatic content.

The heterochromatic nature of Y makes it likely to have the high repetitive DNA content. The correlation between repetitive DNA and heterochromatin has been well eleborated (Flavell 1980, 1882, Bennett 1982, Sharma 1983, 1985). The analysis of repetitive DNA in Y chromosome of *Coccinia indica* would be worth investigation. It would be desirable to know the extent to which structural gene involved in expression of maleness is associated with repeat DNA content in this chromosome.

Radiation study so far carried out has led to the occurrence of subgynoecious plant in the species (Roy and Roy 1971). It has been suggested that in the Y chromosome there is a strong female suppressor. In the species of *Melandrium* similar behaviour has been recorded. Critical radiation studies coupled with identification of segments of the Y chromosome in the pregeny, undergoing breakage, are necessary. The heterochromatic nature and increased amount of DNA in the Y chromosome have been demonstrated in the present investigation. The analysis of radiation induced breaks in specific loci and the associated sex related phe-

notypic changes and estimation of the repeat DNA content may give an indication of the structural and repeat sequences in Y chromosome.

DNA content. chromosomal length and plant habit

An estimation of the nuclear DNA content of the herbaceous annuals and woody perennials has revealed that the latter in general have more DNA content than the former (Bennett 1982, Ohri and Khoshoo 1986). This is, however, been related to the duration of mitotic cycle, generation time and the duration of period for favourable growth (Mukherjee and Sharma 1990).

Both *Trichosanthes dioica* and *Coccinia indica* are herbaceous species. Of the two species *C. indica* is a perennial climber wherea the habit of *T. dioica* is trailing, the vegetative phase only extending upto 3 months during summer. However, the thin root stock of *T. dioica* remains and can perennate underground. Despite being herbaceous, the two species differ remarkably from each other in the mode and nature of growth, one behaving as perennial, the other as an annual. The plants of *T. dioica* are visible only for a short period completing the vegetative and fruiting season within 3–4 months.

The DNA value of both male and female plant of *Trichosanthes dioica* is comparatively higher than those of *Coccinia indica.* The total chromosome length too reflects this difference. The average chromosome length of *T. dioica* is also higher than that of *C. indica.* From all these evidences it is clear that *T. dioica* has longer chromosome and higher DNA value as compared to that of the other. Despite this difference in favour of *T. dioica* it is *de facto* annual in habit as compared to the perennial *C. indica.* This high DNA value in an annual as compared to that of a related perennial genus and not *vice versa* is of special interest. The reverse situation is met with in most other species. However, this difference may be due to their distinct generic difference. The parameters of chromosome size and DNA content are under strict genetic control. The difference between the two is principally attributed to their different genotype. It would be worthwhile to investigate the period taken for the completion of mitotic cycle in both species having difference in amount of nuclear DNA.

Summary

Analyses of the structure and behaviour of chromosomes and estimation of 4C nuclear DNA in both male and female plants of *Coccinia indica* and *Trichosanthes dioica* were made. In male and female plants of *T. dioica* had identical chromosome numbers. There was remarkable similarity between sexes in chromosome morphology, number of secondary constrictions and total chromosome length, as well as the amount of 4C DNA. In absence of any detectable chromosomal differences it may be argued that the sex determination in this species is principally controlled at the autosomal level. The karyotypes of male and female individuals were not markedly different in *C. indica*, except for the sex chromosomes. The total chromosome length was slightly more in male as compared to female plant. There was a corresponding difference in 4C DNA values. The differences in chromosome size between sexes of *C. indica* reflected additional chromatin rather than differential condensation. The heterochromatic nature of long sex chromosome was also indicated in its staining behaviour. The total chromosome length, average chromosome length and the 4C nuclear DNA values of both males and females of annual *T. dioica* were comparatively higher than those of the related perennial, *C. indica.*

Acknowledgement

Financial assistance from University Grants Commission and Indian National Science Academy are gratefully acknowledged.

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