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Research Article

ANALYSIS OF KARYOTYPE OF THE SEX FORMS OF *TRICHOSANTHES BRACTEATA* AND *THLADIANTHA DUBIA*

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ABSTRACT
Chromosome studies were carried out in two dioecious species of cucurbits, viz., *Thladiantha dubia* and *Trichosanthes bracteata* for a detailed comparison of the karyotype of their respective sexes. While the somatic chromosome number of each sex form of *Thladiantha dubia* was recorded as 2n = 18, the somatic chromosome count of male and female plants of *Trichosanthes bracteata* was found to be 2n = 22. In both genera the karyotype of male and female plants showed high homogeneity and no heteromorphicity was recorded in relation to sex. However, in comparison to symmetrical nature of chromosomes of *Thladiantha dubia*, a progressive asymmetry was observed in *Trichosanthes bracteata.*

Keywords: *Dioecious, Karyotype, Heteromorphicity, Asymmetry.*

INTRODUCTION
Cucurbitaceae is one of the most genetically diverse groups of plants approximately having 130 genera and 800 species (Jeffrey, 2005; Renner and Pandey 2013) many of which are economically important. Dioecism exists in a number of cucurbitaceous genera including *Trichosanthes* and *Thladiantha*, but presence of the sex chromosomes are reported only in few species like *Coccinia indica* and *Bryonia dioica* (Chakraborty, 1948; Chattopadhyay and Sharma, 1991; Correns, 1903; D'Curz *et al.*, 1972; Gala´n, 1946; Grant *et al.*, 1994; Mayer and Charlesworth, 1992; Ming *et al.*, 2011; Oyama *et al.*, 2009; Roy and Roy 1971; Sen and Datta 1977; Sinha *et al.*, 1997; Westergaard, 1958). The term sex chromosome is generally applied to a pair of chromosomes on which the sex determination locus resides regardless of whether they are morphologically distinguishable (heteromorphic) and non-recombining, or morphologically indistinguishable (homomorphic) and recombining along their length (Kejnovsky *et al.*, 2009). In the flowering plants, approximately 4-6% or 14,000 species in 960 genera and 200 families are dioecious (Guttman and Charlesworth, 1998; Renner and Ricklefs, 1995). *Trichosanthes bracteata* (Lamk.) Voigt, a vegetatively propagated dioecious perennial species, commonly grows in moist thickets in the Eastern Himalayas in India, Bangladesh, Southern China, Southern Japan, Malayasia and tropical Australia (Bhandari *et al.*, 2008, Rahman *et al.*, 2006). *Trichosanthes bracteata* is an important medicinal plant in several traditional systems (Dubey *et al.*, 2012) and its seeds also contain high amount of punicic acid (Laxminarayan *et al.*, 1988). *Thladiantha dubia* is another vegetatively propagated dioecious perennial species and is distributed in the Assam, North Bengal and E. Himalayas regions in India, apart from Bangladesh, Thailand, Malaya, China and Europe (Alegro *et al.*, 2010; Deb, 1981). This plant has been used in Chinese folk medicine for treatment of various diseases, such as scrofula, carbuncle, lactation deficiency etc. (Wang *et al.*, 2011). In both species male and female plants strictly maintain their respective sexual phenotypes and every year sprouting occurs, from their respective vegetative reproductive structure i.e., the tuberous root without failing, to reproduce their own kind. Limited cytogenetic studies had so far been carried in the plant *Trichosanthes bracteata* and there is a record of polyploid series from diploid to hexaploid (Verghese, 1971, 1972; Rangaswami, 1949; Singh and Roy, 1973; Thakur, 1973). A few workers reported the somatic chromosome number of *Thladiantha dubia* as 2n=18 chromosomes (Delay, 1947; Mra'z, 2005; Probatova and Sokolovskaya, 1988). However, comparative analysis of the detailed karyotype of the sex forms of both species was not carried out till date probably due to its ecological distribution and extreme difficulty in obtaining suitable chromosome

spreads needed for thorough analysis. The present work deals for the first time, with the comparative chromosomal study of the sex forms of *Thladiantha dubia* and *Trichosanthes bracteata*.

MATERIALS AND METHODS

The tuberous roots of the sex forms of *Trichosanthes bracteata* {herbarium voucher - 1047(a), 1047(b)} and *Thladiantha dubia* {herbarium voucher - 1048(a), 1048(b)} growing in wild condition, collected from west Tripura, were grown in the experimental garden of the Department of Botany, Tripura University for research purposes. Young leaf tips of the sex forms of *Thladiantha dubia* and *Trichosanthes bracteata* were pre-treated in saturated solution of para-dichlorobenzene at 10-15^{o}C for 4 hours followed by over-night fixation in 1:3 acetic-ethanol mixture. The leaf tips were then stained overnight in 2% aceto-orcein after hydrolysis in 5(N) HCl at cold for 30 minutes which were finally squashed in 45% acetic acid.

RESULTS AND DISCUSSION

In both *Trichosanthes bracteata* and *Thladiantha dubia*, the male and female flowers differ from each other and it has been observed that the male flowers of both species are in racemes and have bracts whereas the female flowers are solitary and without any bract (Figs. 1. A, B, C, D).

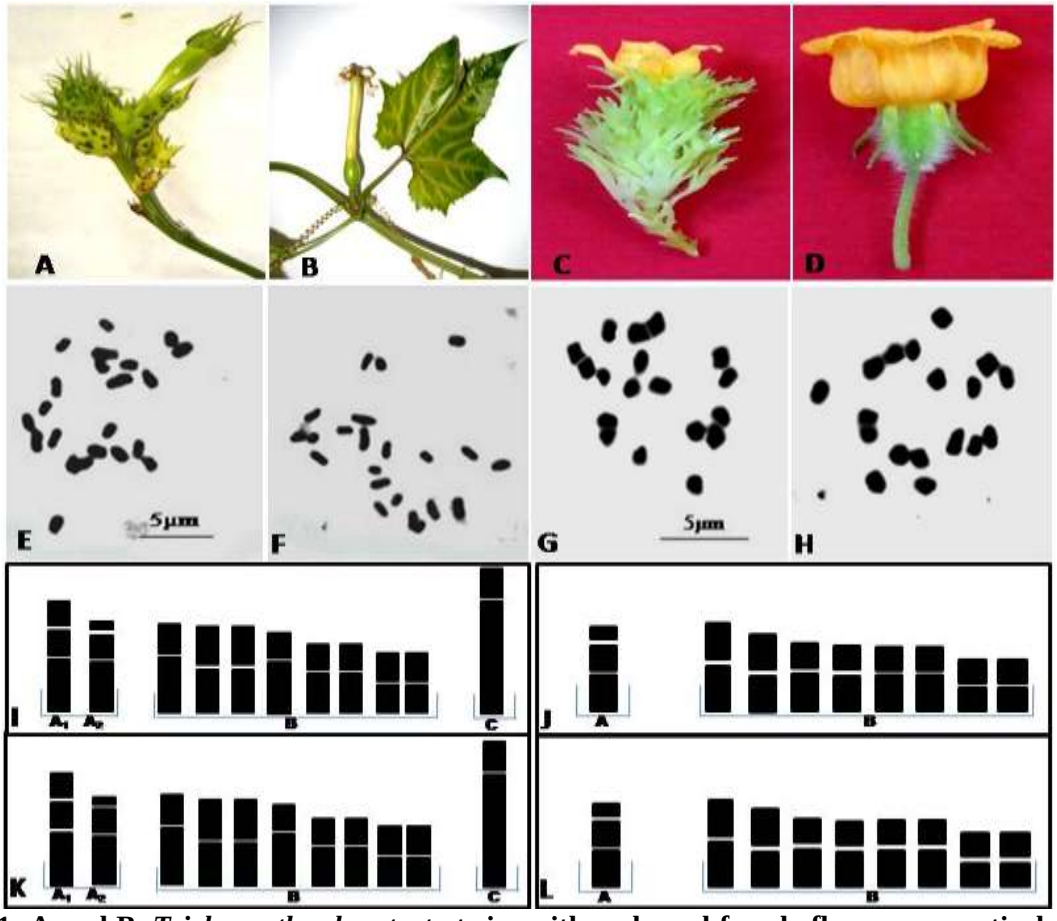

Figure 1: A and B. *Trichosanthes bracteata* twigs with male and female flowers respectively. C and D. *Thladiantha dubia* male and female flowers respectively. E and F. Somatic metaphase plates of male and female plants of *Trichosanthes bracteata* showing 2n = 22 chromosomes. G and H. Somatic metaphase plates of male and female plants of *Thladiantha dubia* showing 2n = 18 chromosomes. I and K. Ideogram of male and female plants of *Trichosanthes bracteata* respectively. J and L. Ideogram of male and female plants of *Thladiantha dubia* respectively.

The somatic chromosome number was found to be 2n=22 (Figs E, F) in both sexes of *Trichosanthes bracteata* having two pairs of chromosomes bearing secondary constrictions and can be classified (Levan *et al* 1964) into 4 distinct morphological types.

In male and female plants of *Thladiantha dubia*, the somatic chromosome count was found to be 2n=18 (Figs G, H) having one pair of chromosomes bearing secondary constrictions. However, in contrast to *Trichosanthes bracteata*, the somatic chromosomes can be classified into 2 distinct morphological types only.

Type **A_1:** Chromosomes are short (2.13µm) having 2 constrictions, primary and secondary, both are nearly sub-median in position.

Type **A_2:** Short chromosomes (1.68µm – 1.46 µm) bear 2 constrictions, primary and secondary, one is nearly sub-median and the other is sub-terminal in position.

Type **B:** Short chromosomes (1.06µm – 0.97µm), the constriction of chromosomes are median and or nearly median in position.

Type **C:** The chromosomes (2.96µm) are short in size and the constrictions are nearly sub-terminal in position.

The largest chromosome of the somatic chromosome complements of *Trichosanthes bracteata* is a medium sized acrocentric chromosome with sub-terminal constriction and does not bear any secondary constriction as reported by Verghese (1972). The TF% of male and female plants is 36.07 and 35.97 respectively. In the karyotype of male and female plants of *Trichosanthes bracteata*, one pair of chromosome was found to be acrocentric. The TF% of male and female plants of *Thladiantha dubia* is 42.80 and 42.56 respectively and, moreover no acrocentric or telocentric chromosomes were observed in the karyotype of male and female plants of *Thladiantha dubia*. . Somatic chromosome count 2n=22 and 2n=18 are found constant in the respective sex forms of *Trichosanthes bracteata* and *Thldiantha dubia* which corroborates the findings of Verghese (1971) and Mar'z (2005). The karyotypes (Figs. I, K, J and L) of male and female plants of both species exhibit a gross similarity in the types of chromosome present, number of chromosomes with secondary constriction, TF%, chromosome arm symmetry index and total chromosome length (Table-1).

Table 1: Comparative analysis of karyotype of male and female *Trichosanthes bracteata* and *Thladiantha dubia*

Name of the species	Somatic chromo-some number	Karyotype formula	No. of chromosomes with secondary constriction	Total chromosome length (µm ± S.E)*	TF%	Chromoso me arm symmetry index	Stebbin's categorization
Trichosanthes bracteata (M)	22	$2A_1$+ $2A_2$ + 16B + 2C	4	36.26 ± 0.003	36.04	56.34	2B
Trichosanthes bracteata (F)	22	$2A_1$+ $2A_2$ + 16B + 2C	4	36.36 ± 0.003	35.93	56.10	2B
Thladiantha dubia (M)	18	$2A_2$+ 16B	2	22.88 ± 0.004	42.56	74.35	1A
Thladiantha dubia (F)	18	$2A_2$+ 16B	2	22.84 ± 0.004	42.80	74.12	1A

The diploid males are homogametic and no heteromorphic pair of chromosome is recorded in relation to sex. The chromosome complements, therefore, do not show XY mechanism in relation to sex. It seems that the sex expression in *Trichosanthes. bracteata* and *Thladiantha dubia* is under genic control.

Stebbins (1971) analysed the degree of asymmetry of flowering plants by recognizing three degrees of difference between the largest and smallest chromosome complements and four degrees with respect to the proportion of chromosome which are acro- or telocentric. In the light of this knowledge, chromosomes of the sex form of *Thladiantha dubia* are symmetric and fall under category 1A. On the other hand, the karyotype of male and female plants of *Trichosanthes bracteata* falls under category 2B. Thus a progressive asymmetry is observed in *T. bracteata* which suggests evolutionary advancement of *Trichosanthes bracteata* over *Thladiantha dubia*.

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REFERENCES

Alegro A, Bogdanovic S, Restnic I and Borsic I (2010). *Thladiantha Dubia* Bunge (cucurbitaceae), new alien species in Croatian Flora. *Natura Croatica* **19** (1) 281-286.
Bhandari S, Dobhal U, Sajwan and Bisht NS (2008). *Trichosanthes tricuspidata*: A Medicinally Important Plant. *Trees for Life Journal* **3**(5) 1.
Chakraborty AK (1948). Cytology of *Coccinia indica* W and A with reference to the behaviour of its sex-chromosomes. *Proceedings of the Indian Academy of Science, Section* **B** (27) 74-86.
Chattopadhyay D and Sharma AK (1991). Sex Determination in Dioecious Species of Plants. *Feddes Repertorium,* Berlin **102** 29-55.
Correns C (1903). Weitere Beitrage zur Kenntnis der dominierenden Merkmale und der Mosaikbildung der Bastarde. *Berichte der Deutschen Botanischen Gesellschaft* **21** 195-201.
D'curz R, Vyahalkar GR and Ugale SD (1972). Cytogenetic studies in tetraploid *Coccinia indica* W. and A. *Caryologia* **25** 505-512.
Deb DB (1981).*The Flora of Tripura State* (Today and Tomorrow's Printers and Publishers) 259.
Delay C (1947). Recherches sur la structure des noyaux quiescent chez les Phanerogames. *Rev. Cytol. Cytophysiol. Ve'g.* **9** 169-222.
Duvey BK, Goyel R, Parashar B, Verma D, Dhameja H and Sharma D (2012). *Trichosanthes tricuspidata*: Exploration of it's medicinal value. *Asian Journal of Pharmacy and Technology* **2**(1) 26-28.
Gala´n F (1946). Sur la g´enetique de la monoecie et dioecie zygotique chez *Ecballium claterium* Rich. *Crit. Rev. Hebd. Seances Acad. Sci.* Paris **222** 1130-31.
Grant S, Houber A, Vyskot B, Siroky J, Pan WH and Macas J (1994). Genetics of Sex Determination in Flowering Plants. *Developmental Genetics* **15** 214-230.
Guttman DS and Charlesworth D (1998). An X-linked Gene with a Degenerate Y-linked Homologue in a Dioecious Plant. *Nature* **396** 263-266.
Jeffrey C (2005). A new system of Cucurbitaceae. *Botanicheskii Zhurnal* **90** 332–335.
Kejnovsky E, Hobza R, Cermak T, Kubat Z and Vyskot B (2009). The role of repetitive DNA in structure and evolution of sex chromosomes in plants. *Heredity* **102** 533-541.
Lakshminarayana G, Rao KS, Klttur MH and Mahajanshetty CS (1988). Occurrence of punicic acid in Trichosanthes bracteata and Trichosanthes nervifolia seed oils. *Journal of the American Oil Chemist's Society* **65(**3) 347-348.
Levan A, Fredga K and Sandbery AA (1964). Nomenclature for Centromeric Position on Chromosomes. *Heriditas* **52** 201-220.
Mar'z P (2005). Chromosome number and DNA ploidy level reports from Central Europe – 2. *Biologia* **61** 115-120.
Mayer SS and Charlesworth D (1992). Genetic Evidence for Multiple Origins of Dioecy in Hawaiian Shrub Wikstroemia (Thymelaeaceae). *Evolutions* **46** 207-215.
Ming R, Bendahmane A and Renner SS (2011). Sex Chromosomes in Land Plants. *Annual Review of Plant Biology* **62** 485-514.

Oyama RK, Volz SM and Renner SS (2009). A sex-linked SCAR marker in *Bryonia dioca* (Cucurbitaceae), a dioecious species with XY sex-determination and homomorphic sex chromosomes. *Journal of Evolutionary Biology* **22** 214-224.
Probatova NS and Sokolovskaya AP (1988). Chromosome numbers in vascular plants from Primorye Territory, the Amur River basin, north Koryakia, Kamchatka and Sakhalin. *Botanicheskii Zhurnal* (Moscow and Leningrad) **73** 290–293.
Rahman AHMM, Anisuzzaman M, Ainsuzzaman M, Alam MZ, Islam AKMR and Zaman ATMN (2006). Taxonomic studies of the cucurbits grown in the northern parts of Bangladesh. *Research journal of Agriculture and Biological Science* **2**(6) 299-302.
Rangaswami AK (1949). Sex Chromosome of *Trichosanthes palmata* Roxb. *Proceeding of the 36th Indian Science Congress* **3** 137.
Renner SS and Pandey AK (2013). The cucurbitaceae of India: Accepted names, synonyms, geographic distribution, and information on images and sequences. *Phytokeys* **20** 53-118.
Renner SS and Ricklefs RE (1995). Dioecy and its correlates in the flowering plants. *American journal of Botany* **82**(5) 596-606.
Roy RP and Roy PM (1971). Mechanism of sex determination in *Coccinia indica*. *Journal of the Indian Botanical Society* **50A** 391-400.
Sen R and Datta BK (1977). Chromosomal basis of sex expression in some dioecious cucurbits. *Nucleus* **22** 241.
Singh AK and Roy RP (1973). Cytological Studies in *Trichosanthes palmata* Roxb. A Natural Hexaploid. *Science and Culture* **39** 505-506.
Sinha S, Debnath B and Sinha RK (1997). Differential Condensation of Chromosome Complements of Dioecious *Momordica dioca* Roxb. in Relation to DNA Content. *Indian Journal of Experimental Biology* **35** 1246-1248.
Stebbins GL (1971). *Chromosomal Evolution in Higher Plants* (Addison – Wesley Publ. Co., Melno Park (Calif)).
Thakur GK (1973). A Natural Tetraploid in the Genus *Trichosanthes* from Bihar. *Proceeding of the 60th Indian Science Congress* **3** 324.
Verghese BM (1971). Cytology of *Trichosanthes palmata* Roxb. *Cytologia* **36** 205-209.
Verghese BM (1972). Cytology and Origin of a Tetraploid *Trichosanthes palmata* Roxb. *Genetica* **43** 292-301.
Wang L, Zhao D, Di L, Cheng D, Zhou X, Yang X and Liu Y (2011). The anti-hyperplasia of mammary gland effect of *Thladiantha dubia* root ethanol extract in rats reduced by estrogen and progestogen. *Journal of Ethnopharmacology* **134**(1) 136-140.
Westergaard M (1958). The Mechanism of Sex Determination in Dioecious Flowering Plants. *Advances in Genetics* **9** 217-281.