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Karyotype analysis and biochemical estimation of *Coccinia indica*: An ethnomedicinal dioecious cucurbit of Tripura, North East India

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Abstract
Coccinia indica Wight and Arn. Commonly used as leafy vegetable in Tripura, North East India. Young leaves and fruits are used as vegetable in different forms of recipes of tribal and rural communities of this region. The cucurbit also widely used for its ethnomedicinal properties among them. The utility of the vegetable as good source of protein and total amino acids with low level of phenolics and sugar clearly indicated its significance as useful health diet and alternative food supplement to the common people. The somatic chromosome number and karyomorphological details were also investigated in these dioecious forms. Somatic chromosome number was found to be 2n=24 with karyotype formula $A_2B_{20}C_{X1}D_{Y1}$ and $A_2B_{20}C_{X2}$ in male and female sex forms. Diploid males are heterogametic with 22+XY and females are homogametic with 22+XX. The somatic chromosomes are generally short with median and submedian constrictions except the metacentric X and Y chromosome. X chromosomes are larger than other chromosome complements in both the sex forms and Y chromosome is conspicuously large. The chromosome count and constancy of the karyotype with biochemical analysis recorded in the present study.

Keywords: *Coccinia indica*, dioecious, somatic chromosome, biochemical estimation

1. Introduction
Tripura is one of the smallest State of North Eastern parts of India and inhabited by the Tribal of Tibeto-Burman stock with as many as 19 different tribal communities and also represented by other communities like Bengali, Manipuri and Nepali since king dynasty in the State [1]. This ethological diversity has not only reflected their sociological and cultural aspects but also exposed unique traditional food habit and rural-herbal therapy [2]. The state is also rich in floristic diversity. Many of these ethno-botanical species and their traditional knowledge of uses associated with health care system are to be explored scientifically [3].
Coccinia indica Wight and Arn. is the dioecious vegetatively propagated species of cucurbitaceae which shows wide spread distribution in the tropical and sub-tropical regions of the world [4]. As a medicinal plant it is well-known to the herbal doctors of Tripura and other states of North-Eastern region. The tribal people of Tripura cultivate this plant both as vegetables as well as medicinal plant. They prepare various tasty dishes with this vegetable. Aqueous extract of this plant is used in the treatment of diabetes, jaundice, sores of tongue, diarrhoea with bleeding, epileptic convulsions, earache etc. The paste of this plant is applied in leucorrhoea [2]. The plant is considered as refrigerant and astringent [5]. It is used to treat fever by applying the infusion of the whole plant on the body of the patient. Juice of the stem is dropped into ear to relieve earache. Roots are employed in late stage of syphilis. The powder root is applied over the nose to treat Syphilitic ulcer of the nose while decoction is taken to treat Syphilis [6].
Earlier workers have found that a series of polyploidy (=24,36,48) exists in *Coccinia indica* with distinct heteromorphic pair of sex chromosomes [7-10] in which Y chromosome plays a decisive role in the differentiation of the male sex [11]. Considering its versatile activities, an effort has been made to study karyomorphological nature of the species at cytological level along with the biochemical activity of the plant.

2. Materials and Method
The tuberous roots of the sex forms of *Coccinia indica* grown (Fig.1a) in wild condition collected from West Tripura were grown in the experimental garden of Women's College,

Agartala. The plant was identified consulting authentic floristic literatures like Flora of Tripura [12], Flora of Assam [13] and Flora of British-India [14]. Cross examination of the specie was done consulting with Dr. Nalini Kanta Chakraborty, Ex-Reader, Department of Botany, M. B. B. College, Agartala. Finally, voucher specimen was prepared following conventional methods [15] deposited in the Departmental Herbarium.

Fig 1: *C. indica* twigs growing in wild condition

2.1 Study of Chromosome

To analyse the somatic chromosome shoot tips were pre-treated in saturated solution of paradichloro benzene for 4h at 10-15 °C followed by overnight fixation in 1 : 3 acetic acid-ethanol mixture. The shoot tips were stained overnight in 2% aceto-orcein after hydrolysis in 5N Hcl at 10 °C for 15 min and finally squashed in 45% acetic acid.

After staining the shoot tips were squashed in 45% acetic acid and studied under compound microscope. At least 15 observations were made from such treatments. Chromosomes of the plates were drawn using oil immersion objective (x 1460). Measurement of chromosomal complements like absolute length of the chromosome, length of short arm and long arm and F% are carried out for each complement with 15 different metaphase plates. Detailed karyotype table was constructed from the well spread metaphase plates using total length, short arm, long arm and F% of the chromosome complements and types of the chromosome complements. The F% was determined by using the following formula:

$$F\% = \frac{\text{Short arm length}}{\text{Total length of chromosome}} \times 100$$

Total Forma percentage (TF %) of chromosomal complements [16]. was also calculated.

To analyse the meiotic chromosomes young flower buds of male sex form of *Coccinia indica* was fixed in 1: 2 glacial acetic acid - ethyl alcohol mixture for 6h, then in 1:3 acetic-ethanol mixture for overnight and finally transferred to 70% ethanol for 7days. Then the buds were treated with 45% acetic acid for 20 min, followed by warm hydrolysis in 1 N Hcl for 20 min at 50°C. Finally after thorough washing in distilled water flower buds were stained in 2% aceto-carmine for 3h and smeared in 45% acetic acid. As the cytoplasm takes colour, smearing in 45% acetic acid gives desired result.

2.2 Biochemical study

Fresh apical young leaf, young shoot, mature leaf and young fruits of *C. indica* was used for biochemical analysis. The fresh materials were washed with double distilled water repeatedly and were used for analysis. Nutritional value as biochemical characters like protein, phenol, sugar and total free amino acids were estimated following the standard methods of Lowry *et al.* (1951) [17], Swain and Hills(1959) [18], Hodge Hofreiter (1962) [19] and Sadasivam and Manickam (1992) [20] respectively.

Fig 2: *C. indica* twigs with male and female flowers respectively.

3. Result and Discussion

The sexual phenotypes of *Coccinia indica* differ and it has been observed that the male flowers of *C. indica* are in solitary racemes whereas female flowers are solitary (Fig. 2b, c).

In the present investigation the Somatic chromosome number $2n = 24$ (Fig. 3d, e) was found to be constant in the male and female plants of *Coccinia indica* as was reported by earlier researchers [21]. In both sexes there were 11 pairs of metacentric and one pair of sub-metacentric chromosome having secondary constriction. The present cytological investigation also revealed the presence of a distinct heteromorphic pair of sex chromosome in male plants. Diploid males are, therefore, heterogametic with 22 + XY and the females are homogametic with 22 + XX. The male determining Y chromosome is conspicuously large, nearly metacentric and does not bear secondary constriction as reported by Singh [22].

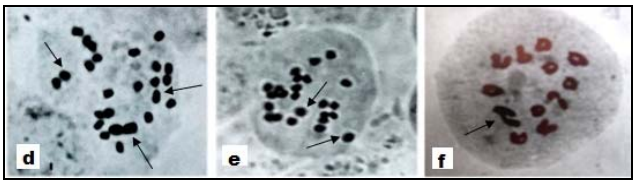

Fig 3: Somatic metaphase plates male and female plants of *C. indica* respectively showing 2n=24 chromosomes (Arrow indicates the X and Y chromosome). Meiotic metaphase-I showing distinct bivalents (Arrow indicates the heteromorphic bivalent).

This paradoxical situation can be explained by the fact that in *Coccinia indica* the maleness determining Y chromosome (Fig.3d) is conspicuously large and is nearly 3-4 times larger than majority of the chromosomes present in somatic chromosome complement of the male plantas well as female plant also. Whereas in both the sex forms of *C. indica* the X chromosomes are larger than other somatic chromosome complements (Table 1). Detailed karyological studies suggest that apart from the sex chromosomes, the karyotypes of male and female plants exhibit similarity in the types of chromosome present, number of chromosomes with secondary constriction, chromosome arm symmetry index (SI) and TF% etc. (Table 1). Relatively less size differences between the chromosomes of the complement suggests its karyotype is to be considered as slightly asymmetric one and according to degree of asymmetry, the karyotype of the species belongs to the category 1A and 1B(Table 2) in

dioecious *C. indica* [23].

Meiotic study from flower buds of male sex form show 12 pairs of distinct bivalents where the heteromorphic bivalent shows homology (Fig.3f) exists only between the tips of X and Y chromosomes [24].

Biochemical characters of the apical young leaf, young shoot, mature leaf and young fruit were analyzed and revealed high protein and total free amino acid with low level of phenolic and sugar content (Table 3).

Table 1: * Karyomorphological characteristics of both the sex forms of *Coccinia indica*

Sex	Somatic Chromosome number	No. of chromosome with secondary constriction	Total chromosome Length (µm)	Total F%	Chromosome Symmetry index (SI)	Length of X-chromosome (µm ± SD)	Length of Y-chromosome (µm ± SD)	Range of Somatic chromosome (µm ± SD)
Male	24	2	41.75	45.41	84.18	2.16 ± 0.42	4.92 ± 0.74	1.92 ± .024 -1.22 ± 0.43
Female	24	2	39.54	46.16	73.15	2.16 ± 0.56	-	1.92 ± 0.43 -1.20 ± 0.47

*Mean of 15 metaphase plates

Table 2: Stebbins categorization of karyotype *Coccinia indica*

Ratio Largest/Smallest	Proportion of Chromosome with arm ratio<2:1			
	0.00	0.01-0.50	0.51-0.90	1.00
	1A	2A	3A	4A
<2:1	*C. indica* (Male)			
2:1- 4.1	1B			
	C. indica (Female)			

Table 3: Biochemical estimation of different vegetative parts of *Coccinia indica*

Sample used	Biochemical parameters (mg. /g. Fr.wt.)			
	Protein	Phenol	Sugar	Total free amino acids
Apical young leaf	21.34± 0.24	1.16± 0.14	1.43± 0.53	105.45± 0.71
Young Shoot	16.32± 0.37	1.25± 0.25	1.97± 0.33	96.22± 0.36
Mature Leaf	29.33± 0.54	0.96± 0.21	2.22± 0.37	185.12± 0.43
Young Fruit	22.23±0.22	0.76±0.31	0.82±0.31	178.32±0.52

4. Conclusion

Karyomorphological account of the taxon with a karyotype formula $A_2B_{20}C_{X1}D_{Y1}$ and $A_2B_{20}C_{X2}$ in both the sex forms are described in the present communication. The somatic chromosomes are short with median and submedian constriction except the metacentric Y chromosome which is conspicuously large. The X chromosome is larger than other somatic chromosome complements may be determined from Camera Lucida drawing and mean of measurement. The preliminary information on biochemical analysis showed that the leafy vegetable may be used as health diet and alternative food supplement.

5. Acknowledgement

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