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### RESEARCH ARTICLE

#### KARYOMORPHOLOGICAL AND STOMATAL STUDIES IN *TINOSPORA CORDIFOLIA*(WILLD.)

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#### Abstract

Among the vast library of important medicinal plants, *Tinospora cordifolia* (Willd.) is a deciduous climbing shrub which belongs to the family Menispermaceae. It has hypoglycemic, antipyretic anti-allergic, Antineoplastic, anti-inflammatory, anti-oxidant and immuno-modulatory properties. The karyotype analysis and stomatal studies form an important tool for understanding the Phylogenetic relationship, in taxonomic identification and to improve breeding program. Keeping these views in mind, the present investigation was undertaken to disentangle the stomatal index and karyomorphological data. The Stomatal character and detailed Karyotype analysis of the plant *Tinospora cordifolia*(Willd.) were performed. The leaf had Anomocytic type of stomata. Stomatal index were higher at the apex portion of leaf. *Tinospora cordifolia*(Willd.) under investigation was diploid with  $2n = 26$ . Normal mitotic division were observed in all the examined cells with one pair of chromosome bearing satellite. The chromosomes were measuring  $2.42 \pm 0.18$  to  $6.82 \pm 0.02$ . Gradient index is more than 30.

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#### Introduction:-

The world health organization (WHO) estimated that upto 80% of people still rely mainly on traditional remedies such as medicinal plants for their medicines. Among the vast library of important medicinal plants, *Tinospora cordifolia*(Willd.) is a deciduous climbing shrub which belongs to the family Menispermaceae [C Bharti et al, 2018]. It is widely used as a unique ingredient of various natural medicine and traditionally use for numerous ailments like fever, vomiting, diabetes, jaundice, anaemia, polyuria and skin diseases etc. It has hypoglycemic, antipyretic anti-allergic, Antineoplastic, anti-inflammatory, anti-oxidant and immuno-modulatory properties. A variety of constituents have been isolated from different parts of *Tinospora cordifolia*(Willd.), mainly contains alkaloids like Aporphine alkaloids, clerodone, diterpenes, berberine, palmatine, tembertarine, tinosporin, magniflorine, choline, and glycosides like Tinocordiside, Tinocordifolioside, steroids essential oils, mixture of fattyacids and polysaccharides [ Minu Bhan, 2016].

Nevertheless the great importance of this species only a few reports are available about cytogenetic of *Tinospora cordifolia*(Willd.). Realising the fact that the conventional breeding techniques with modern biotechnological methods are necessary to broaden the genetic base of the *Tinospora cordifolia*(Willd.). A standardization of the karyotype of *Tinospora* species will be extremely important not only to understand the evolution of the genus, but

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also to assist breeding programmes. Earlier it was observed that the haploid chromosome number of *Tinospora cordifolia*(Willd.) to be  $n=12$  [Joshi and Rao,1935]but, later some scientists studied on the chromosome of dioecious plants, examined the pollen mother cells of *Tinospora cordifolia*(Willd.) and reported a new haploid number for this plant was  $n =13$  and  $2n= 26$ [Abraham, 1942]. Later in 1958, whereas some reports showed 13 bivalent chromosomes during meiosis in *Tinospora cordifolia*[Mathew PM,1958]. It is suggested that the existing basic chromosome numbers in the species are 12 and 13, of which 13 is the most common[Joshi AC,1934]. Recent cytological data showed that *Tinospora cordifolia* was diploid with the chromosome number  $2n = 22$  and basic chromosome number is  $n = 11$ . [Richa Jain and Bheem Prasad,2014].The karyotype analysis and stomatal studies form an important tool for understanding the phylogenetic relationship, in taxonomic identification and to improve breeding program. Keeping these views in mind, the present investigation was under taken to disentangle the stomatal index and karyomorphological data [Kumar and Abdali,2015].

The Stomatal character and detailed Karyotype analysis of the plant *Tinospora cordifolia*(Willd.) performed first time in Jharkhand.

### Material and Methods:-

The plant *Tinospora cordifolia*(Willd.) were collected from Birsa Agriculture University Ranchi, Jharkhand, India. The fresh stem cuttings were grown in 1 part vermicompost, 1 part sand, and one part soil mixture. After 10 to 15 day roots were emerged. The plant can be also grown by the help of rootex powder. The rootex powder were applied at the base of stem and kept into water in a container for 7-8 days. The roots were emerged. The root apices of about 1-4 min in length were excised between 1:00 to 2:00 pm under sunlight and were pretreated with 1, 4 paradichlorobenzene for 8-9 hrs. The pretreated root apices were transferred to fixative 1:3 acetoalcohol (cranoy's fluid) for 24 hours. After that the root apices were transferred to 70% alcohol for preservation. Slides were prepared and photomicrographs were taken. The data were statistically analyzed and karyotype classification was made according to Abraham and Prasad (1983). The Total form percentage i.e the average degree of symmetry over the whole karyotype was calculated according to Y. Huziwar. (1962).

The orderly placement of stomata on leaf surface is called stomata patterning. The stomatal studies were done by usual method of scrapping epidermis of fresh leaves. A sharp blade is used for peeling of leaf surface. The epidermis was cut across the leaf and scrapped away together with the mesophyll cells until only the epidermal layer of the leaf remained on the slides [Khan et al]. Both abaxial and adaxial surface of leaves were prepared and observed under the light microscope. The scrapped epidermis were stained with aqueous safranin and mounted by glycerin. The stomatal index was calculated by counting the number of stomata and the epidermal cells. Length and width stomata were measured with the help of ocular and micrometer. The stomatal index (S.I) and guard cell area (A) were calculated as per Willkinson (1979) and Fronco (1939) respectively.

$$\text{Stomatal index (S.I)} = \frac{S}{S+E} \times 100$$

Where S= number of stomata per unit area

E= number of epidermal cells in the same unit area above.

The length and width of Stomatal aperture was calculated by ocular micrometer and the area of stomatal aperture was calculated by the formula(Metcalf CR, Chalk L., 1950 and Hedge et al,2015),

$$A = \frac{\pi}{2} \times l \times b \mu\text{m}^2 \text{ (Since it is a semicircle)}$$

Where, A = Area, l = length, b = width,  $\frac{\pi}{2}$  = constant .

### Results:-

The leaves of *Tinospora cordifolia*(Willd.) was heart shaped. The stomata type was anomocytic. Stomata are only present at abaxial surfaces(Table-1, Fig.-5). Stomatal index( $30.85 \pm 2.569$ ), length( $11.6 \pm 0.382\mu$ ), width( $8.2 \pm 0.344\mu$ ) and Area ( $150.09 \pm 9.37\mu\text{m}^2$ ) of *T. cordifolia* was highest at the apex. It was lowest at the base (SI =  $24.153 \pm 0.791$ ), (L =  $10.6 \pm 0.401\mu$ ), (B =  $7.5 \pm 0.382\mu$ ), (A =  $125.31 \pm 8.36\mu\text{m}^2$ ), [Table-1, Fig-1, Fig - 2].(where SI = Stomatal Index, L= length of stomata, B= width of stomata, A = Area of stomata). The plant *Tinospora cordifolia* was diploid with  $2n=26$ (Fig.- 6). The chromosomes were measuring  $2.42 \pm 0.188$  to  $6.82 \pm 0.02$ [Table2, Fig - 3]. Nearly median, Median, and nearly subterminal chromosomes were focused[Table 2] . The karyotype formula is  $11nm+1m+1nst(+)$ . The Total haploid chromatin length was  $49.2 \mu$  [Table 3]. The Total

form percentage , Gradient index, Symmetry index, Disparity index and Centromeric index are depicted in the table 3 (Fig – 4).The Gradient index of *T. cordifolia* was more than 30.

### Discussion:-

As a basis for causal analysis data from stress physiology research, studies on functional leaf anatomy are becoming more important for evaluating the performance of plants in certain environmental conditions for tracing down its relation with eco- physiology ,ecophysiological adaptations and biosystematics as well [ Vijay Paul et al,2017].The leaf had Anomocytic type of stomata i.e the stomata were not surrounded by any subsidiary cells, only epidermal cells were present along with stomata.Leaves were hypostomatic as stomata were only present on abaxial surface(Metcalf CR, Chalk L., 1950). P.B. Tomlinson said that leaf epidermis is the secondmost important character after cytology for solving taxonomic problem .

*Tinospora cordifolia*(Willd.) under investigation was diploid with  $2n = 26$ [fig. 6]. Normal mitotic division were observed in all the examined cells with one pair of chromosome bears satellite. The plant *T. cordifolia* under investigation have high symmetrical index. Values thus indicating the tendencies towards symmetry. Gradient index is more than 30 which shows highly symmetrical karyotype, considered as Primitive.

### Conclusion:-

On the basis of above findings, it may be concluded that in *Tinospora cordifolia*(Willd.) leaves were hypostomatic, stomata was Anomocytic type and chromosomes were symmetrical showing primitiveness.

### Acknowledgements:-

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**Table 1:-** Stomatal index, Length and width( $\mu$ )of stomata in Abaxial surface.

surface	Apex portion of leaf				Middle portion of leaf				Base portion of leaf			
	S.I	L ( $\mu$ )	B ( $\mu$ )	Area ( $\mu\text{m}^2$ )	S.I	L ( $\mu$ )	B ( $\mu$ )	Area ( $\mu\text{m}^2$ )	S.I	L ( $\mu$ )	B ( $\mu$ )	Area ( $\mu\text{m}^2$ )
Abaxial Surface	30.85	11.6	8.2	150.09	25.63	11.3	8.1	144.45	24.15	10.6	7.5	125.31
	$\pm$ 2.569	$\pm$ 0.382	$\pm$ 0.344	$\pm$ 9.37	$\pm$ 1.375	$\pm$ 0.321	$\pm$ 0.223	$\pm$ 7.67	$\pm$ 0.791	$\pm$ 0.401	$\pm$ 0.382	$\pm$ 8.36

Chromosome No.	Arm length		Chromosome length in $\mu$	Arm ratio LA/SA	R.L	F%	TCI	CI	Classification
	Long (LA)	Short (SA)							
I	3.99 $\pm$ 5.6 7	2.83 $\pm$ 0.1 6	6.82 $\pm$ 0.02	1.40 $\pm$ 8.2 1	100	41.4 9	13.8 6	58.5 0	nm
II	2.64 $\pm$ 0.0 7	2.58 $\pm$ 0.0 5	5.22 $\pm$ 0.12	1.02 $\pm$ 0.0 1	78.5 3	49.4 2	10.6	50.5 7	nm
III	2.44 $\pm$ 0.0 3	2.44 $\pm$ 0.0 3	4.88 $\pm$ 0.01	1.00 $\pm$ 0.01	71.5 5	50.0 0	9.91	50.0 0	m
IV	2.20 $\pm$ 0.0 5	2.1 $\pm$ 0.04 9	4.30 $\pm$ 0.08	1.48 $\pm$ 0.0 2	63.0 4	48.8 3	8.73	51.1 6	nm
V	1.90 $\pm$ 0.0 2	1.84 $\pm$ 0.0 3	3.74 $\pm$ 0.06	1.03 $\pm$ 0.0 1	54.8 3	49.1 9	7.60	50.8 0	nm
VI	1.78 $\pm$ 0.0 1	1.72 $\pm$ 0.0 3	3.50 $\pm$ 0.04	1.03 $\pm$ 0.0 1	51.3 1	49.1 4	7.11	50.8 5	nm
VII	1.70 $\pm$ 0.0 2	1.68 $\pm$ 0.0 1	3.38 $\pm$ 0.04	1.01 $\pm$ 8.9 6	49.5 6	49.7 0	6.86	50.2 9	nm
VIII	3.00 $\pm$ 0.0 2	0.34 $\pm$ 0.0 3	3.34 $\pm$ 0.04	9.18 $\pm$ 0.7 1	48.9 7	10.1 8	18.6 5	89.8 2	nst(+)
IX	1.92 $\pm$ 0.0 4	1.4 $\pm$ 0.07 5	3.32 $\pm$ 0.11	1.38 $\pm$ 0.0 4	48.6 8	42.1 6	6.74	57.8 3	nm

X	1.54±0.0 2	1.52±0.0 1	3.06±0.03	1.01±0.0 1	44.8 6	49.6 7	6.21	50.3 2	nm
XI	1.36±0.0 2	1.34±0.0 2	2.70±0.04	1.01±0.0 1	39.5 8	49.6 2	5.48	50.3 7	nm
XII	1.18±0.0 4	1.14±0.0 3	2.52±0.17	1.07±0.0 3	36.9 5	45.2 3	5.12	46.8 2	nm
XIII	1.14±0.0 3	1.08±0.0 4	2.42±0.18	1.06±0.0 3	35.4 8	44.6 2	4.91	47.1 0	nm

**Table 2:-** Karyomorphological data related to *Tinospora cordifolia* (willd.).

R.l. = Relative Length

F%= Form Percentage

TCI=Total Chromatin Index

CI = Centomeric Index

nm = nearly medium

m = median

nst(+)=nearly subterminal

**Table 3:-** Data related to karyotype of *Tinospora cordifolia* (willd.).

TCL in $\mu$	TF%	GI%	SI%	DI%
49.20	44.74	36.36	82.16	47.62

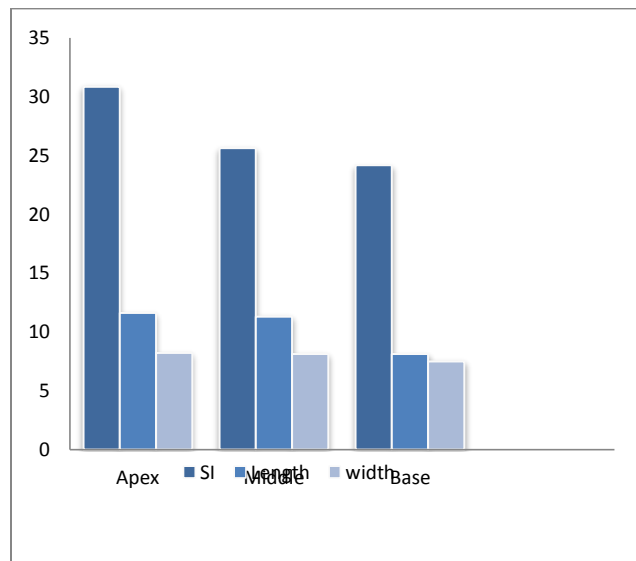
TCL= Total Chromatin Length

TF% =Total Form Percentage

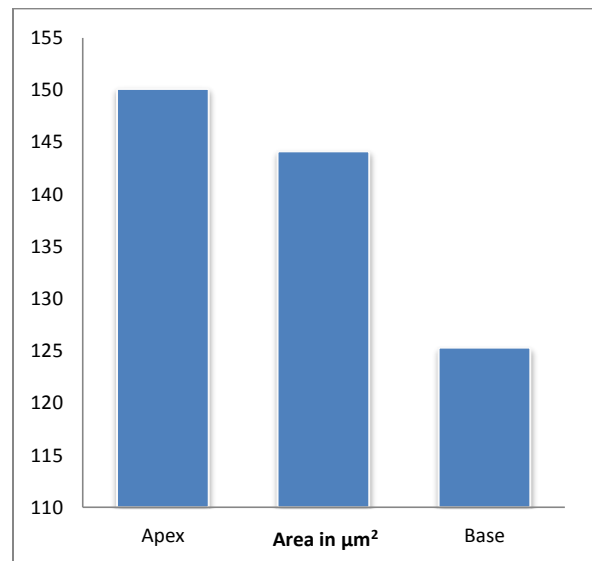
DI% = Disparity index

GI% = Gradient Index

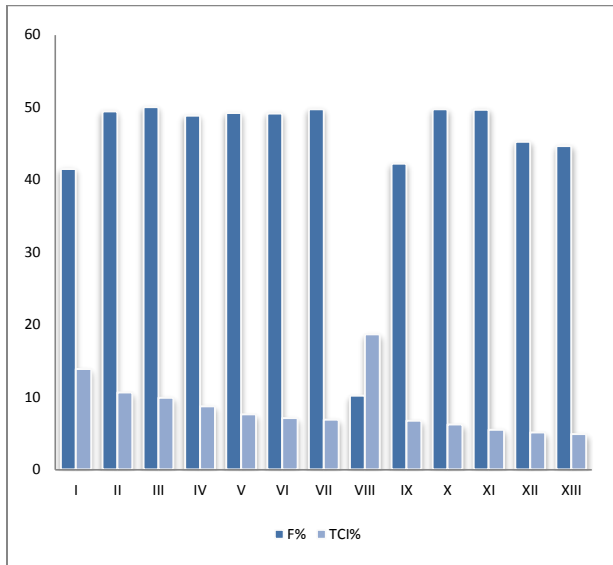
SI% = Symmetry index



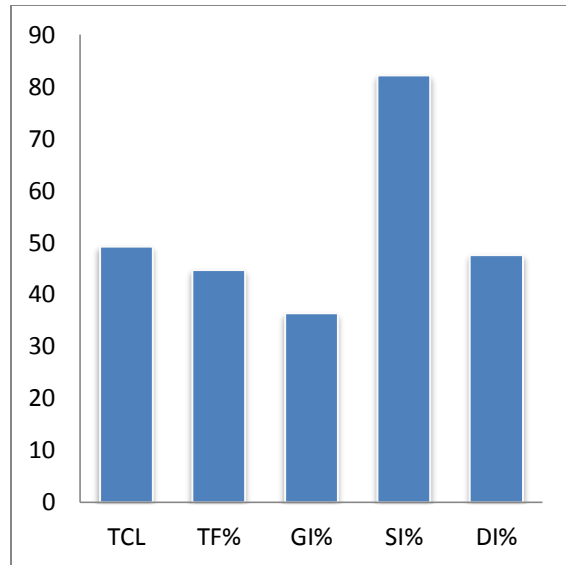
**Fig 1:-** Column graph showing stomatal index, length, width (in  $\mu$ ) of stomata on the abaxial surface of *Tinospora cordifolia* (Willd.).



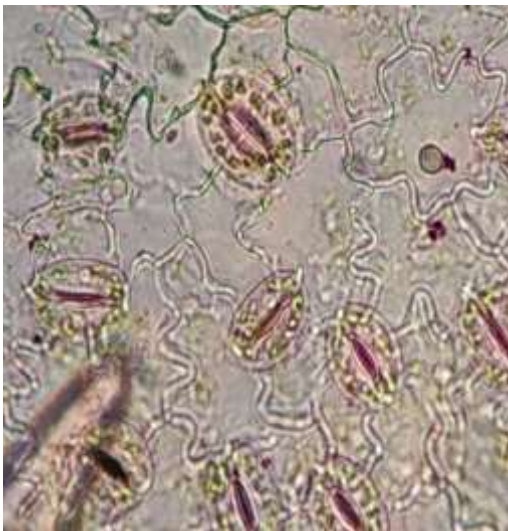
**Fig 2:-** Column graph showing Area (in  $\mu^2$ ) of stomata on the abaxial surface of *Tinospora cordifolia*(Willd.).



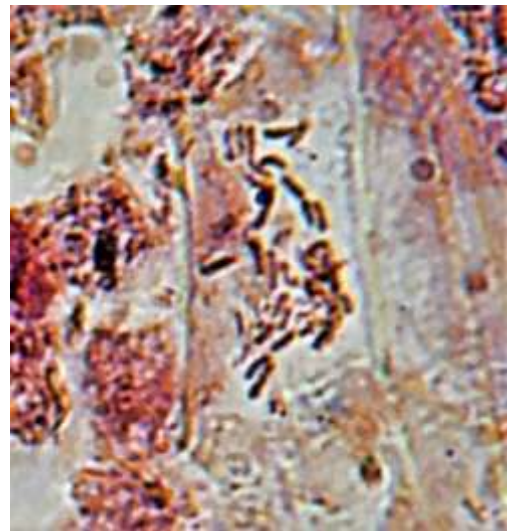
**Fig 3:-** Column graph showing Total Chromatin length(in),TF%,GI%,SI% and DI% in *Tinospora cordifolia*(Willd.).



**Fig 4:-** Column graph showing Total chromatin length(in),TF%,GI%,SI% and DI% in *Tinospora cordifolia*(Willd.).



**Fig 5:-** Stomata on abaxial surface of leaf.



**Fig 6:-** Chromosomes at metaphase Stage.

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