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KARYOTYPE ANALYSIS AND CHROMOSOME NUMBER CONFIRMATION IN *TINOSPORA CORDIFOLIA* (WILLD.) MIERS. EX HOOK. F. AND THOMAS. A.

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ABSTRACT

Karyotypic analysis of *Tinospora cordifolia* (Willd.) was carried out to determine the chromosomal classification by using “Aceto-orcine stain”. The plant is diploid with 2n=22 (18m+2Sm+2St) chromosomes and characterized in eleven pairs of chromosomes. The chromosomes are very short with a mean length 2.64 µm. and the total length of haploid complements was found 29.04 µm. The ideogram of *Tinospora cordifolia* (Willd.) was prepared for the first time and it was found to be asymmetric. The ideogram confirms the karyotypic formula K (n=11) = 9M+1Sm+1St.

KEYWORDS: ***Tinospora cordifolia,*** **chromosome, aceto-orcine, ideogram, karyotype.**

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INTRODUCTION

Guduchi [*Tinospora cordifolia* (Willd.) Miers. ex Hook. F. and Thomas. A.] belonging to the family Menispermaceae is an endangered medicinal climber, which is distributed throughout the tropical Indian subcontinent and china [1,2]. It is Outspread in tropical India ascending to an altitude of 1000 feet from sea level. Guduchi is widely used in veterinary folk medicine and Ayurvedic system of medicine due to different bio-active compounds like alkaloids, glycosides, diterpenoids, lactones, steroids aliphatic compounds and polysaccharides etc. [3]. *Tinospora cordifolia* having various notable medicinal properties such as anti- diabetic, [4] anti-cancerous, [5,6] anti-inflammatory, [7] anti-HIV [8,9] and immunomodulatory activities [10,11] found in different parts of the plant, which is proved in various clinical and experimental trials [12]. Nevertheless the great importance of this species, only a few reports are available about cytogenetic of *Tinospora cordifolia.* Realizing the fact that conventional breeding techniques with modern biotechnological methods are necessary to broaden the genetic base of the *T. cordifolia.* A standardization of the karyotype of *T. cordifolia* species will be extremely important, not only to understand the evolution of the genus, but also to assist breeding programs. Joshi and Rao [13] reported that the haploid chromosome number of *Tinospora cordifolia* to be n=12 but, Abraham [14] found in his studies on the chromosome of dioecious plants, examined the pollen mother cells of *Tinospora cordifolia* and reported a new haploid number for this plant was n=13 and 2n= 26. Later then in 1958, Mathew [15] reported 13 bivalent chromosomes during meiosis in *Tinospora cordifolia*. On the basis of all available cytological data on *Tinospora cordifolia*, [14,15,16] it is suggested that the existing basic chromosome numbers in this species are 12 and 13, of which 13 is the most common. Recent cytological investigation showed that *Tinospora cordifolia* was diploid with the chromosome number 2n = 2x = 22 and the basic chromosome number is n=11. [1.17] Therefore, the chromosome measurements in the proposed study will allow to establish precise ideogram and to discriminate most of the chromosome pairs of the selected strains of *Tinospora cordifolia*. Some reports are demonstrated that there is a high level of genetic diversity present into the different accessions of *T. cordifolia* or the accessions may be genetically heterogeneous in nature [17,18,19].

MATERIALS AND METHODS

Method of fixation

The seeds of *Tinospora cordifolia* were collected from one year old mature plant; grown in departmental greenhouse and germinated in petri-dishes (Borosil) lined with moist filter paper (Whatman filter paper 1) at 25^0C. When root tips were 1-2 cm. long, they were cut in the morning and pretreated with 0.05% colchicine solution for 2 hours at 26^0C. The pretreated root tips were transferred to freshly prepared fixative 3 parts absolute alcohol and one part glacial acetic acid (3:1 v/v) for 24 hours. After fixation root were stored in 70% alcohol at 4^0C for further studies.

Chromosome preparation

Roots were washed in distilled water with the help of fine brush in order to remove the different reagent particles and then hydrolyzed in 1N HCl for about 30 minutes. Before staining, root tips were again washed with distilled water and staining with 2% aceto-orcine for 20 minutes at 60^0C. Root tips were then squashed on slides under the cover slip.

Chromosome observation

The prepared slides were observed under a photomicroscope (Leica microscope, Germany) under oil immersion lens (100X). Selected plates with desirable clarity of all chromosomes at metaphase will be photographed for karyotype analysis. Each chromosome was cut from the photograph and measured in millimeters (mm), and can be divided by the magnification factor to get the original length of the chromosome in micrometer (µm) [20]. The mean value of 10 metaphases was recorded to proceed further. The karyotype analysis will be carried out following Lavan *et al.*[21]. The length of long and

short arm, arm ratio, centromeric index and relative chromosomal length were measured/calculated (Table 2). Chromosomes were classified according to Levan, *et al.,* [21] methods (Table 1).

RESULTS AND DISCUSSION

Chromosome studies have shown that *Tinospora cordifolia* (Willd.) was diploid with 2n=22 chromosomes (fig. 2 A & B). These findings are in agreement with some reports on *T. cordifolia* [1,17]. Joshi and Rao [13] in their studies of microsporogenesis in two menispermaceae have counted the haploid chromosome numbers of *T. cordifolia.* and *Ccocuulus villosus* n=12 but, later in 1942, Abraham [14] observed n=13 chromosome in meiotic division of pollen mother cells of *T. cordifolia*, collected from Poona. Most of its chromosome were metacentric (9) and had a karyotype formula of 9 M+1Sm+1St. The chromosome size ranged from 1.33-3.80 µm. The arm ratios ranged from 1.22 -4.32 µm and the total haploid length of haploid complement (T) was 29.04 µm. Chromosomes were numbered from 1 to 11 in order of their decreasing total length. The ideogram is composed of metacentric (M), Sub-metacentric (Sm) and Sub-acrocentric (St) chromosome pairs with some variation in lengths among chromosomes and the presence of secondary construction has not been noticed in this species. The mean length of the chromosome was 2.64 µm. *Tinospora cordifolia* (Willd.) showed nine pairs of metacentric chromosome, one pair of sub - metacentric chromosome and one pair of sub-acrocentric chromosome. (Table- 2, figure 1 & 2). There was no precise ideogram and karyotype known for *Tinospora cordifolia* previously, but, difference in chromosome size was also studied in various endangered medicinal plant species as in *Psophocarpus tentragonolobus* [22], *Plantago arabica, Plantago cylindrical* [23], *Ammodendron persicum* [24]. However, the plant was showing asymmetrical karyotype in the present studies. As investigated in many plant studies [25,26,27,28,] the root tips are the best part for studying mitotic division, identification of chromosome arrangement and preparation of karyotype, because mitotic division is very fast in this area and lack of chlorophyll in root make it easy for studying the cytogenetic characteristics [29].

Table 1
Classification of individual chromosomes on the basis of their arm ratio and centromeric position.

S. No.	Arm Ratio	Centromeric position	Chromosome type
1	1.0	Median point	M (metacentric)
2	1.0-1.7	Median point	M (metacentric)
3	1.7-3.0	Submedian point	Sm (submetacentric)
4	3.0-7.0	Subterminal point	St (subacrocentric)
5	7.0-∞	Terminal region	t (acrocentric)
6	∞	Terminal point	T (telocentric)

Table 2
Measurement and classification of somatic metaphase chromosome of Tinospora cordifolia (Willd.).

Chromosome No.	Length of Long arm (µm) L	Length of Short arm (µm) S	Total chromosome length (µm) T=L+S	Arm ratio R=L/S	Relative length (%) T/H*100	Centromeric index (%) I=S/T*100	Centromeric position
1	2.09	1.71	3.80	1.22	13.09	45	Metacentric
2	1.90	1.46	3.36	1.30	11.57	43.45	Metacentric
3	1.82	1.26	3.08	1.44	10.61	40.91	Metacentric
4	1.69	1.26	2.95	1.34	10.16	42.71	Metacentric
5	1.57	1.24	2.81	1.27	9.68	44.13	Metacentric
6	1.51	1.12	2.63	1.35	9.06	42.59	Metacentric
7	1.54	0.96	2.50	1.60	8.61	38.40	Metacentric
8	1.53	0.82	2.35	1.87	8.09	34.89	Submetacentric
9	1.29	0.91	2.20	1.42	7.58	41.36	Metacentric
10	1.15	0.88	2.03	1.31	6.99	43.35	Metacentric
11	1.08	0.25	1.33	4.32	4.58	18.80	Subacrocentric
Total length of haploid complement (H):			***29.04 µm***				

Figure 1
Idiograms of T. cordifolia (Willd.) based on chromosome length.

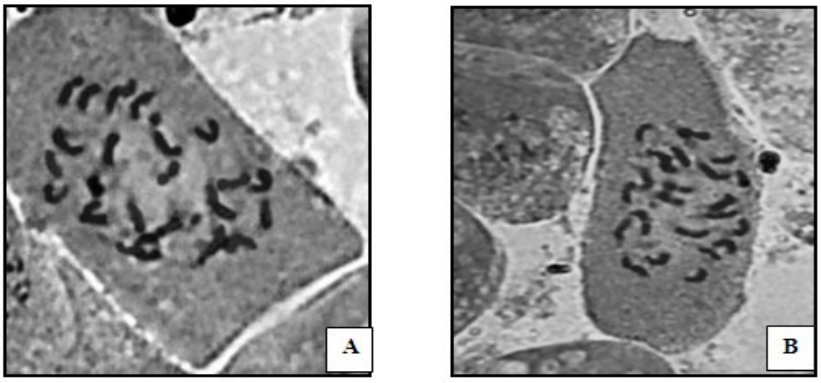

Figure 2
Somatic metaphase chromosomal spread in root tip cell of Tinospora cordifolia (2n=22).

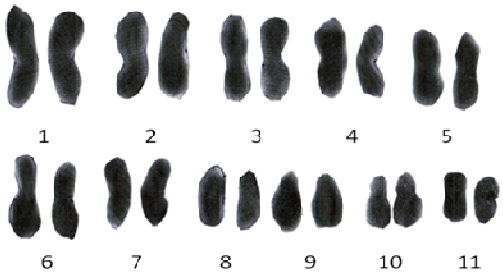

Figure. 3
Chromosome pairs of Tinospora cordifolia (Willd.) arrange in a serial manner.

CONCLUSION

Knowledge of karyotypic relationships is an important prerequisite for effective plant Cytogenetics and breeding programs and has played an important role in the identification and designation of chromosome in many other plant species like *Tinospora cordifolia*. A standardization of the karyotype of *T. cordifolia* species will be extremely important for understanding the phylogenetic relationship and to improve breeding program. The chromosome number and karyotype of *T. cordifolia* is being reported for the first time in India. So, it is unwise to make a conclusion, much more work on the chromosome of *T. cordifolia* is can be expected for further development in breeding strategies.

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