

Karyological study of an endemic anti-fluorosis plant species (*Cassia tora*) of Rajauli block of Nawada District, Bihar

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Abstract: Fluorosis is a serious health problem causing disorders like Dental, Skeletal and Non-skeletal fluorosis due to intake of high levels of fluoride above permissible limit, i.e., more than 1.0 mg/l. Fluoride can reach human body through natural sources like water, soil and air. It is well established fact that ingestion of higher amounts of fluoride causes metabolic disorders by interacting with various cellular processes such as gene expression, cell-cycle, respiration, metabolism, ion-transport, necrosis, stress and disrupts the antioxidants defence system in human body. Fluoride safe water and health promotion through nutritional supplementation are the two universally accepted mitigation programmes. Leaves of plant *Cassia tora* of family Caesalpinaceae are very good source of dietary supplements for fluorosis patients as it contains maximum amount of Calcium along with Iron, Vitamin - C and antioxidants. We are working extensively on cytogenetical aspect of this plant to know the various aspects of their chromosomal behaviour. Mitotic studies indicate diploid chromosome number $2n = 26$. Variations in chromosomes number have also been recorded. Karyotype is asymmetrical type indicating that this plant is not fully established in nature. Total form (T.F %) of chromosome and their Genomic formula have also been calculated to know the actual cause of variation in chromosome number. Such study will help to explore the resistance capacity of the plant and to extract much amount of nutritional products like Calcium, Vitamin - C, Iron and antioxidants.

(Keywords: Fluorosis, *Cassia tora*, Caesalpinaceae)

Introduction

Cassia tora (L.) Rox. is a common weed which belongs to a dicot family Caesalpinaceae and is a highly medicinal plant. It is a good source of dietary supplements for fluorosis patients as it contains maximum amount of Calcium along with Vitamin-C, Iron and Antioxidants which make it very effective in abating the health problems of fluorosis patients (Ref-1). Popularly known as Chakoda Bhaji, Chakoda, Coffee pod or Sickie pod are available even in the remote areas and are easily eatable in form of vegetables. Our studies are mainly concerned to mitigate fluorosis through plants, as medicinal plants play an important role in the social health system. Fluorosis a serious health problem causing disorders like Dental, Skeletal and Non-skeletal fluorosis due to excess accumulation of fluoride above permissible limits i.e. more than 1.0 mg/l. Fluoride can reach human body through natural sources like water, soil and air and also through food stuff.

Our studies are mainly confined to the three villages of Nawada district of Bihar named as Kachhariadih, Hardia and Muslimtola which are severely affected by fluorosis. Centre for Fluorosis Research, A.N. College, Patna in collaboration with UNICEF, Bihar, CSIR-NEERI,

Nagpur, ICMR-NIRTH, Jabalpur and PHED, Government of Bihar has undertaken an “Integrated Fluorosis Mitigation (IFM) Programme in Fluorosis endemic selected villages of Nawada district Bihar” in 2017 and still we are getting very positive results.

A perusal of the previous works indicates that Cytological and Genetical situation in this species of *Cassia tora* has received no considerable attention. All information are of preliminary nature dealing with its Chromosome count (Ref – 2). The present study is a detailed Karyotypic analysis from biosystematic point of view to know the various aspects of chromosome behaviour. Mitotic studies indicate diploid chromosome number as $2n = 26$. Variations in chromosome number have also been reported. Karotype is asymmetrical type indicating that plant is not fully established in nature. Total form of chromosome (T.F %) and their genomic formula have also been calculated to know the actual cause of variation in chromosome number. Such study will help for artificial production of polyploidy of this species to extract many amounts of nutritional supplements for fluorosis patients.

Experimental

Materials for the present investigation *Cassia tora* were collected from Kachhariadih of Nawada district. Young and healthy root tips were pre-treated with paradichlorobenzene (PDB) for 2.30 to 3.00 hours and were fixed in 1:3 acetoalcohol with few drops of ferric chloride as mordant. Root tips were stained and squashed in 2% in acetocarmine. The detailed structure of chromosome was made out from enlarged microphotographs of temporary slide preparations. Total form (T.F. %) has been calculated as given by Huziwara (1962). (Ref. - 3).

$$T.F \% = \frac{\text{sum total of short arm length}}{\text{sum total of chromosome length}} \times 100$$

Observation

The population was found to be cytological quite variable. A very striking point

was noted about the range of variation in chromosome number. The Somatic (Mitotic) chromosome number observed at metaphase varied as $2n = 26$ (figure - 1) and $2n = 28$ in dividing cells. The somatic cells consisting of $2n = 26$ were completely scattered at metaphase and were easily countable. Therefore, such frequently occurring cells were taken for the karyotypic analysis.

The details of the study showed that the chromosomes were mainly of medium size and varied in their relative lengths from 4.2μ to 8.0μ . The detailed karyotypic analysis showed that out of 26 pair of chromosomes, 5 (five) pairs were of medium type (M), 7 pairs of sub – median type (Sm) and one pair of sub-terminal type (St). All 13 pairs of chromosomes are clearly represented in idiogram (figure – 2). Secondary constriction and satellite chromosomes were not recorded in any of the dividing root tip cells.



Figure – 1: $2n = 26$ chromosomes at metaphase stage of mitosis in *Cassia tora*

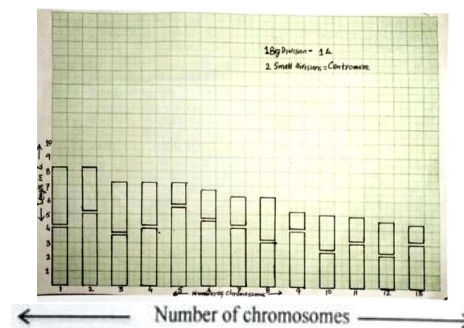


Figure – 2: Idiogram (Graphical representation) of mitotic chromosomes

The Detailed Karyotypic measurements are summarized in Table - 1
Table - 1 Somatic Chromosomes of *Cassia tora* 2n = 26

Nos. of Chromosome pairs	Chromosome Pairing	Positions of the constrictions		Length of the component portion in μ .		Total length in μ .	T.F. %
		Primary	Secondary	Long arm	Short arm		
01	1 + 14	M	x	4.0 μ	4.0 μ	8.0 μ	
02	2 + 15	Sm	x	5.0 μ	3.0 μ	8.0 μ	
03	3 + 16	M	x	3.5 μ	3.5 μ	7.0 μ	
04	4 + 17	Sm	x	4.0 μ	3.0 μ	7.0 μ	
05	5 + 18	St	x	5.5 μ	1.5 μ	7.0 μ	
06	6 + 19	Sm	x	4.5 μ	2.0 μ	6.5 μ	
07	7 + 20	Sm	x	4.0 μ	2.0 μ	6.0 μ	
08	8 + 21	Sm	x	3.0 μ	3.0 μ	6.0 μ	
09	9 + 22	Sm	x	3.8 μ	1.2 μ	5.0 μ	
10	10 + 23	M	x	2.4 μ	2.4 μ	4.8 μ	
11	11 + 24	Sm	x	3.0 μ	1.7 μ	4.7 μ	
12	12 + 25	M	x	2.2 μ	2.2 μ	4.4 μ	
13	13 + 26	Sm	x	3.0 μ	1.2 μ	4.2 μ	

$$\text{T.F. \%} = \frac{30.7}{78.6} \times 100 = 39.05\%$$

Total Chromatin length is 78.6m

The karyotypic formula of cassia tora for this locality is 5(M) + 7 (Sm) + 1 (St).

Results and Discussion

The critical study of morphology of chromosomes has been done in *Cassia tora* to know the actual cause of variation in chromosome number and their role in evolution and speciation. This plant is highly effective in mitigation of fluorosis as it is the best source of nutritional supplements for fluorosis patients.

The karyotype is the morphological aspect of the chromosome complement as seen at mitotic metaphase. The comparative morphology of karyotype develops the concept of symmetry versus asymmetry (Ref-4). Asymmetrical karyotype is one in which the chromosomes are of all approximately the same size and have median or sub-median centromere. Increasing asymmetry can occur either through shift of centromere position from median to sub-terminal or terminal or through accumulation of differences in relative size between the chromosomes of the complement,

thus making the karyotype more heterogeneous (Ref-5). Thus, besides the basic chromosome number, other phylogenetically important characteristics of the karyotype are found in gross morphology of the chromosomes (Ref - 6). The idiogram of this population showed increase in the number of sub-metacentric (Sm) chromosome and decrease in the number of sub-terminal chromosomes (St). The karyotype of this species is, therefore, asymmetrical (Ref. -7). The asymmetrical karyotype provides further proof that sufficient heterogeneity exists - between the pairs of chromosome (Ref 8). Thus, asymmetry in karyotype with structural specialization appears to be associated with important innovation in morphology and breeding system of the species, while colonizing at different places.

Karyotype symmetry has been determined on the basis of size and morphology of chromosomes. According to Levan (1935) Advance karyotype have more sub terminal

chromosomes (St) while primitive ones possess metacentric (M) and sub-metacentric chromosomes (Sm). Such analysis of karyotype will also provide correlation among the species. *Cassia tora* with a chromosome number $2n=26$ has the total chromatin length 78.6μ which indicates that it possesses an average size of chromosome. This is also evident from the range of chromosome length. Thus it may be presumed that a repatterning of chromosomes involving translocation or pericentric inversion in addition to elimination or acquisitions of heterochromatin are the probable modes of diversification in the karyotype (Ref-9) Although genetic significance of heterochromatin is yet to be clearly understood, its association with gene action has long been recognised (Ref 10) Total form value (TF %) mentioned i.e. 39.05 % indicates that chromosomes have more similarities with asymmetrical karyotype. Thus, the above discussions have led to the findings that the species under study must have diverged from the other in which $2n=28$ chromosomes are recorded. It is a fact that once a chromosomal readjustment has achieved a particular adaptive value, the evolutionary line bearing it can radiate into many habitats including relative stable ones, thus

making the beginning of a new cycle of genetic ecological speciation. It is also known that high concentration of fluoride interrupts with mineralization process of bones as well as teeth and alters the normal functioning of various soft tissues viz Liver, Kidney, adrenal glands, Lungs, heart, brain and gastro-intestinal system. Karyotypic analysis is a genetical parameter, so such study will help to solve the above problems (Ref-11). Thus detailed karyological studies of *Cassia tora* have revealed the dissimilarities in the length and quality of chromatin (chromosomes) which have been supposed to be due to asymmetrical karyotype. The climatic condition and repatterning of chromosomes have also been accounted for diversification and asymmetry of the karyotype. These results will encourage to produce the polyploidy of this plant and the protocol reported here could also be used for conserving this plant for mass scale clone propagation which will be helpful for extraction of calcium, Iron, Vitamin - C and antioxidants.

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