



## Colchicine Induced Polyploidy in *Cassia tora* (L.) Rox.

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**Abstract:** An Induction of artificial polyploidy by 0.4% aqueous solution of Colchicine has been successfully made in *Cassia tora*. The data obtained from the experimentally treated materials have been further analysed for all the implications to understand the effect of Colchicine on the Chromosome structure, and function. The tetraploids showed  $\underline{n} = 26$  bivalents and different combinations of univalent and multivalent, the percentage of quadrivalents being the highest Chromosome irregularities in PMCs of polyploids has been detected more frequently than normal diploid. The polyploids thus, raised did show autopolyploidy in nature. The chemical environment within the cell can change the meiotic behaviour of a species. The tetraploid plants showed a slower rate of growth, delayed flowering, thicker, darker and wider leaflet, and decreased stomatal frequency per unit area. This also showed larger stomata, increase in height, increase in number of lateral branching, bigger fruit, seed size and increase in the number of pollen grains with absence of exine. The pollen sterility increases as compared to diploid. It is thus clear that experimental induction by Chemical means (Colchicine) holds a clue for understanding the adaptability of the species in different environmental conditions.

**Keywords:** Colchicine treatment; *Cassia tora*; polyploidy.

### Introduction

*Cassia tora* (L.) Rox. is a common weed which belongs to family Caesalpinaceae and highly medicinal plant. It is a very good source of dietary supplements for fluorosis patients because it contains the maximum amount of Calcium along with vitamin-C and Iron and thus highly effective in abating the health problems of fluorosis patients (Mondal, 2018). Popularly it is known as Chakvad, Chakoda Bhaji, Sickle pod, Coffee Pod. They are available even in remote areas and easily eatable in the form of vegetables.

A perusal of previous work indicates that the Cytological and genetic situation in this species has received no considerable attention. All information is of preliminary nature dealing with chromosome count as  $\underline{n} = 13$  bivalents

(Chaulagain and Sakya, 2002). The present paper deals with chromosomal behavior of the Colchiploid during meiosis and production of tetraploid plants with vigorous growth which can be well exploited in several ways including nutritional supplements to Fluorosis patients. The main aim to use this Chemical was to produce tetraploid plants of *Cassia tora* for the benefit of human beings.

### Materials and Methods

Young seedlings of *Cassia tora* were collected from A. N. College campus, Patna and Hardia village of Nawada district and sown in the pots. The transplanted seedling was left for twenty days under uniform conditions to adjust. Seedlings grown in this way we're treated at their apical growing points. The apical buds

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were exposed and then treated with 0.1, 0.2, 0.3, 0.4 and 0.5% aqueous solution of colchicine. The apical buds were covered with cotton soaked in colchicine solution. The duration of treatment was fixed- 6 hours daily for each concentration up to 5 days as shown in Table 1. After every treatment, the buds were thoroughly washed. The cotton was frequently resoaked. The treated plants were transferred to the field for further observation after treatment. The cytological preparation and meiotic behavior of Colchiploid plants were studied from anther squash preparations. Fixation of floral buds was made in 1:3 acetoalcohol with a few drops of ferric chloride as mordant. All the stages of meiosis were obtained by squashing anthers in two percent (2%) acetocarmine. Microphotographs

were taken from temporary slide preparation. Studies of pollen grains fertility and sterility test were made on the basis of their stainability in acetocarmine.

## Results

The seedlings of *Cassia tora* were treated with different percentage of aqueous solution of colchicine for different periods. The resulted seedlings grew very slowly with thick and curled leaves showing the primary effect of colchicine. Some of the seedlings died at different stages of growth before reaching maturity. The percentage of mature plants classified as normal, affected and tetraploid with concentration and duration of treatment is given in Table 1.

**Table 1.** Apical bud treatment of seedlings, plant survival and percentage of tetraploid produced after colchicine treatment in *Cassia tora*.

Concentration of colchicine in percentage	Duration (6-hours daily)	No. of seedlings treated	Normal Plants	Affected Plants	% of tetraploid produced
0.1	4 days	20	20	0	0
	5 days	20	20	0	0
0.2	4 days	20	18	02	0
	5 days	20	15	05	0
0.3	4 days	20	14	06	0
	5 days	20	10	10	0
0.4	4 days	20	05	15	10
	5 days	20	02	18	15
0.5	4 days	20	0	20	All Died
	5 days	20	0	20	All Died

The seedlings after apical bud treatment of *Cassia tora* were generally slow in growth. However, the tetraploid plants were obtained from apical bud treatment of seedlings with 0.4 percent aqueous solution of colchicine. But the highest percentage of tetraploids were obtained from apical bud treatment of seedlings with 4 percent of colchicine for six hours daily for five days. It was found that treatment of higher concentration and longer durations, the mortality increased. The lower concentration of 0.3% showed only certain morphological abnormalities but there was no change in the chromo-

some number. However, the meiotic abnormalities in such plants were of much prevalence in comparison to the control plants.

The preliminary criteria used for detecting polyploidy were the size and frequency of stomata and the size of pollen grains. The plants of  $C_1$  generation (colchicine treated 1<sup>st</sup> generation plants) differed strikingly in several morphological characters from the control (Fig. 1 and 2). At first such plants showed stunted growth with slightly thicker but shorter internodes and more lateral branches arising from the inter-

nodal portion. The Lamina was dark green, thicker, broader and vein and veinlets were more prominent. The number of stomata per unit area was less but the size of stomata was bigger as compared to the control (Fig. 3 and 4). Every part of the tetraploid flower and fruit

showed enlargement. The seed setting was quite good and showed an increase in size and dark in colour. The observations of important morphological characters of diploid (control) and tetraploid plants are given in Table 2.

**Table 2.** Morphological differences between Control (Diploid) and treated plants of *Cassia tora* with 0.4 % colchicine solution for 6 hours daily for 5 days.

S.No.	Character	Diploid (Control plant)	Treated plant (Tetraploid)
1	Height of plant	30 to 40 cm	50 to 60 cm
2	Branches	1 to 2	5 to 6
3	Internode length	3.0 ± 0.20 cm	5 ± 1 cm
	Leaflet (Lamina)	Obovate	Obovate
	(a) Shape	2 to 3 cm	4 to 6 cm
	(b) Length of Leaflet	1.5 to 2.5 cm	3 to 5 cm
4	(c) Breadth of Leaflet	20	12
	(d) Number of Stomata per field of microscope	Length 15.12 μ	25.30 μ
	(e) Size of Stomata (in μ)	Breadth 12.10 μ	20.02 μ

**Cytological observations**

The tetraploid of *Cassia tora* has been raised for the first time. A gametic number  $n = 26$  was made out from meiotic studies. The meiotic division was found to be highly non-synchronised. Most of the pollen mother cells at diakinetik stage showed scattered bivalents and some quadrivalents (Fig. 5). At metaphase Ist bivalents and some quadrivalents were also recorded (Fig. 6). Metaphase Ist also showed

varying number of multivalent with different configurations (Fig.7). Some of the PMCs were observed with trivalent, different configurations of hexavalent but quadrivalents were of more frequent nature (Fig.8). Clumped chromosomes with precocious separation of chromosomes were observed in few pollen mother cells (Fig.9). The nature and frequency of Chromosome association at metaphase I are summarized in Table 3.

**Table 3.** Nature and frequency of Chromosome association at metaphase I of *Cassia tora* with 0.4 %colchicine solution for six hours daily for 5 days

←—————Chromosome Association—————→							
OCTA VIII	HEXA VI	PENTA V	QUADRI IV	TRI III	BI II	UNI I	Frequency of PMCs
0	0	0	0	0	26	0	25
0	01	0	05	0	13	0	05
0	0	0	01	0	20	08	06
01	01	0	03	0	15	04	05
01	0	0	01	05	10	05	05
0	01	0	04	0	15	0	04

At anaphase I, the number of normal PMCs was fairly not regular with equal distribution of chromosomes at the two poles (Fig. 10).

The anomalies like lagging chromosomes delayed disjunction of bivalents, unequal separation of Chromosomes and chromosomal bridges

were also observed (Fig. 11). Besides all the mentioned anomalies, an interesting case of Transmigration (Chromosomal migration) from one PMC to another PMC has also been recorded (Fig. 12). Some of the PMCs showed tendency of complete migration of chromatin matter from one cell to adjacent cell, while in another migration of a part of chromatin matter into adjacent cell has been recorded. The presence of C-mitosis has also been observed in some of the somatic cells of the wall layer of the

anther (Fig. 13). The later stages of meiosis also showed irregularities like dyads, triads, pentads with micronuclei at the quartet stage. The pollen grains were bigger in size than that of diploid (Fig. 14). However, the absence of exine in some of the treated pollen grains has also been recorded (Fig. 15). Pollen sterility in this autotetraploid (Fig. 16) has been estimated to be 15 percent (Table 4) which has been higher than the normal diploid (13.04%).

**Table 4.** Pollen analysis of Control (2n) and treated (4n) plants of *Cassia tora* with 0.4 percent Colchicine solution for 6 hours daily for 5 days.

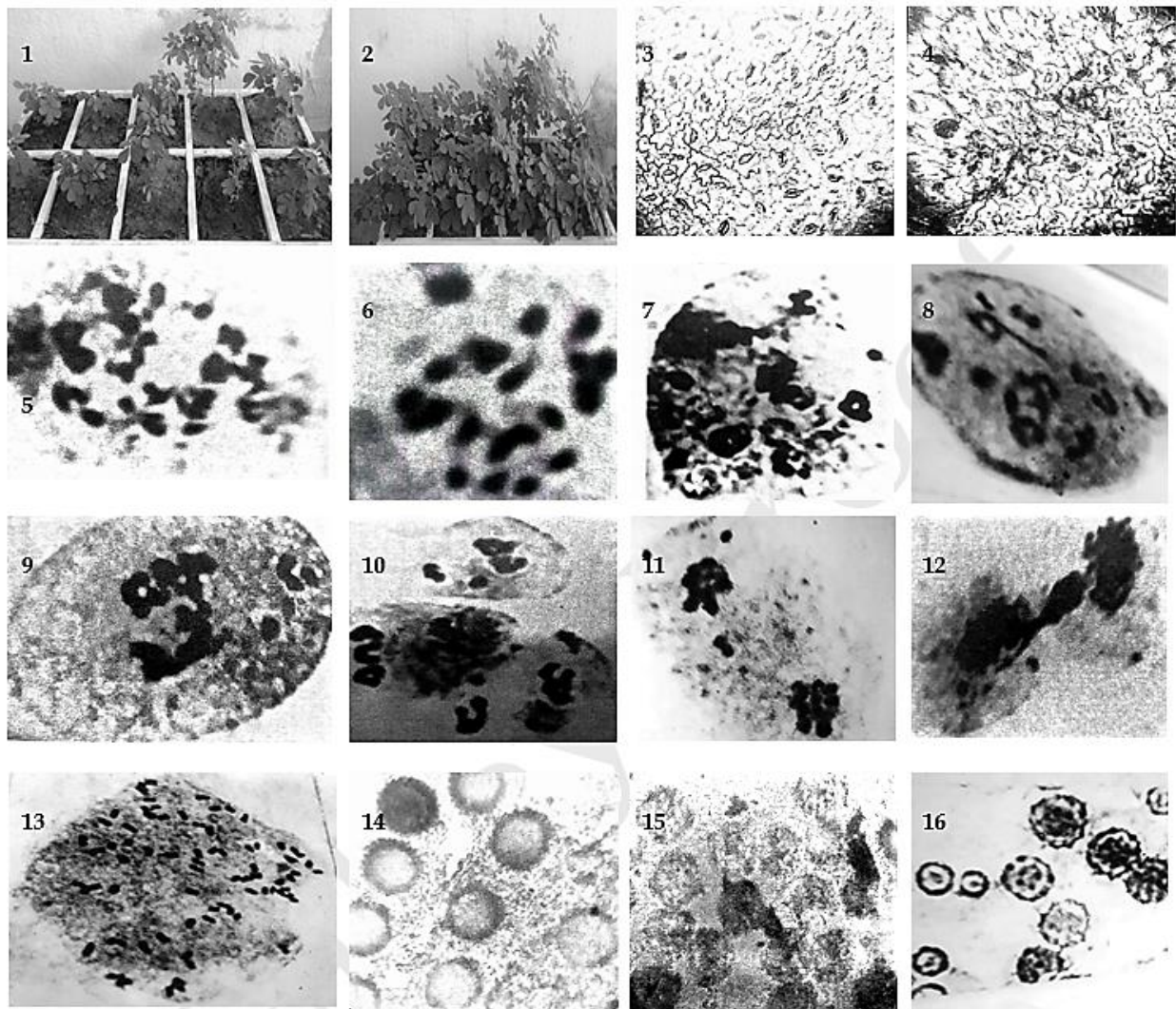
Plants of <i>Cassia tora</i>	No. of Pollen Studied	No. of Normal Pollen	No. of Sterile Pollen	% of sterile Pollen
Diploid (2n)	1955	1700	255	13.04 %
Tetraploid (4n)	2000	1700	300	15 %

## Discussion

Inspired by the meiotic irregularities in the previous studies of *Cassia tora* and to know the cause other than hybridization, induction of artificial polyploid by Colchicine was employed. However, increase in size of leaf, thickness of stem and formation of more lateral branch in such produced autotetraploid can be well exploited to get an increased amount of Calcium, Vitamin C and Iron. Many workers have successfully used this chemical on different plants (Trivedi and Trivedi, 1987; Tulay and Unal, 2010). The Colchicine applied by Cotton plug method was effective in doubling the chromosome number.

This is the first report of the production of autotetraploid in *Cassia tora*. The tetraploid has been morphologically quite different from the normal one, the details of which have earlier been given in Table 2. In the early stage of treatment, the growth of seedling has been found to be stunted due to chemical effect of Colchicine. After a few days, the effect has been found to suppress gradually and the plant showed gigantism. The gigantic growth is considered to

be due to increase in genetic material present in the polyploids. The increase in the cell size with doubling of Chromatin material was associated with a reduced rate of cell division. Similar observations have also been made by Siddiqui (1981) and Trivedi and Trivedi (1984). Delayed flowering has been attributed to the metabolic physiological phenomenon. It could be also due to stunted growth in their earlier phase of the development just after Colchicine treatment (Roy and Trivedi 1976). The tetraploids also showed higher values with regard to the size of stomata and pollen grains as compared to diploids. Cytologically an autotetraploid provides information on chromosome behavior during meiosis. The inhabitation of spindle fibre formation and cytokinesis by the action of Colchicine, may lead the cell to have more chromosomal complements and large size of cell (Dhamyanthi and Gotmare 2010). The appearance of univalents may be due to the precocious separation of the chromosomes or due to environmental stress or due to desynapsis (Rao and Arundhati 1983).



**Figure 1.** Plants of *Cassia tora* before colchicine treatment (Control) Diploid.

**Figure 2.** Plants of *Cassia tora* after colchicine treatment tetraploid.

**Figure 3.** Stomata of diploid plant showing smaller size and more numbers.

**Figure 4.** Stomata of tetraploid plant showing larger size and less numbers.

**Figure 5.** PMC at diakinesis showing  $n = 26$  bivalents and quadrivalents.

**Figure 6.** Metaphase 1<sup>st</sup> having bivalents and multivalents.

**Figure 7.** Metaphase 1<sup>st</sup> showing varying number of multivalents with different configurations.

**Figure 8.** Metaphase 1<sup>st</sup> Showing bi, trivalents and hexavalents.

**Figure 9.** Metaphase 1<sup>st</sup> showing clumped chromosomes of equator with precocious chromosomes.

**Figure 10.** Anaphase 1<sup>st</sup> with unequal distribution of chromosomes.

**Figure 11.** Anaphase 1<sup>st</sup> showing lagging chromosomes.

**Figure 12.** PMCs showing transmigration

The occurrence of univalents and distinctive manifestation of chromosome association at successive stages in the first division suggest different affinity and the attendance of factors which may be of genetic nature. It is probable

that the genotype of the diploid species of *Cassia tora* has a decisive influence in suppressing the diploidizing effects (Kumar and Tiwary, 2013). The number of quadrivalents up to five has been usually observed in addition to low

frequency of multivalents. The formation of multivalents may be assigned due to homology of chromosomes but the presence of trivalents in the autotetraploid shows the structural dissimilarities between the chromosomes (Raghuvanshi, 1978). The presence of higher number of bivalents may be due to preferential pairing or due to smaller size of Chromosomes which causes interference in pairing at any point (Fawzia and Alam, 2011). Multipolar orientation of Chromosomes was observed in some of the pollen mother cells, which may be due to the formation of spindle fragments of single spindle (Mehra and Sood, 1974). Both genic and chromosomal causes arise from the polling of partially homologous genomes and complementary genes may govern the pollen sterility. The presence of C-mitosis of the auto tetraploids leads to believe that Colchicine has changed the Cellular environment causing polysomaty (Gupta and Gupta, 1976). The common irregularities found at anaphase I was formation of simple bridges, laggards and un-equal separation of chromosomes at the two poles. Simple bridges and lagging bivalents are may be due to the failure of terminalisation of Chiasmata or stickiness of chromosome ends (Mehra, 1977). This situation indicates that even within a cell different chromosome may often have different meiotic rhythms showing lack of coordination between the chromosomes. Such conditions are supposed to be the outcome of hybridity and ultimately lead to sterility. The other meiotic irregularity was transmigration of chromosomes from one PMC to other PMC. Such irregularities may be responsible for producing aneuploids or there is possibility of origin of polyploids (Thakur and Singh, 1978). Thus meiotic behavior confirms that the induced tetraploid in *Cassia tora* is autote-traploid in nature and also that chemical environment with in the cells can change the meiotic behavior of a species in natural population.

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