

Induced polyploid in *Eclipta alba* for their medicinal and ecological importance

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Abstract: *Eclipta alba* (L.) Hassk commonly known as Bhangraya which belongs to a large and highly natural family Asteraceae. This family is highest in scale of evolution among angiosperms. The plant is highly medicinal and commonly used in scorpion sting, jaundice, in preparation of hair dye, headache, and elephantiasis. It is a fast colonizing weed and commonly grows in different environmental conditions in form of a small prostrate or a fully erect plant. This is species is highly plastic and capable of forming populations through the adjutant of their genetic system according to the environment in which they happen to grow. An induction of artificial polyploid by 0.4 % aqueous solution of colchicine was successfully made in *Eclipta alba* for the first time. The data obtained from the experimentally treated materials have further been analyzed with all implications to understand the effect of colchicine on chromosome structure, behavior and doubling the number. The artificially produced tetraploid showed $n = 22$ bivalents as compared to normal bivalents $n = 11$ in diploid plant. Different combination of univalent, quadrivalents and other multivalent were also recorded. Morphologically tetraploid plants showed slower rate of growth, thicker and wider leaves, swollen stem base, increase in the height of plant and larger seeds. Decreased stomatal frequencies were also observed. The data of chromosomal association, chiasma frequency and half chiasma per chromosome clearly indicate their plastic nature to form new population, showing their role in ecological adaptation. However, the polyploid thus raised did slow autopolyploidy in nature.

The main aim of such research project is

- * To know the cause of morphological variations like prostrate and erect form of *Eclipta alba* which may be an outcome of environmental influences or some genetical factor is associated with such variations?

- * To produce polyploid of *Eclipta alba* for the benefit of the human being which can be well exploited in several ways including their medicinal properties.
- * The result of experimental manipulation will strengthens the contention that this species is highly plastic and capable as forming populations and holds a clue to understand adaptability of the species in different environmental condition.
- * That the chemical environment within the cell can change the meiotic behavior of a species

(Key words : Polyploidy, *Eclipta alba*, Medicinal, Ecological)

Introduction

Eclipta alba (L.) Hassk is highly medicinal weed of family Asteraceae and highest in scale of evolution among angiosperm¹. It is an annual herb which commonly grows on roadside, wasteland, drains, orchards, gardens and grazing field. Its several forms exist in nature which differ strikingly in many morphological features, specially prostrate and erect form. It is known to contain various alkaloids and chemicals constituents² of diverse nature which are used to cure several diseases including scorpion stings, to prepare hair dye, neuropharmacological, and act as an antioxidants. It is found useful for building and binding soil-against erosion³. Inspired by medicinal properties, variable morphological features and to check soil erosion, an induction of polyploid by colchicine was employed.

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The present paper deals with morphology and chromosomal behaviour of colchiploid during meiosis. Several workers have successfully used colchicine on different plants for artificial production of polyploid⁴. The colchicine applied by cotton plug method was effective in doubling the chromosome number.

Experimental

The young seedling of *Eclipta alba* were collected from A. N. College, Patna campus and sown in pots. After 15 days of adjustment, they were treated with aqueous solution of colchicine at their apical growing points. The apical buds were covered with cotton soaked in colchicine solution of 0.1 to 0.5 percent daily for 6 hours up to 5 days. The cotton was frequently resoaked. After each treatment, the buds were thoroughly washed. The plants were transferred to the field for further observation after a week of treatment.

Meiotic behaviour of colchiploid plants was studied from anther squashed preparation. Fixation of floral buds was made in 1:3 aceto-alcohol with a few drops of ferric chloride as mordant. All the stages of meiosis were obtained by squashing anther in two percent aceto carmine.

Studies of pollen grains fertility and sterility test were made on the basis of their stainability in acetocarmine.

Observation

The seedlings of *Eclipta alba* were treated with different percentage of aqueous solution of colchicine for different periods. The resulted seedling grew slowly with thick and curled leaves. 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 – I.

Table - 1
Apical bud treatment of seedlings, plant survival and percentage of tetraploid produced after colchicine percentage in *Eclipta alba*.

Concentration of colchicine in percentage	Duration (6 hours daily)	No. of seedlings treated	Normal Plants	Affected Plants.	Percentage 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 were generally slow in growth. The best result of tetraploid plant of *E. alba* was obtained from apical bud treatment of seedlings with 0.4%

aqueous solution of colchicine for six hours daily upto 5 days was found that- treatment with higher concentration and longer duration, the mortality increased.

The preliminary criteria used for detecting polyploidy were the size and frequency of stomata and fertility of pollen grains. The plants of C1 generation strikingly differed in many morphological characters from the corresponding control (Fig-1A + B). All such plants showed stunted growth with slightly thicker and shorter internodes and more lateral branching. The

lamina was dark green, thicker, broader with prominent veins as compared to control (Fig-2A+B). Every part of tetraploid (4n) flower (Fig-3A+B) and seeds showed enlargement. The observations of important morphological characters of diploid and tetraploid are given in Table – 2.

Table - 2
Comparison of morphological characters of diploid and tetraploid plant of *Eclipta alba*

Characters	Diploid	Tetraploid
1. Height of plant (main branch)	8 to 10 cm.	18 to 19 cm.
2. Number of Lateral branch	4 to 5	10 to 12
3. Length of Lateral branch	6 cm to 7 cm	10 cm., 12 cm
4.Distance between nodes	2 to 3 cm	1 to 1.5 cm
5. Laminar length.	2 to 4 cm	4 to 8 cm
6.laminar breath	3 to 4 mm.	6 to 8
7. Flower size	2 to 3 cm in dia mt	4 to 6 cm in dia mt
8. Seed	Small- dark brown	Large dark black
9. Stomata per field of microscope	8 to 9	4 to 5
10. Length of stomata	15.12	25.30 μ .

Cytological Observations

The tetraploid of *Eclipta alba* has been raised for the first time. The gametic number in $n = 22$ bivalents was made out from the meiotic studies. Meiotic division was highly non-synchronised. Most of the PMCs showed multivalents configuration of different nature of diakinesis and metaphase 1st (Fig-4A+B) Quadrivalents were of

more frequent in nature as compared to trivalents and univalents (Fig-5). Few PMCs showed 32 univalents and 6- bivalents (Fig-6). This may be due to partial desynapsis. Clumped chromosomes with precocious separation of chromosomes were also observed (Fig-7). The nature and frequency to chromosome association at 50 PMCs at metaphase 1st have been summarised in Table -3

Table - 3
Nature and frequency of chromosome association at metaphase-1st treated with 0.4/ chlchicin

Chromosomal Association						Frequency of PMCs
VI	V	IV	III	II	I	
0	0	0	0	22	0	16
0	0	02	0	10	16	08
0	0	02	0	02	32	14
0	0	04	0	08	12	04
0	0	03	0	12	08	02
0	0	05	0	10	04	06

At Anaphase Ist, the numbers of normal pollen mother cells were fairly regular with equal distribution of the chromosomes at the two poles. The anomalies like laggards formation, chromosomal bridges and unequal separation of chromosomes (Fig. 8 and 9) were also recorded. Interesting cases of transmigration was also

recorded (Fig-10). 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 however; pollen sterility in autotetraploid was estimated to be 15 percent, which was higher than the normal diploid (Table - 4)

Table - 4
Pollen Analysis in diploid and tetraploid plant of *Eclipta alba*

Plants	No. of Pollen Studied	No. of Normal Pollen	No. of Sterile Pollen	Parentage of Sterile Pollen
Control (Diploid)	1955	1700	255	13.04
Treated (Tetraploid)	2000	1700	300	15.00

Results and Discussion

An induction of the artificial polyploidy by colchicine was employed to know the cause of morphological variations and meiotic irregularities in *Eclipta alba*. The gigantic growth is considered to be due to increase of genetic material in the polyploids. The increase in cell size with doubling of chromosome number was associated with reduced rate of cell division. Similar observations have also been reported by Siddique⁵ and Trivedi⁶. Delayed flowering has been attributed to the metabolic physiological phenomenon. It could be due to stunted growth of their earlier phase of development just after colchicine treatment as also been made by Roy and Trivedi⁷.

Cytologically an autotetraploid provides information on chromosome behavior during meiosis. The inhibition of spindle fibre formation and cytokinesis by the action of colchicine, may lead the cell to have more chromosomal complements and large size of cell⁸. The appearance of univalents may be due to desynapsis or precocious separation of chromosomes or due to environmental stresses⁹. The distinctive manifestation of chromosome association at successive stages in the first division suggests different affinity and the attendance of factors which may be of genetic nature. It is probable that

the genotype of diploid species of *Eclipta alba* has a decisive influence in suppressing the diplodising effects¹⁰.

The number of quadrivalents has been usually observed in addition to low frequency of other multivalents. The formation of multivalents may be due to homology of chromosomes but the rare presence of trivalents in autotetraploid shows the structural dissimilarities between the chromosomes¹¹. The presence of higher number of bivalents may be due to preferential pairing or due to smaller size of chromosomes which causes no interference in pairing at any point¹². Multipolar orientation of chromosomes may be due to formation of spindle abnormalities¹³. The rare occurrence of C-mitosis of the autotetraploids leads to believe that colchicine has changed the cellular environment causing polysomaty¹⁴. The common anomalies found at anaphase I were formation of simple bridges, lagging bivalents and unequal separation of chromosomes at two poles. These are may be due to failure of terminalization of chiasmata or stickyness of chromosome ends¹⁵. This situation indicates that even within a cell, the different chromosomes may often have different meiotic rhythms showing lack of coordination between the chromosomes. Such conditions are supposed to be the outcome of hybridity and

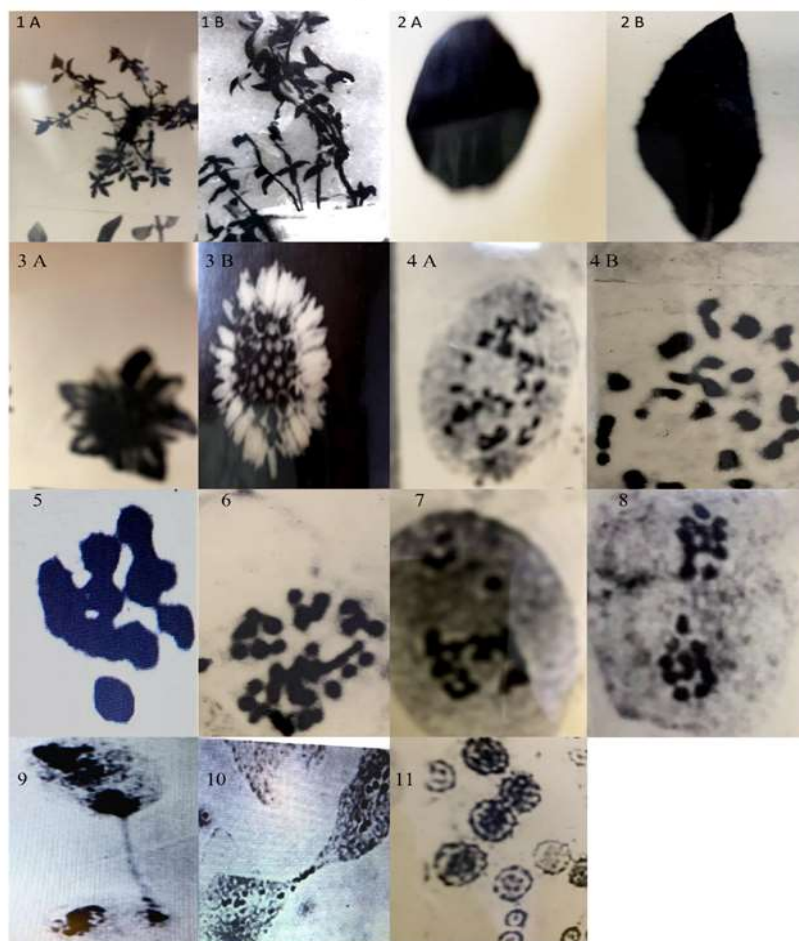
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Fig. – 1. A. Control plant of *Eclipta alba* (2n). B. C1 (Cholchicine treated) plant of *Eclipta alba*. 2. A. Leaf of control plant (Diploid). B. Leaf of treated plant (tetraploid). 3. A. Flower (Inflorescence) of control plant. B. Flower (Inflorescence) of treated plant. 4. PMCs showing 22 bivalents at Diakinesis and Metaphase I of treated plants. 5. PMCs showing quadrivalents of different nature and bivalents of tetraploid. 6. PMCs showing 32 univalents and 6 bivalents. 7. PMCs showing clumped chromosomes and precocious separation. 8. PMCs of Anaphase I showing lagging chromosomes. 9. PMCs at Anaphase I showing chromosomal bridges. 10. PMCs showing transmigration. 11. Pollen grains of different size showing fertility and sterility.

ultimately lead to sterility. The other meiotic irregularities was transmigration of chromosomes from one PMC to another which may be responsible for producing aneuploidy or there is possibility for the origin of polyploids¹⁶. Thus, meiotic behavior studied in induced autotetraploid of *Eclipta alba* confirms that

* Chemical environment within the cells can change the meiotic behavior of the species

in natural population.

* It may be assured that the *Eclipta alba* having prominent morphological variation are subject to various kinds of environmental stresses including temperature fluctuation and cosmic radiations. These are probably responsible for inducing physiological imbalance in a cell as a result such morphological changes is being observed.

However, it requires more study on other plants to know the real cause of variations.

- * Taking such facts into consideration, it can be stated that multiple genetical and environmental factors might have been operating leading to structural differentiation in the populations at *Eclipta alba*.

- * Investigation of such species from Cytogenetical and Ecological point of view appears to be quite interesting as well as rewarding as it will also provide clues to tackle problems related weeds.

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