International Journal in Physical and Applied Sciences
Volume 07 Issue 01, January 2020 ISSN: 2394-5710 Impact Factor: 4.657
Journal Homepage: http://ijmr.net.in, Email: irjmss@gmail.com
Double-Blind Peer Reviewed Refereed Open Access International Journal

EFFECT OF COLCHICINE ON THE CHROMOSOMAL VARIATION OF TRICHOSANTHES ANGUINA L

[1]Madan Mohan Gorain, [2]Amar Das [3]Arbind Kumar Singh, and [4]Sweta,
1. P.G. Department of Botany, Sido Kanhu Murmu University, Dumka Jharkhand.
2. Paredih, Jamtara.
3. Department of Botany, Jamtara College, Jamtara.
4. Department of Botany, J.P. University, Chhapra.

Abstract

Trichosanthes anguina is a district species of cucurbitaceous. It is commonly known as snakeguard (Raman), Kainta (Santhali), Chachinda (Hindi), Chichinga (Bengali), Padwal (Marathi), Padarali (Gujrati) Galartori (Panjabi), Pudal (Tamil), Patavalonga (Assami) and Likaetha (Mundari), it is extensively cultivated in South for vegetable which is North India, it is confined to Kitchen gardens. In the present investigation, although the two species, T. anguina, a cultivated species, and T. cucumarina, which grows wild, have 2n=22 chromosomes, they differ not only in their karyotypes but remarkably in total chromatin length, pollen grain morphology, and in leaf, fruit and seed sizes, flowering behaviour and in number of spots for phenolic compounds in leaves. The total chromatin lengths (*f*Êm} standard error) of the haploid complements of T. anguina (White) and T. anguina (Green-White Stripe) and T. cucumarina are 27.82}0.18, 28.73}0.14 and 40.42}0.20 respectively. In the present investigation, by various methods of colchicines treatment described earlier, one tetraploid plant was raised. From this stock several triploids were raised by the process of hybridization. The tetraploid in T. anguina showed the gigas habit, i.e. vigorous vegetative parts like broader and darker leaves, bigger floral organs and stomata pollen grains etc.
Key Words: Trichosnathes anguina, Colchicines, Diploid, Triploid, Tetraploid, Polyploidy.

Introduction: *Trichosanthes* is predominantly an indo-Malayan genus. It is a large genus with about 48 species of which 24 occur in India; 18 in Malaya; 4 in China and Japan and 4 in Australia. *Trichosnathes anguina* (kaita) is a well known species of this area which has high medicinal value (Chopra et.al.1969). The plant is a monoecious climber with up to 2 feet sized fruit. The medicinal properties of the plant can be enumerated as follows by the tribal and local patients. Fruits of the plants as vegetable by the tribal people. It is cultivated in rainy season between July and August. This stem is long, slender, furrowed, sub glabrous, tendril 3 fid; leaves broad, 8-14 cm long, slightly lobed, hairy, petiole slender, lamina orbicular, reniform and distinctly denticulate. Flowers white, raceme made male and female flowers, peduncle 5-15 cm long, bearing 8.15 near apex, petals such fringed at the margin, petals much fringed at the margin. Fruit long up to 2 feet size, cylindrical, green or whitish green, sometimes with long white stripes (Kirtikar & Basu 2000). Seed ovate, compressed margin and slightly emarginated. The artificially raised tetraploid plant differed in several characters from that of the diploid (Hartmair, 2003).

Materials and Methods: The materials for the present investigation include two vaarietiesof cucubitaceae Trichosanthes anguina Linn., chosen after consultation of local vaidyas of Rajmahal areas, having medicinal importance. The members of Cucurbitaceae generally grow in plains as

well as on hills as tropical annual climbers. Most of the species are wild and growing during rainy season only. Rainy season variety of plants growing between July and October, were taken for the present investigation. Seeds from ripe fruits of T. anguina were colleted from areas. Next year in the month of July seeds of T. anguina were Grown in the Botanical Garden of S.P. College, Dumka for cytomorphological and biochemical studies.

Cytological Techniques: Cucurbitaceae is a difficult material for cytogological studies. It is difficult to make satisfactory cytological preparations, because of –

(a) Chromosome are small, morphologically indistinct and do not easily separate from one another (Kongtun et.al.2009).
(b) They are not easily stainable as, its cytoplasm also takes stain making very poor differentiation between cytoplasm, and chromosome.
(c) The pollen masses do not separate easily. In spitge of these handicaps, satisfactory preparations were made and cytological investgigations were carried outg in normal and experimentally raised poplyploids and hybrids of some species under consideration.
(d) Seed were sown in small pots between July and August; after 12 to 15 days, healthy young root tips were taken for mitotic studies. Plants were then transferred from post to experimental ground for meiotic studies and breeding. The best time for somatic metaphase and meiotic metaphase were between 10:30 AM to 12:30 PM and 10:15 AM to 12:00 noon, respectively, on sunny days under field condition. The division and development were found non-synchronous in the buds of approximately same size. The divisions and development, differ in immature to fully developed pollen grains and even, same anther showed PMCs, with different stages of division.

Mitotic Studies: Pretreatments: The Root tips were cut and thoroughly washed in tap water to remove soil particles. They were preserved for varying duration, in any one of the following chemicals-

a. Saturated aqueous solution of para dichloride benzene for one to one and half hours;
b. 0.05% aqueous solution of colchicines for 45 minutes to one hour;
c. 0.002M solution of 8-hydroxy- quinolene;
d. Saturated aqueous solution of colchicines gave better result as compared to other pre treating chemicals.

1. **Fixation:** The pre treated tips were thoroughly washed in running water for 2 minutes. Then they were soaked in the folds of filter paper and then fixed in 1:3 V/V acetic acid and absolute alcohol with two or three droips of ferric chloride (5%) as mordant. The fixation was done for 48 to 78 hours. In hot weather, fixed materials were kept at 15^0c to 20^0c. In order to store the materials for longer period, they were then transferred to 70% alcohol at 15^0c.
2. **Staining:** After fixing the materials for at least 48 hours, they were heated in 2% aceto-carmine till boiling, this process was repeated three to four times and then they were transferred to fresh stain. A small portion of root tip was taken on the slide and then squashed in 1% aceto-carmine till boiling, this process was repeated three to four times

and then they were transferred to fresh stain. A small portion of root tip was taken on the slide and then squashed in 1% aceto-carmine. Some times the root tips were left in fresh stain for 12 to 16 hours, for better staining. Once chromosomes separated satisfactorily by repeated tapping and pressing, the slides were sealed with paraffin's wax for detailed studies or for microphotography. Microphotography was taken using the same lenses. The slides were made permanent by following buty- alcohol method as described byh Celarier (1956). Other stains such as aceto-orceine and leuco basin were also tried but results obtained with these stains were not satisfactory. 1.5% propino-carmine when used, gave almost the same result as 2% aceto- carmine.

Meiotic Study: The flower buds of proper size were fixed for 72 to 96 hours directly in a mixture of aceto-alcohol (1:2 and 1:3 v/v) in which a few drops of ferric chloride were added as mordant. After several trials it was found that, fixation for 96 hours in the mixture of aceto-alcohol (1:3) gave better result. The processes of staining, and squashing and other techniques were the same as used for mitotic studies.

Colchicines Treatment And Inducation Of Polyplodiy: The following methods were tried for inducation of polyploidy in T. anguina by colchicine treatment.

Method I: 16 hours continuously water soaked seeds were treated in 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, and 1% aqueous solution of colchicines for variable duration. After that treatment, seeds were sown without washing with water in different post for further studies. The control plants were also raised in each case.

Method II: Seedling sown in the pots were treated, at their apical points, when they were 2-6 days old. The apical buds were exposed and covered with cotton, soaked in colchicines solution of 0.1%, to 0.7% daily for 5 hours, upto 2 to 4 days. The cotton was frequently re soaked. After every treatment the plants were transferee to the field for further studies and observations.

Method III: The roots of young seedlings were dipped in 0.025 to 0.3% of colchicines solution for 6 to 10 hours and then after washing with water, they were sown in the posts. This treatment was, however not successful. In the present investigation only first method gave polypoloid plants.

Results and Discussion: Natural tetraploid in this species has not been reported so far. In this present investigation tetraploid in the species was raised to study the biochemical compositions or the plant besides by seed soaked treatment method but tetraploid could not be obtained by this methods (Kowatani, et.al.2014). The artificially raised tetraploid plant differed in several characters from that of the diploid.

Tetraploid trichosanthes anguina (2n = 44): Tetraploid Trichosanthes anguina had 2n = 22. This number was confirmed in the somatic plates (Fig. No.8). Mitosis was suited normal. Meiosis was also normal. Eleven clear bivalents were observed at metaphase I of mitosis. The bivalents ware of both rod and ring types but ring bivalents were more frequent. This contines the earlier reported on this species by. The tetraploid of this species had 2n=44 in somatic cells Metaphase and anaphase were regular with equal distribution of 44 chromosmes to every daughter cells. Mitotic studies revealed the presence of varying number of varying number of univalents, bivalents, trivalents and guadrivalents at metaphase. Altoghether 25 were analysedx in which the frequency of univalents

ranged from 0-11, bivalents 0-10, trivalents 0-5 and quadrivalents from 3 to 11. The mean value of univalents, brivalents, rivalents and quadrivlenta was 3.36, 3.80, 1.36 and 7.24 respectively per cell. The chaisma frequency ranged from 52-58 with mean to 53.7 per cell (Laws, 2007). Anaphase I was irregular. Out of 25 PMCs analysed for anaphase I distribution, 20% had regular distributions of 22-22 chromosomes to each pole. 36% of the PMCs were without laggards and rest 64% PMCs had laggards which ranged from 1 to 4 per cell. 25 PMCs were also analysed for ther study of anaphase 11. This stage was also irregular. 84% cell had regular distributions of chromosomes. Only 16% PMCs had regular distribution with 22-22 chromosomes at each pole. Laggards were also noted in 60% of PMCs.

Triploid Trichosanthes anguina (2n=33): The triploid of T-anguina showed 33 chromosomes in the mitotic plates. The division was quite normal with equal distribution of chromosomes to each. No daughter cell with irregular chromosomes number was observed. For meiotic 25 PMCs were examined at metaphase I (Narayanankutty, et.al. 2006). Complete pairing with trivalent formation was counted in only 12% of the PMCs (Rahman, et.al.2002). A varying number of univalent's, bivalents and trivalent was scored in 88% of the PMCs (Shimamura, 2009). The univalents ranged from 0-13 with a mean 4.68, bivalents 6-8 with mean 2.28 and trivalents 3-11 with a mean. Distributions of chromosomes at anaphase I was extremely irregular. A large number of laggards and bridges was noted (Vidhysekharan, & Kandaswamy, 1972). The number of laggards ranged from 1-5.

Conclusion: Tetraploid has 2n = 44. The chromosome in these plants after colchicines treatment were apparently looked smaller than the diploid. The analysis 25 PMCs at metaphase I of meiosis showed bivalents from C- 11 with a sean 3.36, bivalenmts 0-10 with mean 3.80 trivalents 0-5 with 1.36 and quasrivalents ranged from 3 to 11 with mean 7.24. Triploid T. anguina had 2n = 33. Meiosis showed a great range of univalents i.e. 0-13 with a mean 4.68; where as the bivalentgs mean was 2.28. The trivalent frequency was quite high. It ranged grom 3 to 11 with mean 7.92. The anaphase sepatatio was more irregular because of the add number of chromosomes in triploid 16:17 distribution of chromosome was scored in 16% of the PMCs. 28% of the PMCs also showed 16 : 16 separation with one chromosome lost. As a result of the highly irregular, most of the pollens were abortive and the pollen fertility was reduced to 27.2 % only. At anaphase I the distribution of chromosomes in equal number is found in 20% of PMCs. Almost a similar percentage of PMCs showed 22:20 chromosome distribution.

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