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THE MITOTIC STAGES OF PARASITIC PLANT, CUSCUTTA REFLEXA ROXB OF MANIPUR

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ABSTRACT

The chromosomes movement during the somatic division in all flowering plants followed certain conservative pattern. The centromeric regions with kinetochore are the sites for spindle binding and pull towards the opposite poles. Do a parasitic plant chromosomes follow this movement? For the above query the shoot tips of parasitic plant, Cuscuta reflexa**,** Roxb were collected, fixed in Carnoy's solution for 24 hours from Indian plum**,** Ziziphus mauritiana Lam., but the plants have found to parasite on other plants**.** The fixed shoots were macerated in the solution of glacial acetic and 1 N HCl and squashed and observed under the microscope. The chromosome number of the plant was 32 with monocentric chromosomes. The pattern of the movement of the chromosome was observed to be quite acquainted as in other plants. The nuclear architecture of the interphase nuclei and dividing cells are quite conventional. The patterns of prophase to cytokinesis stages are quite stable as in other angiosperms. As expected the heterochromatic content in the interphase nuclei known as chromocenters were huge and distinct. The shoots of 3 cm to 7 cm could be utilized for mitotic studies. The future works are to determine the chromatin organization of the plant and study the germinal chromosomes and compare them with the other species.

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INTRODUCTION

Cuscuta reflexa or dodder plant or devil's hair, witch's hair, love vine, amarbel or akashabela is a parasitic plant of family Convolvulacea (Siani *et al*., 2015; Story *et al*., 1958) and is common in the Indian Subcontinent and the Greater Himalayas O'Neill and Rana (2019). Cuscuta is group of 100-170 species of yellow, orange, red or, rarely green parasitic plant. Many reports are pouring in for its medicinal activity for the treatment of headache, labour pain, bone fracture, fever, rheumatism, etc. (O'Neill and Rana, 2019). Lupeol isolated from *C. reflexa* is a pharma-cologically active tri- terpenoids and posse's antimicrobial, anti-inflammatory, antitumor, antiprotozoal, and chemo-protective properties (Gallo *et al*, 2009).

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Parasitic plants are important in research, especially on the loss of photosynthesis and the co-dependency of functional, genetic and lifestyle changes Yang *et al*., (2015), Wicke, *et al*., (2016), Wicke and Naumann (2018), Chen *et al*., (2020).
In the present study, the applicability of shoot of the dodder plant in the cytological research as well as the practical experiment, for the students in UG and PG classes as the plant abundantly found in the area and usually consider as waste. Moreover, it is learned that the regenerative tissues in animals and plants do have large chromocenters. It applies to the parasitic plant was to check. The heteropycnotic blocks of this plant could be used to study nuclear architecture in the future.

MATERIALS AND METHODS

Plant material used to study the Mitosis: Shoots of *Cuscuta reflexa* of 20 -30 cm in lengthfrom tips were collected in the month of April, 2021 from the two locality-one from Elangbam Leiaki and one from Manipur University (Fig. 1) in different time 3.40 pm, 1.0 pm and 10.30 pm and fixed in

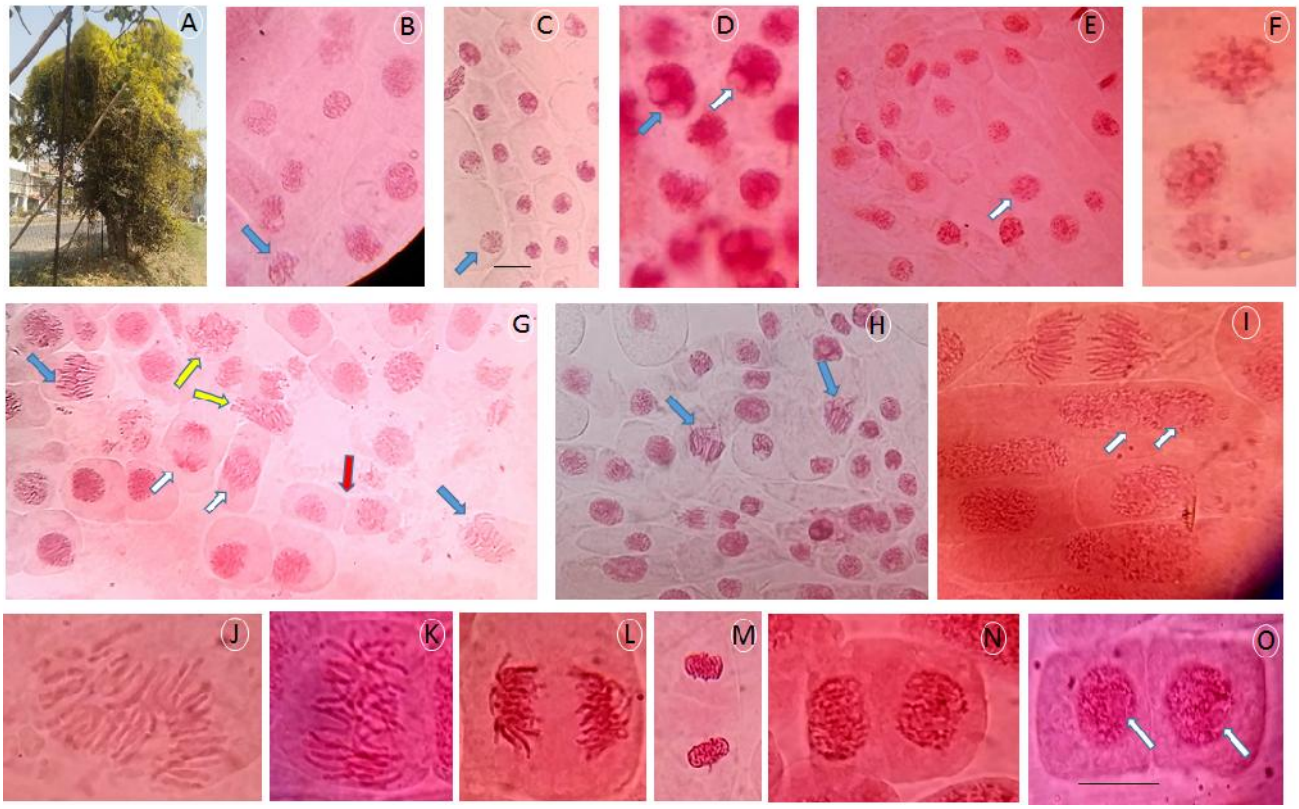

Figure 1. The plant and mitotic division from shoot meristematic tissues of *Cuscuta reflexa* Roxb. A) the plant grown on the Indian plum, B-D) the interphase nuclei with one to three nuclear vacuoles, blue and white arrows, E) C banded nuclei with distinct chromocenters, F) the prophase nuclei with prominent heterochromatic, G) the microscopic view of the stages of mitotic division, yellow arrows indicate prophases, white anaphase, blue the telophase, H) the metaphase staged nuclei, I) the white arrows indicate G_2 phase nucleus with two reddish nucleoli just below the mid-anaphase, J) the prophase staged plate with clear centromeres, K) metaphase staged plate with peculiar metaphase chromosome arrangement at equatorial plane, L) anaphase staged nucleus, M) late anphase, N) the early telophase where the de-condensation started and O) the two daughter nuclei after cytokinesis with prominent nucleoli (white arrows). Bar represents 10 micron meter

fixative (3:1 ethanol and glacial acetic acid by volume) for 24 hours and preserve in 70% ethanol.

Slide preparation: In a test tube, 1 ml of Acetocarmine (2%, Merck, India- C. I. No. 75470, S. No. 1381) mixed with 20µl each of 45% glacial acetic acid and 1NHCl were taken along with seven shoot tips of thespecimens and were warm for 10 minutes over sprit lamp. Meristematic cells from the soften tips were used for micro slides preparation by squashed method to obtain the different stages of mitotic division.

Fifty cell plates were used for each stage and photographs of best 5 were selected for each stages starting from Interphase, Prophase, Metaphase, Anaphase and Telophase. Nuclear architecture is studied from 200 non-dividing cells.

Banding: The shoot tips of 2 mm of plant were warmed in the solution of one milliliter each of 2XSSC and Acetocarmine (2%) in a test tube with few grains of Barium hydroxide over sprit lamp for five minutes and slides were prepared as stated above.

Dividing and non-dividing nuclei were screened C banding. All the observations were done in 40X/100X objective lens of the Olympus light microscope and microphotographs were taken with MI 7 pro Camera through the eyepiece of the microscope.

RESULTS

The chromosome number of the plant was seen to be 32 as in prophase and anaphase all with monocentric chromosomes (Fig. 1 J). But as of now, we cannot differentiate the respective chromosomes with types. The acetocarmine-stained meristematic nuclei showed well-defined characteristic mitotic divisions. In the experiment, the shoots of 3 cm to 7 cm of Cuscuta reflexa Roxb could be utilized for mitotic studies. The interphase nuclei with one to three nuclear vacuoles of the nucleoli were distinct in G1 and G2 stages (blue and white arrows, Fig. 1. B-D) but prominent in G1 as expected. Again the C banded the nuclei showed distinct chromocenters in interphase (Fig. 1. E). C banded the prophase nuclei with prominent heterochromatic blocks (Fig. 1. F). The conventional characteristics of mitotic cell division could be examined from the tissues (Fig. 1. G, yellow arrows indicate prophases, white anaphase, blue the telophase, H. I, J, K, L, M, N, and O).

DISCUSSION

According to Kaul and Bhan (1977), the chromosome number of *Cuscuta reflexa* with 2n=32 and 48 were referred as tetraploid and hexaploid respectively base on the identical features of the respective chromosomes.

Due to the unavailability of the clear distinct metaphasic plate, the number of and types of the chromosomes could not be ascertained but the prophase and anaphase count corresponded to 32. So further intense work on this matter will be the first goal in future study. The plant with 2n=32 was observed to grow parasitically on Bouganvillea glabrachoissy**,** the race with 2n=48 was found growing on Cleredendron inerme (Kaul and Bhan, 1977) but the host plant in the present study is not either of these, hence future works should include the host plant beside the chromosome numbers. The interphase nuclei with one to three vacuoles/lacunae were reported in the Allium cepa ascalonicum in our laboratory. The finding leads to the conclusion that the vacuole/lacunae are the actual space occupied by the nucleolus. It seems most likely that the chromosomes are floating above the nucleolus during the interphase while attaching to the nuclear membrane and reestablished after the anaphase-telophase stage in the succeeding cycle. The lacunae are the spaces leftover by lateral arms of the chromosomes (two lateral vacuoles/lacunae) and telomeres of the sixteen chromosomes. The present studies strongly convinced to speculate the studies on nucleolus of the plant as well as animals will be partial not the whole of the nucleolus. Our results were by per under (Guerra and Gercia, 2004) who reported that nucleolus was observed in hematoxylin stained cells. It was always single in meiotic and endoreplicated cells, being very large in the latter. In meristematic cells, there were one or two nucleoli whereas in tapetal cells, there were up to six smaller ones per nucleus. So the outlook on nucleolus should be reevaluated altogether for a better understanding of this nuclear organelle. In future works, this point of nuclear architecture particularly plants will be the focal point and try to establish in the animal also.

Ever since Heitz (1928) introduced the term heterochromatin for a specific portion of a chromosome, it has been a mysterious subject in cytogenetics (Cumings, 1972). Centromeric heterochromatin appears to be involved in changes in chromosome morphology through breakage and re-fusion. Constitutive heterochromatin has been regarded to have a distinct role in evolution (Sharma, 1985) and also it is believed to play a significant role in the evolution of the karyotype in plants and animals (White, 1973). As our findings, Guerra and Gercia (2004), the chromocenters and heteropycnotic blocks were seen slightly differently at interphase, however, the number of heteropycnotic blocks were smaller, probably owing to fusions. After C banding or DAPI staining, they were more deeply stained and contrasted. The CMA+ heterochromatin was observed as small dots associated with DAPI+ chromocenters. The same kind of heterochromatin arrangement seen in the diploid interphase was observed in highly endoreplicated nuclei of glandular cells of the ovary walls and sepals. These cells were remarkably large and their giant nuclei had chromocenters similar in number to those of diploid nuclei but several times larger. In such nuclei, the small blocks of CMA+ heterochromatin were more clearly observed (Guerra and Gercia, 2004). Future works could be to study the nuclear architecture mainly the distribution of heterochromatin during interphase.

The present study concludes that: a) the chromosomes in Cuscuta reflexa Roxb from Manipur are monocentric, and meristematic tissue could be utilized for cytogenetic practical classes and research, b) the heterochromatic blocks could be used to study the nuclear architectures, and c) identification of the plants on basis of chromosomes.

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