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Research Article

CYTOLOGICAL STUDIES OF *CANNABIS SATIVA* IN SHIMLA HILLS OF HIMACHAL PRADESH

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ABSTRACT
Chromosome counts made in *Cannabis sativa*, clearly depict that the genus is diploid with 2n= 20. On the basis of the position of centromere, they can be represented as 10M + 6SM + 4ST i.e., 10 median, 6 sub-median and 4 sub-terminal. Polyploidy were also observed in some of the tetraploid cells with 4n=40. The root-tips showing polyploidy were thicker in appearance than those showing diploid number.

Key Words: *Cannabis sativa, Hyperploid, Hypoploid, Centromere, Karyotype*

INTRODUCTION
Cannabis is one of the popular plants among common people since times immemorial due to its various uses and abuses. It is known by various names in different parts of the world, e.g., Bhang, ganja,charas, hashish, kif etc. Partnership of *Cannabis* and man has existed probably for ten thousands years, since the discovery of agriculture in the old world and it is considered as one of the oldest cultivated plants (Mohan Ram1964,Maheshwari 1983). It is believed to have been under Chinese cultivation more than 4,500 years ago.According to Chakravorty 1983, hemp might have had originated in a diffuse manner in a huge area extending from Caspian and Himalayas to China and Siberia.Inspite of the various harmful effects, the consumption of the drug has, however, increased alarmingly over the past few years throughout the world, especially in the industrialized countries (Hammond, and Mahlberg 1983).

A large section of the people especially in the interior of Himachal Pradesh live in villages, remote forests or sometimes in small cities, still depend upon folk medicines and household remedies. *Cannabis sativa* plants grow abundantly throughout the Himachal Pradesh at all the altitudes, ranging from 1,000 ft. to 9,000 ft., so such a high range of distribution provides a good scope for study.

For the cytological studied the chromosomes are the most significant components of the cell. They control most of the cell biological and genetical activities of a species. They contain the genetical material, the DNA which ultimately influences all the biological phenomena at molecular, physiological and gross morphlogical levels (Maheshwari, 1983).

One of the most impotent characteristics of the living cells is their power to grow and divide. When a cell reaches the limit of its size peculiar to its species, it divides into two parts. These parts grow to their full size and they divide again. In the cell division chromosomes occupy a central position (Fried, 1980). According to Hammond and Mahlberg1977, one of the basic characteristic of mitotic cell division which is meant for growth due to multiplication is that it gives rise to two daughter cells, which resembles each other and also the parent cell qualitatively and quantitatively. The mitosis composed of two apparatuses, viz., chromatic apparatus which includes the chromosomes and the nucleolus and the achromatic apparatus which in its turn includes the centrioles and spindle (Hammond and Mahlberg 1973).According to Pandey and Sharma 1983 mitosis helps in the maintenance of an equilibrium in the amount of DNA and RNA in the cell. The mitosis provides the opportunity for the growth and development to plants (Krishnamurthy 1974)

The present study was undertaken to carry out the cytological analysis of *Cannabis sativa* in Shimla district of H.P state. The study was undertaken with the following objectives:-

1. To study the no of chromosome and karyotype of somatic cell in *Cannabis sativa* and also identify any abnormality during mitosis division.
2. Measurement of chromosome and position of centromere in them.

MATERIALS AND METHODS

Following procedures were followed:

(a) Pre-treatment and fixation of Materials: Seeds were collected and germinated by keeping them in Petri-dishes on moist blotting sheets at 28^0 C. Then these seedlings were treated with 0.25% colchicine solution for 3 hours at a temperature of about 20 $\pm$ 2^0C. After pre-treatment the roots were excised, washed thrice with distilled water and fixed in 1:3 acetic alcohol for 24hrs. after which they were preserved in 70% alcohol.

(b) Preparation of stain: Belling's (1926) iron acetocarmine method was used, however, it was modified slightly to get best results. one gram of carmine powder was refluxed for 3 hrs. in 45 cc. glacial acetic acid. After refluxing 55cc. of distilled water was added to it. It was again refluxed for one hour. After cooling, the solution was filtered and stored in bottle. Smear preparations were then made in 1% acetocarmine and HCI (in the ratio of 9 : 1) by heating the root-tips upto 60-70^0C. for 15minutes. Slides were made permanent using Darlington and La Cour method (1962). Desirable preparations were selected and microphotographed.

RESULTS AND DISCUSSION

The somatic compliment of *Cannabis sativa* invariably consisted of 20 chromosomes, as determined by large number of metaphase plates (Figs. 1a, 1b, 3a, 3b, 3c and 4a). The disparity in the size of the chromosomes was well-marked and they constituted a graded series. On this basis, they may be represented as n= 10, 5L + 4M+ IS,Where L represents long, M Median and S Short-sized chromosomes.

Karyotypically (Fig.2), they are classified as one pair of large chromosomes, being longest in the compliment, with median primary constriction (chromosome No. 1).Four pairs of long sized chromosomes one with sub-median centromere (Chromosome No.2), two with sub-terminal centromeres (Chromosome No. 2), two with sub-terminal centromeres (Chromosome No. 3 and 4) and one with median centromeric position (Chromosome No. 5).Four pairs of medium-sized chromosomes, with two median (Chromosome numbers 7 and 8) and two sub-median types (Chromosome numbers 8 and 9). one pair of short chromosomes with median centromere (Chromosome No. 10.). Polyploidy was also observed in some of the cells. Figs. (4b and 4c) depict tetraploid cells with 4n=40. The root-tips showing polyploidy were thicker in appearance than those showing diploid number. Hyperploid cells were also commonly observed (Figs. 5a, 5b and 5c). The Karyotype possessed 10 median, 6 sub-median and 4 sub-terminal chromosomes. Secondary constrictions were not noticed in any of them. Chromosome size ranged from 1.0um- 4.0 um. The length of the long arm, short arm and their ratios are given in Table I.

The number of nuclei varied from 1-7 (figs.6a,6b,6c,7a) but most of them were with 2 nuclei. These cells were rounded in appearance. Some of the cells showed darkly stained permanent nucleolus with very granulated material (fig. 7b).At anaphase stage most of the cells show unequal number of chromosomes moving towards the poles (Fig.7c).

In telophase or resting stage, there were micronuclei and meganuclei. Aneuploidy is more frequent with hyper and hypoploid cells (Figs. 5a, 5b, 5c) showing 11 and 18 chromosomes. Generally seed germinated after 24 hours but some seeds which germinated after 72 -96 hours,the root tip were thick and rounded. On analysing them cytologically, it was found that those seedlings with pointed tips had diploid cells, i.e., 2n=20, while the seedlings with thick root-tips showed variations in chromosome number (ranging from 10-20) and morphology. A few cells with tetraploid number of chromosomes (4n=40) were also observed. Most of the cells in these abnormal seedlings were rounded and multinucleate with more chromatin material and were aneuploid. Aneuploidy observed in the cells may be due to spindle abnormalities or due to some other factor. The observations depict that the presence of THC contents or alkaloids may have inhibited the proper functioning of spindle in these abnormal root-tips as was earlier reported by Kabariti et.al (1980), that the alkaloids present in *Cannabis sativa* are responsible for the induction of C- tumour and polyploidy.

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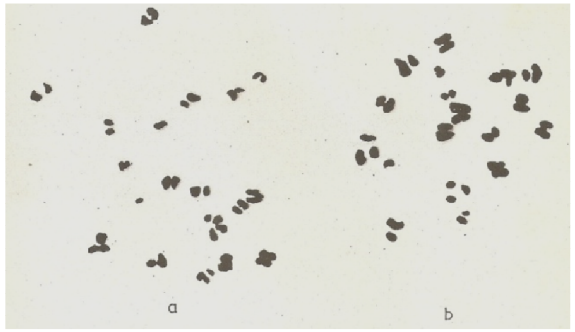


Figure 1: Showing 2n = 20 in root-tip mitosis.

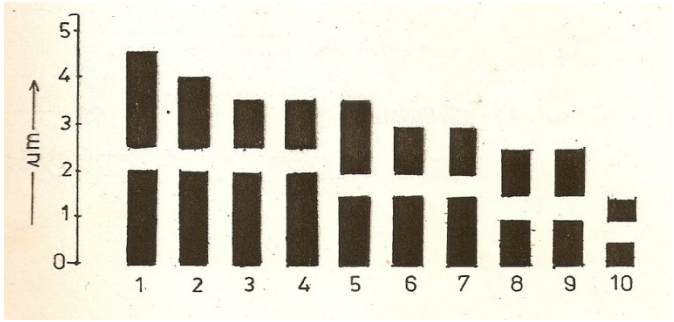
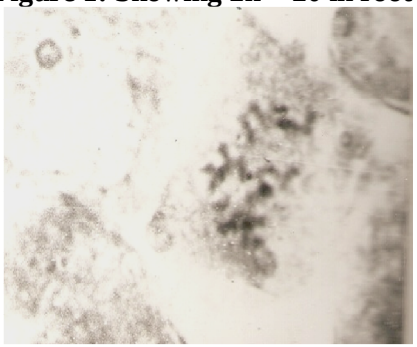
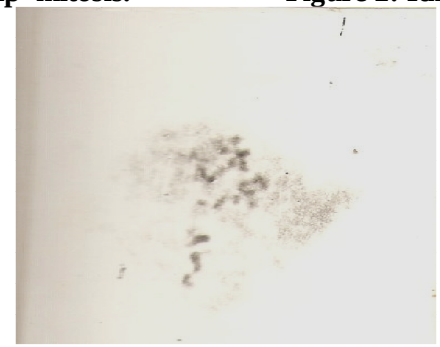


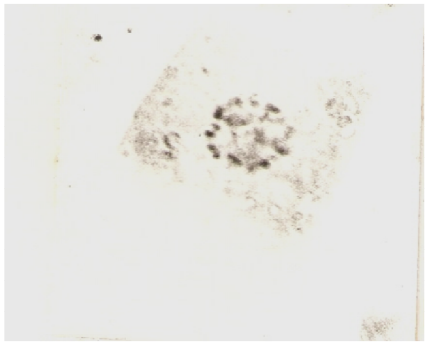
Figure 2: Idiogram of Cannabis sativa with n= 10



(3a)

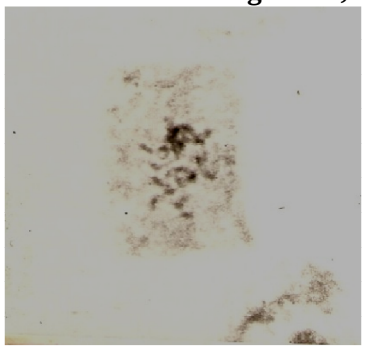


(3b)

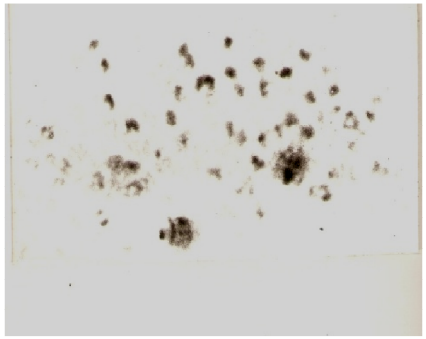


(3c)

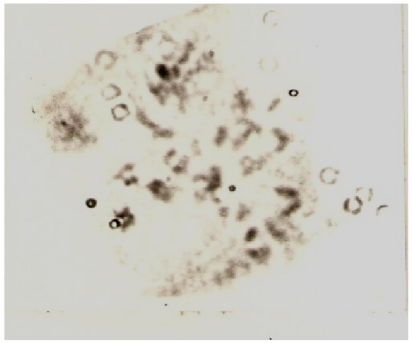
Figure 3a, 3b, 3c: Mitosis root-tip cells showing 20 chromosomes.



(4a)

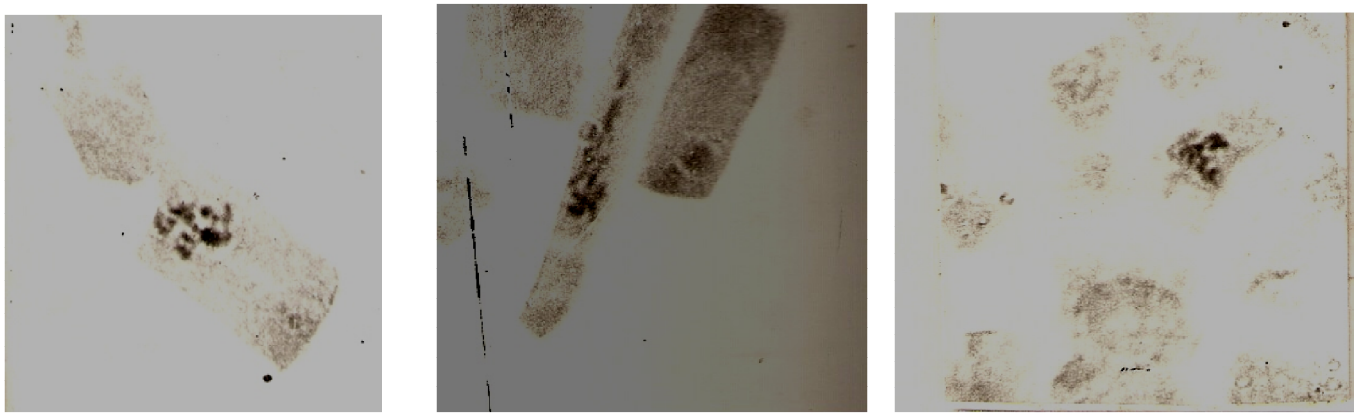


(4b)



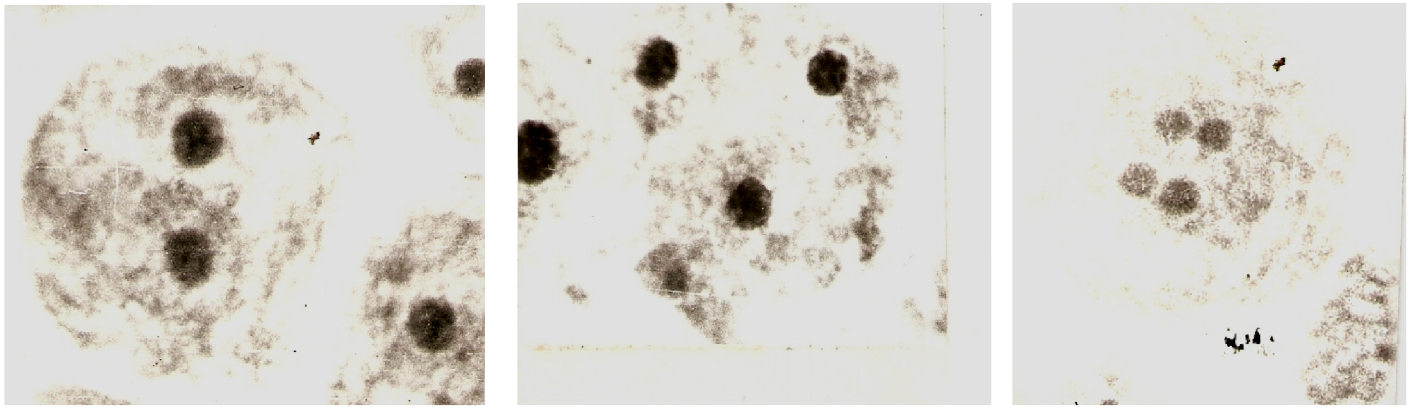
(4c)

Figure (4a): A root-tip cell with 20 chromosomes, (4b,4c): Mitosis root-tip cells showing 4n = 40 or tetraploid cells



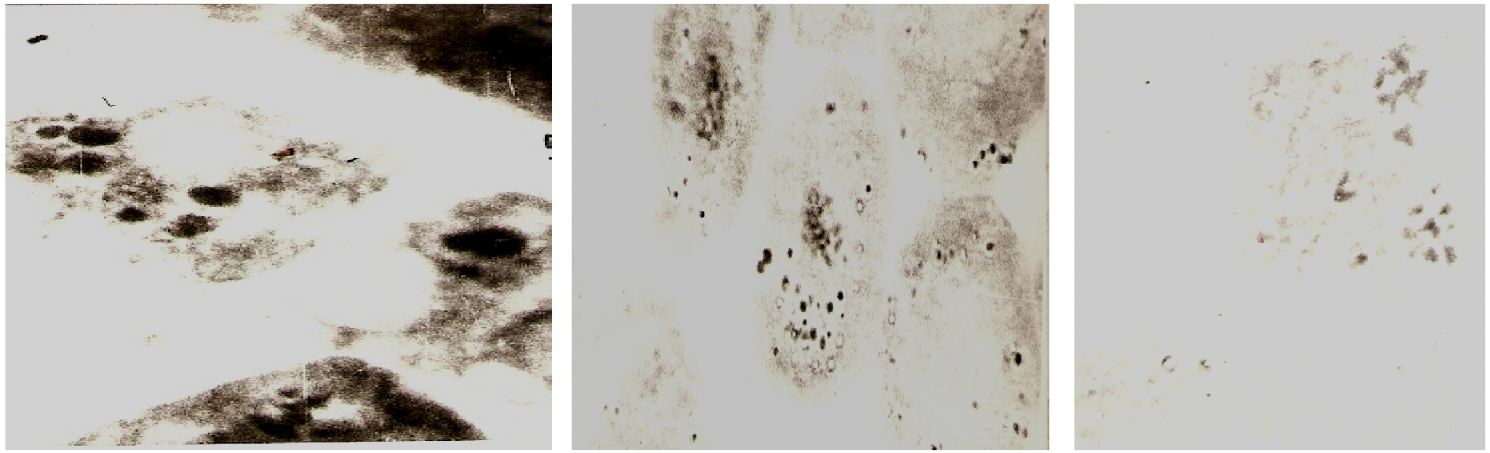
(5a) (5b) (5c)

Figure (5a, 5b, 5c): Cells showing less number of chromosomes or hyperploid cells.



(6a) (6b) (6c)

Figure (6a, 6b, 6c): Abnormal and rounded large cells from the thick tumour like root-tips with two, three , four nuclei respectively



(7a) (7b) (7c)

Figure (7a): Abnormal and rounded large cells from the thick tumour like root-tips with seven nuclei. Figure (7b) Cells with granulated chromatin material. Figure (7c) Anaphase stage with unequal number of chromosomes moving at the two poles. Leggards can be clearly seen.

Table 1: Measurement and position of centromere of somatic chromosomes in *Cannabis sativa*

Chromosome pair	Long arm of Chromosome 'L'	Short arm of Chromosome 'S'	Short arm of Chromosome 'S'	Arm ratio 'R'	Position of Chromosome
1	2.0	2.0	4.0	1:1	M- Madian
2	2.0	1.5	3.5	4:3	SM- Sub-median
3	2.0	1.0	3.0	2:1	ST-Sub-terminal
4	2.0	1.0	3.0	2:1	ST-Sub-terminal
5	1.5	1.5	3.0	1:1	M-median
6	1.5	1.0	2.5	3:2	SM- Sub-median
7	1.5	1.0	2.5	3:2	SM- Sub-median
8	1.0	1.0	2.0	1:1	M-median
9	1.0	1.0	2.0	1:1	M-median
10	0.5	0.5	1.0	1:1	M-median

n = 10 (5M +3SM +2ST)

From the chromosome counts made so far, it is obvious that the genus is diploid with 2n = 20, with the presence of few tetraploid cells having 4n = 40. Tetraploid tissues arise regularly in certain structures as an adaptation. In *Cannabis* also, the periblem cells are generally tetraploid, having 40 chromosomes instead of the 20 found in plerome (Breslawetz, 1925, 1935). However, it may be admitted that observations made are incomplete as we have investigated only the root-tips of the seedlings which is mixture of chromosome compliment of male and female cells, and it is quite possible that the detailed meiotic studies of male plants may show some variations in their number if they are investigated separately.

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