

## Karyological Studies in Root-Tip Cells of *Cannabis sativa* var. *indica*

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**Summary** Chromosomal study was carried in the dividing root-tip cells of *Cannabis sativa* (Family: Cannabinaceae). The diploid chromosome number of this species is  $2n=20$ . Karyotype analysis reveals that all the 9 chromosomes in female somatic cells are metacentric and 1 chromosome is sub-metacentric whereas the X and Y chromosome in male cells is sub-metacentric. The Y chromosome is longer than X chromosome. The third pair bears the satellite.

*Cannabis sativa* var. *indica* is a narcotic species. Leaves, pistillate flowers and resin of this species yield bhang, ganja and charas, respectively. The psycho-active principle in all these preparations is  $\Delta^9$  tetrahydrocannabinol. Cultivation of *cannabis* is illegal in India. Punishment is severe for keeping ganja and charas but less severe for bhang. *Cannabis sativa* is a dioecious species in which pistillate and staminate flowers appear on different plants which can be easily distinguished by their characteristic morphology. However, determining sex of plants is difficult when they are young. Therefore, it is desirable to develop a technique that can unambiguously differentiate male and female plants at juvenile phase of development. Identification of sex chromosomes and development of sex-specific DNA probes would be useful for establishing sex of individual seedlings, during analysis of Forensic samples.

Breslavetz (1926) reported that the nuclei of *C. sativa* contain 20 chromosomes that are difficult to individualise due to minute size. Mc Phee (1926) suggested that *C. sativa* has XY type of sex determining mechanism. Breslavetz (1932) recognized X and Y chromosomes in both  $2n$  and  $4n$  cells of the young root-tips. Later, Mackay (1939) found unequal sex chromosome pair (XY) in two varieties of *C. sativa*. Warmke and Davidson (1944) suggested that the male and female determining genes are present in sex chromosomes, not on autosomes. Yamada (1943) found XX chromosomes in female plants and XY chromosomes in male plants, Y chromosome being longer than X. Present paper describes the karyotype of *C. sativa* var. *indica*.

### Materials and methods

The seeds collected from Regional Forensic Science Laboratory, Raipur, were dried and stored in cool place. For germination, the seeds were presoaked for 24 h in water, transferred to Petri dishes in wet filter paper, and then placed under natural photoperiod at ambient room temperature. The root-tips were excised and pretreated with saturated para-dichlorobenzene for 3 h at 4°C, fixed in glacial acetic acid : ethanol (1 : 3) for 24 h at 4°C, and preserved in 70% ethanol at 4°C. The root meristems fixed at 1 h interval from 7:30 AM to 2:30 PM showed high frequency of metaphase cells during 9:30 AM to 11:30 AM. Therefore, for chromosome study, root-tips were finely cut and kept in pretreatment solution during this period only. To determine the sex of root-tips each seedling was numbered and same number was given to the excised root-tips. After removing the root-tips, the seedlings were planted in earthen pots in a wire-mesh house. Flowering revealed that approximately

50% of these plants were male and 50% female, very rarely monoecious plants appeared in this population. Squashes of corresponding male and female root-tips were prepared separately to study the karyotypes.

For removing cytoplasmic contents, preserved root-tips were hydrolysed for 5–7 min in 1N HCl at 60°C. This was followed by a thorough washing in deionised water to remove the traces of acid. The root tips were then warmed in 2% aceto-orcein staining solution for 3–5 sec and subsequently kept in staining solution for 48 h. The tip portion was finally squashed in a drop of glacial acetic acid. Cells with good spread of chromosomes were photographed. Karyotype of *C. sativa* was made by cutting out individual chromosomes and arranging them in homologous and heterologous pair in order of length and arm ratio. Arm ratios were calculated by dividing the length of long arm by that of the short arm, and the conventions proposed by Levan *et al.* (1964) were used to refer to the different chromosomes—ratio 1.0 to 1.7 metacentric (m), 1.7 to <3.0 submetacentric (sm), 3.0 to <7.0 subtelocentric (st) and >7.0 telocentric (t) chromosomes. Karyotype asymmetry has been estimated using the equation of Huziwara (1962)— $F\% = (\text{Sum of short arm length} / \text{Sum of total chromosome length}) \times 100$ . The Gradient index (GI) and Symmetry Index (SI) was calculated by using the following formula (Pritchard 1967)

$$GI = (\text{Length of shortest chromosome} / \text{Length of longest chromosome}) \times 100$$

$$SI = (\text{Total length of short arms} / \text{Total length of long arms}) \times 100.$$

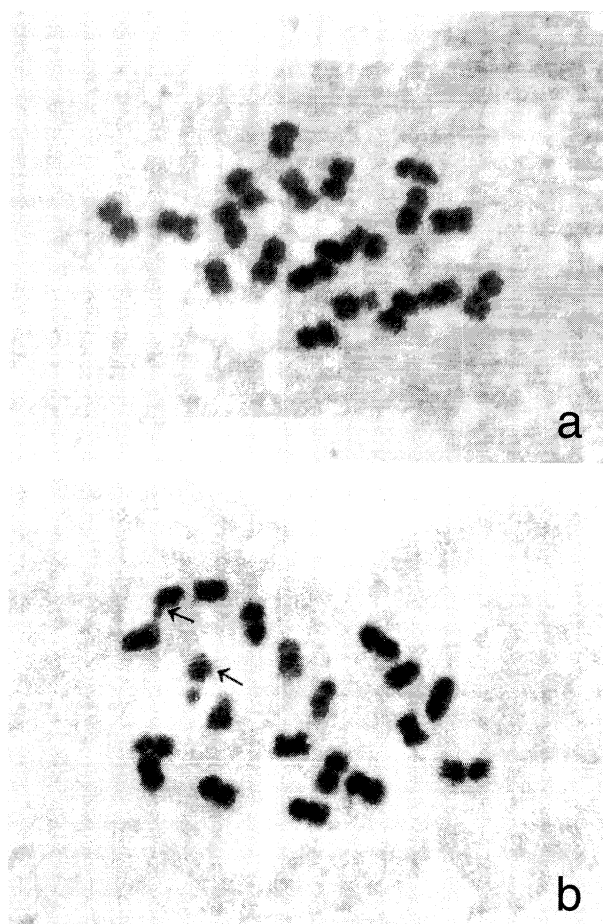


Fig. 1. Cytological analysis of *Cannabis sativa*. Metaphase plate in female (a) and male (b). The sex chromosomes X and Y are indicated by arrow.

Table 1. Averages of chromosomes measurements and classification of obtained observations on 10 cell spreads, of *Cannabis sativa*

| Chromosome number | Chromosome length ( $\mu\text{m}$ )    |                           |                        | Relative length (%)        |                           |                        | Arm ratio         | Centromeric index  |               | Chromosome type |
|-------------------|--|---------------------------|------------------------|----------------------------|---------------------------|------------------------|-------------------|--------------------|---------------|-----------------|
|                   | Short arm<br>Mean $\pm$ SE             | Long arm<br>Mean $\pm$ SE | Total<br>Mean $\pm$ SE | Short arm<br>Mean $\pm$ SE | Long arm<br>Mean $\pm$ SE | Total<br>Mean $\pm$ SE |                   | Mean $\pm$ SE      | Mean $\pm$ SE |                 |
| I                 | 1.000 $\pm$ 0.017                      | 1.114 $\pm$ 0.022         | 2.114 $\pm$ 0.033      | 5.365 $\pm$ 0.095          | 5.976 $\pm$ 0.120         | 11.341 $\pm$ 0.181     | 1.114 $\pm$ 0.024 | 47.303 $\pm$ 0.843 | m             |                 |
| II                | 0.858 $\pm$ 0.039                      | 1.174 $\pm$ 0.040         | 2.032 $\pm$ 0.026      | 4.603 $\pm$ 0.210          | 6.298 $\pm$ 0.220         | 10.901 $\pm$ 0.141     | 1.368 $\pm$ 0.114 | 42.224 $\pm$ 1.929 | m             |                 |
| III               | 0.890 $\pm$ 0.020<br>(0.17 $\pm$ 0.72) | 1.019 $\pm$ 0.009         | 1.909 $\pm$ 0.085      | 4.774 $\pm$ 0.109          | 5.466 $\pm$ 0.052         | 10.241 $\pm$ 0.460     | 1.144 $\pm$ 0.023 | 46.621 $\pm$ 1.066 | m+sc          |                 |
| IV                | 0.781 $\pm$ 0.025                      | 1.098 $\pm$ 0.026         | 1.879 $\pm$ 0.022      | 4.189 $\pm$ 0.134          | 5.890 $\pm$ 0.129         | 10.080 $\pm$ 0.119     | 1.405 $\pm$ 0.070 | 41.564 $\pm$ 1.331 | m             |                 |
| V                 | 0.800 $\pm$ 0.020                      | 1.036 $\pm$ 0.028         | 1.836 $\pm$ 0.026      | 4.291 $\pm$ 0.112          | 5.557 $\pm$ 0.151         | 9.849 $\pm$ 0.141      | 1.295 $\pm$ 0.064 | 43.572 $\pm$ 1.148 | m             |                 |
| VI                | 0.732 $\pm$ 0.021                      | 1.064 $\pm$ 0.025         | 1.796 $\pm$ 0.027      | 3.927 $\pm$ 0.115          | 5.708 $\pm$ 0.136         | 9.635 $\pm$ 0.149      | 1.453 $\pm$ 0.064 | 40.757 $\pm$ 1.198 | m             |                 |
| VII               | 0.760 $\pm$ 0.032                      | 0.998 $\pm$ 0.020         | 1.758 $\pm$ 0.033      | 4.077 $\pm$ 0.174          | 5.354 $\pm$ 0.150         | 9.431 $\pm$ 0.179      | 1.313 $\pm$ 0.077 | 43.230 $\pm$ 1.844 | m             |                 |
| VIII              | 0.719 $\pm$ 0.014                      | 0.967 $\pm$ 0.026         | 1.686 $\pm$ 0.025      | 3.857 $\pm$ 0.079          | 5.187 $\pm$ 0.143         | 9.045 $\pm$ 0.137      | 1.344 $\pm$ 0.053 | 42.645 $\pm$ 0.875 | m             |                 |
| IX                | 0.696 $\pm$ 0.010                      | 0.948 $\pm$ 0.028         | 1.644 $\pm$ 0.030      | 3.733 $\pm$ 0.058          | 5.085 $\pm$ 0.151         | 8.819 $\pm$ 0.165      | 1.362 $\pm$ 0.045 | 42.335 $\pm$ 0.667 | m             |                 |
| X                 | 0.476 $\pm$ 0.046                      | 1.262 $\pm$ 0.056         | 1.738 $\pm$ 0.064      | 2.553 $\pm$ 0.250          | 6.770 $\pm$ 0.381         | 9.324 $\pm$ 0.613      | 2.651 $\pm$ 0.085 | 27.387 $\pm$ 2.685 | sm            |                 |
| Y                 | 0.800 $\pm$ 0.070                      | 1.435 $\pm$ 0.067         | 2.235 $\pm$ 0.119      | 4.291 $\pm$ 0.381          | 7.698 $\pm$ 0.363         | 11.990 $\pm$ 0.644     | 1.793 $\pm$ 0.151 | 35.794 $\pm$ 3.177 | sm            |                 |

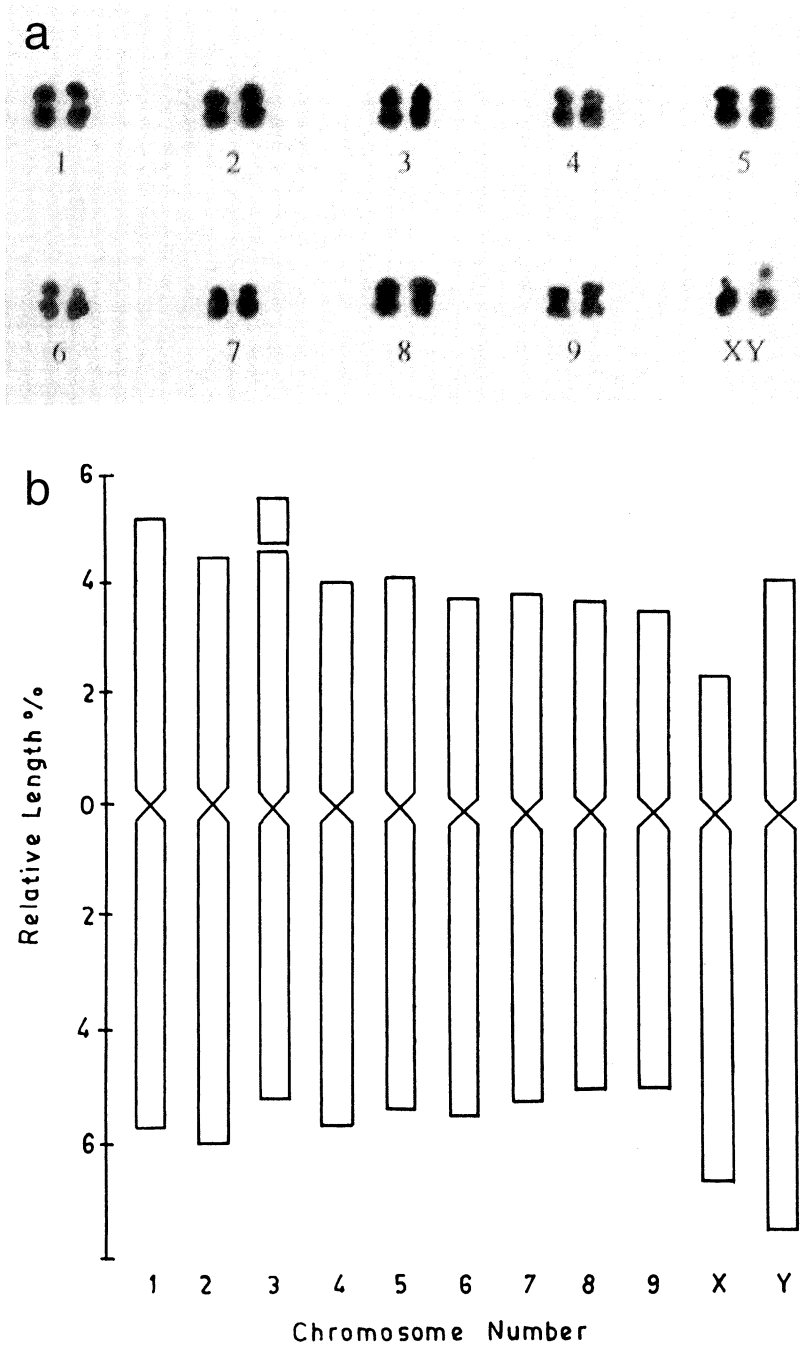


Fig. 2. a) The Karyotype of a male plant. b) Idiogram of *Cannabis sativa*.

## Results

In all 20 diploid metaphase spreads were selected for karyotype analysis, 10 metaphase spreads from 10 male root-tips and 10 female root-tips. Diploid root-tip cells of *C. sativa* var. *indica* showed 20 chromosomes (Fig. 1a, b). On the basis of karyotype data (Table 1) and idiogram (Fig. 2a, b), the chromosomes of male *C. sativa* can be divided into 3 groups: (a) 8 pairs of metacentric, non-satellite, homologous chromosomes (b) 1 pair of metacentric, satellite chromosomes and (c) 1 pair of heterologous, XY chromosomes. All the 9 pairs of chromosomes in female and 9 pairs of chromosomes in male are metacentric with arms ratio ranging from 1.114  $\mu\text{m}$  to 1.453  $\mu\text{m}$ , relative length % from 8.819 to 11.341 and centromeric index from 40.757 to 47.303. And one pair of chromosomes in female and X and Y chromosomes in male are sub-metacentric. The arms ratio, relative length % and centromeric index of one pair of chromosomes in female and X chromosomes in male are 2.651, 9.324 and 27.387 whereas the arms ratio, relative length % and centromeric index of Y chromosome are 1.793, 11.990 and 35.794 respectively. The Y chromosome in male is longer than X chromosome. The former is  $2.235 \pm 0.119 \mu\text{m}$  long, and latter is  $1.738 \pm 0.064 \mu\text{m}$  long. Moreover, their long and short arms also differ considerably. The values of F%, GI and SI are 42.24, 73.55 and 73.13, respectively. The metaphase cells in both female and male root-tip showed a considerable number of polyploid cells.

## Discussion

The results of this study confirm the chromosome count in *C. sativa* var. *indica*. Earlier workers have reported 20 chromosomes in root meristem cells (Breslavetz 1926) and leaf tip cells (Bir and Sidhu 1980), and 10 pairs of chromosomes in PMC's (Bir and Sidhu 1980) of *C. sativa*. Similarly, the 4n cells appear to be of common occurrence in root meristems. Breslavetz (1926) found tetraploid cells in the primary cortex and in the secondary root cells. It appears that gene amplification is required for differentiation of cells in the primary cortex and in the secondary root cells. However, these results supports the observation of Yamada (1943) who reported that Y chromosome is longer than X chromosome. In this study male and female root tips were fixed separately and then their idiograms were prepared. Sub-metacentric Y chromosome was found to be longer than X chromosome and could be identified unambiguously. Sakamoto *et al.* (1995) have developed probes for male-dependent DNA sequences of *C. sativa*. Karyological analysis may be useful for chromosome identification with specific DNA-probes and also for analysis of seedling samples of Forensic significance.

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