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NUCLEAR PHENOTYPE AND HETEROCHROMATIN PERCENTAGES IN SOMATIC CELLS ON FIVE SPECIES OF *CROTALARIA* L.

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Abstract

Crotalaria L. is a genus of flowering plants in the family Fabaceae (subfamily Faboideae). Mitotic index is proportionately relevant to cell division rate; mitotic index was found higher (10.38±1.08%) in *C. spectabilis* and lower (9.43±0.304%) in *C. juncea*. The nuclear organizations were observed chromocentric in all examined species. The interphase chromosome volume was highest in *C. juncea* (0.596±0.27 μ^3 in meristematic cell and 0.408±0.21 μ^3 in differentiated cell) and lowest in *C. saltiana* (0.205±0.31 μ^3 in meristematic cell and 0.16±0.25 μ^3 in differentiated cell) which do not correlated with the somatic chromosome number but may be related with environmental condition. The largest cell size was found in *C. juncea* (20.47±0.49 μm^2 in meristematic cell and 106.47±0.63μm^2 in differentiated cell) and the smallest in *C. saltiana* (14.43±0.39 μm^2 in meristematic cell and 45.86±0.21 μm^2 in differentiated cell). It was also observed that, the mean value of cell size was larger in differentiated cell compared to meristematic cell in all the studied species of *Crotalaria*. Chromocenter numbers were found highest (13.66±0.14**)** in *C. saltiana* and lowest (9.50±0.32) in *C. juncea* which are considered as more primitive and more advance in nature, respectively. Percentages of heterochromatin per nuclear area were found highest (14.75±0.71**)** in *C. saltiana* and lowest (9.67±0.71) in *C. juncea* which are considered as more primitive and more advance in nature, respectively.

Key words: Cell size, *Crotalaria*, chromocenter, heterochromatin, mitotic index, nuclear phenotype.

Introduction

Crotalaria L. is commonly known as rattlepods (Everist 1979*).* The genus includes over 700 species of herbaceous plants and shrubs (Muli et al. 2022). Several species of *Crotalaria* are cultivated as crops to be consumed by human populations throughout the world. To ensure the survival and optimal cultivation of these plants, they are often selected for yield and nutritional quality as well as resistance to diseases. Some species of *Crotalaria* are grown as ornamentals. In plantations, the genus *Crotalaria* is utilized as green manure and cover plants. They're also fed to livestock as a source of nutrition. Many of the species are known to be nodulated by soil Rhizobia and are also capable of fixing nitrogen from the atmosphere. Soil fertility management is crucial to achieving sustainable crop production and cover crops play a key role in soil fertility by reduction in synthetic nutrients applied (Yaradua et al. 2018). Several species of *Crotalaria* are currently being cultivated for suitable traits that are not directly related to human consumption. *Crotalaria juncea*, also known as sunn hemp, is currently grown throughout the tropics and subtropics as a source of green manure, lightened fiber, and fodder. *Crotalaria juncea* is also being considered as a potential source of cellulosic ethanol for biofuel (Morris et al. 2013).

The chromosome number and cytology of many *Crotalaria* species are already recorded. Most of the species are diploid (2n = 2x = 16), some are polyploid, and with predominance of tetraploids (2n = 4x = 32), and a few are diploid (2n = 2x = 14) (Mondin et al. 2007). Lafontaine and Luck (1980) suggested that plant cell nuclei are two types of structural organizations, this structure are reticulate and chromocentric. Studies on interphase nucleus have indicated several interesting features like nonrandom arrangement of chromosome, somatic association, and orientation of chromosomes maintaining their telophasic configurations (Patankar and Ranjekar 1984). Chromocentric nuclear organization was observed in *Phaseolus* species by Patankar and Ranjekar (1984) and in *Cicer* species by Kabir and Singh (1989). The present study deals the determination of mitotic index, interphase nuclear structure; cell size and percentage of heterochromatin (%) in five species of *Crotolaria* L. The major objectives of the study are- i) to observe the mitotic index, nuclear volume and interphase chromosome volume of five *Crotalaria* species and ii) to observe the chromocenter number and heterochromatin estimation of five *Crotalaria* species.

Materials and Methods

Five species of *Crotalaria* L. *viz*. *C. pallida, C. saltiana, C. verrucosa, C. spectabilis* and *C. juncea* were investigated during this study. Seeds of these germplasm were placed on petridis with wet tissue paper to germinate and it took about 2-3 days for germination. Root tips about 1.5-2 cm long were collected and then fixed in 1:3 aceto-alcohol for 48 hours prior to store in 70% ethanol in refrigerator for further use.

For all parameters temporary slide were prepared by heamatoxylin method following the Bstergren and Heneen (1962). Nuclear volume of meristematic and differentiated cell was observed from temporary slides by oculometer. The mitotic index, nuclear volume and interphase chromosome volume, were calculated as described by Fiskesjo (1993) and Nayar et al. (1971), respectively. Numerous techniques were followed to observe chromocenter number and heterochromatin percentage estimation. According to planimetry method, 10 nuclei of each type were examined under microscope along with their chromocenters prediction from the prepared slide. Nagl and Fusenig (1979) stated that chromocenters assemble to heterochromatin.

Analysis of data

Mitotic index $(\mathrm{MI}) = \dfrac{\text{No. of dividing cells}}{\text{No. of total cells}} \times 100$

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Nuclear Volume (NV) = $\frac{4}{3}\left[\pi r^3\right]$

Interphase nuclear volumes (ICV) = $\frac{\text{NV}}{\text{2n no. of chromosome in somatic cell}}$

Cell size = cell length× cell breadth

In terms of $\overline{X} \pm$ S. E.

Where,

SE = Standard Error,

$\overline{X}$ = Mean, $\overline{X}=(\frac{\sum fixi}{N})$

Fi = Individual observation of frequency

xi = Individuals observation of mid values

N = Total no of observation.

S^2 = Mean variance, $S^2 = (\frac{\sum(\overline{x}-x_i)^2}{N})$

Standard Deviation, S.D. = $\sqrt{s^2}$,

Standard Error, S.E. = $(\frac{SD}{\sqrt{N}})$

Results

Mitotic index

The mitotic index (MI), total number of cell and number of dividing cells were counted under microscope and photomicrographs were taken from five species of *Crotalaria* L. MI was found highest (10.38±1.08%) in *C. spectabilis* and lowest in *C. juncea* (9.43±0.304%) in the root tip cells of *Crotalaria* which shown in (Table 1 and Fig. 1). MI was found 10.33±0.677%, 10.11±0.650% and 9.67±1.193% in *C. pallida*, *C. verrucosa* and *C. saltiana*, respectively. High mitotic index value indicates that the division rate is high and low mitotic index value indicates relatively lower divisional rate.

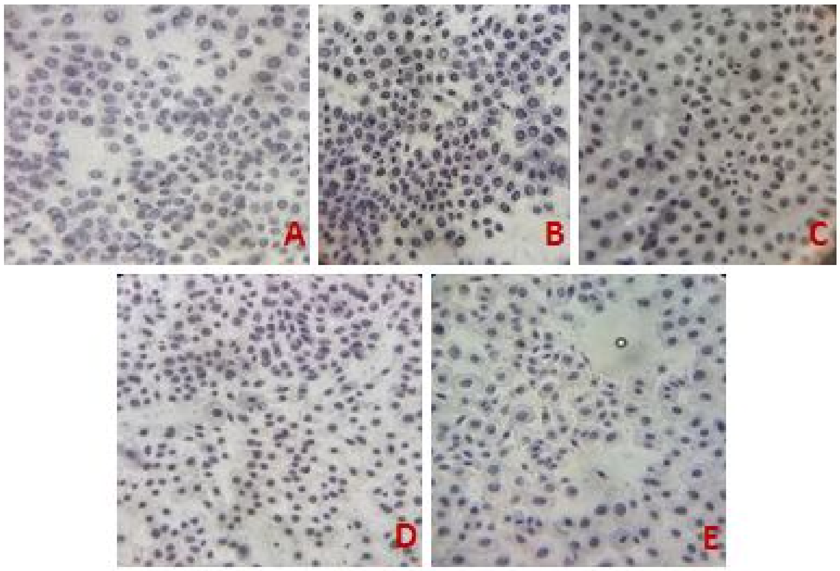

Fig. 1 (A-E): Observation of mitotic index in meristematic cells of five species of *Crotalaria* L. (A: *C. pallida*, B: *C. saltiana*, C: *C. verrucosa*, D: *C. spectabilis*, E: *C. juncea*).

Table 1. Mean values of mitotic index, nuclear volume and interphase chromosome volume of five species of *Crotalaria* L.

Species	Mitotic index (%) $(\bar{X} \pm S.E.)$	Nuclear volume (μ^3) $(\bar{X} \pm S.E.)$		Interphase chromosome volume (μ^3) $(\bar{X} \pm S.E.)$	
		Meristematic cell	Differentiated cell	Meristematic cell	Differentiated cell
C. pallida	10.33±0.677	5.69±0.46	4.13±0.28	0.253±0.29	0.392±0.37
C. saltiana	9.43±0.304	9.54±0.30	6.54±0.27	0.596±0.27	0.408±0.21
C. verrucosa	10.11±0.650	3.28±0.33	2.56±0.22	0.205±0.31	0.16±0.25
C. spectabilis	10.38±1.08	7.91±0.27	5.07±0.35	0.494±0.23	0.316±0.25
C. juncea	9.67±1.193	4.06±0.17	2.81±0.23	0.355±0.34	0.258±0.23

Interphase nuclear structure

Data were recorded for nuclear volume from interphase stage of mitotic cell division in root tips. In meristematic cells, the highest mean value of NV 9.54±0.30 μ^3 in *C. juncea* and 3.28±0.33 μ^3 in *C. saltiana* was examined and the ICV was found highest 0.596±0.27 μ^3 and lowest 0.205±0.31 μ^3 in *C. juncea* and *C. saltiana*, respectively. In differentiated cells, the highest mean value of NV was found 6.54±0.27 μ^3in *C. juncea* and 2.56±0.22 μ^3 in *C. saltiana* and the ICV was found highest 0.408±0.21 μ^3 and lowest 0.16±0.25 μ^3 in *C. juncea* and *C. saltiana* was examined, respectively. In this investigation the highest value of nuclear volume and interphases chromosome volume was found in *C. juncea* and lowest mean value of interphase chromosome volume was found in *C. saltiana* (Table 1).

Meristematic cell size (length × breadth)

Among the five examined species of *Crotalaria* L. the highest (9.72±0.45 μm) and lowest (4.44±0.82 μm) meristematic cell length were found in *C. pallida* and *C. saltiana* respectively; the breadth of meristematic cells was found highest (3.90±0.64 μm) and lowest (2.01±0.52 μm) in *C. juncea* and *C. pallida* respectively; and the highest (20.47±0.49 μm^2) and lowest (14.43±0.39 μm^2) cell size was found *C. juncea* and *C. saltiana*, respectively (Table 2).

Table 2. Meristematic cells length, breadth and cell size in five species of *Crotalaria* L.

Species	Cell length (μm) ($\bar{X} \pm S.E.$)	Cell breadth (μm) ($\bar{X} \pm S.E.$)	Cell size (length × breadth) (μm^2) ($\bar{X} \pm S.E.$)
C. pallida	9.72±0.45	2.01±0.52	19.54±0.65
C. saltiana	4.44±0.82	3.25±0.73	14.43±0.39
C. verrucosa	4.55±0.62	3.40±0.86	15.47±0.47
C. spectabilis	7.11±0.59	2.53±0.69	17.99±0.84
C. juncea	5.25±0.52	3.90±0.64	20.47±0.49

Differentiated cell size (length × breadth)

Crotalaria L. differentiated cell lengths were found highest (33.44±0.64 μm) and lowest (11.7±0.31 μm) in *C. pallida* and *C. verrucosa*, respectively; and differentiated cell breadth was found highest (4.20±0.30 μm) and lowest (2.10±0.41 μm) in *C. pallida* and *C. saltiana,* respectively (Table 3) among the examined species.

Table 3. Differentiated cells length, breadth and cell size in five species of *Crotalaria* L.

Species	Cell length (µm) ($\bar{X} \pm S.E.$)	Cell breadth (µm) ($\bar{X} \pm S.E.$)	Cell size (length × breadth) (μm^2) ($\bar{X} \pm S.E.$)
C. pallida	33.44±0.64	2.80±0.32	93.63±0.62
C. saltiana	21.84±0.36	2.10±0.41	45.86±0.21
C. verrucosa	11.7±0.31	4.20±0.30	49.14±0.59
C. spectabilis	24.3±0.29	3.25±0.28	78.98±0.33
C. juncea	27.3±0.61	3.90±0.21	106.47±0.63

Among the five examined species of *Crotalaria* L. the cell size were found highest (106.47±0.63 μm^2) and lowest (45.86±0.21 μm^2) in *C. juncea* and *C. saltiana*, respectively (Table 3).

Chromocenter numbers and heterochromatin

Chromosome number in each of the five studied species of *Crotalaria* L. were reported to be 2n=16 which was confirmed in the present investigation. In the present study all species of *Crotalaria* shown a lower number of chromocenters compared to their respective diploid chromosome number. The root tip cells of five species exhibited different number of chromocenter in meristematic cells were found in the range from 9.50±0.32 to 13.66±0.14.

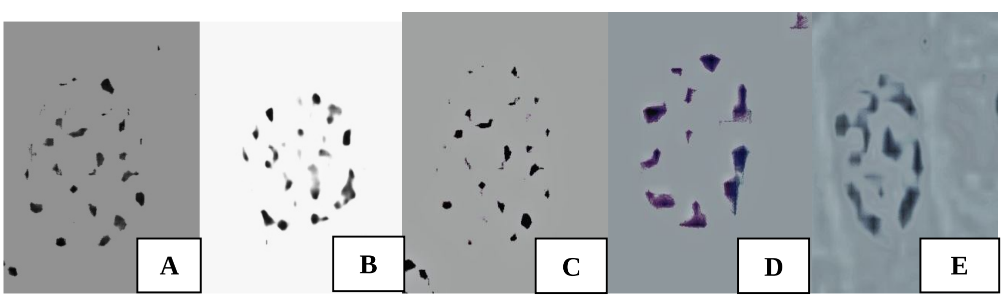

Fig. 4 (A-E). Photomicrograph of interphase nuclear structure and heterochromatin in meristematic cells of five species of *Crotalaria* L. (A: *C. pallida*, B: *C. saltiana*, C: *C. verrucosa*, D: *C. spectabilis*, E: *C. juncea*).

Table 4. Number of chromocenters and heterochromatin percentage per nuclear area in five species of *Crotalaria* L.

Species	Number of chromocenter ($\bar{X} \pm S.E.$)	Heterochromatin per nuclear area (%) ($\bar{X} \pm S.E.$)
C. pallida	12.50±0.98	12.75±0.29
C. saltiana	13.66±0.14	14.75±0.71
C. verrucosa	10.75±0.29	12.75±0.83
C. spectabilis	11.66±0.04	12.80±0.61
C. juncea	9.50±0.32	9.67±0.71

Chromocenter in meristematic cells were found in the range from 9.50±0.32 to 13.66±0.14. It was observed that the chromocenter number in root tip of five studied species of *Crotalaria* were less than their total chromosome number 2n = 16. As chromocenter corresponds to heterochromatin, the value for nuclear area and chromocenter area were used to determine the heterochromatin percentage. It was observed that in meristematic cell the highest (13.66±0.14) and lowest (9.50±0.32 %) heterochromatin content per nuclear area were found in *C. saltiana* and *C. juncea* (Table 4 and Fig. 4).

Discussion

Mitotic index refers to the ratio of number of cells in the dividing phase to the total number of cells observed. The mitotic index characterized by Fiskesjo (1993) demonstrates the cells dividing ability. In this study, mitotic index were found 10.33±0.677% in *C. pallida*, 9.67±1.193% in *C. saltiana*, 10.11±0.650% in *C. verrucosa*, 10.38±1.08% in *C. spectabilis* and 9.43±0.304% in *C. juncea*. Mitotic index was different in all examined species of *Crotolaria* L. Mitotic index was found highest 10.38±1.08% in *C. spectabilis*. The lowest 9.43±.304% mitotic index was found in the root tip cells of *C. juncea*. Mitotic index provides a measure of the capacity of cells to divide and of the rate of cell division. A high mitotic index value shows the higher growth patterns of plants than others. The variation of those values could also vary on root collection time, environmental factors or their cellular activity.

In meristematic cell, the highest mean value of NV 9.54±0.30 μ^3 in *C. juncea* and 3.28±0.33 μ^3 in *C. saltiana* was examined and the ICV was found highest 0.596±0.27 μ^3 and lowest 0.205±0.31 μ^3 in *C. juncea* and *C. saltiana* respectively. In this investigation the highest value of NV and ICV was found in *C. juncea* and lowest mean value of NV and ICV was found in *C. saltiana*. The research findings suggest that, the nuclear volume and interphase chromosome volume do not correlated with the somatic chromosome number but may be related with environmental condition. More or less same result was examined by Yamakawa and Sparrow (1965, 1966). It is believed that the nuclear size increases during the interphase stage. Nuclear volume and interphase chromosome volume is a useful parameter to understand chromosomal morphology. Two types of structural organizations in plant nuclei, namely chromocentric and reticulate have been suggested by Lafontaine (1974). The nuclear organizations were observed chromocentric structure in all examined species of *Crotalaria*. Interphase nuclear phenotype and chromosomal characterization are very useful cytogenetical parameters for distinguishing cytotypes, accessions and even other taxonomic status of germplasm of a plant species (Huang et al. 2014).

The largest cell size (20.47±0.49 μm^2 in meristematic cell and 106.47±0.63 μm^2 in differentiated cell) was found in *C. juncea* and the lowest (14.43±0.39 μm^2 in meristematic cell and 45.86±0.21 μm^2 in differentiated cell) was found in *C. saltiana*. It was also observed that, in each five examined species of *Crotalaria* L. the mean value of cell size was larger in differentiated cell compared to meristematic cell. Cell size is another characteristic that were observed and measured in five species of *Crotalaria*. Meristematic cell capable to divide and provides new cells for expansion and differentiation of tissues and the initiation of new organs, providing the basic structures of the plant body. These cells continue to divide until a time when they get differentiated and then lose the ability to divide. Differentiated plant cells generally cannot divide or produce of new cells. Usually, the cell changes to a more specialized type. Differentiation happens multiple times during the development of a multicellular organism as it changes from a simple zygote to a complex system of tissues and cell types.

In heterochromatin percentage, it was observed that the chromocenter number in root tip of five species of *Crotalaria* L. were less than their total chromosome number 2n = 16. The reduction in the number of centromeres in all the species might be due to overlapping of chromocenters indicating the somatic association of chromosomes. However, the number of chromocenters is considered to be controlled genetically and is therefore a species-specific character (Dayal 1975, Dayal and Prasad 1983). Heterochromatin percentage is one of the key indicators to determine the advance and primitive nature of plants (Lavania and Sharma, 1983, Stebbins, 1950). Lowest heterochromatin percentages (9.67±0.71%) were found in *C. juncea* may be considered as advance in nature and highest (14.75±0.71%) in *C. saltiana* may be consider as primitive.

Conclusion

The present study deals with determination of mitotic index, interphase nuclear structure; cell size and percentage of heterochromatin in five species of *Crotolaria.* The main objectives of this study was to determine the mitotic index, nuclear volume and interphase chromosome volume and the chromocenter number and heterochromatin estimation of five *Crotalaria* species. The interphase chromosome volume was found to be the highest in *C. juncea* and lowest in *C. saltiana*. The largest cell size was observed in *C. juncea* and the smallest in *C. saltiana*. It was also observed that, the mean value of cell size was larger in differentiated cell compared to meristematic cell in all the studied species of *Crotalaria*. Chromocenter numbers were found highest in *C. saltiana* and lowest in *C. juncea* which are considered as more primitive and more advance in nature, respectively. Percentages of heterochromatin per nuclear area were found highest in *C. saltiana* and lowest in *C. juncea* which are considered as more primitive and more advance in nature, respectively.

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Contribution: Authors contributed equally in the research and writing of this article.

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