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Cytological study on pollen grain mitosis of *Setcreasea purpurea* Boom

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Abstract

Setcreasea purpurea Boom (Syn. *Tradescantia pallida* Rose D.R. Hunt Cav) is widely distributed as an ornamental plant. It is a member of Commelinaceae family which is identified and poorly understood taxa. The objective of this present investigation is to study the cytology of pollen grain mitosic of *Setcreasea purpurea* Boom. The study based on the nuclear phenotype, characterization of karyotypes using total chromosome length (TCL), Total frequency (TF) in pollen grai*n of setcreasea purpurea* Boom which will help to understood the taxa. In order to study nuclear phenotype, nuclear structure, nuclear volume, mitotic index were estimated. Nuclear phenotype and karyotype analysis was made from the pollen grain of *setcreasea puurpur*ea Boom. To estimate the chromosome number and karyotype analysis anther of *setcreasea purpurea* were treated with acetocarmin. Cytological studies of pollen grain mitosis have shown that the expected haploid chromosome number was n=12. In this present investigation the karyotype formula was found to be $2L^m+6L^m + 4L^{st}$. Present investigation showed that the karyotype was bimodal and assymetrical with reticulate chromosome. Presence of 4 sub-terminal chromosomses shows it advanceness and process of undergoing structural evolution.

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Introduction

Setcreasea purpurea Boom is a member of the tribe Tradescantieae of the family Commelinaceae. Originally it was named *Setcreasea pallida* by Joseph Nelson Rose, in 1911. This plant was grown as an ornamental for its striking purple foliage. This family is diverse in both the Old World tropics and the New World tropics with some genera. Well known genera including *Commelina* (dayflowers) and *Tradescantia* (Spiderwort).

The variation in morphology especially that of the flower and inflorescence is considered to be exceptionally high amongst the angiosperm comprising approximately 90 species confined of the Neotropics and inhabiting Mexico and Southern USA as its diversity center (Hunt 1975, 1980, 1986b; Faden 1998; e Monocot 2010; The plant list, 2013). This plant is native to a wide area of Mexico from Tamaulipas east toVeracruz and south of the Yucatan peninsula. The members of Tradescantieae and predominantly Mexican origin and based on x=6 fairly symmetrical Chromosomes (Owens, 1981), Xerophytes of the genus *Tradescantia* showing their greatest morphological diversity are located along the Gulf of Mexico. It is now widely grown as an ornamental and houseplant in many tropical and subtropical regions including North, Central and South America, the West Indies, South Africa, the Canay Islands, Maderia and Mayanmar (Govaerts, 2012; USDA-ARS, 2012). It is naturalized in the West Indies, Canary Islands, Southeastern USA, Argentina, Nicaragua and Honduras (USDA-ARS, 2012).

In Puerto Rico and the Virgin Islands fruits and seeds are unknown and plant always propagated by cuttings (Acevedo-Rodriguez and Strong, 2005). This species should be planted in fertile, sandy soil with good drainage. Optical growth occurs in moist, well-drained soil, but it is drought tolerant. Leaf color is most vivid when grown under full sun, but light shade is also acceptablehttps://florafaunaweb.nparks.gov.sg/specialpages/plantdetail, *Tradescantia pallida)*. Pollen grains are male micro gametophytes of seed plants; produce male gametes have a hard coat made of spore pollen in that protects the gametophytes during the process of their movement from the stamens to the pistil of flowering plants. In plant pollen grain transfers the haploid male genetic material from anther to stigma, both between flowers (cross-pollination) and within the same flower (self-pollination). Pollen grain mitosis has been studied in a large number of angiosperms and also in some gymnosperms (Muntzing, 1946; Mehra, 1946; Newcomer, 1954; Abraham and Mathews, 1962; Bhaduri and Majumder, 1965; Khoshoo, 1965).

The karyotypes were distinguished based on their position of centromere in each chromosome median and sub-median region. Therefore, the chromosome number of this plant is 2n = 12 (Bose 1958) and 2n = 24 (Mehra *et al.* 1961), Mitsukuri and Kobayashi 1962). This study is to analysis the karyotypic chromosomes in the gametic cells (pollen grain) of *Setcreasea purpurea* Boom.

Materials and methods

Experimental materials

The experiment was conducted at Professor Sultanul Alam Cytogenetics Laboratory, Department of Botany, University of Rajshahi, Rajshahi-6205, Bangladesh.

The selected experimental model was *Setcreasea purpurea* Boom flower. The plant of these species was collected from 3rd science building, Department of Botany, University of Rajshahi, Rajshahi-6205.

Nuclear phenotype

In order to study the nuclear phenotype (Mitotic Index and Nuclear Volume) at first collect inflorescence from *Setcreasea purpurea* Boom and immediately washed by distilled water. Then young anther was placed on a clean slide.

Mitotic Index (MI)

Mitotic Index values were expressed in percentage were calculated by this following formula:

$$MI = \frac{No.of\ dividing\ cells}{Total\ number\ of\ cells} \times 100.$$

Nuclear volume (NV)

The Nuclear Volume (NV) was measured by oculo-meter and the values were calculated by using the formula for a where, $NV = \frac{4}{3}\pi r^3$.

Karyotype analysis

For the karyotype analysis at first, we collected the inflorescence. Then staining the mature anthers and prepare the desire slide to perceive the photomicrography for chromosome to be study. There after measurement the length of chromosome, arm ratio, centromere position and the length of chromatin.

Collection of inflorescence

During the proper growth of the plant and at the stage of flowering, young inflorescence were collected from *Setcreasea purpurea* Boom in different time like (7.00 am, 8.00 am, 9.00 am, 10.00 am, 12.00 pm, 2.00 pm, 6.00 pm, 7.00 pm).

Staining of mature anthers and preparation of temporary slides

For study pollen grain mitosis temporary slides were prepared by 2% aceto-carmine smear technique according to the schedule mentioned as follows. Young anther was placed on to a clean slide and a drop of 2% acetocarmine was added.

The anther wall was then crushed by a curved dissecting needle and the anther wall was removed. Then the pollen grains were covered with a cover glass. Warmed gently over an alcohol frame and a light pressure was extracted by thumb on fingertip. Additional heating was applied as needed until the cytoplasm become clear. Then observe it under the microscope and Photomicrographs of well spread metaphase chromosome of *Setcreasea purpurea* Boom were taken by camera photomicroscope.

Measurement of chromosome length

Chromosomes were measured from photomicrographs of metaphase plates with the help of a divider and a millimeter scale. The values for chromosome size (mm) were converted into mili-microm (µm) with the help of stage micrometer.

Arm ratio

For making the analysis chromosome were classified according to the position of the centromeres. Arm ratios were calculated by dividing the length of short arm by that of the long arm.

Centromeric position

Centromeric position of chromosomes was determined as followed by Kutarekar and Wanjari (1983).

Type	Arm ratio
Sub-terminal (st)	less than 0.50
Sub-median (sm)	between 0.51 and 0.75
median (m)	above 0.75

Chromatin length

Ideograms of chromosome pairs were prepared side by side according to their length (from longer to shorter) keeping the short arm in each case pointing upwards and the centromere at the same plant.

The total frequency (TF %) was calculated by using this formula of Huziwara, (1962).

$$TF\% = \frac{\text{Total sum of short arm}}{\text{Total sum of chromosome length}} \times 100.$$

Results

Determination of nuclear volume (NV)

In the present investigation nuclear volume and mitotic index were determined from the pollen grain of *Setcreasea purpurea* Boom flower were shown in Fig. 1 (a) and (b). From this study represents nuclear volume 39.35µ^3. Frequency of Nuclear Volume (NV) withstandard error, in pollen grain cells of *Setcreasea purpurea* Boom was shown in table-2.

Table 1. Mitotic Index and Nuclear volume of *Setcreasea purpurea* Boom.

Name of the species	Number of chromosome (2n)	Gametic Chromosome number	Mitotic Index (MI) %	NuclearVolume (NV) µ^3
Setcreasea purpurea Boom	24	12	19.5	39.35±0.11

Mitotic index

MI is an important parameter to identity the region of most mitotic activities. It helps to quantify the cell division. Mitotic observation from the pollen grain of treated anther show different mitotic activity. If the mitotic index is high then it can be said that thedivision rate will be high. The observed MI from this experiment was shown in fig.1 (b). The calculated mean values of MI was 19.05%. Frequency of mitotic Index with standard error, in pollen grain cells of *Setcreasea purpurea* Boom was shown in table-2.

Table 2. Individual chromosome length, arm ratio, centromeric position and chromosome type of *Setcreasea purpurea* Boom.

Name of the species	Somatic chromosome number (2n)	Gametic chromosome number (n)	Individual chromosome	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Setcreasea purpurea Boom	24	12	Long arm (□m)□SE	4.87 □0.15	5.31 □0.60	4.38 □0.47	4.44 □0.38	4.55 □0.25	4.55 □0.31	4.33 □0.35	4.76 □0.53	3.9 □0.31	4.12 □0.46	3.25 □0.46	3.25 □0.30
			Short arm (□m)□SE	4.01 □0.39	3.03 □0.35	3.36 □0.29	3.14 □0.089	2.925 □0.32	2.6 □0.29	2.49 □0.08	1.95 □0.31	2.38 □0.18	1.62 □0.15	1.46 □0.23	1.46 □0.13
			Total length (□m)□SE	8.88 □0.54	8.33 □0.95	7.74 □0.76	7.58 □0.47	7.58 □0.57	7.15 □0.60	6.82 □0.41	6.72 □0.84	6.28 □0.47	5.74 □0.58	4.71 □0.69	4.71 □0.43
			Arm ratio (SA/LA) □SE	0.82 □0.05	0.57 □0.017	0.77 □0.03	0.71 □0.04	0.64 □0.02	0.57 □0.02	0.58 □0.04	0.41 □0.02	0.61 □0.02	0.39 □0.04	0.45 □0.01	0.45 □0.02
			Centromeric position	m	sm	m	sm	sm	ms	sm	st	sm	st	st	st
			Type	L	L	L	L	L	L	L	L	L	L	L	L

Karyotypic formula = $2L^{m} + 6L^{sm}+4L^{st}$.

Karyotype analysis

Karyotype analysis was made from the pollen grain of *Setcreasea purpurea* Boom and the results are presented in Table-3. gametic chromosome in this species was found to be n=12. Fig 3(A-C) illustrates the gametic metaphase and karyotypes of the studied plant. Table-3 gives a comparison of the mean lengths and types of chromosomes. The pair ordering was based on the total length of the chromosomes.

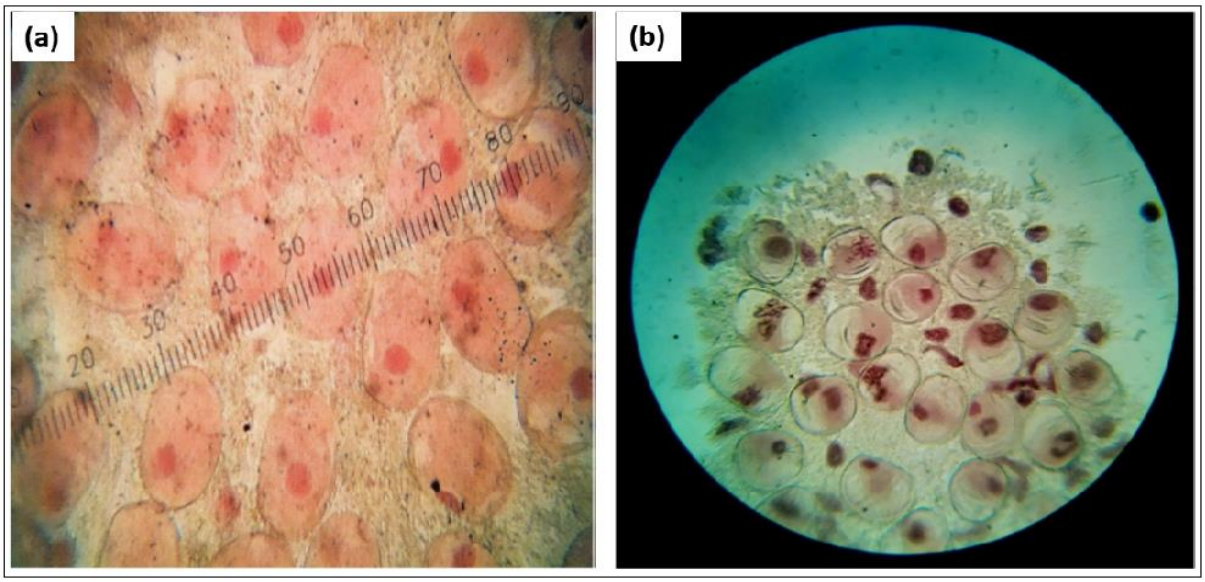

Fig. 1. The Schematic diagram represents (a)-Nuclear Volume (NV) and (b)-Mitotic Index.

There were two (I and III) metacentric, 6 sub-metacentric (II, IV, V, VI, VII, and IX) and four sub geocentric (VIII, X, XI and XII chromosomes. The largest chromosome was 8.88□0.54 □m and the shortest chromosome was 4.71□0.43□m with a TCL of 82. 145□□7.31□m. TF% was found to be 37.07%. The karyotype formula of this species was found to be $2L^{m} + 6L^{sm}+4L^{st}$.

Discussion

In order to study the nuclear phenotype; nuclear structure, nuclear volume, mitotic index (MI) were estimated. To estimate the chromosome number, the pollen grains were stained with aceto-carmine. Lafontaina (1974) and Nagl and Fusening (1979) stated that the structural organization in plant cell nuclei are two types, chromocentric and reticulate. In the present study nuclei of pollen grain of *Setcreasea purpurea* were found to be reticulate. Nuclear volume is an important parameter in this study.

In this studynuclear volume were found to be 39.35±0.11μ^3. However, Nuclear Volume (NV) and Interphone Chromosome Value (ICV) were found to be dependent proportionally on the number and size of chromosomes in cells. According to Lafontaina (1974) and Nagl and Fusening (1979) chromocentric nuclear organization was assumed to be governed by small size of chromosmes and low DNA content.

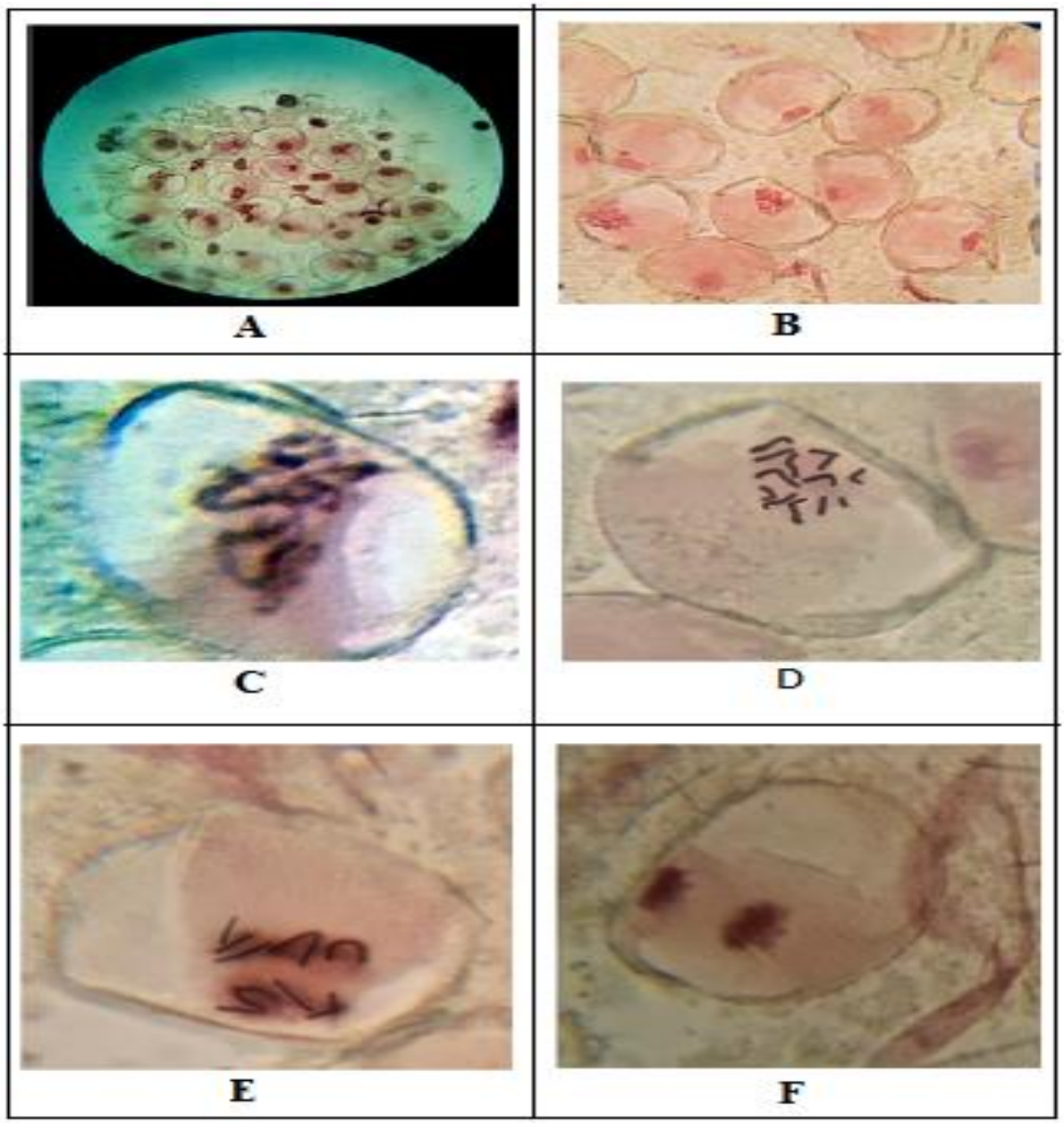

Fig. 2. (A-F). Different stages of pollen grain mitosis found in *Setcreasea purpurea* Boom. A. A single focus under microscope showing different stages of pollen grain mitosis, B. Poor stained nuclear chromosome, C. Prophase, D. Metaphase, E. Anaphase, F. Telophase.

This statement holds true chromocentric structure but in this investigation the number and size of chromosomes were fund to be longer with reticulate structure. Mitotic Index (MI) is an important parameter. Mitotic index is defined as the ratio of number of cells the dividing phase and the total number of cells. Total number of cells and number of dividing cells were counted under microscope. Mitotic index in this investigation were found to be19.03%. Study of karyotype is an important field of investigation for understanding generic or specific interrelationship and evolutionary trends (Anderson and Sax, 1936; Faruqi *et al.* (1962, 1967); Bhatt and Dasgupta, 1976; Gupta, 1978; Faden, 1980).

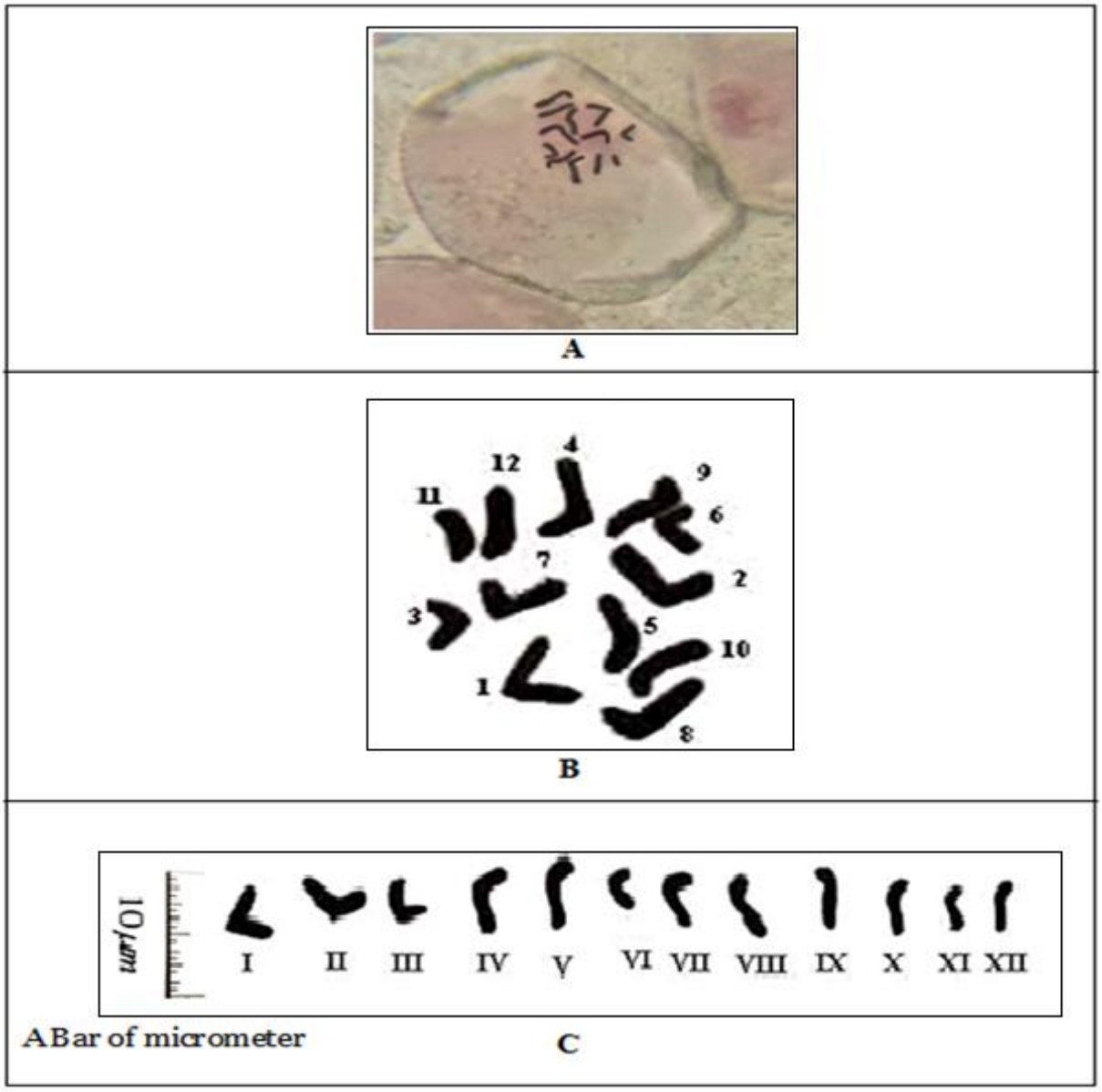

Fig. 3. (A-C). Metaphase chromosomes obtained from pollen grain mitosis and determination of its karyotypic analysis, A. Metaphase chromosome, B. Camera Lucida drawing, C. Ideogram.

In order to carry out the karyotypic analysis of anther of *Setcreasea purpurea* were treated with aceto-carmin. Among the gymnosperms, Mehra (1946) was the first to make karytypic analysis based on pollen mitosis in *Ephedra*. Newcomer (1954) was able to identify two types of pollen in *Ginkgo*. The two types of karyotypes were aptly correlated by him with the dioecism in *Ginkgo*. Similarly, Abraham and Mathews (1962) discovered the same phenomenon of karyotype form pollen mitosis in wheat species and variety.

The objective of this present investigation was karyotype analysis based on pollen grain mitosis in *Setcreasea purpurea* Boom.

The gametic chromosome number determined in this study for the *Setcreasea purpurea* Boom equals n=12; so that 2n=24. This result is in agreement with the data reported by Mehra *et al* (1961), Mitsukuri and Kobayashi (1962), Guervin and Le Coq (1966). It differs from that given by Bose (1958) which was2n =12. In the present study the chromosomes in general were graded being mostly large in size and constrictions were either sub-terminal (St), sub-median(sm) or median (m) in position. While Guervin and Le Coq (1966) reporting 2n=24 and Bose (1958) giving 2n=12, both found median and sub-median chromosomes in the studied karyotype.

The chromosome length recorded in this study for *Setcreasea purpurea* Boom were found to be 4.71-8.8µm which is similar to the mean values given by Guervin and Le Coq (1966) for this species (around-8µ). The karyotype of *Setcreasea purpurea* Boom characterized by the presence of chromosomes that had considerably different length and variously positioned centromere such as sub-terminal (st), sub-median (sm) and median (m) position. That can be described as asymmetric karyotypes. If the chromosomes having centromere only in the median position, can be described assymmetric chromosome.

The occurrence of symmetric karyotypes in higher plants is a primitive trait (White 1966). When the karyotype assymmetry is taken into consideration the asymmetrical karyotypes are supposed to be more advanced than the symmetrical ones (Stebbins 1950). As the karyotype of *Setcreasea purpurea* in this present investigation were found to be asymmetric so this species may be regarded as advanced.

The karyotype formula was fund to be 2m+6sm+4st and due to the presence of 4 sub-terminal chromosomes in its gametic cell this might be showing its advanceness and process of undergoing structural evolution.

Conclusion

The present investigation was carried out of study the nuclear phenotype and chromosome morphology (karyotype) of pollen grain mitosis in *Setcreasea purpurea* Boom. Inflorescence were collected in different times as experimental material and mitotic index, nuclear volume were studied from pollen grain.

In this investigation nuclear volume was found to be 39.35±0.11µ^3 and mitotic index was found to be 19.05%. In this investigation the karyotype was bimodal and asymmetrical with reticulate chromosome.

The length of long arm ranged from 3.250.30µm to 5.31±0.60µm and the length of the short arm ranged from 1.46±0.13µm to 4.01±0.9µm. Total length of chromosome ranges from 4.71±0.43µm to 8.88±0.54µm with a TCL of 82.145±7.31µm and the Total Forma percentage (TF%) was 37.04%. The karyotype formula of this investigation was 2Lm + 6Lsm+4Lst consisting two median, six sub-medians and four sub terminal chromosomes.

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