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Research Article

CHROMOSOME ARCHITECTURE OF *CRINUM DEFIXUM* KER-GAWL.

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ABSTRACT
Number of Chromosomes in the root meristem cells of *Crinm defixum*, Ker-Gawl is 2*n* =22. Atypically, different root tip cells show aneusomatic chromosome number ranging between 19 and 36. Karyotype analysis indicated that the extra chromosomes are actually duplicates of some of the chromosomes belonging to the set of 11 and the cause of aneusomaty is apparently endoduplication of chromosomes. C-banding studies showed that the amount of heterochromatin varied from 2 and 4.2 in the chromosomes. Regarding heterochromatin content, it was found that polymorphism is common for number and size of heterochromatic bands. Chromosomes were mostly sub-medium, three of them with satellites.

Key Words: *Crinum Defixum, Karyotype, Chromosome Architecture, Aneusomaty, Aneuploidy, Heterochromatin and C-Bandin*

INTRODUCTION
Crinum defixum Ker Gawl is a common weed of the family Amaryllidaceae with underground large bulbous stem and cauline erect leaves growing mostly in well inundated agriculture fields or pastures. Growth of the linear, lanceolate leaves occurs usually in response to the swelling water during late rainy season and the plant produces elongated flowering scape after rains to produce white attractive flowers. It has been reported that *Crinum* species hybridize freely in nature and produce interspecific hybrids with high degree of chromosomal heterozygosity and sterility (Bailey, 1963). Hybrids are sexually sterile with high degree of genetic differentiation (Stebbins, 1973). Different workers (Sharma and Ghosh, 1954; Khushboo and Raina, 1976; Raina and Khushoo, 1971) reported wide range of variation in chromosome number in *Crinum* species. This report presents architecture of the somatic chromosomes of *Crinum defixum* Ker Gawl in relation to their heterochromatic C-bands.

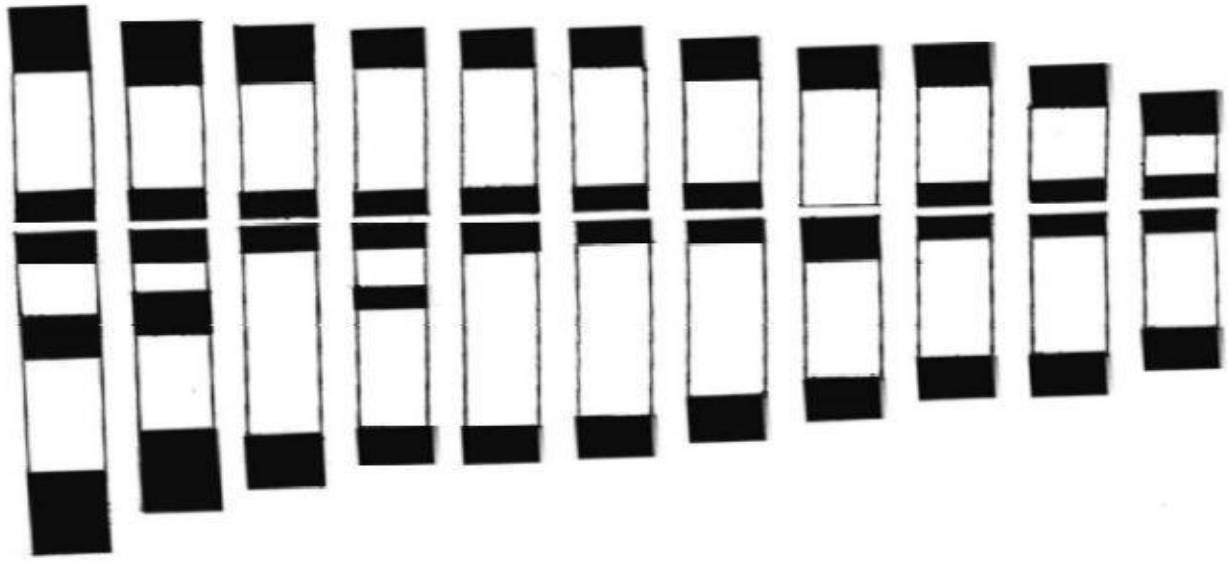
MATERIALS AND METHODS
Mitotic studies were carried out in the meristem cells of the root tip of *Crinum defixum*. Root tips were collected from the bulbs of the living plants growing in the natural habitat or experimental garden between 11:30 am to 12:40 pm in sunny days. Collected root tips were washed to remove soil and root cap and pretreated insaturated aqueous solution of paradichlorobenzene for 2.5 hours at 10-15^{0}C. After the pretreatment, they were fixed in 1:3 aceto-alcohol with a drop of FeCl3. 1.5% acetocarmine was used for staining the mitotic preparations. Slides were made permanent though acetic acid - ethyl alcohol series (1:1, 1:2, 1:3 and absolute alcohol). Photo micrographs were taken fromtemporary and permanent slides using collapsable 100:1 objective lens by Ricoh 10 M auto camera. Giemsa stain was used for C-banding of the mitotic chromosomes. 1.5-2.5 cm long root tips were pretreated with ice water for 20 hours and fixed in Carnoy's I fluid for more than 1 hours. Squash preparations were made in 45% acetic acid using gentle heat, slides placed on dry ice until frozen after cover-slip being removed, followed by their transfer into 99% ethanol for overnight. Next day, the slides were air dried for several minutes, then incubated for 1 minute in 0.2 N HCl in water bath at 60^{0}C, washed briefly in distilled water and incubated again for 7 minutes in saturated $Ba(OH)_2$ at room temperature. Washed again in distilled water, slides were kept in 2 x SSC in a water bath at 60^{0}C for 30 minutes. Slides were directly moved from 2 x SSC into 10% solution of Giemsa stain for 45 minutes to 1 hour. Stained slides werefinally rinsed in distilled water and air dried for final viewing.

RESULTS AND DISCUSSION

Photo-micrograph of a metaphasic plate of the somatic chromosomes of *Crinum defixum* Ker Gawl is shown in Figure 1. Figure 2 presents the karyotype generated from photo-micrographs of the chromosomes. Analysis of a typical karyotype with 2*n* = 22 with location, amount and % of hetrochromatin in the chromosomes is shown in table -1. The most common basic chromosome number in Amaryllidaceae is *x* = 11 (Flory, 1977). The next most frequently encountered basic chromosome number is *x* = 6 found in at least 62 species of the family (Flory, 1977). Meerow and Snizmann (2001) reported basic chromosome number as *x* = 10 for some species of Amaryllids. Apparently, karyotype evolution in the family has taken place along two or three lines. *Crinum* is the genus with a considerabledegree of stability in the karyotype. Similar chromosome morphology is reported to be shown by different species of this genus (Tandon and Mathur, 1966; Meerow, 1995). The present investigation has clearly established *x* = 11 for *Crinum defixum* as all the metaphasic spreads showed 2*n* = 22 or some aneuploid number arising out of the original basic 11 chromosomes. In some extreme cases, almost all the 11 chromosomes were found to have duplicates with 1 or 2 chromosomes having as much as 3 – 4 extra homologues. Endoduplication of the chromosomes was confirmed by the absolute and relative size of the chromosomes and their organization in terms of location, amount (in µ) and % of the heterochromatic C-bands. Aneusomaty is rather common in plants and reported in several taxa (Greilhuber and Weber, 1975). It has been suggested that asymmetrical karyotype is more advanced than symmetrical karyotypes (Stebbins, 1973) and that the changes in symmetry are normally associated with chromatin loss (Bakhshi and Ebrahim, 2000). As regards the karyotype of *Crinum defixum,* there is 1 median chromosome, 1 median chromosome with satellite, 5 sub-median chromosomes, 2 submedian chromosomes with satellites and 2 sub-terminal chromosomes. The chromosomes also show conservative distribution of heterochromatin ranging from 30% to 44% approximately of the total chromatin length (or DNA content) of the chromosomes. This karyotype may be assumed to be rigid with little tendency towards asymmetry. Propagation through emergency food storage in bulb is the survival strategy for the plant which probably might overshadow the strategies for dynamic chromosome evolution.

Figure 1: Metaphase of mitosis in a root tip cell of *Crinum defixum*

Figure 2: Karyotype of *Crinum defixum*



Heterochromatic regions:

Chromosome 1: 4.2 µ (31.1%)	**Chromosome 2:** 3.8 µ (31.9%)	**Chromosome 3:** 3.4 µ (30.0%)
Chromosome 4: 3.4 µ (32.0%)	**Chromosome 5:** 3.3 µ (31.42%)	**Chromosome 6:** 3.2 µ (30.77%)
Chromosome 7: 3.0 µ (30.61%)	**Chromosome 8:** 3.0 µ (33.3%)	**Chromosome 9:** 3.0 µ (34.88%)
Chromosome 10: 2.9 µ (36.25%)	**Chromosome 11:** 2.8 µ (43.75%)	

Figure 3(c): bands in the chromosomes of *Crinum defixum*

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