Cytogenetics of Amaryllidaceae VI. Karyomorphology and meiotic behaviour of *Crinum zeylanicum* L.—a new cytotype

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Crinum is a genus of bulbous ornamentals with a large number of species. Chromosomes of *Crinum* are fairly large and this perhaps stimulated interest in karyotype analysis of a number of species (Sharma and Bhattacharya 1956, Bose 1965, Khoshoo and Raina 1968, Jones and Smith 1967, Raina and Khoshoo 1971, Patwary and Zaman 1975 and Zaman *et al.* 1977a, b). The difficulty one encounters in studying meiosis in *Crinum* species, however, has been long recognized (see the above reports).

Reports on mitotic chromosomes of *C. zeylanicum* L. are conflicting. Sharma and Bhattacharya (1956) found $2n=16$, 20 and 23 as aneuploid numbers, a chromosome formula $2L+8M+6M^s+6s$, centromeric types as $4M+6NM+12SM$ and observed secondary constrictions in three pairs (six chromosomes). Raina and Khoshoo (1971), however, found no aneuploid numbers, obtained a chromosome formula $2L+10M+2M^s+8S$, centromeric types $14L (SM)+2Ln+6J(NAC)$ and secondary constrictions in one pair (two chromosomes). These disagreements and the lack of a report on meiosis of *C. zeylanicum* stimulated our interest in a re-examination of its karyotype and an investigation of its meiotic behaviour.

Materials and methods

The plants (Fig. 1) which have been growing for the past few years in the Dacca University Botany garden, are bulbous herbs with 6-10 leaves. Leaves 0.6 to 1.00 meter long and 7 to 10 cm wide, are gradually pointed towards the apex with slightly wavy margins; scape is of same length as the leaf. Umbels are 10 to 15 flowered; flowers are white, back side of the petals contains a pink stripe at the middle; perianth tube is curved and 7 to 10 cm long. Plants usually flower in May.

The method of studying mitosis in root tip cells (RTCs) was the same as previously reported (see Patwary and Zaman 1975, Zaman *et al.* 1977a, b). For meiosis young buds were collected by cutting the underground bulbs during the first week of April, fixed in 1:3 acetic alcohol for 12 hours and preserved in 70% ethanol. Bud collections were made at half hourly intervals from 8.00 AM to 11.30 AM. Buds

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fixed at 10 AM and 10.30 AM gave a large number of pollen mother cells (PMCs) with division stages. Permanent slides have been retained in our laboratory.

Figs. 1-2. 1, *Crinum zeylanicum* L., F-Fruits. (roughly 1/16th of natural size). 2, mitotic meta-phase showing $2n=22$ chromosomes. 2', same as Fig. 2, but added to show different level of chromosomes under microscope.

Figs. 3-4. 3, karyotype prepared from Fig. 2 showing 11 (I to XI) homologous pairs. Magnification as per scale. 4, idiogram prepared from the average length of chromosomes from 10 RTCs. Magnification as per scale. For convenience in preparing the idiogram, the actual $0.32 \mu\text{m}$ centromere and $0.24 \mu\text{m}$ secondary constriction were taken as 0.50 and $0.30 \mu\text{m}$ respectively.

Table 1. Length of chromosomes (in micron) in RTCs of *Crinum zeylanicum*. Each value represents mean of ten different RTCs

Chromosome pairs										
	I	II	III	IV	V	VI	VII	VIII	IX	XI
T	14.52	10.72	10.32	9.52	9.47	9.42	9.37	7.62	6.77	5.92
	12.12	10.72	10.32	9.52	9.47	9.42	8.72	7.02	6.77	5.92
l	7.50±0.15	7.70±0.12	7.70±0.12	3.10±0.20	6.65±0.15	6.40±0.28	6.70±0.22	4.80±0.26	3.75±0.23	3.10±0.20
	6.30±0.12	7.70±0.12	7.70±0.12	2.46±0.05	6.65±0.15	6.40±0.28	6.10±0.16	4.20±0.12	3.75±0.32	3.10±0.20
				5.80±0.25						2.75±0.23
s	6.70±0.20	2.70±0.15	2.30±0.19	3.40±0.11	2.50±0.21	2.70±0.15	2.30±0.19	2.50±0.13	2.70±0.15	2.50±0.13
	5.50±0.24	2.70±0.15	2.30±0.19	3.40±0.11	2.50±0.21	2.70±0.15	2.30±0.19	2.50±0.13	2.70±0.15	2.50±0.13
l/s	1.12	2.85	3.35	1.71	2.66	2.37	2.91	1.92	1.39	1.24
	1.15						2.30	1.68		1.15
M	NAC			SM			NAC	SM	NM	M
							SM			

T = Total length; l = Long arm; s = Short arm; l/s = Arm ratio.

Photomicrographs were prepared by using a Kodak 35 mm microscopic camera and a black and white OROW 80 ASA film.

Results

Observation on 36 well spread mitotic metaphases showed $2n=22$ chromosomes (Figs. 2 and 2'). The karyotype (Fig. 3) prepared from a typical plate (Fig. 2) showed 11 chromosome pairs (I to XI) with apparent homology in their morphological features excepting pair I and IV where heteromorphism was prominent. An idiogram (Fig. 4) prepared by averaging the measurements (Table 1) from ten different RTCs showed chromosome lengths ranging from 5.47 to 14.52 μm . Based on size (Fig. 4 and Table 1) the chromosomes were grouped into the following categories:

- L (Large): One pair, (pair I) of large chromosomes (12.12 to 14.52 μm);
- M (Medium): Six pairs (pair II to VII) of medium chromosomes (8.72 to 10.72 μm);
- S (Small): Four pairs (pair VIII to XI) of small chromosomes (5.47 to 7.62 μm).

This grouping could be represented as $2L + 12M + 8S$ for the diploid complement. The centromeric types of chromosomes (Fig. 4 and Table 1) determined by l/s ratio (see Zaman *et al.* 1977, b) are:

- M (Metacentric): Three pairs (I, X and XI);
- NM (Near metacentric): One pair (IX);
- SM (Submetacentric): Four pairs (IV to VI and VIII and one chromosome of pair VII);
- NAC (Near acrocentric): Two pairs (II and III) and one chromosome of pair VII.

The overall centromeric formula thus stands as $6M + 2NM + 9SM + 5NAC$. Heteromorphism on the basis of length was found in four pairs (I, VII, VIII and XI) of which in two pairs (I and XI) it is prominent (Figs. 3 and 4). Chromosomes of pair VII are heteromorphic with respect to both centromeric type and length. In both VIIth and VIIIth pair the longer arms of one homologue were always shorter. Secondary constriction was found in the larger arm of a chromosome of pair IV.

Study of 104 PMCs at MI revealed the presence of 11 bivalents (IIs) in each cell (Figs. 5 to 7); 99.56% IIs were ring and the rest were rod-shaped. The percentage of terminal and interstitial chiasmata are 39.76 and 60.24 respectively. The maximum number of chiasmata observed in the largest bivalent was four and the minimum number in this II was three. The other IIs had two to three chiasmata in each except the rod-shaped one (0.44%) where it was only one. The mean chiasma frequency per II ranged from 2.55 to 2.91 with a sample mean of 2.79 ± 0.05 (Table 2). In about 3% MI plates one acentric fragment (shown by f in Fig. 7) forming a ring was found. Most of the anaphase plates showed 11:11 disjunction (Fig. 8) but a few (4%) had a single (Fig. 9) or double (Fig. 10) bridge without fragment. In about 2% of the late AI plates one chromosome in each had not reached the pole. Roughly 5% of the PMCs at TI had one (Fig. 11) or two (Fig. 12) laggards. About 95% of the pollen grains stained blue in a mixture of lactophenol and cotton blue.

Figs. 5-12. 5 to 7, MI showing 11 bivalents. f=acentric fragment forming a ring. Approx. 715, 718 and 875 $\times$ respectively. 8, AI showing 11:11 disjunction. Approx. 1050 $\times$. 9, single bridge at AI. Approx. 610 $\times$. 10, double bridge at AI. Approx. 1274 $\times$. 11, one lagging chromosome at TI. Approx. 786 $\times$. 12, two lagging chromosomes at TI. Approx. 712 $\times$.

Seed formation (Fig. 1, indicated by F) was apparently reasonable.

Discussion

The presence of $2n=22$ chromosomes in RTCs and $n=11$ chromosomes in PMCs of *C. zeylanicum* confirms earlier reports on its diploid number (Sharma and Bhattacharya 1956 and Raina and Khoshoo 1971). In the lack of aneusomaty the present report is in disagreement with Sharma and Bhattacharya (*l.c.*) but agrees with Raina and Khoshoo (*l.c.*). Jones and Smith (1967) studied a good number of *Crinum* species and having found no irregularity like aneusomaty concluded that the irregularities observed by Sharma and Bhattacharya (*l.c.*) may be due to drastic squash technique and perhaps mistaken counts.

It is possible from our study to classify, based on length, the diploid chromosome complement as $2L+11M+1Ms+8S$. This stands in agreement with the haploid karyotype $1L+5M+M^s+4S$ used by Satô (1938), Dolcher (1950) and Jones and Smith (1967) for the genus *Crinum* in general and Raina and Khoshoo (*l.c.*) for *C. zeylanicum* in particular. On the other hand, Sharma and Bhattacharya (*l.c.*) classified *C. zeylanicum* as $2n=2L+8M+6M^s+6S$. The previous reports on centromeric formula in *C. zeylanicum* as $14L(SM)+2Ln$ (SM with secondary

Table 2. Chromosome association, distribution of chiasmata and chiasma frequency at MI in the PMCs of *Crinum zeylanicum* L.

Bivalents/PMC.		Chiasmata/PMC.		Range of mean x-ma freq./II.	No. of PMCs studied
Ring	Rod	Terminal	Interstitial		
10-11	00-1	11-14	16-20	2.55-2.91	104
% 99.56	0.44	39.76	60.24	$\bar{X} \pm S.E. = 2.79 \pm 0.05$	

constriction)+6J (NAC) by Raina and Khoshoo (*l.c.*) and as $4M+6NM+12SM$ by Sharma and Bhattacharya (*l.c.*) contrast ours which is $6M+2NM+9SM+5NAC$. In having only one chromosome with secondary constriction our results differ from those of Sharma and Bhattacharya (*l.c.*) who reported secondary constrictions in six chromosomes (pair I to III) and Raina and Khoshoo (*l.c.*) who noted secondary constriction in both the members of pair VII. In this respect, however, our finding agrees with Jones and Smith (*l.c.*) on several other species of this genus. We observed prominent heteromorphicity in pair I and XI (Figs. 3 and 4, Table 1) while Raina and Khoshoo (*l.c.*) found only pair I heteromorphic. Our l/s ratios differ from those of Raina and Khoshoo (*l.c.*); for a quick comparison we present both sets of values below:

	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
Raina and Khoshoo	1.1	3.7	2.4	3.04	2.3	3.5	1.8	1.2	1.6	2.0	1.5
Present work	1.12	2.85	3.35	2.13	2.60	2.37	2.91	1.92	1.39	1.24	1.14
	1.15			1.71			2.30	1.68			1.15

The present report on meiosis, probably the first for *C. zeylanicum*, shows re-

gular meiosis with 11 IIs at MI (Figs. 5 to 7) with a few anomalies such as bridge without fragment (Figs. 9 and 10), delayed disjunction and laggards (Figs. 11 and 12). The former is probably due to the pairing of incompletely homologous chromosomes (Fig. 3) showing delay in disjunction (Darlington 1938, Murthy and Swaminathan 1956). Presence of laggards and apparent presence of delayed disjunction may be related to the lack of chromosome features which are thought to favour normal disjunction such as metacentric chromosomes with more or less uniform length and terminally localized chiasma (Burnham 1956).

The consistent presence of $2n=22$ chromosomes in the RTCs, regular formation of 11 IIs with a high chiasma frequency/II (Table 2) and reasonable matching of homologous chromosomes in the karyotype indicate that the plant is a disomic ($2n=2x$) with $x=11$ chromosomes. But, as noted above, our material appears to differ in many features from those used in previous reports and hence it may represent a different cytotype. The occurrence of bridges without fragments, high chiasma frequency per bivalent and the absence of interchange complexes, might give a possible explanation of the heteromorphicity observed here; the heteromorphicity may be due to possible deletion or duplication or both rather than to a possible hybrid origin of the plant.

Summary

Consistent presence of $2n=22$ chromosomes in RTCs apparently forming 11 homologous pairs, $n=11$ chromosomes in PMCs and high chiasma frequency indicate that *Crinum zeylanicum* studied here is a basic diploid ($2n=2x$). The somatic chromosomes, 5.47 to 14.52 μ m in length, had a centromeric formula of $6M+2NM+9SM+5NAC$. The heteromorphicity in two pairs may be related to deletion or duplication or both rather than to hybrid origin of the plant. The present results being different in many features from those reported by others on *C. zeylanicum* seem to favour the idea that the plant studied by us may represent a new cytotype.

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