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Research article

A comparative karyomorphological analysis of *Crinum asiaticum* L. and *Crinum latifolium* L. from Paschim Medinipur district of West Bengal, India

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Abstract: *Crinum asiaticum* and *C. latifolium* are two ornamental plant species with medicinal importance. These species have a host of biomolecules of pharmaceutical uses. The chromosomal study is a very basic one in characterizing the genetic material of a species. Earlier reports on such studies have shown both of 22 and 24 to represent the diploid number of chromosomes in the somatic cell of *Crinum* sp. The present study confirmed the 2n number as 22 for both of the species. However, these two species differ in respect of different parameters. Chromosome types are 10 metacentric and 12 submetacentric in *C. asiaticum*, while 10 metacentric, 6 submetacentric and 6 subterminal chromosomes in *C. latifolium*. Considerable variations are also evident in the total chromosomal length of the haploid set, symmetric index, degree of karyotype asymmetry, mean centromeric asymmetry, coefficient of variation of chromosome length, coefficient of variation of the centromeric index as well as the asymmetric index. These variations provide the chromosomal identity of these two species and also the nature of the relationship in them.

Keywords: Chromosome study - Karyomorphology - Ideogram - *Crinum* species.

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INTRODUCTION

The genus *Crinum* L. belongs to the family Amaryllidaceae, one of the largest families of monocotyledons containing 85 genera and 1100 species of the tropical and subtropical region of the world (Willis 1973). All the species of this genus are geophytes; as a result, they are propagated mainly through vegetative mode. Many of the species are approved as horticultural plants for their large and attractive flowers. The disputed systematic position of many genera of this family has twisted the interest to cytological study. The importance of the study of karyotype is being more appreciated in framing a natural and phylogenetic system of classification. Chromosomal morphology of plants in vogue of their taxonomy has been established for a long time back (Babcock *et al.* 1937, Stebbins 1938, Gunderson 1950).

Somatic chromosomes of both the species are of average size and amenable to analysis. Enumeration of chromosome number for some diploid species in the past revealed different chromosome numbers like *2n* = 24 (Tomita 1931, Sugiura 1936) and *2n* = 22 (Sharma & Ghosh 1954, Sharma & Bhattacharya 1956, Bose 1965). Infraspecific variation in chromosome number has been described earlier by Sharma & Bhattacharya (1956). A comparative account of chromosomal features of two species of *Crinum* L. has been presented here.

MATERIALS AND METHODS

The species *Crinum asiaticum* L. and *Crinum latifolium* L. both has been collected from three different sites of the Midnapore forest range of West Bengal, India (Table 1).

Cytological studies

All bulbs were rooted by placing on moist sand soil mixture; tips from 4–5 mm long root were excised and

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pre-treated with an aqueous solution of paradichlorobenzene for 4 hours at about 10°C. The roots were fixed in a mixture of glacial acetic acid: ethanol (1:3 v/v) and kept for a couple of hours. Roots stained with 2% aceto-orcein stain. The stain was squashed on clean slides with a drop of 45% acetic acid. Cells with scattered metaphase chromosomes were examined and photographed by Leica DM 1000 microscope camera at X 1000 magnification. Chromosomes from ten metaphase plates for each species were considered for measurement.

Table 1. Name of the studied species and their location.

Name of Species	Location	Latitude (ºN)	Longitude(ºE)
Crinum asiaticum L.	Midnapore forest area of West Bengal	22.430889	87.321491
Crinum latifolium L.	Midnapore forest area of West Bengal	22.430889	87.321491

Karyotype analysis

Karyotype analysis was carried out using Ideokar 1.2 software. For analysis and comparison of the karyotype, the chromosomes of the species of *Crinum* were categorized on the basis of their length and centromeric position. Karyotype asymmetry was estimated by many different methods, Arano index of karyotype asymmetry (AsK %), the total form percent (TF %), the r-value, relative length of chromosome (RL %) and asymmetry index (AI), the intra-chromosomal asymmetry index (A1) and inter-chromosomal asymmetry index (A2), degree of asymmetry of karyotype (AI index). The categories according to Stebbins (1971), equations and calculations of these parameters and their references were shown in table 2.

Table 2. Morphometric analyses of chromosomes of *Crinum asiaticum* L. and *Crinum latifolium* L.

Chromosome parameters	*Crinum asiaticum*	*Crinum latifolium*
No. of chromosome (2n)	22	22
Average of Long arm(µm)	13.81	10.85
Average of Short arm (µm)	07.93	04.90
Arm ratio (AR)	01.92	02.41
Average chromosome length (µm)	21.30	15.39
r-value	00.60	00.54
Relative length of chromosome (RL %)	04.54	04.54
Centromeric index (CI)	00.36	00.33
Chromosome types	10 m + 12 sm	10 m + 6 sm + 6 st
Form percentage of chromosome (F %)	01.62	01.43
Total chromosome length of the haploid complement (HCL)	468.55	338.67
Total form percentage (TF %)	35.76	31.47
Class asymmetry index (Stebbins)	2B	2B
Arano index of karyotype asymmetry (AsK%)	64.24	68.52
Intrachromosomal asymmetry index (A1)	00.39	00.46
Interchromosomal asymmetry index (A2)	00.31	00.24
Symmetry index (S%)	30.22	42.85
Mean centromeric index (xCI)	00.36	00.33
Degree of karyotype asymmetry (A)	00.27	00.34
Mean centromeric asymmetry (xCA)	27.36	33.59
Coefficient of variation of chromosome length (CVCL)	31.12	24.67
Coefficient of variation of centromeric index (CVCI)	24.45	33.81
Asymmetry index (AI)	127.22	72.97

RESULT AND DISCUSSION

The somatic chromosome numbers for the two species, understudy, have been noted to be 22. This number complies with an earlier report (Sharma & Ghosh 1954, Raina & Khoshoo 1971, Patwary & Zaman 1975), however, it contradicts with other reports claiming the number 33 (Vijayavalli & Mathew 1992, Ahmed *et al.* 2004). The presence of such variation proves the role of both euploidy and aneuploidy to play in course of evolution of the species under this genus. Some earlier workers (Bose 1965, Sharma & Bhattacharya 1956, Raina & Khoshoo 1971) had also reported on the existence of polyploidy in the species of this genus. Moreover, such variations express the presence of two base number for this genus as x = 11 and 12. The chromosome length varied in *C. asiaticum* and *C. latifolium* 21.30 µm and 15.39 µm while their r-value is 00.60 and 00.54 respectively. On the position of the centromere, the chromosomes were classified into the different names (Table 2). The total genomic chromosome length was found to be 468.55 µm in *C. asiaticum* and 338.67 µm in *C. latifolium*. 22 somatic chromosomes of *C. asiaticum* were noted to be comprised of 10 metacentric chromosomes and 12 sub-metacentric chromosomes, whereas, in the case of *C. latifolium* was shown 10 metacentric, 6 sub-metacentric and 6 sub-telocentric chromosomes. The karyotype formula of both of the

species has expressed their diversity in chromosome morphology, as it is also apparent in figures 1 & 2 showing ideograms.

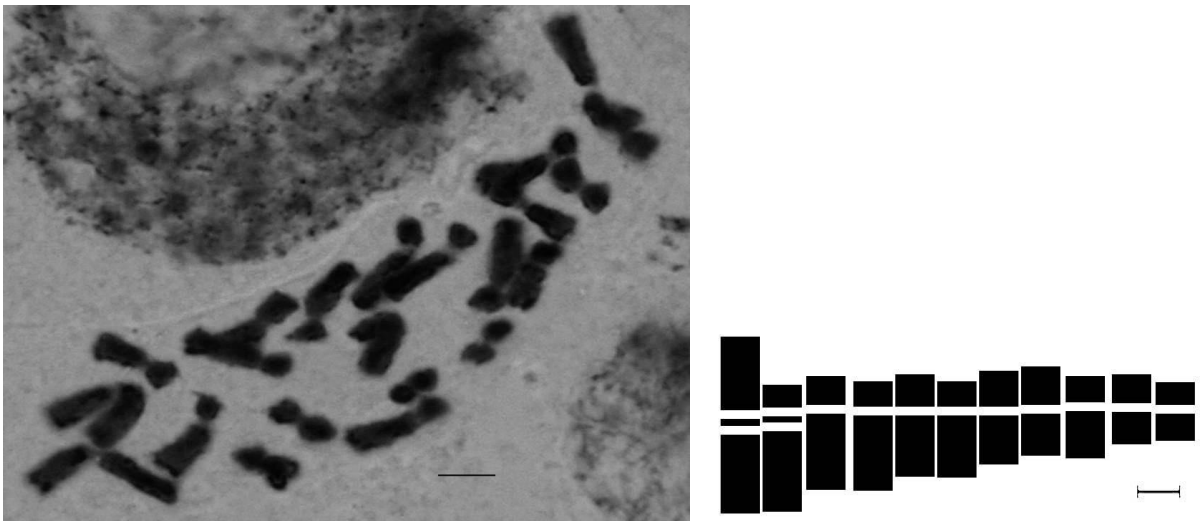

Figure 1. Scattered mitotic metaphase plate of *Crinum asiaticum* L. with respective ideogram. [Bar is 10 µm]

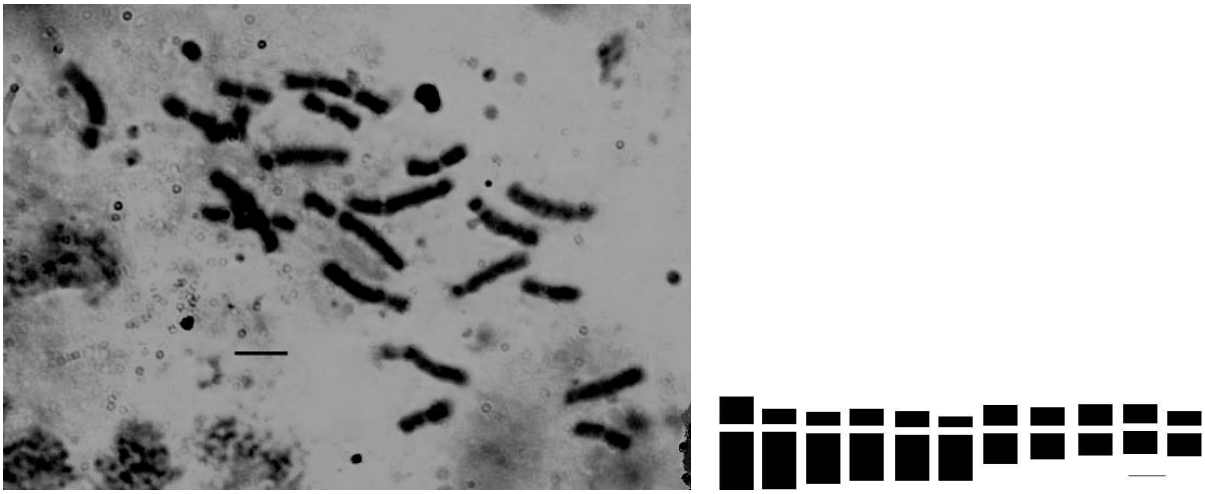

Figure 2. Scattered mitotic metaphase plate of *Crinum latifolium* L. with respective ideogram. [Bar is 10 µm]

In both the species, karyotypes were moderately symmetrical and both fell into the 2B category of Stebbins (1971) (Table 2). Most of the karyotypes in the genus *Crinum* L. fall in 2B and only a few in 3B category (Raina & Khoshoo 1971) which depicted that karyotypes are moderately or reasonably symmetrical. The karyotypes of *C. asiaticum* and *C. latifolium* also fall in 2B category.

There is a consistent difference in the size of two members of the longest pair in both, *C. asiaticum* and *C. latifolium*. Chromosomes of *C. latifolium* show heteromorphicity in respect of length. Such heteromorphicity indicating heterogeneity seems to have an evolutionary significance. A constancy in the chromosome number in two species with subtle morphological variations purports that certain nominal changes in chromosome morphology might have played role in speciation within the genus.

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