

 <p>ISSN NO. 2320-5407</p>	<p>Journal Homepage: - www.journalijar.com</p> <p>INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)</p> <p>Article DOI: 10.21474/IJAR01/12514 DOI URL: http://dx.doi.org/10.21474/IJAR01/12514</p>	 <p>INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR) ISSN 2320-5407</p> <p>Journal Homepage: http://www.journalijar.com Journal DOI: 10.21474/IJAR01</p>
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RESEARCH ARTICLE

COMPARATIVE KARYOMORPHOLOGICAL STUDIES OF TWO SPECIES OF *CRINUM*

Shahla Firdaus¹ and Kamini Kumar²

1. Research Scholar, University Dept. of Botany, Ranchi University, Ranchi, Jharkhand (India).
2. University Professor, University Dept. of Botany, Ranchi, Jharkhand (India).

Manuscript Info

Manuscript History

Received: 23 December 2020
Final Accepted: 25 January 2021
Published: February 2021

Abstract

Crinum asiaticum L. and *Crinum latifolium* L. are members of the family Amaryllidaceae. Karyomorphological studies of these two species had been performed by using different quantitative and qualitative parameters to know the karyotype asymmetry, which defines the advanced and primitive nature of these two species. The study revealed that *Crinum asiaticum* L. and *Crinum latifolium* L. are diploid with $2n=22$ chromosomes. based on Total Chromatin Length, Arm Ratio, Gradient Index, Form Percentage, Total Form Percentage, Karyotype Asymmetry Index, Symmetry Index, and Stebbin's classification, *Crinum asiaticum* L. is more advanced than *Crinum latifolium* L.

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Introduction:-

Crinum is an important genus of the family Amaryllidaceae with wide geographical distribution throughout the tropics, subtropics, and warm temperate regions of the world (Mabberly, 1990). This genus *Crinum* was first established by Linnaeus in 1737. The name *Crinum* is derived from the Greek word 'Krinos' which means trailing hair or comet-tail (Hannibal, 1992; Hannibal and Williams, 1998). Formerly this genus was kept in the family Liliaceae. But, later according to APG III classification, the genus *Crinum* was placed in the new family Amaryllidaceae (Nordal, 1977).

Morphologically, the *Crinum* species shows a little variation. Identification of any specimen up to the species level of *Crinum* is difficult. Thus, cytological features are an important aspect to identify the different species of *Crinum*. The study of the chromosome is an implementation to reveal the karyotypic variation, systematic relationships, phylogeny, and evolution of the related species of *Crinum*.

Materials And Methods:-

Two species of *Crinum* – *C. latifolium* L. and *C. asiaticum* L. were collected from home garden Ranchi, Jharkhand, India. Fresh young roots of both the plants were cut and pretreated with a saturated solution of Paradichlorobenzene and fixed in 1:3 acetoalcohol (Carnoy's fixative) for 24 hours, after that the root tips were preserved in 70% alcohol for further studies. The root tips were hydrolyzed for 5-7 minutes in 1 N HCl at 60°C and these were stained with 2% acetoorcein. For the preparation of slides, the squash technique had been adopted and the measurement of chromosomes had been recorded from ten well-separated metaphase plates by using ocular and stage micrometer. The photographs were taken by using Nikon DSLR Camera. Seven parameters were measured in each metaphase plate-length of the long arm (L), length of the short arm (S), total chromosome length (TL), Arm ratio (L/S),

Relative Length of chromosome(RL), Chromosome Form Percentage (F%) and Centromeric Index (CI). Their formulas are as follows ---

$$TL=L+S$$

$$RL=\frac{TL}{\Sigma TL}$$

$$\%=\frac{S}{TL} \times 100$$

$$CI=\frac{S}{TL}$$

Karyotype asymmetry was examined by Total Chromatin Length (TCL), Gradient Index (GI; Levitsky, 1931) the total form percentage (TF%; Huziwara 1962,) Asymmetrical Karyotype Percentage (AsK%; Arano,1963), Symmetry Index (SI; Greihuber and Septa;1976) and Dispersion Index (DI; Lavania and Srivastava 1999) and Stebbins classification (1971). Their formulas are as follows ---

$$TF\%=\frac{\Sigma S}{\Sigma TL} \times 100$$

$$GI=\frac{s}{l} \times 100$$

Where, s =Length of the shortest chromosome of the complement

l = Length of the longest chromosome of the complement

$$SI=\frac{\Sigma S}{\Sigma L} \times 100$$

$$AsK\%=\frac{\Sigma L}{\Sigma TL} \times 100$$

$$CG=\frac{Lmc}{Sm} \times 100$$

Where, CG= Centromeric gradient

Lmc=length of the median chromosome, and

Sm=length of the median short arm

$$CV=\frac{SD}{\overline{TL}} \times 100$$

Where, CV= Coefficient of variation for chromosome length

\overline{TL} = mean

SD= standard deviation

$$DI=\frac{CG \times CV}{100}$$

Table 1:- Stebbins' classification.

Ratio	Proportion of chromosomes with arm ratio < 2:1			
Largest/ Smallest	1.00 (1)	0.99–0.51 (2)	0.50–0.01 (3)	0.00 (4)
< 2:1 (A)	1A	2A	3A	4A
2:1–4:1 (B)	1B	2B	3B	4B
> 4:1 (C)	1C	2C	3C	4C

Chromosomes were classified using the nomenclature of Levan et al (1964) and Chromosomes were categorized based on their length as follows: Type A=13.00 μ m and above, Type B=11.00 μ m-12.99 μ m, Type C=9.00 μ m-10.99 μ m, Type D=7.00 μ m-8.99 μ m, Type E=5.00 μ m-6.99 μ m, Type F=3.00 μ m-4.99 μ m, Type G= 1.00 μ m- 2.99 μ m and Type H=0.99 and below.

Result:-

The karyomorphological data of *C. latifolium* L. and *C. asiaticum* L. are statistically analyzed first time in Jharkhand, a state of India and these data are recorded in table 2,3 and 4. The maximum total chromatin length was recorded in *C. latifolium* L. with 83.19 μ m and in the case of *C. asiaticum*, it is 75.73 μ m. The size of the chromosome complement ranged from 2.43 μ m to 13.30 μ m in *C. latifolium* L. and in the case of *C. asiaticum* L. the chromosome complement size ranged from 6.54 μ m to 9.55 μ m. Based on the centromeric position the chromosomes of *C. latifolium* L. were classified as 8 submedian and 3 subterminal whereas in *C. asiaticum* L. it was classified as 5 submedian and 6 subterminal. Thus, the karyotypic formula for *C. latifolium* L. and *C. asiaticum* L. were represented as 8sm+3st and 5sm+6st respectively. According to the length of chromosomes, the karyotype formula are 2C+8D+1E and 1A+1B+1C+2D+2E+2F+2G for *C. latifolium* L. and *C. asiaticum* L. respectively. As per karyotype asymmetry, the karyotype of *C. latifolium* L. and *C. asiaticum* L. comes under 1A and 1C according to Stebbin's classification. In *C. latifolium* L., TF% was 30.69, GI was 68.55, Ask% was 78.33 and SI was 39.21 which are less as compared to TF%(23.74), GI(18.27), Ask% (76.29), and SI (31.31) of *C. asiaticum* L. Maximum DI (9.46) was recorded in *C. asiaticum* L. and minimum DI (3.84) was observed in *C. latifolium* L.

Table 2:- Karyomorphological data related to *C. asiaticum* L.

Chromosome No.	L(μ m)	S(μ m)	TL(μ m)	L/S	RL	F%	CI
I	10.15 \pm 1.63	3.15 \pm 1.12	13.30 \pm 2.75	3.22 \pm 0.33	17.74	23.68	0.24
II	9.15 \pm 1.75	1.95 \pm 0.41	11.10 \pm 2.16	4.69 \pm 0.16	14.81	17.56	0.18
III	7.40 \pm 1.44	1.70 \pm 0.41	9.35 \pm 1.85	3.79 \pm 1.31	12.47	20.85	0.21
IV	6.30 \pm 1.20	1.95 \pm 0.37	8.00 \pm 1.57	3.70 \pm 0.45	10.68	21.25	0.21
V	5.65 \pm 1.25	1.65 \pm 0.37	7.30 \pm 1.62	3.42 \pm 1.33	9.74	22.60	0.23
VI	5.30 \pm 1.08	1.60 \pm 0.38	6.90 \pm 1.46	3.31 \pm 1.26	9.20	23.18	0.23
VII	4.25 \pm 1.13	1.60 \pm 0.39	5.85 \pm 1.52	2.65 \pm 0.44	7.80	27.35	0.27
VIII	3.40 \pm 0.69	1.55 \pm 0.35	4.95 \pm 1.07	2.19 \pm 0.29	6.60	31.31	0.31
IX	2.60 \pm 0.66	1.25 \pm 0.35	3.85 \pm 1.01	2.08 \pm 0.46	5.14	32.46	0.32
X	1.85 \pm 0.36	0.85 \pm 0.36	2.70 \pm 0.72	2.17 \pm 0.31	3.67	45.14	0.45
XI	1.70 \pm 0.38	0.73 \pm 0.14	2.43 \pm 0.52	2.32 \pm 0.33	1.90	51.04	0.50
			75.73				

Table 3:- Karyomorphological data related to *C. latifolium* L.

Chromosome No.	L(μ m)	S(μ m)	TL(μ m)	L/S	RL	F%	CI
I	6.87 \pm 0.23	2.67 \pm 0.24	9.55 \pm 0.47	2.57 \pm 0.48	11.47	28.04	0.28
II	6.63 \pm 0.21	2.73 \pm 0.25	9.36 \pm 0.46	2.43 \pm 0.23	11.25	29.17	0.29
III	6.36 \pm 0.22	2.61 \pm 0.21	8.97 \pm 0.44	2.44 \pm 0.44	10.78	29.09	0.29
IV	6.33 \pm 0.20	2.55 \pm 0.22	8.88 \pm 0.42	2.48 \pm 0.35	10.67	28.71	0.29
V	6.03 \pm 0.17	2.70 \pm 0.19	8.73 \pm 0.36	2.23 \pm 0.19	10.49	30.92	0.30
VI	5.88 \pm 0.22	2.70 \pm 0.19	8.58 \pm 0.41	2.17 \pm 0.30	10.31	31.46	0.31
VII	5.82 \pm 0.26	2.52 \pm 0.19	8.34 \pm 0.46	2.30 \pm 0.18	10.02	30.21	0.30
VIII	5.61 \pm 0.19	1.86 \pm 0.19	7.47 \pm 0.39	3.01 \pm 0.18	8.97	24.89	0.25
IX	5.43 \pm 0.20	1.86 \pm 0.19	7.29 \pm 0.39	2.91 \pm 0.45	8.76	25.51	0.25
X	5.28 \pm 0.20	1.72 \pm 0.17	7.00 \pm 0.37	3.06 \pm 0.13	8.41	24.57	0.24
XI	4.93 \pm 0.20	1.61 \pm 0.17	6.54 \pm 0.43	3.06 \pm 0.23	7.87	24.61	0.24
			83.19				

Table 2:- Data related to the karyotype of *C. asiaticum* L. and *C. latifolium* L.

Species	TCL(μ)	TF%	GI	DI	Ask%	SI	Karyotype	Stebbin's
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	m)						formula	type
<i>C. asiaticum</i> L.	75.73	23.74	18.27	9.46	76.29	31.31	5sm+6st	1C
<i>C. latifolium</i> L.	83.19	30.69	68.55	3.84	78.33	39.21	8sm+3st	1A

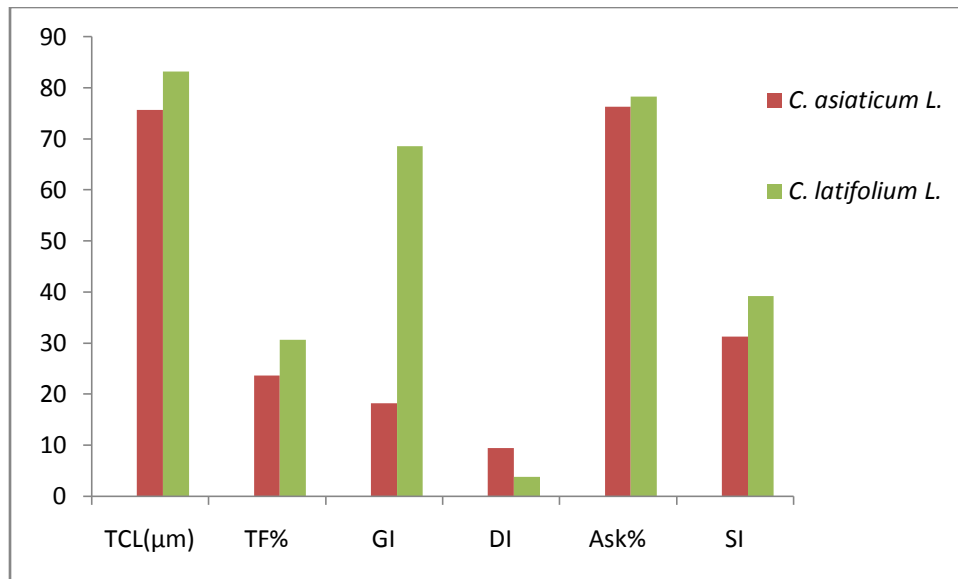


Fig 1:- column graph showing comparative total chromatin length(TCL), Total Form Percentage (TF%), Gradient Index (GI), dispersion Index (DI), Asymmetrical Karyotype Percentage(Ask%) and Symmetry Index(SI) of *C. latifolium* L. and *C. asiaticum* L.

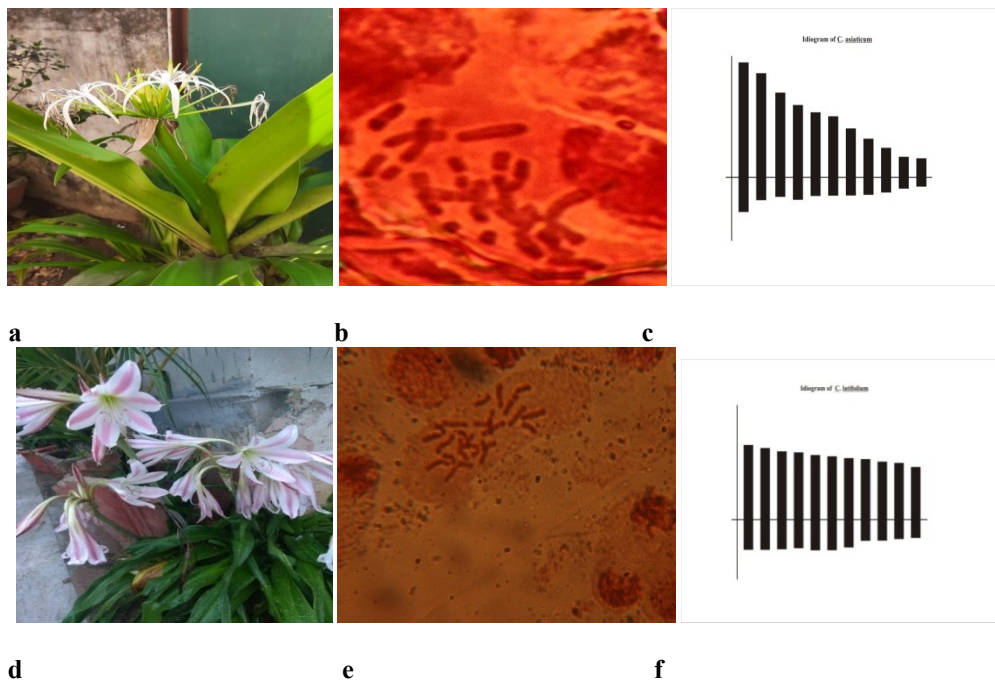


Fig 2:- a- Photograph of Plant of *C. asiaticum*, b- Photomicrograph of mitotic metaphase of *C. asiaticum*, c- ideogram of *C. asiaticum* d- Photograph of Plant of *C. latifolium*, e- Photomicrograph of mitotic metaphase of *C. latifolium*, f- ideogram of *C. latifolium*

Discussion:-

The present investigation was an attempt to find a relative affinity of the considered species of genus *Crinum*, based on the karyoevolutionary parameters. Normal mitotic divisions were found in all the cells and no satellites were

recorded in any chromosome. The initial reports on chromosome number of some species of genus *Crinum* were $2n=24$ (Tomita 1931, Sugiura 1936) but after this record, many researchers have found diploids to be $2n=22$. The earlier reports were considered as inaccurate and $x=11$ is observed as the basic chromosome number of this genus (Inariyama 1937, Sato 1938, Gouws 1949, Dolcher 1950, Sharma And Ghosh 1954, Sharma And Bhattacharya 1956, Fernandes And Neves 1961, Bose 1965). In the present investigation, both the species of *Crinum*, *C. latifolium*, L. and *C. asiaticum* L. were found as diploids having $2n=22$ chromosomes. Karyotypic asymmetry was measured based on differences identified using either a qualitative classification method or quantitative indices. Thus, according to Stebbins (1971), the karyotype of *C. latifolium* L. studied in the present report was located in group 1A, which is classified as symmetric and karyotype of *C. asiaticum* L. seizes 1C class, classified as a relatively asymmetric, or more developed karyotype. The karyotype of the studied species is mostly comprised of submetacentric to subtelocentric chromosomes, indicated by their mean arm ratio. The arm ratio ranges between 2.17 ± 0.30 to 3.06 ± 0.13 in *C. latifolium* L. and 2.08 ± 0.46 to 4.69 ± 0.16 in *C. asiaticum* L. The arm ratio values indicate the degree of asymmetry. The high value represents asymmetrical karyotype. Increasing chromosomal asymmetry occurs because of the shifting of the centromere from median or submedian to the subterminal or terminal position (Stebbins, 1971). Comparing species with each other, the chromatin length of *C. latifolium* L. and *C. asiaticum* L. was observed as $83.19\ \mu\text{m}$ and $74.93\ \mu\text{m}$ respectively. *C. latifolium* L. is considered primitive having the greatest chromatin length and *C. asiaticum* L. is advanced having the lowest chromatin length (Stebbins, 1971). The arm ratio and chromatin length cannot fulfill the criteria of primitiveness and advancement.

Due to the evolutionary process, the DNA per genome can be increased or decreased and it can be found in the whole complement in equal amount or the quantitative proportional to the chromosome length or it may be restricted to one or more chromosomes (Jones, K. 1984). When GI is less than 30, karyotype shows asymmetry. Asymmetrical karyotypes are considered advanced ones. (Levitsky, 1931). Hence, *C. asiaticum* L. is advanced as its GI value (16.27) is less than 30 and the GI of *C. latifolium* L. (68.55) is more than 30, shows its primitiveness. The advancement of *C. asiaticum* L. has been further supported by TF%, F%, AsK%, and SI. The TF% gives an estimate of the mean position of centromere in different chromosomes while F% is another estimate of arm ratio. When TF% is taken for analyzing the asymmetrical nature of karyotype, it should be observed that the plant species which has fewer TF% value are considered more asymmetrical. In the present case, the TF% of *C. asiaticum* L. (23.74) was recorded as less than the TF% of *C. latifolium* L. (30.69) (Huziwaru, 1962). Karyotype asymmetry percentage (Arano, 1963) was recorded 76.29 and 78.33 in *C. asiaticum* L. and *C. latifolium* L. respectively. The lower values observed for these Parameters also indicate that the level of asymmetry is higher in *C. asiaticum* L. Both the species have a lower SI value, showing the tendencies towards asymmetry. The SI of *C. asiaticum* L. is lower than the SI of *C. latifolium* L. It shows *C. asiaticum* L. is more asymmetrical.

Conclusion:-

Based on the above findings, it may be concluded that the karyotype of *C. asiaticum* L. is more asymmetrical which represents its advancement.

Acknowledgment:-

We are grateful to the University Department of Botany, Ranchi University, Ranchi, for providing the laboratory facilities.

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