

III- Materials And Methods

Genus Crinum L. is represented by about 7 species in Maharashtra of which '3' are endemic to the state [Table.1] Localities of occurrence of these species were noted by referring taxonomic works on the flora of Maharashtra (Cooke, Kulkarni, 1988, Almeida, 1990), and herbarium of BSI. The bulbs of the species were collected in vegetative and flowering stages from natural localities and planted in botanical gardens. Morphology of these species was studied in the field as well as of plants grown in Botanical garden. Careful observations were made on vegetative characters of the plants during rainy season and reproductive characters during summer. In spite of several visits and careful search in type locality for Crinum eleonorae, author failed to collect the species.

To make a comparative account of morphological characters at least 25 individuals of each species from each locality were examined. The important characters are represented in the form of polygraphs. Pollen morphology was studied by fixing pollen grains in glacial acetic acid and then processing by acetolysis method (Erdtman 1952, Nair 1966). Shape and size of atleast 25 pollen grains for each species was determined. To determine pollen fertility pollengrains were stained in 2% T.T.C. solution. The pollen fertility for each species indicated in text is based on at least 1000 pollen grains. The pollengrains with distinctly stained generative cell and

vegetative nucleus were taken as fertile pollengrains. Size frequency classes of pollen grains are based on measurement of atleast 1000 pollen grains.

Anatomy of leaf was studied by using hand cut sections of fresh leaves. Cuticular studies were made by using peels of fresh as well as preserve materials. The leaf thickness was determined with the help of 'Mitutoyo's thickness meter. Stomatal index was determined by following formula

$$\text{Stomatal index (SI)} = \frac{S}{S + E} \times 100$$

Where,

SI = Stomatal index

S = Number of stomata mm^{-2}

E = Number of epidermal cells
per unit area

To study vessels, the roots about 1 - 2 cm in length were treated in Jeffrey's fluid (mixture of equal amount of 10% chromic acid and 10% nitric acid.), at 60°C in oven for 1 - 3 hours. The material was washed thoroughly in water and then spread on slide with the help of needles. The material was stained with 1% saffranin and studied under microscope.

For karyotypic studies excised root tips of water cultured bulbs were pre-treated with saturated solution of Para-dichloro-benzene (PDB) or 0.2% colchicine or combination of these two for 4 - 6 hours

at $10 \pm 2^\circ\text{C}$. Then the root tips were hydrolysed in 1N hydrochloric acid at 60°C in oven for 5-10 minutes, and then squashed in 2% propionic orcein or aceto-orcein stain. 2% propionic orcein was found to be most satisfactory. The slides were made permanent by passing through usual grades of acetic acid-Butanol. Materials were mounted in DPX. Minimum 20 mitotic plates were analysed for each species. The karyotypic studies of tetraploid of Crinum species are based on 10 mitotic plates. For karyotypic analysis the nomenclature recommended by Levan et al. (1964) for centromeric position has been adopted. Symmetry of karyotype has been analyzed using Stebbin's (1958) system of classification. F% and TF% were calculated as given by Huziwara (1962), while TCL%, S% and relative length of chromosome were determined by using following formulae -

$$\text{TCL}\% = \frac{\text{Length of the chromosome}}{\text{Absolute length of the complement}} \times 100$$

$$\text{S}\% = \frac{\text{Length of shortest chromosome}}{\text{Length of Longest chromosome}} \times 100$$

$$\text{Relative length of chromosome} = \frac{\text{Length of chromosome}}{\text{Length of longest chromosome in the compliment}}$$

$$\text{F}\% = \frac{\text{Short arm Length of a chromosome}}{\text{Total Length of a chromosome}} \times 100$$

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

Most of Crinum species of Maharashtra flower during April-May. To study meiosis in the species the bulbs were obtained from natural habitats as well as from garden in Last week of February or first week of March. The scales were removed carefully and young inflorescences were fixed in Cornoy's Fluid (45 ml absolute ethyl alcohol + 15 ml glacial acetic acid). Inflorescences were washed well in water, hydrolysed in 1 N HCl and smeared in 2% propionic orcein. Slides were made permanent by usual method using acetic acid-Butanol grades.

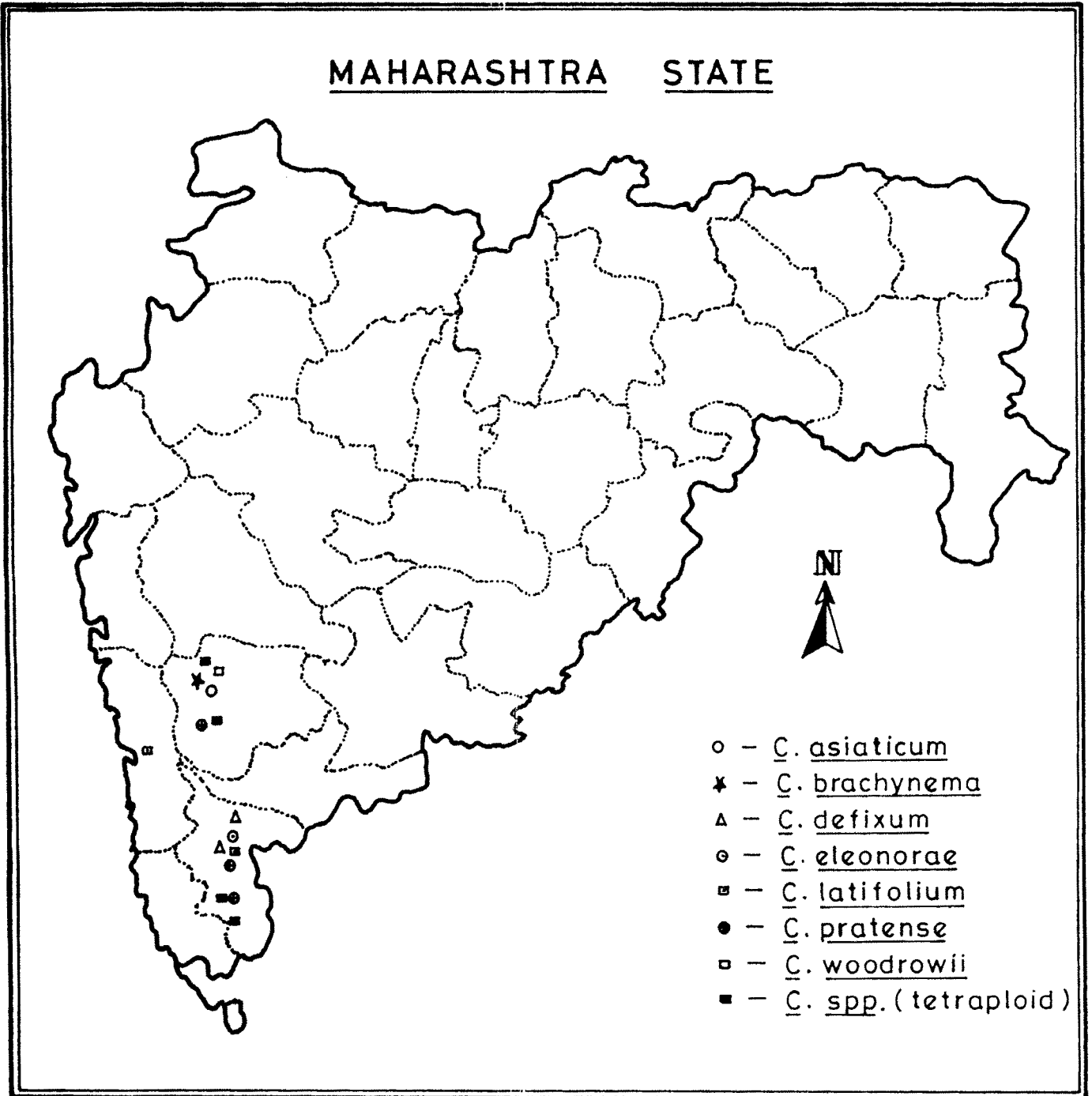
The various species were brought to flowering more or less in the same period by manipulating watering. Interspecific hybridization was tried in month of April for two consequents years. Undehisced anthers from the buds going to bloom on that day were removed in morning hours. Anthers were kept in small vials with labels.

The buds open in evening when stigmas were pollinated by pollens collected in the morning. Interspecific crosses were tried. The entire inflorescence was bagged during night time. The seeds obtained through interspecific crosses were sown separately in earthen pots. The hybrid plants are under observation.

Table No.5. Distribution of Crinum species in Maharashtra (collected and cultivated)

Sr. No.	Name of the Species	Locality	Year of Collection	Remark
1.	<u>Crinum asiaticum</u> Linn.	Kolhapur	Sept.1992	Cultivated in many public gardens
2.	<u>Crinum species</u> (Tetraploid)	Kas, Chandoli, Radhanagari, Phonda, Ramghat	1991,92	The species resembles with <u>C. asiaticum</u> but significant difference in morphology are seen. Leaves are large, broad glaucous, tetraploid, reproduce sexually.
3.	<u>Crinum brachynema</u> Herb.	Mahabaleshwar	March 1993	Only found on the slopes of hills around Mahabaleshwar, endemic.
4.	<u>Crinum defixum</u> Ker.Gawl.	Kolhapur, Gaganbawada, Belgaum, Panhala, Vajreshwari	1992; Feb. March 1993	It grows along the margin of river bed and water courses.
5.	<u>Crinum eMonorae</u> Blatt.	Mahabaleshwar	----	Endemic reported from hills of Mahabaleshwar
6.	<u>C. latifolium</u> Linn.	Bhatia, Ratnagiri, Ramghat, Kolhapur, University, Botanical garden	1992,1993	Cultivated ingradens as well as wild
7.	<u>C. pratense</u> Herb.	Kolhapur, Radhanagari, Barki plateau, Kas plateau	1992, 1993	Grows on plateaus at higher altitudes and Konkan.
8.	<u>C. woodrowii</u> Bak.	Mahabaleshwar	----	Endemic found only on hills of Mahabaleshwar.

MAHARASHTRA STATE



Map No.1- Distribution of Crinum species in Maharashtra .

Map showing places of collection of Crinum species .

Drawings of anatomical structures were made by using Hamburg microscope and Eram's Camera Lucida at suitable magnifications. Photomicrographs were taken by using MFAK's system of JENEVAL Carlzeiss Microscope. All the slides prepared during present investigation and voucher specimens are deposited in the Botany department of the University.