V- Discussions

Most of the Crinum species found in Maharashtra are found growing along sahyadri ranges and Konkan. Crinum asiaticum and C. latifolium are found under cultivation in gardens as well as widely growing in forest areas. Crinum defixum is mainly found growing in marshy places along river beds, streams and similar situations. Crinum pratense grows in rocky places on lateritic plateaus at higher altitudes along western ghats. Crinum brachynema C. eleonorae and C. woodrowii grows on hill tops and hill slopes around Mahableshwar.

During present investigation Tetraploid crinum species was collected from various places along western ghats of Maharashtra. It grows along hill-slopes, has dark-green glaucous leaves. The leaf and flower characters are similar to and overlap with C. asiaticum. As there is no qualitative character to distinguish Crinum species from C. asiaticum and over-lapping quantitative vegetative and flower characters, it is difficult to distinguish and delimit the species, however it is very distinct from C. asiaticum and could be a new species. All the populations of crinum species collected from localities such as Kas, Chandoli damb, Radhanagari, Fonda ghat, Ramghat are tetraploid with 2n=44 and show seed formation by sexual means. Even meiotic studies have shown that the meiosis is normal and polen fertility is above 90%. . All these facts support that it is a distinct species of Crinum mistaken for C. asiaticum by previous workers.

Genus Crinum mainly distributed in tropical and subtropical regions of both hemispheres is represented by about 100 species (Willis, 1985). A large number of species are found in tropical Africa, Asia, Austrelia and America. About 57% of species occur in Africa and it seems to be the centre of origin and dispersal for the genus Crinum. The number of species reported for the genus varies from 60 to 165 (Wealth of India, 1950). Various workers have reported different numbers of species viz. 60 (Hooker, 1884), 164 (Koshimizu, 1928), 80-100 (Baiky 1949), 165 (Wealth of India, 1950), 75 (Cooke, 1958), 148 (Traub, 1962), 100 (Lawerence, 1968), 80-90 (Thistleton-Dyer, 1979), 150 (Wahlstrom and Laane, 1979), 70 (Kirtikar and Basu, 1984), 100-110 (Willis, 1985) which indicate the problem of species delimitation and recognition in the genus.

The genus Crinum is represented by about 12 species in India (Karthikeyan, et al. 1989) of which three species viz. C. brachynema, C. eleonorae and C. woodrowii are endemic to Maharashtra restricted to their type locality, Mahabaleshwar. There are about 7 species found widely growing in Maharashtra (Blatter and McCann, 1928, Cooke, 1958) viz. C. asiaticum, C. defixum, C. latifolium, C. pratense, C. brachynema, C. eleonorae and C. woodrowii.

Critical observations on vegetative and flower characters indiated that there are only few characters of taxonomic value. Bulb size, length of neck, leaf size, petal size, corolla shape and stamen characters are of taxonomic value. Character, especially leaf size, seems to be fairly constant for identification of some species and this character is used by most of the workers to distinguish the species (Hooker, 1984, Cooke, 1958, McCann and Blatter, 1928, Gamble, Kulkarni, 1988, Almeida, 1990). Present observation indicate that instead of leaf length and breadth, the leaf length- breadth ratio and leaf length X leaf breadth values are more useful for delimiting the species (Table. 7, Polygraph fig. I)

Shape of corolla is useful in distingushing some species. Crinum latifolium can be easily distinguished by its funnel shapped corolla, curved tube and bent stamens, and grayish-white pollen, while remaining all other species have orange coloured pollens. Crinum brachynema and C. eleonoral are very distinct from rest of species in having stamens with very short filaments (Nordal, 1979). Probably it is a close relative of C. brachynema and C. eleonorae found in India.

C. asiaticum is mainly cultivated in Gardens. It has long neck, very long broad leaves and inflorescence usually with many flowers (15-50). Crinum spp (tetraploid) closely resembles C. asiaticum but differs mainly in smaller, dark-green glaucous leaves, narrow neck

and its natural habitat of occurence. A more reliable key to identify Crinum species is provided on basis of critical observations and analysis of morphological characters.

All the species of Crinum show same phenological events except C. asiaticum which is cultivated in gardens. It shows vegetative growth throughout year and is grown as ornamental plant, mainly for its long dark green foliage and white flowers. It shows flowering almost throughout year, however, peak flowering is seen during May to August. Remaining species shows vegetative growth after flowering from June to September. By the end of September the aerial parts start drying and bulbs go into dormancy period. Studies on meiosis in Crinum species indicated that inflorescence initation starts somewhere during December - January and meiosis in meiocytes occur during February in most of the species.

Observations on cultivated plants of all the species of Crinum under study revealed that they are night blooming. The flower buds to be going to open on the day, bent down from remaining young flower buds in morning. Anthesis takes place in bent flower bud during 2-4 PM and flower open by 6-7 PM in evening. Flowers remain open for a night and start closing and withering by next morning. During night, observations were made on pollinators. It was obsered that Hawk-moth visits flower of crinum

species arround 8-9 PM and bring about pollination (Phoplate III, Fig.-11). It has long haustella of about 6 cm. It seems that all the species of Crinm under study are month pollinated. Crinum flaccidum flowers are reported to be classic phalenophilous (moth pollinated) type by faegri and Vander Pill (1979). Howell and Prakash (1990), ford et al (1979) recorded that birds some times feed on Australian Crinum flowers.

According to Howell and Prakash (1990), Spingid moth pollination is likely in the Crinum flaccidum. A Crinum pollen were found on the haustellum one spingid species. Similarly a correlation between corolla tube and haustellum lenths could be expected in adapted species (Howell and Prakash, 1990).

In all the species of Crinum under study the hypanthium (corolla tube) get filled with nectar by evening. Cruden and Herman (1983) reported that sucrose is the main constituent of the nectar of spingid pollinated flowers. In Crinum flaccidum sucrose formed a significant fraction of the nectar solids (Howell and Prakash, 1990). According to Armstrongs (1979) and Faegri and Vander Piji (1979), as compaired to the large energy requirements of these moths for their powerfull flight, the nectar is surprisingly dilute. Spingid moth, unlike hymenopterans, donot label the previously visited flower (Faegri and Vander Piji, 1979). According to Hannibal (1966) and Howell and Prakash (1990) the strong floral

adour may mask any label or any olfactory hint of a nectar reward.

In all the species of the Crinum under study only 2-4 flowers on an inflorescence open per day. According to Southwick et al (1981), the search for nectar is frequently futile but punctuated with lucky breaks of occassional nectar rich flowers. These trades as well as restricted number of open flower on any one inflorescence, force any potential pollinator to visit a large number of flowers on different plants, thus facilitating out crossing.

Observations on pollen morphology indicated that all the species have orange coloured-Pollen Except Crinum latifolium in which Pollengrains are grayish white inall the population of C. latifolium (Table 8). The pollengrains of different species of Crinum do not show much variation in general morphology (Table 8 Fig. Photoplate IV). The pollen grains are elliptic, dizonocolpate and echinulate as observedby others (Suita, 1937, Dutt, 1962, Dahlgren and Clifford, 1982; Zavada, 1983; and Nayar, 1990). Although pollengrains are generally monosulcate in family Amaryllidaceal (Dahlgren and Clifford, 1982), they are disulcate in the tribe Amaryllideae including all the species of Crinum investigated except for C. americanum (Zavada, 1983). SEM studies on pollen of Crinum flaceidum revealed that the

grains are free, spheroidal, disulcate echinulate with small nodules between echinae and are dispersed in two called condition.

While largest pollen grains were found in C. Woodii, remaining species showed little variations in pollen size (Table 8). High pollen fertility was observed in all the species except in one cultivated species which was triploid. Tetraploid species showed about 90% pollen fertility.

Anatomical studies (Table 9, photoplate 5,6) on the leves revealed that the leaves of all the species are isobilateral and amphistomatic, they are thick and show xerophytic characters. Gross anatomical characters are similar in all the species and only minor variations are found with respect to leaf thickness, stomatal size, stomatal index and stomatal density. Thus anatomical characters are of little importance in taxonomy of the genus and species.

Chromosome number 2n= 22 reported in present investigation for C. latifolium (Table 11) agrees with previous reports of Inariyama (1937), Suita, (1937,) Sato, (1938; Dolcher, (1950; Khoshoo and Raina, 1968; Raina and Khoshoo, 1971a, Fujishima, 1975, however Stenar, 1925 and Tomita, 1931, reported n= 12 for the species. Triploids are also reported in C. latifolium (Jones and Smith, 1967); Chromosome number 2n = 22 and n= 11 is reported for

first time for C. brachynema and C. pratense (Table 10 and 12). Crinum species with dark green glaucous leves was found to be tetraploid with 2n = 44 and n = 22. This tetraploid showed high pollen fertility, normal meiosis and formation of viable seeds through sexual means. The tetraploid is closely related to Crinum asiaticum and may prove to be a new species of Crinum.

Literature survey shows that most of the Crinum species have diploid chromosome number 2n = 22, and n= 11. Triploids, tetraploids and hexaploids are not uncommon in crinum (Table: 2). Cytological studies on Crinum species have shown that the basic chromosome number for the genus is X= 11, and polyploid forms show the somatic chromosome number in multiple of basic number i.e. X= 11, (Sharma and Bhattacharya, 1959; Jones and Smith, 1967; Raina and Khoshoo, 1971). Present studies on Crinum brachynema and C pratense also support that the basic chromosome number for the genus is X=11. In one populaltion of Crinum ornatum, the diploid chromosome number is reported to be 2n= 24, which mislead (Base, 1965) to suggest another basic number for the genus i.e. X= 12, however, later studies indicated that n=12 in C. ornatum may be derived from X=11 (Raina and Khoshoo, 1971a). Therefore noted it is agreed that the genus Crinum has basic chromosome number X= 11 (Sharma and Bhattacharya, 1956; Raina and Khoshoo, 1971a).

Karyotype of species under study (Table 10 to 13) matches with the basic Karyo type suggested for genus Crinum by Sato (1938, 1942), Dolcher, 1950; Jones and Smith, 1967; and Raina and Khoshoo 1971a). The basic Karyotype suggested by them consists of four types of chromosomes Viz. one long, five medium, one medium SAT chromosome and four short chromosomes. The chromosomes are catagorised into long, medium, and short chromosomes. The position of primary constriction varies in different taxa. The karyotype of various species shows close similarities and variations are found only in position of primary constriction and length of chromosomes.

The Karyotypic formulae for Crinum species under study is shown in table 14. From the table it is evident that there is pair of long chromosomes (9.95 - 13.3 u) with primary constriction in the mediam (m) region. In Crinum brachynema, C. latifolium and C. pratense, two pairs of same types of long chromosomes are also found in tetraploid Crinum species. This long pair of metacentric (m) chromosomes is characteristic to all the species of Crinum so far studied (Sato, 1938; 1942; Dolcher, 1950; Jones and Smith, 1967; Raina and Khoshoo, 1971a; Vijayavalli and Mathew, 1992).

A pair of SAT chromosome of medium size (7.12 - 9.36 u) with primary constriction in the submedian (sm) region is also characteristic to genus Crinum. This pair is found in diploid species under study Viz. Crinum brachynema, C. latifolium and C. pratense, however, SAT

chromosome were not detectable in tetraploid species of Crinum. Probably due to difficulties faced in getting good somatic plates in tetraploid. As this pair of SAT chromosomes is characteristic to all the species of Crinum so far studied, better preparation of somatic plates of tetraploid may reveal the presence of SAT chromosomes. The general Karyotype formula of tetraploid matches with diploid species.

Remaining five pairs of medium chromosomes in diploid and 13 pairs in tetraploids show-variations in position of centromeric position and length of chromosomes in Crinum species under study as well as in species investigated (Jones and Smith, 1967; Raina and Khoshoo, 1971a; Vijayavalli and Mathew, 1992). Under this catagory three types of chromosomes are recognised, Viz. chromosomes with median(m), submedian (sm) and subterminal (st) primary constriction. Medium sized chromosomes with constriction in the submediam region predominates in all the crinum species studies. Pesence of the above three types of centrometric positions in medium sized chromosomes is observed in other species of Crinum (Jones and Smith, 1967; Raina and Khoshoo, 1971a), however the short chromosomes are only of two types Viz. median (m) and submedian (sm) tyupe.

There are 4 pairs of short chromosomes in diploid Crinum species under study and 7 pairs in

Tetraploid Crinum species. These chromosomes are of of median of submedian type. Similar number and types of short chromosomes are observed in other species of Crinum (Jones and Smith, 1967; Raina and Khoshoo, 1971a, Vijayavalli and Mathew, 1992).

Therefore present and previous studies on chromosome morphology support that there is general similarity in karyotype of various species of Crinum. The gross similarity in external morphology and chromosome complement of various Crinum species indicate that the genus Crinum is in all possibliity, represent a homogeneous assemblage (Sharma and Bhattacharya, 1956).

The basic chromosome number X = 11; indicate an independent line of evolution distinct from all other general of Amaryllidanceal except genus Amaryllis and Nerine to which Crinum is closely related. Even intergeneric hybrids Viz. C. moorei and A. belladona related are reported (Sato, 1938) indicating their close genetic relationship.

Karyomorphological data of the three diploid species (Table 14) shows that C. brachynema with lowest values of the total haploid chromatin length (71.13 u) followed by C. pratense (78.9 u) and C. latifolium (90.65 u). The average chromosone length was found to be least in C. brachynema (6.46 u) followed by C. pratense (7.17 u). Tetraploid Crinum spp (7.26 u) and C. Latifolium

(8.24 µ). Average chromosome length in other species Viz. C. asiaticum, C. giganteum and C. moorei are found to be 8.36 to 9.16 µ; 11.63 - 12.09 µ; 11.23 - 12.18 µ respectively by Vijayavalli and Mathew (1992). Karyotype of C. latifolium and C. pratense with pair of subterminal chromosomes were found to be more assymetrical. In C. brachynema and Tetraploid Crinum Sp. Sm-type chromosomes predominated and with no St-type chromosomes were observed. St-type chromosomes are reported in C. asiaticum while they were found to be absent in C. giganteum (Vijayavalli and Mathew, 1992).

In present investigation, a fertile tetraploid Crinum species has been collected from various localities along western ghats. Although it resembles with C. asiaticum, it is a distinct species. Prior to works of Bose (1965), Jones and Smith (1967), Raina and Khoshoo (1971a), the incidence of polyploidy in genus Crinum was reported to be very low and the highest ploidy level reported to be very low and the highest ploidy level reported was triploidy (Miege, 1962). Presently the ploidy level upto 8% has been reported and nearly 21.3% taxa are ploypoid (Raina and Khoshoo, 1971a). The ploidy is noted both at interspecific and intraspecific level (Table 2). The morphology, pollen fertility, normal meiosis and formation seed through sexual reproduction in present tetraploid Crinum species cmplies that two different genotypes are involved and the species presents

case of allotetraploidy. Karyotype and meiotic studies in C. angustum have shown that it is a allotriploid (Raina and Khoshoo, 1971a). Similarly in tetraploids of C. polyphyllum the morphology of the chromosomes indicated their alloployploid origin (Raina and Khoshoo, 1971a). Detailed analysis of Karyotype and meiosis of Indian species is essential to understand the origin of tetraploid Crinum species.

Populations of Crinum pratense growing on rocky lateritic plateaus at higher altitudes showed B-chromosomes ranging between 1-5. Accessary chromosomes rangig from 1-9 are reported in number of species of Crinum (Table-3). Assessary chromosomes ranging from 1-6 B in C. longifolium (Inariyama, 1937, 2-6 B in C. macowanii Wahlstrom and Laane, 1979). The present report of presence of 1- 5B chromosomes in Crinum pratense forms a first report for the species. The occurrence of B-chromosomes in populations growing in more xericplateaus at higher altitude indicate some role of these chromosomes in adaptability of the population to such habitat.

Meiotic studies in C. defixum revealed haploid chromosome number 2n = 11 for the species and also few peculiar events such as bridge configuration and laggards were observed.

Meiotic studies in C. latifolium also revealed the normal stages of meiosis, however certain events such

as grouping of some chromosomes and laggards were observed during diakinesis and metaphase I respectively. Similarly some meiocytes showed DNA starvation in addition to sexual means of reproduction.

Normal meiotic stages in tetraploid species of Crinum are suggestive of a stable nature of species. Certain events like laggards and chromosomal bridges with laggards indicate translocations in its chromosome compliment. Stages of meiosis were found to be very normal producing viable pollens. A cytokinesis was found to be of simulataneous type and pollen tetrads were mainly isobilateral. Prelijinary experimental work on interspecific hybridization showed that Crinum latifolium and tetraploid Crinum species cross with each other, similarly Crinum asiaficum and tetraploid Crinum species can be crossed. Crinum pratense and Crinum defixum also cross with each other. These observation indicate existance of loose interspecific incompatability. Even intergeneric crosses with related genus Amaryllis is possible (Sato, 1938). Cross between Crinum moorei and Amaryllis belladona is possible (Sato, 1938). Crinums are known to hybridize freely (Hannibal, 1962; Bailey, 1963) and this may be responsible for least part of the karyotype heterozygosity at deploid as well as at polyploid level ((Raina, 1978). Crosses between tetraploid and diploid plants of C. macowanii resulted in maternal seeds (Nordal et al, 1977; Wahlstrom and Laane,

1979). The occurence of sterile and semisterile hybrids in Crinum (Hannibal, 1962, 1964) is also indication of the chromosomal differentation within the genus (Raina and Khoshoo, 1971a)

Loose interspecific incompatibility, sexual sterility and means of vegetative propagation account not only for heterozygosity but also make problem of species recognition and delimitation more complicated in genus Crinum.