POLLEN MITOSIS STUDIES IN ORCHIDS WITH PARTICULAR REFERENCE TO CYMBIDIUM ALOIFOLIUM SW.

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KARYOTYPING of orchids is an important factor for elucidating taxonomic and phylogenetic relationships as well as in the breeding of better horticultural types, as has been emphasized by a number of workers.1,2

Root tip cytological studies of orchids have many difficulties especially in the epiphytic species; the aerial roots are bulky and possess on the outside mature or immature velamen which interfere with the proper penetration of the chemicals used for pre-treatment and fixation; failure to get full contraction of chromosomes at M1 leads to errors in counting them. Further chromosomes of most of the orchid species are small and fairly numerous and hence difficult to count with complete accuracy. Probably all these factors account for an array of the chromosome number reports in different species by various workers.3

A new technique has therefore been worked out for studying the orchid chromosomes through pollen mitosis which is most convenient for orchids as a large number of flowers are available at a time; further the post meiotic metaphases or pollen mitosis are very well synchronized and provide excellent opportunity for haploid chromosome counting.

The present study deals with the detailed pollen mitosis studies in one of the most common Cymbidium species from Western Ghats, India—C. aloifolium SW.

The pollinia were harvested 5–6 days before opening of the flower and were cultured in small petri dishes containing the medium made up of 5 g sucrose 1 g, Agar and 100 ml distilled water; the cultures were maintained at 25°C with R.H. 70–75%.

After 24 hr, a small portion of the cultured pollinia was taken and smeared in a drop of 1% Aceto-orcein. If the pollinia showed the right stage the remaining portion was immediately fixed in a mixture of 1:1:2 of 95% ethanol, chloroform and glacial acetic acid respectively and squashed and stained in 1% Aceto-orcein.

The nomenclature system of Leven et al4 was used for designating individual chromosomes except for SAT-chromosome in which the length of the satellite was not included in the arm length and centrometric position. The value of r was found by L/S arm ratio. Numbers (1, 2, 3 . . .) were assigned to the chromosomes aligned in descending order.

The haploid chromosome number n was 20 (figure 1) which confirms the earlier reports3 of 2n = 40.

The Karyotype was constructed on the basis of descending arm length and also on the basis of d value and r value.6 It was found that there were 5 chromosomes with Median region centromere type, 14 with sub median-Sm type and 1 with subterminal region centromere-Si type (No. 6) with a satellite (figure 2).

Earlier literature reveals that the species has already been investigated but chromosome number reports were varying5, and both n = 16 and n = 20 have been reported8,9. Further, out of 35 species of Cymbidium studied so far, in only two species, C. sikkimense and C. virescens, chromosome number has been reported to be n = 19, and 2n = 46 respectively3. Both reports are rather unusual as 2n = 40 is the constant number in the genus which as far as cytological data is concerned, represents a clear homogeneous assemblage in which structural rather numerical changes have played a decisive role in evolution2.

The present study clearly indicates the presence of 20 haploid chromosomes in pollen mitosis. Further, based on the distinctive morphology, chr. No. 1, chr. No. 20, and sat chromosomes No. 6 may be considered as good cytological markers for studying cytotoxic relationships of Cymbidium species.

Pollen mitosis studies not only help in karyotypic analysis of different species, due to haploid chromosome number but also help in the detection of any variation in chromosome number including the presence of B chromosomes, etc. Further, the effect of

Figure 1. Pollen mitosis in Cymbidium aloifolium arrow shows the SAT chromosome. × 1350.
certain chemical and physical mutagen agents can be detected even at very low doses through pollen mitosis studies.

In conclusion, it may be mentioned that pollen mitosis studies along with meiotic analysis and breeding behaviour form an excellent adjunct for understanding the mechanism underlying the cytogenetic evolution of different orchid species.

The author is grateful to Dr K. L. Chadha, Director, Indian Institute of Horticultural Research, Bangalore for facilities and encouragement.

22 March 1984; Revised 21 June 1984


THE EFFECTS OF ZINC SULPHATE ON THE ULTRAVIOLET LIGHT SENSITIVITY OF CHLORELLA VULGARIS BEIJERNICK.

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It has been known that zinc chloride enhances the toxic effect of gamma irradiation in the bacterium Bacillus megaterium. The present investigation was undertaken to study the effects of pretreatment by ZnSO₄ on the ultraviolet light sensitivity of Chlorella vulgaris.

The alga, collected from a fresh water pond situated at Sarnath, Varanasi, was maintained on agar plates containing Bold's Basal medium at 22° ± 1°C and illuminated at 2 k lux light intensity from day light fluorescent tubes for 16 hr a day. Irradiation was done with UV light (Philips germicidal lamp) giving main output at 2537 Å with a dose rate of 32 ergs/mm²/sec at a distance of 30 cm for 10, 15 and 20 min. During irradiation the alga was constantly stirred on a magnetic stirrer. Equal amounts of irradiated material were pipetted out and plated on agar plates containing 20 ml of sterilized Bold's Basal medium solidified by 1% agar. The plates containing the irradiated samples were kept in the dark for 24 hr to avoid photoreactivation. They were later exposed to light and transferred to culture chamber. In another series of experiments, the alga was pretreated with different concentrations of ZnSO₄ ranging between 2 to 100 ppm for 60 min and then irradiated as above.

Table 1 shows that ZnSO₄ alone at the concentration of 2 and 5 ppm has no effect on the percentage