Material and Methods

MATERIALS AND METHODS :

Aponogeton satarensis is found on plateaus of Western Ghats in Satara district while **Wiesneria triandra** is found in Konkan area of Maharashtra. Both the species are rare aquatic herbs restricted in their distribution. Materials of both the species were collected during rainy season from July to September. Plants were collected both in vegetative and reproductive stages. Plants and tubers of **Aponogeton satarensis** were collected mainly from Kas-Plateau in Satara district while plant materials of **Wiesneria triandra** were collected from various localities of Konkan viz. Dapoli, Deogad, Malwan, Ratnagiri and Vengurla.

Living plants of **Wiesneria triandra** and **Aponogeton satarensis** collected during July from Konkan area and Kas-Plateau respectively and then planted in water tubs containing some soil. The plants showed good growth and produced flowers and fruits under artificial irrigation condition. Both the species are maintained in Botanical Garden of the Department.

Morphological characters of each species were studied both in the field and under cultivation. At least 25 plants from each locality were analyzed for each morphological character. Sketches of plants were made from fresh plant materials. To determine the variations in floral characters, about 2000 flowers from about 100 plants were analyzed for **Wiesneria triandra.** Mean of all quantitative characters with standered deviation is given in the text. **Aponogeton satarensis** does not show variation in floral characters and therefore only range of quantitative characters of flower is given in the text.

During karyomorphological studies on **Wiesneria triandra**, healthy excised root tips of water cultured plants were pretreated with saturated solution of Para-dichlorobenzen (PDB) for 3 hrs. at 8°C. The root tips were hydrolyzed in hydrochloric acid and squashed in 2% aceto-orcin solution which gave satisfactory results. For meiotic studies, young inflorescences were fixed in Cornoy's fluid (3 parts of absolute alcohol + 1 part of glacial acetic acid) for 1 hour and then washed well in water. The anthers were squashed in 2% aceto-orcin. the slides were made permanent after passing through usual acetic acid-Butanol grades. DPX was used as a mounting medium. For classification of Karyotype asymmetry the scheme of Stebbins (1971) was used and the nomenclature recommended by Levan et al. (1964) for centromeric position was adopted.

For palynoligical studies, pollen grains of both the species were collected in acetic acid. These pollen samples were used for acetolysis. The acetolysis method for pollen preparation as given by Erdtman (1952) and modified by Nair (1966) was followed. Scanning electron microscop of pollen grains was made with the help of palynology laboratory, Wadia Institute of Himalayan Geology, Dehradun. SEM photomicrographs of pollen grains were taken on philips 515 SEM microscope. SEM photographs of **Aponogeton satarensis** were also made available from J.Bogner, München, Germany. For pollen size, at least 100 pollen grains were measured for each species and at least 1000 pollens were analyzed for pollen fertility.

For anatomical studies of various plant parts, conventional technique of fixation, dehydration, infiltration, embeding, sectioning

and staining as given by Johansen (1940) was followed. The healthy materials were fixed in FAA (5 cc Formaline + 5 cc Acetic acid + 90 cc of 70% alcohol). Sometimes fresh materials were also used for hand cut sectioning. Anatomical characters of various plant parts were studied by microtome sections as well as handcut sections. Aqueous saffranin and fast green in clove oil was found to be most suitable stain for both the species. For Floral anatomy of **Wiesneria triandra,** usual paraffin method was followed. Due to aquatic habit and great reduction in conducting tissue, it was difficult to trace the traces to floral organs, however, staining with aqueous crystal violet with erythrosin B in rectified spirit as a counter stain was found to be very satisfactory in study of Floral anatomy. Similarly clearing with lactic acid or KOH was found to be very useful for both the species in study of sclereids, distribution of tannin cells, vascular supply to various parts of flower and leaf venation.

For cuticular studies, peels of both fresh and preserved leaves were used. The peels were taken from various parts of the leaves. Fresh peels were taken under water. They were dehydrated, stained with saffranin and fast green and made permanent by passing through usual grades of Alcohol-xylol. It was sometimes difficult to obtain peels of leaves. Treatment with 10% KOH facilitated the peeling process. Peels both from fresh materials and preserved materials were used to determine stomatal size, stomatal density and stomatal index. At least 50 readings were taken for each stomatal parameter and mean of all these readings with standered deviation is represented in the text. Stomatal index was determined by following formula.

$$\text{Stomatla Index (SI)} = \frac{S}{S + E} \times 100$$

Where S = Number of stomata per unit area (mm^{-2})

E = Number of epidermal cells per unit area (mm^{-2})

To study the changes in anatomical character of fruit wall of **Wiesneria triandra**, seeds of the species right from zygot to mature embryo stage were fixed in FAA. They were sectioned by usual paraffin method and stained with saffranin and fast green which gave satisfactory results. To study fruit, wall elements, Jeffrey's macerating fluid (Jaffrey 1928) was used which gave better separation of cells.

For embryological studies, inflorescences at various growth stages were fixed in morning before 10 A.M. or in evening after 4 A.M. in FAA (5 cc Formaline + 5 cc Acetic acid + 90 cc of 50% or 70% ethyl alcohol). Similarly post-fertilization stages were fixed in same fluid. They were stored in same solution at laboratary conditions. The materials were dehydrated through usual grades of alcohol, alcohol xylol and infiltrated with 52 - 54°C or 54 - 56°C paraffin wax. The sections were cut at 8-20 μm thickness on Rotary microtome. Egg albumen was used as adhesive. After deparaffining and hydration, the slides were stained with either Delafied's haematoxylene or aqueous saffranin and fast green in clove oil. The slides were stained in Haematoxylene for 10 - 30 minutes and stain differentiation was done with acidified water. It was found that saffranin - fast green combination works very well for microsporogenesis, megasporogenesis and

embryo development in **Wiesneria triandra**. Material of **Aponogeton satarensis** was found to be difficult to stain with many stains, however Delafied's haematoxylene gave satisfactory results. The slides were mounted in DEX.

To study stages of embryo developments, the fertilized young ovaries at various stages of developments, were cleared in Lactic acid. Then the ovaries and ovules were dissected under stereoscopic binocular microscope. The embryos were stained with cotton blue in lactophenol.

Camera lucida Drawings of anatomical and embryological structures were made by using Hamburge microscope and Eram's camera lucida at suitable magnifications. Photomicrography were taken by using MFAK's system of JENEVAL Carlzeiss microscope. Photographs of plants were taken by using Asia Pentax Camera. NP 55 black-white film and coloured film of Kodax or Konica was used for general outdoor photography and microphotography.

All the slides prepared during present investigation and the voucher specimens are deposited in the Botany department of the University.