Materials and Methods

Different species of Dipcadi Medik were collected from various localities of Maharashtra as given in Table 2 and Map - I. D. saxorum was collected from Kanericave Borivali which is its type locality. D. ursulae was collected from Panchagani plateau, Mahabaleshwar which is its type locality and also from Kas plateau, a place in vicinity of Mahabaleshwar. D. montanum was collected from various plateau's viz. Masai (Panhala), Kas (Satara), Panchagani (Mahabaleshwar) and Kartikiswami (Pusegaon). D.concanense was collected from Ratnagiri, Deogad and Malwan area of Konkan. Plants were collected in vegetative, flowering and fruiting stages during June to September. Observations on morphological character, phenology, flowering and fruiting of different species were carefully noted in the field. Minimum 100 bulbs were collected from each locality. After careful study on morphological characters, plants were planted in earthern pots and plots in botanical garden of the department.

For morphological studies at least 25 randomly selected plants were analysed. Minimum 25 reading were taken for each character and the mean of all reading was computed with standard deviation. Morphological attributes of plants grown in botanical garden and from

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natural habitats were studied critically and the changes in quantitative characters have been noted. The changes in quantitative characters are shown polygraphically in Text Fig. I fig. 1-6.

The karyotypic studies of Dipcadi species have been performed from excised healthy root tips of water cultured bulbs after pretreatment with aqueous saturated solution of para-dichloro-benzene and then by applying normal aceto-orcein technique. Minimum 10 and upto 25 bulbs from each locality were used for karyotypic studies. Minimum 20 mitotic plates were analyzed for each species. 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.

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

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

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

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For meiotic studies young flower buds were fixed in Acetic-alcohole (1:3). The anthers were squashed in 2% aceto-orcine after hydrolysis in 1N HCL.

Photomicrographs were taken from temporary and permanent preparations using MFAKs system of JENAVAL Carl. Zeiss microscope. The cytological preparations were made permanent by using usual grades of acetic acid and n-Butonol.

Shape and size of at least 25 hydrated pollen grains for each species was determined accurately. For determination of % of fertile pollen, pollen grains were stained in 1% aceto-orecin solution and minimum 1000 pollen grains for each species were analysed. The pollen grains with distinctly stained generative and vegetative nucleus were taken as fertile pollens. To study wall ornamentation acetolysis method described by Erdtman (1952) and modified by Nair (1966) was tried, however, it was found difficult to get entire pollens even with varying time of acetolysis. Acetolysis badly affects pollen morphology and pollen characters could not be studied satisfactorily.

For anatomical studies of various plant parts usual technique of fixation, dehydration, infiltration, embeding, sectioning and staining was followed (Johansen, 1940). Sectioning was done at various thickness ranging from

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5-15 mu. Section were stained with aqueous safranin and fast green in clove oil, which gave satisfactory staining. Staining with crystal violet in water and erythrosin B in rectified spirit gave better differentation of tissues especially xylem elements. For staining with crystal violet and erythrosin B, section were brought to water passing through usual grades of xylol alcohole, stained in aqueous crystal violet for 10-15 minutes, passed rapidly through alcohole grades and stained with and erythrosin B in rectified spirit and brought to xylol and then mounted in DPX.

For cuticular studies peels of fresh as well as preserved leaves were used. To keep accuracy only middle portions of leaves of each species were used. Fresh peels taken under water were made permanent after staining with safranin or fast green or delafied's Hematoxylene and passing through usual - alcohole - xylol grades. Ocassionally KOH treatment was used to get entire peel of leaf piece. Leaf pieces of suitable size were treated with 10% KOH solution for 1-3 hours which facilitated easy removal of cuticle. Peels mounted in 10% glycerine solution were used to determine size of epidermal cells and stomata. For stomatal density, minimum 50 reading from different regions of peel were taken from both upper and lower epidermis. Stomatal index was determined by the following formula.

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

Where,

SI = Stomatal index,

S = Number of Stomata mm^{-2}

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

Anatomy of leaf was studied by both hand cut and microtomcut sections. Many times in microtome sections the epidermis get separated and therefore, hand cut sections were also used to study anatomical details of leaf. For comparative study the sections of middle portions of leaves of each species were studied. Leaf thickness was measured at base, middle and apical portion of leaf with Mitutoyo's thickness meter. Leaf sections were double stained either with fast green and aqueous safranin or crystal violet and erythrosin B. The latter staining was found to be more suitable for differentation of tissues. For every measurable anatomical character at least 25 observations were taken and the mean with standard deviation is presented in the text.

For embryological studies young inflorescence, buds, flowers and fruits of various growth stages of D.concanense were fixed in FAA(50% or 70% alcohole 90 ml + 5 ml glacial acetic acid + 5 ml neutral formaline) in morning hours between 8-10 a.m. as well as in evening after 4 p.m. The material

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was preserved in same solution in specimen bottles at laboratory conditions. The selected young inflorescence, buds and fruits were passed through usual alcohole-xylol grades and infiltrated with 52-54 or 58-60° C paraffin wax (Johansen, 1940). Section were cut at 8 to 20 mu thickness on rotary microtome. The fresh egg albumen was used as adhesive. After dewaxing slides were brought to water by passing through normal grades of xylol - alcohole. The slides were stained with Delafied's hematoxylene for 10-30 minutes. Differentiation of stain was done with acidified water. The slides were passed through usual grades of xylol - alcohole series and made permanent using DPX as a mounting medium. Delafied's hematoxylin gave satisfactory staining.

Some of the embryological slides of microsporogensis and megasporogensis were processed to localize total carbohydrates by periodic acid schiff's (PAS) reaction (Jensen,1958). These sections of FAA fixed material were deparaffinized, hydrated and brought to water. The slides were placed in 0.5% periodic acid in distilled water at room temperature for 10 minutes. The slides were stained in schiff's reagent for 15 minutes at room temperature. The slides were rinsed in water and treated with 2% sodium bisulphite for 1-2 minutes. The slides were dehydrated by passing through usual grades of alcohole series, cleared in xylol and mounted in

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DPX. Carbohydrate take an intense purplish red color.

Total insoluble proteins were localised by Mercury bromophenol blue reaction (Bonhag, 1955 - modified form Mazia, et al. 1953). F.A.A. fixed materials were deparaffinised and brought to water. They were kept in Mercury bromophenol blue for 5 minutes, followed by rinsing in 0.5% acetic acid for 5 minutes. The sections were then transfered directly into tertiary butyl alcohole for two hours. The slides were cleared in xylol and mounted in DPX. Proteins take a deep clear blue color. Controls were not tried.

The young seeds of different growth stages were treated with 10% KOH solution for different time intervals. The seeds were washed wells in water and stored in 70% alcohole. Then the young embryos were dissected either under dissecting microscope or zoom - binocular, stained with 1% Aniline blue and mounted in Lactophenol. The slides were made semipermanent by sealing with paraffin wax.

Drawings of anatomical and embryological structures were made by using Hamburg microscope and Erama's camera lucida at suitable magnification. Photomicrograph's were taken by using MFAK's systerm of JENAVAL Carlzeiss microscope. Photographes of plants, and plant parts were taken by Asia Pentax camera using NP = 55 black-white film and film of Kodak or Konica company.

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TABLE NO.2

SHOWING TIME AND PLACES OF COLLECTION OF DIFFERENT SPECIES OF DIPCADI MEDIK IN MAHARASHTRA

Name of species	Locality	Chromosome No.	Time of collection
1. Dipcadi concanense (Dalz) Bak.	Deogad, Ratnagiri, Malwan.	2n=12	July,1989 August,1990.
2. Dipcadi saxorum Blatt.	Kanericave	2n=12	Sept.1990
3. Dipcadi montanum Bak.	Panhala, Kas, Mahabaleshwar, Kartikiswami	2n=20	August,1989 July,1990.
4. D.ursulae Blatt.	Mahabaleshwar, Kas	2n=20	June, 1989
5. D.maharashtrensis (A form with coriaceous bract)	Mahabaleshwar, Kas	2n=20	August,1989.
6. D.ursulae Blatt. (A form with broad leaves and coriaceous bract,large bulb)	Kas, Mahabaleshwar	2n=20	July, 1989
7. D.montanum Bak. (A form with narrow leaves).	Panhala, Kas	2n=20	June,1989 June, 1990.