MATERIALS AND METHODS

Different species of Urginea Steinh. were collected from various localities in Maharashtra (Table 3). Plants were collected in vegetative as well as flowering and fruiting stages through frequent visits to the places of collection. Observations on vegetative growth, phenology, blooming of flowers and fruiting were carefully noted in field. Minimum 100 bulbs from each locality were brought to laboratory and after morphological studies the bulbs were planted in labelled earthen pots as well as in plots of experimental garden. Different species were grown under natural condition without artificial irrigation except in few cases. Careful observations were kept on vegetative growth, time of flowering, anthesis, pollination, fruiting, sprouting of bulbs etc. of the plants grown in experimental plots and earthen pots and noted from time to time.

For morphological studies plants both from natural habitats and cultivated plots were used. For each morphological character at least 25 randomly selected plants were used and characters were studied critically. Minimum 25 reading were taken for each character and the mean of all readings was computed with standard deviation.

To determine cytological status of each species, excised root tips of water cultured bulbs were pretreated with saturated solution of Para-dichlorobenzene (PDB) for 3 hrs at 8°C and then kept in same solution for 1-3 hrs at room temperature. Then root tips were hydrolysed in 1 N hydrochloric acid at 60°C in

oven for 10-15 minutes and then squashed in 2% aceto-orcein solution which gave satisfactory results. The slides were made permanent after passing through usual Butanol-acetic acid grades and were mounted either in DPX or euparol.

For palynological studies, pollen grains of different species were directly dusted on slides. Shape and size of at least 25 pollen grains for each species was determined. These pollen grains are designated as unhydrated pollens in the text. For pollen size, frequency classes, and pollen fertility, pollen grains were stained in 1% aceto-orcein solution and then size of minimum 1000 pollen grains for each species was determined accurately. To determine pollen fertility, more than 1000 pollen grains for each species from each locality were observed. The pollen grains with distinctly stained generative cell and vegetative nucleus were taken as fertile pollens. To study wall ornamentation acetolysis method described by Erdtman (1952) and modified by Nair (1966) was used. Semipermanent slides of acetolysed pollen grains were prepared by using glycerine jelly and slides were sealed with paraffin wax.

For anatomical studies of various plant parts conventional techniques of fixation, dehydration, sectioning and staining were followed. Size of epidermal cells of scape was measured by taking peel of the scape. To determine fibre length pieces of scape 2-4 mm in thickness were placed in Jefrey's fluid and kept at 60°C in oven for 1-3 hrs. After maceration the pieces were

washed well in water and then fibres were made free on slide with the help of needle and mounted in 10% glycerin solution. Slides were sealed with paraffin wax and made semipermanent.

For cuticular studies peels of fresh as well as preserved leaves were used. To keep constancy only middle portions of leaf of each species were studied. Fresh peels taken under water, were made permanent after staining with safranin or Delafied's hematoxylin and passing through usual alcohol-xylol grades. Sometimes 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 stomatal size, length and breadth of epidermal cells. For stomatal density minimum 50 readings from different region of peel were taken from lower and upper epidermis. 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 (mm^{-2}).

Leaf anatomy was studied by both handcut and microtome cut sections. The leaf sections were cut both at right angle to long axis of leaf as well as parallel to long axis of leaf. In

microtome sections while passing through grades the epidermis get separated and therefore, handcut sections were also used to study leaf anatomy. To keep constancy for all species the sections of middle region of leaf were studied. Leaf thickness was measured at base, middle and apical portion of leaf with the help of Mitutoyo's thickness meter. For every anatomical character, at least 25 observations were taken and the mean with standard deviation is represented in the text.

For embryological studies young inflorescences, buds, flowers and fruits of different growth stages were fixed in FAA (50% alcohol 90 ml + 5 ml acetic acid + 5 ml formaline) in morning between 8-10 A.M. as well as in evening after 4 p.m. The material was preserved in same solution in specimen bottles at laboratory conditions. The selected buds were passed through usual grades of dehydration, alcohol-xylol grades and infiltrated with 52-54°C paraffin wax. The sections were cut at 8 to 20 µm thickness on Rotary microtome. The egg albumen was used as adhesive. After dewaxing and dehydration sections were stained in Delafied's haematoxylene for 10-30 minutes and then stain differentiation was done with acidified water. The slides were passed through usual grades of alcohol and xylol series and made permanent using DPX as a mounting medium. Double stained preparations were tried with safranin and fast green, however, Delafied's haematoxylene was found to be most satisfactory.

Nuclear cycle of female gametophyte was studied by maceration technique (Langer and Koul, 1981). The ovules from

ovaries of various sizes were excised and hydrolyzed in 1 N. HCl. After hydrolysis, ovules were washed with water and then squashed in 2% aceto-orcin. Slides were made permanent by using acetic acid and butanol grades.

For entire embryo-mounting young seeds of different growth stages were treated with 10% KOH solution for different time intervals, then the seeds were washed throughly in water and stored in 70% alcohol. Then young embryos were dissected either under dissecting microscope or Zoom-binocular, stained with 1% Aniline blue and mounted in lactophenol. The slides were sealed with paraffin wax.

Drawings of anatomical and embryological structures were made by using Hamburg microscope and Erma's camera lucida at suitable magnifications. Photomicrographs were taken by using MFAK's system of Jenaval Carlzeiss microscope. Photographs of plants, plants parts were taken with Asai Pentax camera by using NP55-ve film.

Table 3 : Showing time and place of collection and cytological status of Urginea species.

Sr. No.	Name of the species	Locality	Chromosome Number	Time of collection
1.	Urginea congesta	Singnapur(Satara)	2n = 20	October 1985
		Piliv (Satara)	2n = 20	October 1985
		Kartikiswami (Satara)	2n = 20	October 1986 & April 1987
2.	Urginea razii	Dive-Ghat (Poona)	2n = 20	April 1986
				October 1987
3.	Urginea polyantha	Kolhapur	2n=20, 2n=20+1B	1985 - 1988
		Poona	2n = 20	April 1985
		Panchagani(Satara)	2n = 20	October 1983
		Mavashi Plateau (Satara)	2n = 20	September 1986
		Panhala (Kolhapur)	2n = 20	September 1986
		Kartikiswami(Satara)	2n = 20	October 1986
		Yelgaon (Satara)	2n = 20	March 1986
4.	Urginea indica (2n = 20)	Vajreshwari(Thane)	2n = 20	March 1984
		Malvan (Ratnagiri)	2n = 20	March 1986
		Ganapatipule (Ratnagiri)	2n=20 + 2B	March 1986
		Vengurla(Ratnagiri)	2n = 20	April 1988
		Araunda (Ratnagiri)	2n = 20	April 1987 & 1988
5.	Urginea indica (2n = 30)	Alibag (Thana)	2n = 30	April 1983
		Vengurla (Ratnagiri)	2n = 30	April 1988
		Ganapatipule (Ratnagiri)	2n = 30	March 1986
		Goa	2n = 30	March 1982
		Malvan (Ratnagiri)	2n = 30	December 1986

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Table 1 : (Contd...)

Sr. No.	Name of the species	Locality	Chromosome Number	Time of collection	
6.	Urginea indica (2n = 40)	Kagal (Kolhapur)	2n = 40	May	1986
		Singnapur (Satara)	2n = 40	October	1985
		Aurangabad	2n = 40	April	1984
		Dive-Ghat (Poona)	2n = 40	October	1986
		Piliv (Satara)	2n = 40	October	1986
7.	Urginea govindappae	Banglore	2n = 20	July	1987