

## **Introduction**

The family Liliaceae Economically ranks very high as it comprises hundreds of species and varieties that man uses for different purposes. Most of the plants are used as ornamentals. Some are as food and fibre whereas others have medicinal use.

The genus Urginea steinh one of the extremely interesting polytypic genera of Liliaceae is represented by 100 species (Airy shaw, 1966). This perennial bulbous plant grows on sandy coasts of the countries bordering the mediterranean sea (Spain, France, Italy, Sicily, Malta, Greece, Algeria and Morocco), Europe, India, Tropical and South Africa.

It is commonly called as squill which has an ancient and more or less honourable history as a medicinal plant. From the 16th century B. C. the Papyrus Ebers reported it's use for heart diseases. Detailed study of it's history was made by Muller (1931). Early physicians used these bulbs and their extract in treating hydropsy, urine retention and pneumonia (Stoll, 1936). Thus squill bulb represents drug which has been known to mankind since antiquity.

There are two common varieties of squill. One with white or yellowish scales cultivated in Malta, Sicily, Italy, commonly called as white squill (Urginea maritima). Another with red scales, cultivated in Algeria. The white squill (U. maritima) contains two glycosides crystalline scillaren B

and an amorphous complex constituent scillaren B (Stoll et al., 1933). Both are active glycosides. This plant has diuretic properties and digitalis like action on heart. It exerts nausea and vomiting. Various squill preparation now available in market are in the form of squill oxymal, squill syrup, squill ~~tincture~~, squill vinegar and sedatassin, a cough syrup.

Red squill, a variety (U. maritima) contains cardiotonic glucosides and squilliroside. The latter is very toxic to rats and is incorporated in rat paste especially in Europe and north America (Crabtree 1947). Urginea altissima native to tropical and South Africa, is considered dangerous to live stock. Urginea brachystachys is used to make arrow poison. The decoction of U. burkei is used to induce abortion in South Africa (Lewis 1977).

In India genus is mainly represented by Urginea indica (Indian squill or true squill) which is commonly wide spread in sandy places near sea throughout India. It also grows in drier hills of lower Himalayas, Western ghats. It is tunicated bulb commonly called as Kolkanda, Rankanda, Jangli piyaz etc. It contains cardiac glycosides similar to those of U. maritima and possesses antiprotozoal, hypoglycemic and anticancer properties (Dhar et al., 1968). It's bulb powder is mucilaginous in nature and is used extensively to check skin diseases (Malhotra and Moorthy 1973). The powder has good adhesive properties and its 3% solution

in water can be used as paper paste. This paste is used in calicoprinting, as a thickening agent for colours to be used in screen printing of textiles. The sizing gum in bulbs used in textile industries (Seth, 1949).

Recently it is proposed that European squill could be substituted by Indian squill to overcome shortage of European squill. If its cultivation and methods of harvesting are improved it will compete with European squill. During last few years there is steady increase in export trade nearly 21,805 kg. of U. indica plants were exported during the year 1967-68 (Wealth of India, Raw materials, IX 256, 1972). At present pharmaceutical company from Bombay (Boehlinger-Knoll Ltd) is collecting these plants and exporting the crude extract to West Germany.

The glycosides are not manufactured in India. Only M/S Sandoz Ltd. of Switzerland prepares injections, tablets of squill glycosides. One cc injection of "Scillaren" containing 0.5 mg. glycoside priced at Rs.1. At this rate 1 g of it would make 250 Rupees.

The available literature indicates very little physiological work has been done on Indian squill. Most of the papers deal with cytology of U. indica. (Raghavan 1935, Raghavan and Venkatasubban 1940, a, b, Zaman 1978). Sabramanian (1978) has found nine different cytotypes, Naik (1974) reported tetraploid, Sen (1980) reported

hexaploid. Thus U. indica exhibits high degree of multiformity.

The ploidy is known in many economical crop, fibre and medicinal plants. These polyploids providing food (cloathing and medicines to large populations in the world. The cytological investigations have shown U. indica also exhibit polyploidy (Naik, 1974; Subramaniam, 1978; Sen 1980). The polyploids are supposed to be more productive, show vigorous growth and wide adaptability and therefore it is gift to mankind to cultivate them to get maximum production, and it proved true in wheat, cotton, tobacco etc. The man is studying these natural polyploids and trying for inducing the ploidy in other economical plants. (Karpechenko 1927). But he couldnot master that perfection which nature could attain in its own laboratory. The success of artificial evolution of polyploids mainly depends upon basic understanding of cytogenetics, information regarding physiological parameters and environmental conditions.

In the present investigation, therefore, different cytotypes of U. indica are studied cytologically, anatomically and physiologically.

The present work therefore comprises four parts.

- 1) Karyotypic study of different cytotypes of U. indica growing at different localities.

(Kolhapur, Goa, Aurangabad).

- 2) Anatomy of the leaves in different cytotypes.
- 3) Organic and inorganic constituents of different cytotypes and inorganic constituents of soil where these cytotypes are inhabited.
- 4) Stomatal studies and  $^{14}\text{CO}_2$  incorporation in different cytotypes.

The available relevant literature on the subject has been reviewed under the title "Review of literature" at the beginning and followed by morphological description of studied cytotypes under heading "Taxonomy of Urginea indica kunth".



## **Review of Literature**

### Review of Literature

The family Liliaceae is considered to be suitable material for cytological studies. The available literature showed that Taylor, (1925); ~~Fraser~~ and Snell, (1926); Newton, (1927); Raghavan, (1935); Jones and Smith (1967-68) have studied the number and morphology of the chromosomes of most common Liliaceous genera.

Majority of the papers are dealing with the chromosome number reports of Urginea indica kunth (Raghavan, 1935; Capoor, 1937; Hari Kishore, 1951; Battaglia, 1957 a; Miege, 1960; Love, 1964; and Zaman and Khaleque, 1978). The first chromosome analysis of U. indica showed the presence of diasomic ( $2n=20$ ) and a triploid ( $3n=30$ ) cytotype in India (Raghavan, 1935). He studied in detail the chromosome morphology and characteristic nuclear behaviour in this species. From comparative study of diploid and triploid cytotype a positive correlation between gigas characters and chromosome number has been confirmed. Zaman and Khaleque (1978) have studied karyotype of U. indica in detail and showed that all the chromosomes are acrocentric and no SAT chromosome has been found.

Chromosomal variability in U. indica has been reported by Moorthy and Sampat kumar (1968) and found haploid ( $n=10$ ), triploid ( $3n=30$ ), tetraploid ( $4n=40$ ) and octoploid ( $8n=80$ ) cells in addition to normal diploid



( $2n=20$ ) cells in somatic tissue. Nine different cytotypes distinctive in their morphological characters like bulb, shape and size of leaf, inflorescence and flowers were recognized by Subramanian (1978) on the basis of their cytological details.

The B chromosomes in the genus Urginea steinh. have been reported by several authors like Raghavan and Venkatasubban, (1940 a); Martinoli, (1949); De wet (1957); Battaglia, (1957 b, 1958 and 1964); Battaglia and Gaunti, (1968); Ayyangar, (1969) and Sen (1974). The cytological literature on the genus Urginea has been reviewed for occurrence of B chromosomes recognized for the following species.

Name	Chromosome number.	Author
<u>aurantia</u> Ca <u>Linoberg</u>	$2n=20+1B$	Battaglia (1958)
<u>epigea</u> Dyer	$2n=30+2B's$	De Wet (1957)
<u>Faqax</u> var. Major	$2n=20+4B's$	Martinoli (1949)
" " "	$2n=20+6B's$	Battaglia (1964)
" " typica	$2n=20+1B's$	Martinoli (1949)
" " "	$2n=20+2B's$	Battaglia (1957 a)
" " "	$2n=20+0-8B's$	Battaglia <u>et al.</u> (1968)
<u>Indica</u> kunth	$2n=20+1-4B's$	Raghavan <u>et al.</u> (1940)

Name	Chromosome number.	Author
<u>Indica</u> kunth	2n=20+0-7B's	Ayyangar (1961)
" "	2n=20+6 and 7B's	Sen (1974)
<u>altissima</u> Bak.	2n=20 (Nelspruit, Africa)	de Wet (1957) Miege (1960 a) Jones and Smith (1967).
<u>aurantiaca</u>	2n=20, 21, 22 (Morocco, Italy)	Battaglia (1958).
<u>burkei</u> Bak.	2n=20 (Wartburg and Pretoria, Africa)	de Wet (1957)
<u>Coromandeliana</u>	2n=20	Datta (1966)
<u>weight</u>	4n=40 (India)	Naik (1978)
<u>depressa</u> (Bak)	2n=20 and 40 (Krugens drop)	de Wet (1957)
<u>epiquea</u> Dyer	2n=32 (Zulaland)	de Wet (1957)
<u>Fugax</u> (Moris)	2n=20, 21, 20+1-2B	Martinoli (1949)
<u>steinh</u>	2n=24 (sardinia, Italy)	Battaglia (1957 a)
<u>Fugax</u> (moris)	2n=20+6B	Battaglia (1964)
<u>Fugax</u> var. <u>typica</u> .	2n=20+0-8B	Battaglia and Gaunti (1968)
<u>gigantea</u> (Jacboye wde)	2n=22 (W. Africa)	Oyewole (1975)



Name	Chromosome number.	Author.
<u>govindappae</u> Boraiah and Fathima	2n=20 (India)	Boraiah and Fathima (1970)
<u>indica</u>	2n=20 (India)	Raghavan (1935) capoor 37 Harikishore 51. Battaglia 57 a, Miege 1960 a Zaman and Khaleque 78.
	2n=30 (India)	Raghavan 35, Miege 1968.
<u>hydenburgensis</u> Dyer	2n=30+2B's	de Wet (1957)
<u>rubella</u> Baker	2n=40+2B's	de Wet (1957)

Sen (1974) has reported a tetraploid devoid of B chromosome while studying the nature and behaviour of B chromosome in Urq~~idea~~ indica.

### Polyploid

The natural tetraploid of U. coromandeliana was reported by Naik (1973) on the basis of it's karyomorphology and meiosis . Naik (1976) has concluded that U. coromandeliana is a cytodome or more over an autotetraploid. of U. indica. Jha and Sen (1980) reported hexaploid in U. indica with 6 pairs with secondary constriction.

Various degrees of polyploidy and geographical

distribution of polyploids of U. maritima (L) Baker have been reported by Battaglia, (1957, 64 a); Moginia and Maleci, (1974). Diploid biotype ( $2n=20$ ) from Sicily and Calubria, N. Campania, Puglia and sardini, triploid biotype ( $3n=20$ ) from islands of Elba, tetraploid biotype ( $2n=40$ ) from the islands of Monterriasto, Giannutri, S. Lario, and Puglie, Pentaploid biotype ( $5n=50$ ), from Tur KGB, and hexaploid biotype from the islands of Gidnutri were reported by Maugini (1953, 56, 60, and 74) on the Italian peninsula.

It has been <sup>thought</sup> worthwhile to review the literature on chromosome number of various species of the genus Urginea which reveals the ~~ex~~stence of basic chromosome number as 5, 7, 8, and 10. The list is given below.

Name	Chromosome number and source.	Author.
<u>Indica</u>	$2n=30$ (Africa)	Marvey (1966)
	$2n=40$	Sato (1934)
	$2n=40$	Sumitra sen (1974)
<u>langii</u>	$2n=20$ Hammanskraal and Zoutpansberg.	de Wet.
<u>Lydenburgensis</u>	$2n=32$ (Skukurn)	de Wet. (1957)
<u>Macranthum</u> Wr.	$2n=20$	de Wet (1957)

Name	Chromosome number and source.	Author.
<u>Maritima</u> Bak	2n=20, Gr.	Heitz (1926)
	2n=10+rB (12)	Geitner (1929)
	2n=20+1-4B, 30	Raghavan and Venkatasubban. (1940)
	2n=20, 30, 40. Italy	Guiffrida (1950)
	2n=20 Italy	Maugini (1953, 66)
	2n=20, 30, 40, 60 Italy sicily.	Battaglia (1957)
	2n=40	Sato (34)
	2n=40	Martinoli (49)
	2n=40 Israael	Larsen (1960 b)
	2n=40 Israael	Waisel (1962)
	2n=50 Italy	Maugini (1974)
<u>Maura</u> Maire	2n=20 Italy	Battaglia (1957 d)
<u>Maurelii</u> Batt. et. Trab.	2n=54	Neves (1958)
<u>Mulli</u> setosa Bak.	2n=20 pretoria S. Africa.	de Wet (1957)
<u>nigritiana</u> Bak.	2n=20	Miege (1960 b)
<u>Polyantha</u> Blatt	2n=20 (India)	Blatter and McCunn. (1928)
<u>Polyphylla</u> H.K.F.	2n=20 (India)	Raghavan (1940)
<u>pretoriensis</u> Bak.	2n=20 S. Africa	de Wet (1957)
<u>Razii</u> Ansari sp. Nor.	2n=20 (India)	Ansari (1958)

Patil (1981) studied seven different cytotypes (4 diploids, 2 triploids and 1 tetraploid) of U. indica isolated from various collections along the south west coast of India (Alibag to Karwar). With the help of O-banding technique he has shown that there are 3 different groups of cytotypes, where diploids, triploids and tetraploids have separate entities.

### Anatomy

Kambale and Ansari (1977) studied anatomy of leaves and scape of some species of genus Urginea steinh. According to them anatomy of leaf and scape is simple but it is helpful to distinguish different species of the genus Urginea.

### Phytochemistry

De. P. (1927) observed the pharmacological action of scillaren. Stehel et al. (1931) had worked on the action of scillaren B<sub>1</sub> from squill upon heart and blood vessels. Chemical examination of squill has been made by sheshadri and Subramanian (1950) whereas enzymatic hydrolysis was first studied by Stoll and Remz (1950). Chemistry of cardiac glycosides was studied by Reichstein (1951) Rossi (1952), Stoll and Keris (1952).

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Rangaswami and Subramanian (1954) was the first to report an isolation of crystalline glycosides from commercial

Indian squill admixed with Scilla indica. They have extended their studies further and extracted crystalline glycosides.

Scillaren A<sub>2</sub> was isolated by Rangaswami and Subramanian (1955,1958). Chopra et al. (1958) isolated drug from U. indica and named it as Chopra's indigenous drug of India

Waltberg (1964) extracted Scillarenin B glycosides from red squill. El-Keiy et al. (1964, 1965 and 1967) have done pharmacognostic study of bulbs of Urginea species growing in Egypt. Vega et al. (1967, 1971, a, b) studied U. maritima and extracted cardiogenic D glucoside. Garcia Casado et al. (1977) extracted 'proscillaridin' from the squill bulbs.

However Rao, (1967); Dhar et al., (1968); Deri and Pharasi, (1974) studied U. indica for glycosides. Rao and Deri (1964-1965) extracted sterols from South Indian squill.

Vega (1963) studied anthocyanins of the squill. Detailed study of flavonoids from white and red squill has been made by Vega and Fernandez (1964,1969,1972 and 1976) Fernandez et al. (1972,1974,1975,1976 and 1977) studied flavonoides and glycosides from U. maritima.

Garcia- Jalon et al. (1973-1974) studied components of U. maritima particularly the anthocyanins. Patil (1981) has analysed the bulbs of U. indica in respect of organic and inorganic constituents.

**Taxonomy of**  
**Urginea indica Kunth**



The Liliaceae has a large alliance of petaloid monocots with very wide distribution embracing various climates and geographical zones but abundance in warm temperate and tropical regions of the world. Except some xerophytic representatives, members of Lily family do not form dominant or climax vegetation over areas of appreciable extent. The morphology, cytology and taxonomy of family have attracted attention of research workers.

### Taxonomic history of family Liliaceae

The superior nature of the ovary in Liliaceae has largely been taken to distinguish it from the Amaryllidaceae having inferior ovary. Bessey (1915), Diels (1930), and Wettstein (1935) consider that the Amaryllids are derivatives of the Liliales, further more, the Irids are supposed to be derived from Amaryllidaceous stock. Benth and Hooker (1880-83) have treated family Liliaceae under series coronariaceae, while Engler and Prantle (1892) in ~~his~~ <sup>their</sup> "syllabus der pflanzen families" considered it in the order Liliiflorae. Hutchinson (1934) has used the umbellate and often spatheaceous nature of the inflorescence to distinguish Amaryllidaceae from the Liliaceae. In such a process of distinction he transferred or isolated several taxa from Liliaceae, (Krause 1930 in Engler and Prantle) and its allies to the Amaryllidaceae or to new families, thereby reducing the size of Liliaceae considerably. According to Hutchinson (1959) Liliaceae group under the second division corolliferae and the order Liliales.

Though Hutchinson's view now a days are widely accepted, Cronquist (1968) does not separate the family Amaryllidaceae from Liliaceae because he believes that the attempt to substitute the form of inflorescence for the position of the ovary is not satisfactory and has submerged the Amaryllids in the Liliaceae.

Recently Cronquist (1968) and Takhtajan (1969) have classified the family as class Liliatae, subclass- Liliidae, order- Liliales, family- Liliaceae.

The order Liliales contain varying number of families according to different authors. Bessey (1915) treats Lilies, Palms, Aroids as a single group, Hutchinson (1959) includes 7 families under the Liliales while Cronquist (1968) and Takhtajan (1969) accomodate 13 and 20 families in their Liliales.

As family Liliaceae is one of the larger families of seedplants, it is subdivided into eleven sub families in "Genera plantarum" of Benthem and Hooker (1880-83) and earlier editions of Engler and Prantle (1887-1909). On the basis of Schnarf's work on embryology (1929), the scilleae has been separated (Krause in Engler plantle 1930) from the Lillioideae and placed in their own subfamily, the Scilloideae, thus making twelve subfamilies for Liliaceae. 8/

Further embryological researches by Wunderlich (1977) have substantiated this separation.

Twelve subfamilies of Liliaceae are- Melanthioideae, Hereroideae, Asphodeloideae, Alloideae, Lilioideae, Scilloideae, Dracaenoideae, Asparagoideae, Ophiopogonoideae, Aletridoideae, Luxuriatoideae and Smilacaceae.

Phylogenetically the family Liliaceae appears to have originated from the order like Ranles, Helobiales, Triaridales and Commelinales separately (Mitra 1955). It is supposed to be more significant from consideration of origin of the other monocotyledons. Thus the family Liliaceae is thought to represent basic stock for the evolution of many monocotyledonous families through reduction. (Deyel 1955, Kimura 1956, Hutchinson 1959, Cheadle and Tucher 1961, Eames 1961, Cronquist 1968 and Tahhtajan 1969).

#### Brief description of family Liliaceae

Mostly perennial herbs, rarely shrubs or small trees with fibrous roots or with creeping root stock or an underground bulbs, corm, tubers. Some are climbers and xerophytes. Leaves are simple alternate or whorled and sharp pointed, mostly with parallel veins, sometimes basal or cauline or radiate. In a few cases they are tendriferous, functionally replaced by cladodes. Inflorescence scape, racemose, paniculate, spicate, fascicled or umbellate, often few flowered, sometime axillary or terminal solitary flowered. Bracts are usually small and scarious or spathe like when flowers are in umbels. Flowers mostly bisexual, rarely, unisexual, actinomorphic or slightly zygomorphic; Perianths are generally sixparted (rarely 4, 8 or 10), corolla like,

arranged in two series, the segments some times united, inferior, usually imbricate, rarely valvate in buds. Stamens usually 6 rarely 3, 4 or more opposite to the perianth lobes. Filaments free, connate anthers oblong or linear, dorsifixed or versatile carpels three, united, ovary superior or seminferior, 3 or 1 celled with axile or parietal placentation respectively. Ovules one to many anatropous or hemianatropous, rarely orthotropous. Style one to three rarely 0, simple long or short, entire or divided rarely free. Fruit locullicioal capsule, rarely berry, seeds many, globose elongated or flattened, embryo straight or curved or surrounded by abundant copious and fleshy endosperm.

There is no unanimity as to the genera and species of the family Liliaceae. Krause (1930) includes 233 genera, Pool (1941) and Rendle (1959) give 200 genera and 2500 species, Lawrence (1959) gives 240 genera and 4000 species and Airy shaw (1966) give 250 genera and 3700 species of world wide distribution.

Economically the family ranks very high in valuable plants that man uses for many purposes. Hundreds of species and varieties are being used as ornamentals, including well known forms of Tulips, Lilies, Hyacinthus, Scilla etc.

Allium and Asparagus are minor food plants. Phoradendrum, Yucca and Sunseverine yield useful fibres. Similarly Urginea, Aloe, Colchicum, Verbetrum etc. are medicinal, Xanthorrhoea and Dragena yield resins, Chlorogalum is used as soap.

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Juice from most of the Aloe species have more or less purgative action. Colchicum autumnale contains important alkloid known as colchicine.

Sub family - Scilloideae.

### Morphology

Root stock a tunicated bulbs, leaves usually few and in a cluster at the base of the scapose raceme. Some times the tuft of the leaves at the top, Perianth segments free or rarely, united, anthers introse dorsifixed, ovary 3 celled, ovules many or few. Fruit loculicidal capsule, seeds globose, angular or compressed.

Genus Irginea Steinh - Fl. Brit. Ind. vi 147.

The word Irginea stems from the name of an Arabian tribe in Algeria. It's detailed early history has been given by Muller (1931). As the genus in different geographical zones of the world, it has got several names.

Fusifilum Rafin Fl. Tellur 1836, Montassa Salisb. 1866, Physodia salisb. 1836, Pilasia Rafin 1836, Squilla Steinh. 1838, Cypharissu salisb 1866, Tenicron Rafin 1836.

### Morphology

Herbs with tunicate bulbs, leaves radicle, simple, linear and entire. Inflorescence racemose, often appearing

before leaves. Flowers bisexual, pedicels short or long articulate, branch small, perianth petaloid, campanulate, segments six subequal, stamens 6 adnate or near base of perianth lobes. Filaments filiform or thick at base, anthers oblong or linear introse, ovary superior, sessile 3 celled, often trigonous style tapering towards the base, stigma capitate, ovules many in each cell, compressed, testa black, marginally winged, embryo rather large with fleshy endosperm.

The genus represented <sup>by</sup> about 100 species <sup>is</sup> distributed in South Europe, West Asia, Africa especially in the countries bordering the <sup>M</sup>editerranean Sea.

### Distribution

Urginea is well known <sup>as</sup> northern hemisphere genus with some species having poisonous and cardiotoxic properties. However, in Africa the Drimia is almost wide spread and well known. In India, the genus is represented by eight species, viz. U. indica Kunth, U. congesta Wt., U. polyphylla H.K.F., U. Nightiana H.K.F. and U. Coromandeliana H.K.F. (Hooker 1894), U. Polyantha Blutt (1926), U. Govindappa, Boraiah et Fatima (1970) and U. razii, Ansari. (1978). Out of these U. indica is very common and greatly being used in modern therapeutic medicines.

U. indica (Roxb.) Kunth, Enum 4, 333, Gamble fl. Madras, 1527 (1855), 1928.

### Morphology

Tunicated bulb, about 5 to 10 cm. long, ovoid or globose, leaves simple, linear lanceolate, acute appearing after the flowers. Inflorescence scape, erect, slender, 50 to 130 cm. long brittle, flowers bisexual, drooping or spreading, pedicels long, 1-5 cms. bracts minute or soon falling, perianth campanulate, linear, oblong, lanceolate, segments six. Staminal filaments flattened below, anthers versatile, carpels three, syncarpus, superior ovary with axile placentation. Fruit elliptic capsule of about 1.5 cm. long.

### Distribution

Tropical India, western Himalaya and Western Ghats very common in sandy places near sea throughout India.

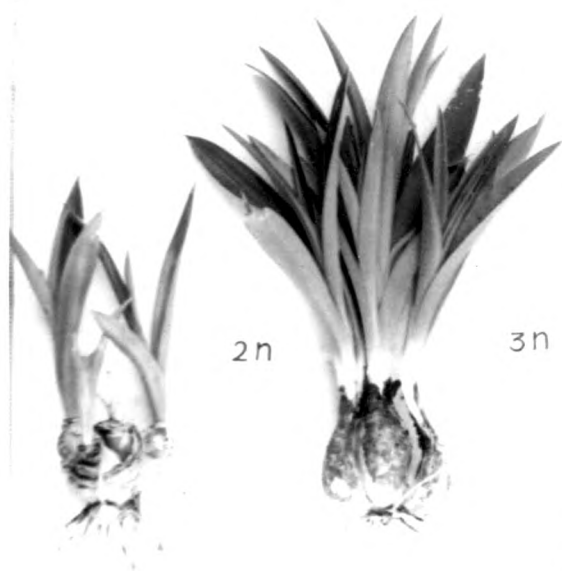
U. indica populations occurring in different geographical regions of India are exhibiting a variety of phenotypic variations in respect of the size of the plants, the underground bulb, the size shape of the leaf. This rather confusing morphological feature of the taxon, on the contrary made the authors to collect the specimens along the south west coast of India (from Ratnagiri to Goa) and from some places of Marathwada and Kolhapur (Maharashtra). During course of study strains isolated from various collections.

1) Diploid - Cytotype  $2n=20$ .

The bulbs globular or obconical,  $18 \pm 1.0$  cm diameter,

Fig. 1 : Morphology of cytotypes of Urginea indica Kunth.





Urginea indica - Kunth



35  $\pm$  2.1 cm. height, 3-4 buds, leaves 30.5  $\pm$  1.5 cm. long, 2  $\pm$  0.4 cm. broad, (Fig. 1) Inflorescence 63  $\pm$  4.4 long, 11  $\pm$  1 flowers fruit 1.5 cm. length.

2) Diploid cytotype  $2n=30$ . ( $3n$  ?)

The bulbs are long 18.7  $\pm$  1.3 cm. diameter, pink brown, 52  $\pm$  3.8 height, 4 buds leaves 48.2  $\pm$  2.5 cm. long 3.1  $\pm$  0.4 cm. broad. (Fig. 1) Inflorescence 112.3  $\pm$  6.0 cm. in length. 29  $\pm$  2.0 flowers pedicels bracts about 4.2 to 1.2 cm. length. This type shows vigour in most of the morphological characters.

2) Tetraploid  $4n=40$ .

Bulbs spherical, medium sized about 14.6  $\pm$  1.0 cm. in diameter yellowish brown 32  $\pm$  1.8 cms. in height. Single daughter Bud. leaves 27.4  $\pm$  1.1 cm. long and 1 cm. broad. (Fig. 1) Inflorescence scape 56  $\pm$  2.4 cm. in height and bear 13  $\pm$  1.0 flowers. Pedicels bracts 2.6 cms.

**Karyotype Analysis  
And Anatomy of  
Urginea indica Kunth**

A> The modern taxonomist who was satisfied to classify his entities on basis of morphology has come more and more to make use of knowlegde to gain in other fields for his evidence of natural relationship of species, genera and families, study of evolution phyllogeny and classification of plants. The importance of cytological study has been widely accepted. The Russian school of cytologists headed by S. Navashin, developed the fundamentals of the Karyotype concept from their observations that most species of living organisms show distinct and constant indivisuality of their somatic chromosomes and that closely related species have more similar chromosomes than those of distantly related ones.

The Karyotype was first defined in 1926 by Delaunay as group of species resembling each other in their morphology and number of their chromosomes. However Lewitsky (1931) defined, it as phenotypic appearance of the somatic chromosomes in contrast to their genotype. The term Karyotype by defination implies morphological expression of somatic chromosomes and an idiogram as its diagramatic expression.

The study of Karyotype in family Liliaceae with large chromosomes and frequent bimodality in size within the compliments have usually made possible, the determination of progress of the chromosomes change and it's consequences.

Urginea indica is well known for its polymorphic nature and characheristic differences the chromosome number and

Karyotype (Raghavan and Venkatsubban 1940, a, b). The concerned literature is already reviewed in chapter "Review of literature". It reveals from previous study that Urginea indica has various cytotypes and it is essential to find out their relationships with each other for further studies. Present approach is in same direction.

## I> Materials and Methods

The bulbs of Urginea indica Kunth, were collected from Kolhapur, Goa and Aurangabad regions. Climatic conditions of these three regions are extremely differing from each other. The collection of plant material was labeled and subsequently grown in Botanical Garden of Botany Department, Shivaji University, Kolhapur.

The somatic chromosome number was determined from growing root tips by following method.

Young root tips from respective bulbs were excised and thoroughly washed, treated with 0.02% colchicine at 12 to 14°C for three hours. Then the treated root tips were washed thoroughly with distilled water and fixed in a mixture of absolute alcohol and glacial acetic acid (3:1). Such pre-treated and fixed root tips were transferred to 70% alcohol for preservation. For further studies the root tips preserved in 70% alcohol were taken, washed thoroughly with distilled water and transferred to a mixture of 2% acetoorcein and 1 N HCL (9:1) and gently heated over a spirit flame for few

seconds, cooled to room temperature and squashed in 2% acetoorcein. Slides were made permanant following the butyl alcohol and acetic acid series and mounted in DPX.

For determining the length of chromosomes 10 plates of each cytotype, collected from Kolhapur, Goa, Aurangabad, were studied and average length of each indivisual chromosome was calculated. For Karyotype analysis the method of Levan et al. (1964) was followed. The camera lucida drawings were made from temporary and permanant slides at 1000 X magnification and microphotographs were taken using Olympus Japan microphotographic equipment at 1000 X magnification. For karyotype analysis 'd', 'r', and 'i' values have/calculated. The total length denoted by 'C' and length of long and short arms are represented as 'l' and 's' respectively. The locations of centromere was expressed as difference  $d=l-s$ . The ratio between the arms calculated as  $l/s$  where as centromeric index  $i = 100 s/C$ .

## II> Observations

The following general morphological types of chromosomes have been recorded.

Type A - Long chromosome with median centromere.

Type A<sub>1</sub> - Long chromosome with submedian centromere.

Type A<sub>2</sub> - Long chromosome with terminal centromere.

Type B - Medium size chromosome with median centromere.

Fig. 2 : Stomatic chromosomes of Urginea indica.

Cytotype  $2n=20$  (Fig. 2,a) ( X 2275)

Cytotype  $2n=30$  (Fig. 2,b) ( X 1100)

Cytotype  $4n=40$  (Fig. 2,c) ( X 925)



Fig. 2a :  $2n = 20$

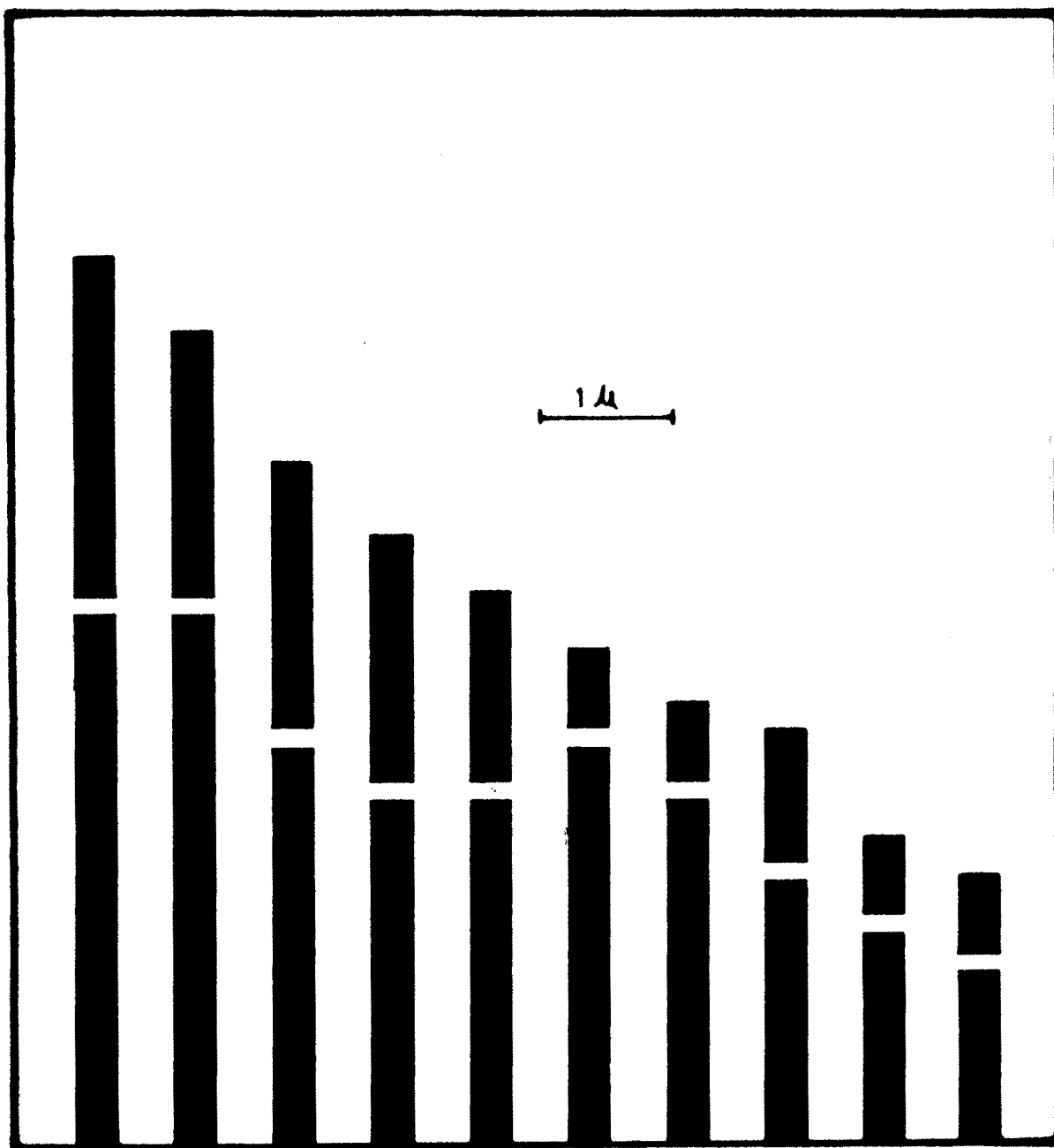


Fig. 2b :  $2n = 30$



Fig. 2c :  $4n = 40$





IDIОGRAM OF URGINEA INDICA KUNTH.

(  $2n = 20$  )

FIG. 3

**Table 1 : Measurement and position of centromere of somatic chromosomes**  
**in cytotype of U. indica (2n=20)**

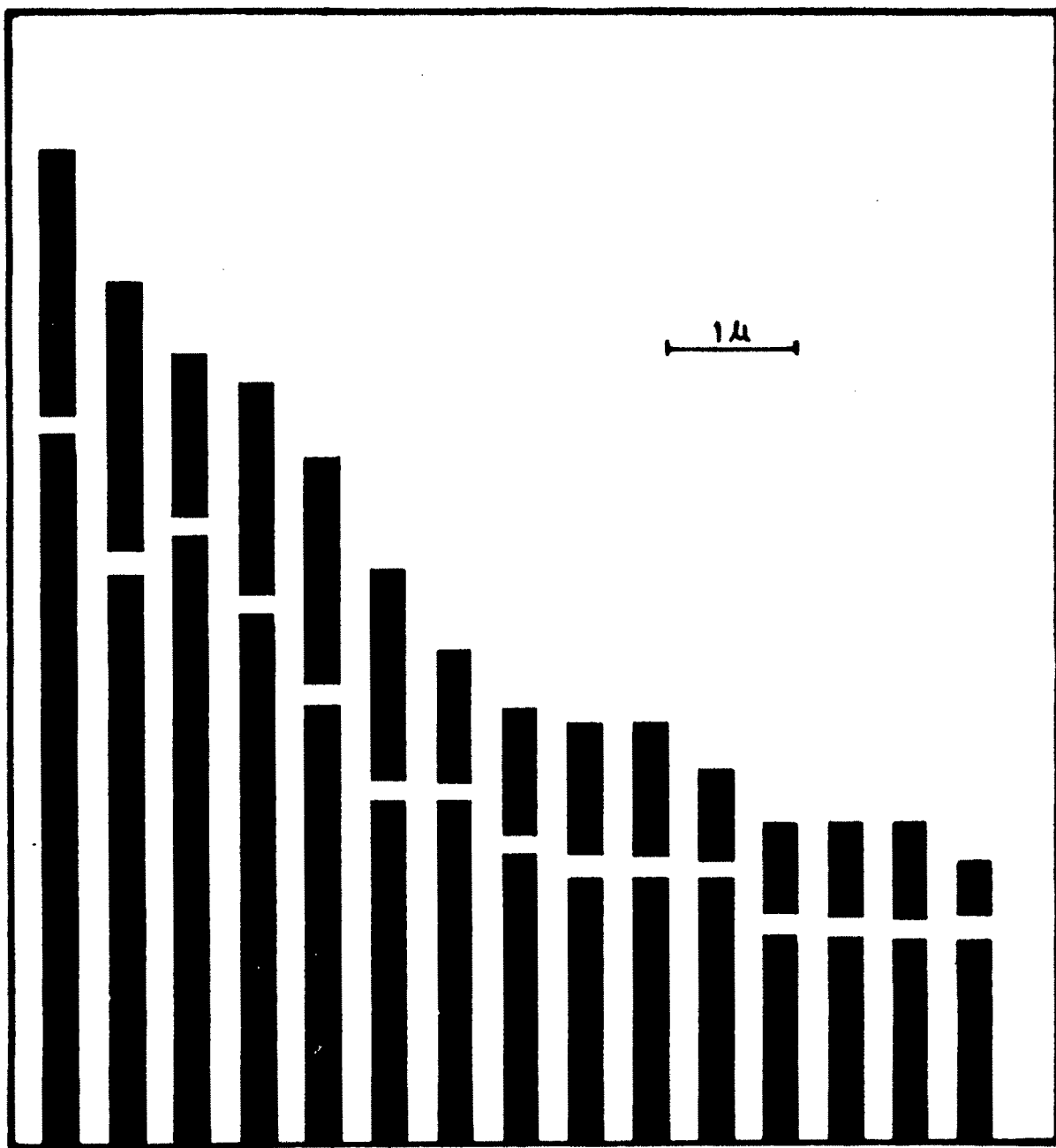
Chromosome pair.	Length of long arm. in $\mu$ (1)	Length of short arm. in $\mu$ (3, 4, 5)	Total length in $\mu$ (1)	d Value	r Value	i Value	Centromere position.
1	4.0	1.5	5.5	1.4	1.0	30.0	m
2	4.0	1.2	5.2	4.0	2.1	33.5	sm
3	3.0	2.0	5.0	1.0	1.5	40.0	L
4	2.0	2.0	4.0	1.0	1.3	45.0	m
5	2.0	1.6	4.2	1.0	1.0	33.5	sm
6	2.0	0.6	3.0	2.0	1.0	16.6	st
7	1.6	0.6	3.0	4.0	4.0	16.6	st
8	2.0	1.0	3.0	1.0	2.0	33.5	sm
9	1.6	0.6	2.5	1.0	2.0	27.0	sm
10	1.3	0.6	1.9	0.7	2.2	32.0	sm

- Type B<sub>1</sub> - Medium size chromosome with submedian centromere.
- Type B<sub>2</sub> - Medium size chromosome with subtelocentric centromere.
- Type C - Short chromosome with median centromere.
- Type C<sub>1</sub> - Short chromosome with submedian centromere.
- Type C<sub>2</sub> - Short chromosome with subtelocentric centromere.
- Type D - Very short chromosome with median centromere.
- Type D<sub>1</sub> - Very short chromosome with submedian centromere.
- Type D<sub>2</sub> - Very short chromosome with subtelocentric centromere.

#### i) Karyotype

The somatic chromosome number in Urginea indica cytotype collected from Kolhapur is  $2n=20$  fig. (2a). In Urginea indica  $2n=20$  the length of chromosome varies from  $6.6 \mu$  to  $1.9 \mu$ . The chromosomes are idiogrammed in Fig (3). The total chromatin lengths in this is  $80.8 \mu$ . The details regarding length of chromosome, position of centromere are given in the table No.1. A perusal of this table indicates that chromosomes can be classified in following 6 types.

- Type A - (Chromosome-1) A pair of chromosomes ( $6.6 \mu$ ) median centromere. This is the largest pair.



IDIОGRAM OF URGINEA INDICA KUNTH.

(  $2n = 30$  )

FIG. 4

Table 2 : Measurement and position of centromere of sonatic chromosomes  
in cytotype of U. indica (2n=20)

Chromosome pair.	Length of long arm. in $\mu$ (l)	Length of short arm. in $\mu$ (s)	Total length in $\mu$ (l+s)	d Value	r Value	l Value	Centromere position.
1	5.3	2.0	7.3	2.7	2.33	27.3	SM
2	4.3	2.0	6.3	2.3	2.12	20.1	SM
3	4.6	1.3	5.9	3.4	2.00	20.0	st
4	4.0	1.3	5.3	3.4	2.00	20.0	SM
5	3.3	1.7	5.0	1.6	1.9	24.0	SM
6	2.6	1.6	4.2	1.6	1.7	23.0	SM
7	2.6	1.0	3.6	1.6	1.6	27.0	SM
8	2.2	1.0	3.2	1.2	1.3	30.3	SM
9	2.0	1.0	3.0	1.0	1.0	33.0	SM
10	2.0	1.0	3.0	1.0	1.0	33.0	SM
11	2.0	0.7	2.7	1.3	2.0	16.0	SM
12	1.6	0.7	2.3	0.9	0.8	30.4	SM
13	1.6	0.7	2.3	0.9	0.8	30.4	SM
14	1.6	0.7	2.3	0.9	0.8	30.4	SM
15	1.6	0.1	1.7	1.2	4.0	20.0	st

- Type B<sub>1</sub> - (Chromosome-2) A pair of chromosome (6.0  $\mu$ ) with submedian centromere.
- Type C - (Chromosome 3 and 4) Two pairs of chromosomes with median centromere. (5.0  $\mu$  to 4.5  $\mu$ ).
- Type C<sub>1</sub> - (Chromosome-5) Chromosome with submedian centromere (4.2  $\mu$ ).
- Type C<sub>2</sub> - (Chromosome 6 and 7) Two pairs of chromosomes with subtelocentric centromere (3.8  $\mu$  to 3.5  $\mu$ ).
- Type D<sub>1</sub> - (Chromosome 8 to 10) Three pairs of chromosomes with submedian centromere (3.0  $\mu$  to 1.9  $\mu$ ).

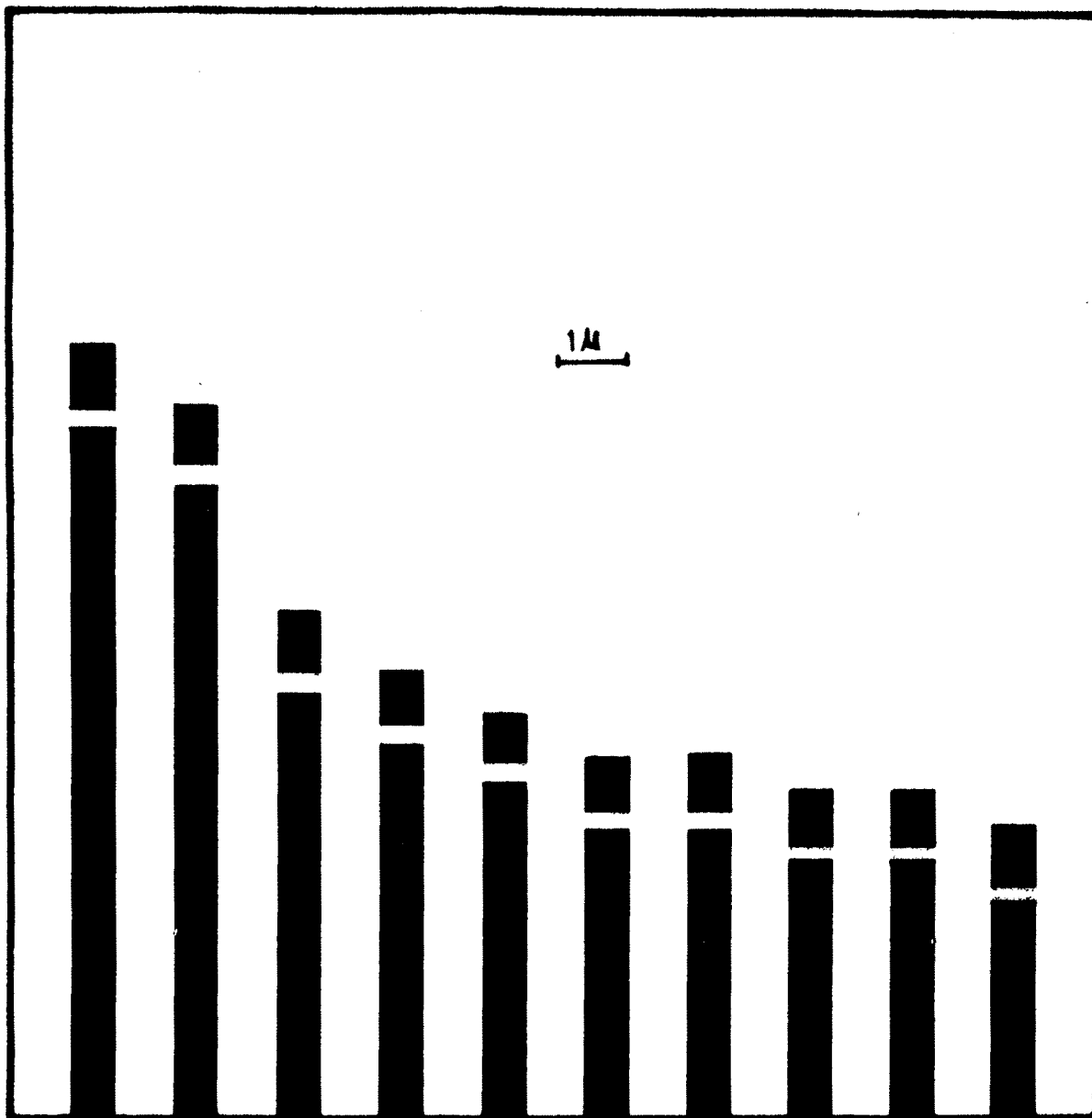
The karyotype formula for P. indica 2n=20 is therefore represented as

$$K(2n):20:2A^m+2B_1^{sm}+4C^{cl}+2C_1^{sm}+4C_2^{st}+6D_1^{sm}$$

#### ii) Karyotype

The somatic chromosome number in P. indica obtained from Jee region has shown 2n=30 (Fig. 2). In this cytotype the length of chromosome varies from 7.3  $\mu$  to 2.0  $\mu$ . The chromosomes are ideogrammed in Fig. 4. The total chromatin length is 119.2  $\mu$ . The details regarding length of chromosome, position of centromere etc. are given in table 2. The chromosomes can be classified in the following types.

- Type A - (Chromosome 1) A pair of chromosome with



IDIOTYPIC OF URGINEA INDICA KUNTH.

(4n = 40)

FIG. 5

Table 3 : Measurement and position of centromeres of somatic chromosomes  
in cytoty. of L. indica (4n=46)

Chromosome	Length of long arm. in (1)	Length of short arm. in	Total length	d Value	r Value	i Value	Centromere position.
1	10.00	0.83	10.83	9.17	12.05	7.66	t
2	9.17	0.83	10.00	8.34	11.05	8.30	t
3	6.17	0.83	7.5	5.84	8.04	11.07	t
4	5.42	0.83	6.25	4.59	6.53	13.28	st
5	4.59	0.83	5.42	3.76	5.53	15.31	st
6	4.17	0.83	5.0	3.34	5.02	16.60	st
7	4.17	0.83	5.0	3.34	5.02	16.60	st
8	3.75	0.83	4.58	2.92	4.52	18.12	st
9	3.75	0.83	4.58	2.92	4.52	18.12	st
10	3.17	0.83	3.0	2.34	3.32	20.75	st



median centromere ( $7.3\mu$ ). This is the largest chromosome.

Type  $A_1$  - (Chromosome 2) A pair of chromosomes with submedian centromere. ( $6.3\mu$ ).

Type  $B_1$  - (Chromosome 3 to 5). 3 pairs of chromosomes with submedian centromere ( $5.8\mu$  to  $5.0\mu$ ).

Type  $B_2$  - (Chromosome 6). A pair of chromosome with subtelocentric centromere ( $4.0\mu$  to  $8.0\mu$ ).

Type  $C_1$  - (Chromosomes 7 to 10). 4 pairs of chromosomes with submedian centromere ( $3.6\mu$  to  $3.0\mu$ ).

Type  $D_1$  - (Chromosome 11 to 15). 5 pairs of chromosomes with submedian centromere ( $2.7\mu$  to  $2.0\mu$ ).

The karyotype formula for U. indica  $2n=30$  is represented as-

$$K(2n):30:2A^m + 2A_1^{sm} + 6B_1^{sm} + 2B_2^{tm} + 8C_1^{sm} + 10D_1^{sm}$$

iii) The somatic chromosome number of U. indica collected from Aurangabad has shown  $2n=40$  fig. No. 2. In this cytotype each chromosome is in quadruplicate therefore it is represented as  $4n=40$ . The length of the chromosome varied from  $10.83\mu$  to  $4.0\mu$ . The chromosomes are idiogramed in fig. No. 5. Total chromatin length is 252.64 . The details regarding of chromosome, position of centromere are given in the table .3.

A perusal of the table indicates that the karyotype can be classified in following 4 types.

- Type A<sub>2</sub> - (Chromosome 1,2) Two pairs of long chromosomes (10.83  $\mu$  to 10.0  $\mu$ ) with terminal centromere.
- Type B<sub>2</sub> - (Chromosome 3) A pair of chromosomes with terminal centromere (7.5  $\mu$ ).
- Type C<sub>2</sub> - (Chromosome 4,5) Two pairs of chromosomes with subterminal centromere (6.25  $\mu$  to 5.42  $\mu$ ).
- Type D<sub>2</sub> - (Chromosome 6 to 10) Five pairs of short chromosomes with subterminal centromere (5.0  $\mu$  to 4.0  $\mu$ ).

The karyotype formula for this cytotype is represented as.

$$K:4n:40:8A_2^t + 4B_2^t + 8C_2^{st} + 20D_2^{st}.$$

### iii) Discussion :

Polysomaty or aneusomaty is significant feature of Liliaceae as it has definite relationship with polyploidy (Sen 1973, Kshoshoo and Raina 1976). During the course of present investigation several aneuploid cells along with normal have been found in all cytotypes of U. indica but there frequency is very very low. Such chromosomal variability was reported by Moorthy and Sampatkumar (1968), Ayyangar (1969), Subramanian (1978), and Patil (1981) in

U. indica. The possible cause behind such variability may be "noncongression" at metaphase and "non disjunction" at anaphase (Cormela, 1950-1951). It is also presumed that such irregularities which have been attributed chiefly to breakages, translocations and reductional separation of chromosomes may be due to nucleic acid disturbance in cell (Sharma and Sharma 1959). The principle cause behind such disturbances is not yet clear. In contrast to this Battaglia (1957), Miede (1960), Zaman and Khaleque (1978) have found that in U. indica the cells are normal disomic ( $2n=20$ ) and without any irregularity.

The chromosome number determined in the present investigation is in conformity with that reported by Ayyangar, (1969); Sen, (1974); Naik, (1974, 1976). Subramanian, (1978); Zaman and Khaleque, (1978) and Patil (1981). However there are reports of B chromosomes in U. indica by various authors (Ayyangar, 1969; Subramanian 1978). The karyotype of U. indica having  $2n=20$ ,  $2n=30$  and  $2n=40$  are asymmetrical in nature. Asymmetry is specialized trend and has also been noted in many other genera and species, (Lewitsky, 1931; Makelveg and Sacs, 1933; Whitaker, 1934; and Sato, 1937). Such plants are usually supposed to be more evolved as compared to those with symmetrical karyotype.

In number of plant species considerable diminution of chromatin matter has been noted to accompany the evolution. The diploid  $2n=20$  cytotype has shown least amount of chromatin whereas  $2n=30$ ,  $2n=40$  has higher amount of chromatin which can be correlated with their number of

chromosomes. It is considered that least amount of chromatin is an indication of evolution (Delaunay 1926, Rohweder 1934). The exact manner through which the chromatin diminution is brought is not yet thoroughly understood.

However, centromeric position is also considered as a criterion for studying karyotype evolution. In present investigation U. indica cytotype showing  $2n=20$  and  $2n=30$  have more number of chromosomes, with median and submedian centromere positions whereas  $4n=40$  has shown the chromosomes only with telocentric and subtelocentric chromosomes. According to Stebbins (1950) and Lewitskys concept of karyotype evolution cytotype  $2n=20$  and  $2n=30$  indicate primitiveness of species. Whereas  $4n=40$  may be considered as an advanced one.

It has been reported by Patil (1981) that the karyotype pattern of cytotype having chromosome number  $2n=30$  may be triploid in nature and cytotype having  $2n=20$  might have contributed up to some extent in this triploid formation. But from present investigation it is clear that the role played by cytotype having  $2n=20$  is mere. However it requires further studies in chromosome banding, hybridization, meiotic analysis, DNA estimations to correlate their genomic inter-relationship. In the present investigation neither secondary constriction nor satellite was observed in U. indica similar observations



have been reported by Naik (1978) and Patil (1981). In contrast sat chromosome in U. indica have reported by Raghvan and Venkatsubban (1940 a) and Subramanian (1978), Zaman and Khaleque (1978).

The variation in respect of relative length and arm ratios of chromosomes of Urginea indica to be attributed to the structural changes operative evolutionary history of karyotypes within the species at Diploid, triploid and tetraploid levels. A comparison of idiograms of different cytotypes of single species or different species of genus or of different genera give an indication of evolutionary role played by different cytological processess, polyploids with their altered heriditary out fits are usually found stabilized in nature. A complete link of polyploid complex in species throws, light on pattern of cytological evolution of taxon. It also indicates the chromosomal evolution of taxon through polyploidy associated with structural changes. Among the species of Urginea in which the chromosome number is known, eg. U. indica (Raghvan, 1935); U. Scilla (Sato 1942), U. Martima (Battaglia, 1957; and Mougini 1966, 1974), U. Coromandeliana (Naik, 1973; and Sen, 1974); are at different ploidy levels, while the rest are diploids. The different ploidy levels of these species might be enlarging their adaptive capacities to compete with other plants in their natural population.

Polyploidy is assumed to have ancient origin if taxon includes only polyploids or very few diploids and it may be relatively of recent origin if diploids are more frequent than polyploids or equal, (Stebbins 1950). In U. indica natural population the two cytotypes  $2n=20$  and  $2n=30$  grow together, along south west coast of India. While  $4n=40$  tetraploid and  $6n=60$  hexaploid<sup>are</sup> sporadic in nature and occurring in restricted geographical area where  $2n=20$  (Diploid) and cytotype  $2n=30$  are not so common. Patil (1981) reported from cytogenetic concepts and morphological study that there are two natural groups one with  $2n=20$  and  $2n=30$  having vigorous growth while second tetraploid ( $4n=40$ ) with slow growth. Present investigation indicates that such type of classification is not feasible as population at Kolhapur region shows only diploid vegetation ( $2n=20$ ). The cytotypes  $2n=30$ ,  $4n=40$  and  $6n=60$  is nothing but adaptation to various stressess such as temperature, water, salinity etc.

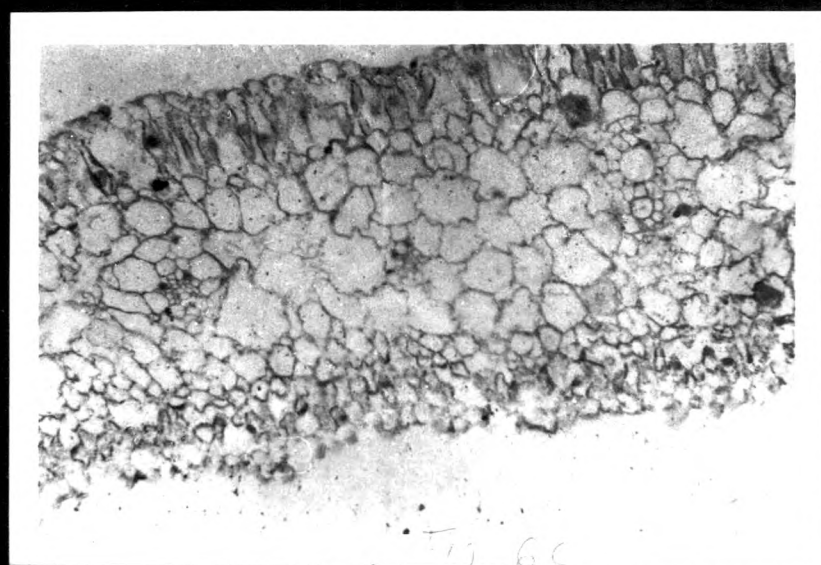
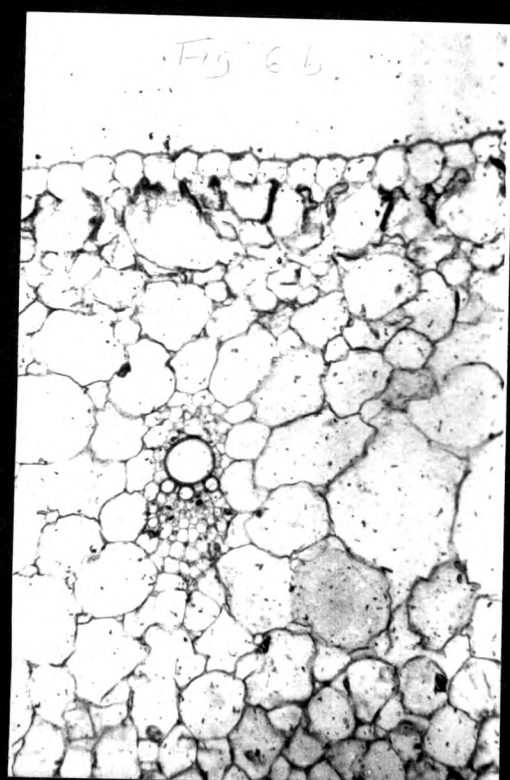
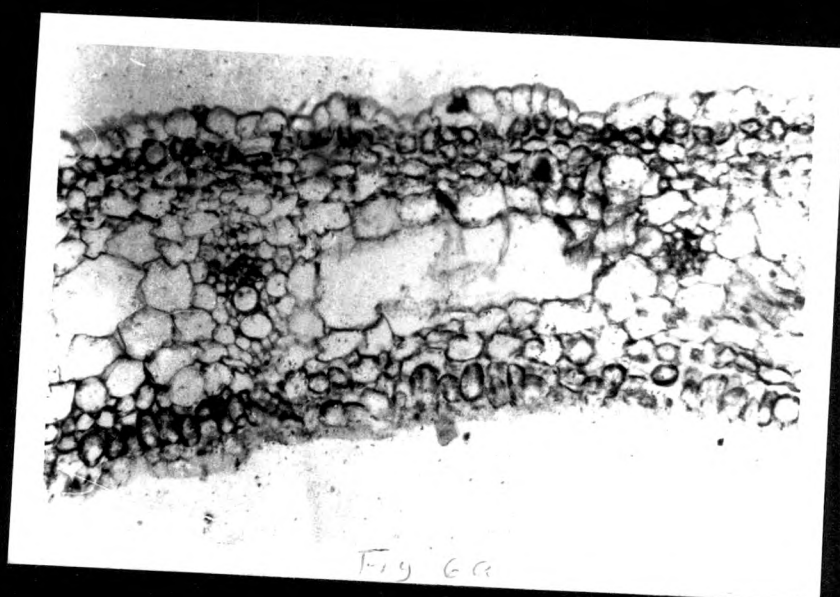
Mookerjee (1955) found chromosomal alterations in Hemerocallis manorana. Similar situation is in Agave (Sharma, 1973). Therefore it was suggested that of abnormal nucleus with numerically or structurally altered karyotype enters into growing apex which gives rise to daughter shoot<sup>by</sup> vegetative means. New shoot will<sup>be</sup> formed of cells containing the altered karyotype thus giving rise to completely new form. Since sexual reproduction is absent such abnormalities are not loosened through gametic

Fig. 6 : Leaf Anatomy ( T. S. ) of Urginea indica.

Cytotype 2n=20 (Fig. 6,a) ( X

Cytotype 2n=30 (Fig. 6,b) ( X

Cytotype 2n=40 (Fig. 6,c) ( X





that U. indica  $2n=20$  thickness of leaf is less as compared to  $2n=30$ , but it is greater than in  $4n=40$ . In all the cytotypes upper and lower layer of epidermis is coated with waxy substances. Sclerides and raphides have been observed in mesophyll tissue of all the cytotypes. The difference between these three cytotypes leaf anatomy is that in cytotype  $2n=20$  the assimilating parenchyma is restricted only for 2-3 layers below the upper and lower epidermis levels, whereas in  $2n=30$  assimilating parenchyma is more below the upper epidermis. The mesophyll tissue in  $2n=30$  is turgid and in more amount than in  $2n=20$  and  $4n=40$ . An increased amount of mesophyll tissue may be correlated with saltstress in cytotype  $2n=30$ . In cytotype  $4n=40$  the situation is quite different. The mesophyll cells are oblong and assimilating parenchyma below the upper epidermis is restricted for two layers, whereas elongated palisade like tissue is developed below the lower epidermis. In all cytotypes vascular bundle pattern in leaf is similar. Morphologically the leaves can be differentiated between  $2n$  and  $4n$  whereas morphological differentiation between the leaves of  $2n=20$  and  $2n=30$  is difficult.