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Research Article

RAPHIDES IN *URGINEA INDICA* KUNTH (LILIACEAE)

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ABSTRACT
Micromorphological traits play a significant role both in systematic and functional aspects of plant survival in response to environmental stimuli. Plant structural trait such as raphides play a vital role in protecting plants from herbivore attack, cause discomfort by physical and chemical irritation by dermal contact to soft tissue thus acts as defensive mechanism. These morphological characteristics may have evolved as response to other environmental stimuli. The presence of raphides (Calcium oxalate crystals) in the anther endothecium represents a rare character, reported in the present study which is of systematic significance. In the present study raphides are present in vegetative and reproductive parts of *Urginea indica*. The mild inflammation and irritation caused when the bulb is rubbed on the skin reveals that it takes part both in mechanical and chemical irritation when they come in contact with tender tissue and protect themselves against herbivore attack. Results indicate that raphide bundle size varies considerably within species. These suggest that, Raphides have some potential to be a useful taxonomic tool. Polarized microscope shows variation in the colouration of raphides.

Key Words: *Idioblastic Cells, Liliaceae, Raphides, Urginea*

INTRODUCTION
Urginea indica Kunth. is a perennial bulbous geophyte native to India, Africa and Mediterranean region and in slopes of hills, in sandy grounds (Gentry *et al*.,1987; Bruneton, 1996; Bellakhdar, 1997). *U. indica* is a winter plant characterized by three phenological stages consisting of inflorescence, leaves and no above ground biomass, leaves first appear after the flowers have wilted in response to first shower during April to May and may remain green till September depending on rainfall and temperature. Some geophytes that flower without bearing leaves in the beginning of April are known as hysteranthous type and some synanthous type bearing leaves and flowers together (Shiva Kameshwari *et al*., 2010).
Plants defend themselves against attack from herbivores has been the subject of considerable interest over many decades (Herms and Mattson, 1992; Agarwal and Fishbein, 2006). The plants that are able to survive in environments where herbivores are common because of their ability to resist or recover from intense herbivore pressure (Hartley and Jones, 1997). Cell inclusions, especially different types of calcium oxalate crystals represent significant taxonomic characters at various taxonomic levels in flowering plants. Calcium oxalate crystals have been recorded in most plant families; the most commonly described being the needle shaped raphides and aggregate in bundles within plant cells and are associated with heavy metal tolerance (Franceschi and Nakata, 2005) calcium oxalate crystals are known to protect tree bark from attack by boring insects (Hudgins *et al.,* 2003) and to act as a foliar defense against both invertebrate (Korth *et al*., 2006) and vertebrate herbivores (ward *et al*., 1997). Ward *et al*., (1997) observed more calcium oxalate crystals in leaves of *Pancratium sickenbergeri* as it is exposed to the highest rates of gazette herbivory. Raphides are also produced in many economically important plants like Palms, Yams, Banana and Pandanus (See review by Crowther in Press).
Raphide crystals are most commonly encountered among monocots (Dahlgren and Clifford, 1982). The development and chemistry of raphides has been subjected to a few detailed, studies and has been reviewed by Franceschi and Horner (1980).
Our primary goal is to trace raphides present in mucilage of bulbs, in leaves, anthers and in roots of *U.indica* as this will help us to evaluate the irritant present in mucilage which plays a significant role in defense mechanism.

MATERIALS AND METHODS

Urginea indica populations were collected from various localities of Karnataka and cultivated in the germplasm of Department of Botany, Bangalore University, Bangalore. Light microscopic studies tissue preparations for anther studies were made by fixing young flower buds in 2.5% gluteraldehyde and 2% paraformaldehyde fixed in 1% oscimum tetraoxide and dehydrating in an ethanol series. Semi thin sections of 1 µm thickness were taken using reichert juang ultra microtome and stained with 0.5% toludine blue O. Photomicrographs were taken in cannon camera attached to nikon microscope.

Maceration of roots were also prepared following Jeffrey's method. The material is treated for one day at 30-40 degree centigrade with mixture of 10% chromic acid and10% nitric acid.

Fresh material sections of the bulbs were prepared and photographed under Polarized Microscope.

RESULTS

Raphides are present in roots bulbs and leaves. *U. indica* Bulbs which grows inside the soil i.e., in darkness generally produces more idioblasts than leaves exposed to light (Figure 1.A). Raphide crystals are initiated very early in plant development. Individual raphides may be formed by calcium oxalate deposition .Raphides idioblast contains bundles of narrow, elongated needle shaped crystals, usually of similar orientation with pointed ends at maturity, one end is abruptly pointed whereas the other tapers to a point or is wedge shaped. There are varying number of crystals in each bundle (10 to 30).

The frequency of Raphides present in endothecium is considerably higher when compared to other reproductive tissues (Figure1.B).

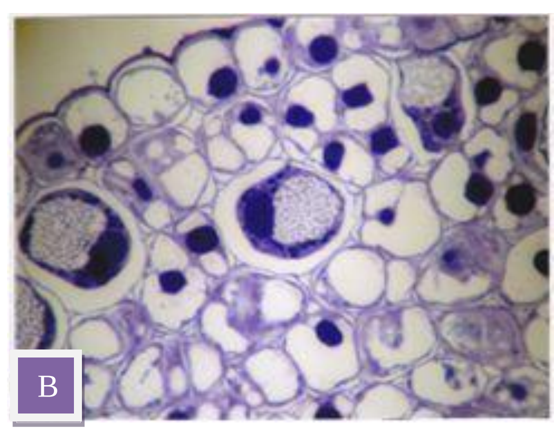
The leaves of *U. indica* are equi-facial i.e., in terms of cuticle, epidermis and spongy cells, which are found on both leaf sides, the lower side of the leaf possesses idioblastic cells containing bundles of raphides .

Raphides occurs within the central vacuole of idioblastic cells in cross section appear foamy and each crystal needle is embedded in a translucent homogeneous substance. In addition, the cell wall of the idioblastic cells contains oil droplets and starch granules (Figure 1.C and D).

The bundles look like a heap of needles in longitudinal section. The length of the raphides vary in different organs (Figure 1.E).

Raphides under polarized Microscope looks like a vibgyor. Each needle like raphides exhibits different colours (Figure 1.F). Bulb sections under the polarized light reveal open bundles of calcium oxalate needles of different sizes. The orientation of Raphide bundles with in a tissue follow the same direction or different direction (Figure 1.G) Sheath walled idioblastic cells containing raphide bundles are present among ordinary cortical cells. Raphides are noticed in both vegetative and reproductive tissues in *U. indica*.

In the macerated root tissues the direction of bundle is parallel to each other (Figure 1.H).



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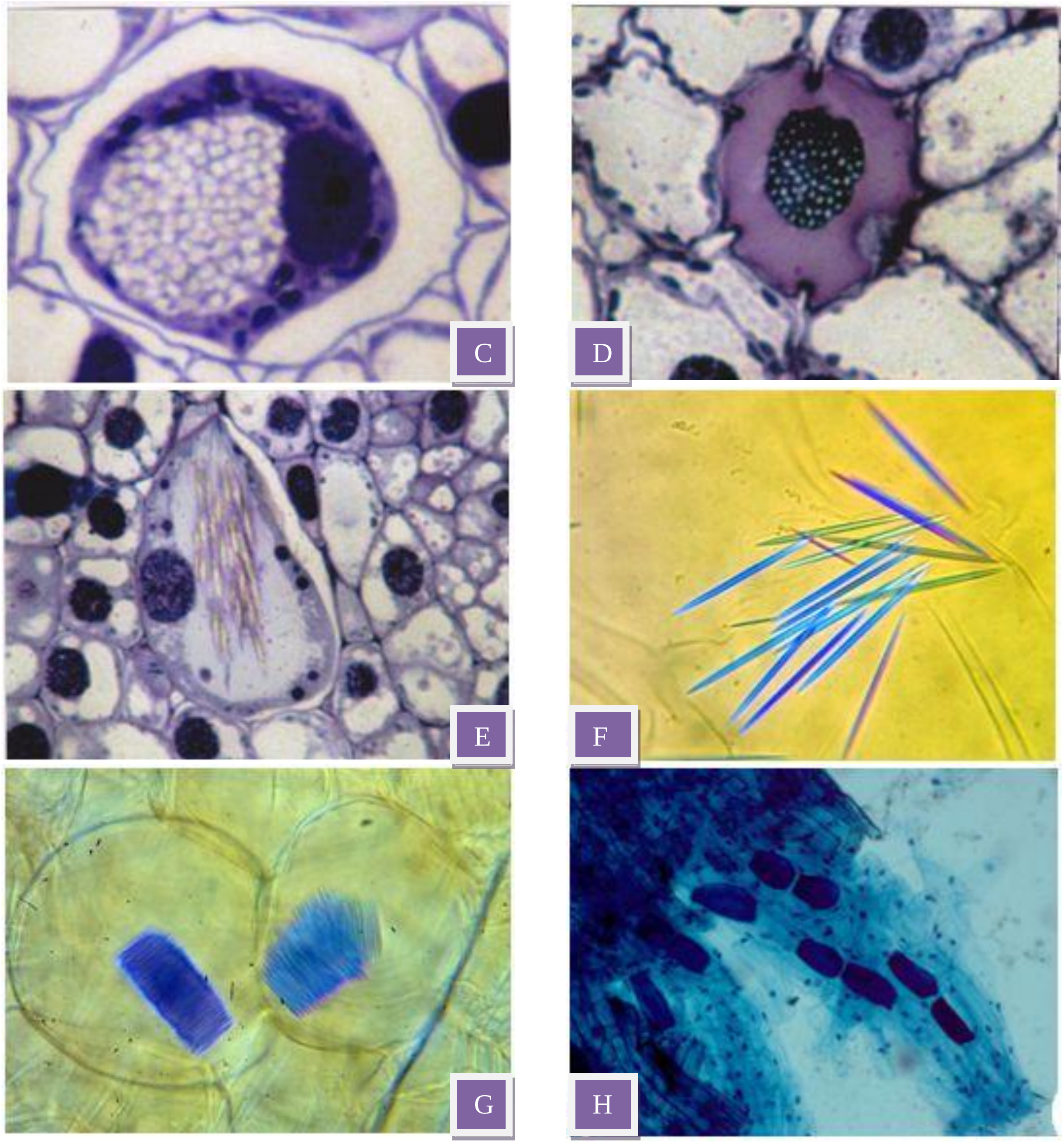

Figure 1: A-*Urginea indica*. **B-**T.S. of anther section showing Raphides in Endothecium. **C-**T.S. of Raphide in endothecial cell. **D-**T.S. of Raphide in middle layers. **E-**L.S.of Raphide in Endothecial cell. **F-**Raphide bundles under polarized microscope. **G-**Bundles of Raphides. **H-**Bundles of Raphides in Macerated root tissue

DISCUSSION

There is little information of *U. indica* from the biological point of view, except for the Morphological and Karyological studies by (Shiva Kameshwari and Muniyamma, 2004; Professor and Speta, 2004) a few Phytochemical studies (Kopp *et al.*, 1996; Krenn *et al.*, 2000) and some Pharmacological studies

(Pascual – Villalobos and Robledo, 1999) It is dominant over wide areas in Tamil Nadu and important for Homeopathic therapy.

Most plants have non cytoplasmic inclusions, such as starch, tannins, silica bodies and calcium oxalate crystals, in some of their cells. Calcium oxalate crystals are widespread in flowering plants, including both dicotyledons and monocotyledons.

They were first discovered by Leeuwenhoek in the 17th Century (Frey, 1929). They have documented using light microscopy (LM) and polarization microscopy and recently using x-ray diffraction infrared and electron Microscopy both scanning (SEM) and transmission (TEM) (Horner and Franceschi, 1981).

The distinct shapes and birefringence, of calcium oxalate crystals, especially raphides and styloids, make them readily observable particularly in young and actively growing plant crystals normally form intra-cellularly.

The value of calcium oxalate crystals to normal plant growth and development, is largely unknown Frances Chi and Horner (1980s).

They may represent storage forms of calcium and oxalic acid, and there has been evidence of calcium oxalate resorption in times of calcium depletion (Sunell and Healey, 1985) They could also act as simple depositories for metabolic wastes which would otherwise be toxic to the cell or tissue. In some plants they have more specialized functions such as to promote air space formation in aquatic plants or help prevent herbivory. The barbed and grooved raphides of some Aracaceae are particularly irritating to mouth and throat tissues when eaten.

Grooves in crystals which have embedded, themselves in animal tissues may allow the entrance of a chemical irritant such as a toxic proteolytic enzyme or a glucoside into the wound (Sakai, Hanson and Jones, 1972; Walter and Khanna, 1972). Calcium oxalate crystals appear in a variety of shapes which are consistent and repeatable from one generation to the next, demonstrating that the physiological and genetic parameters controlling them are consistent.

Crystals may be present in almost every part of both vegetative and reproductive organs, often in crystal idioblasts near veins, possibly due to calcium being transported through the xylem (Frank, 1967). Crystals are present in leaf epidermal cells in addition to 'normal' bundles of raphides in mesophyll cell (Rudall *et al.*, 1998) Many cellular modifications occur during genesis of crystals, which is a highly complex process (Kausch and Horner, 1983) Raphides are absent in some families of Liliales (Rudall *et al.*, 1996).

In the present study, Endothecium raphides in *U. indica* represents a rare character, such studies have been made by Hardy and Stevenson(2000) in tapetal cells of Commelinaceae and Hamann 1966 in Phylidraceae. Hamann (1966) found that in *Philydrum* almost every tapetal cell contained a raphide bundle, upon degeneration of the tapetum these are released between the developing Pollen grains. As the pollen grains mature crystal quantities decreased and diminished in size. Hardy and Stevenson thus postulated that tapetal crystals had been reabsorbed during pollen grain development, thereby releasing free calcium possibly required in the ontogenetic process. Recently Prychid *et al.,* (2003) observed such cell inclusions in Haemodoraceae.

Raphides appear to occur universally as bundles of needle shaped crystals within vacuolar crystal chambers of idioblastic cells of roots bulbs and leaves. In addition, these crystals also have backward oriented surface bulbs capable of increasing damage to the mouths of grazing animals. Such studies have also been made by Sakai *et al.*, (1972).

Raphides of calcium oxalate in *U.indica* responsible for producing mild inflammation and itching when rubbed on the skin Therefore, raphides take part in both mechanical and chemical irritation when they come into contact with tender tissues of soil-living worms and herbivores. The defense mechanisms could be viewed by other stored compounds to act against Microbial agents, herbivores, rodents, fungi and insect studies made by (Hoffman *et al.,* 1993; Sathyamoorthy *et al.,* 1999; Heth *et al*., 2000; Civelek and Wein traub, 2004) revealed similar results.

The presence of copius mucilage in *U.indica* is a possible synapomorphy. The end walls of the idioblasts break down as crystal develop, resulting in elongated, asticulate, containing loose groups of raphides often embedded in mucilage. The same results have been given by cogne *et al.,* (2001) in *Urginea ultissima.*

The idioblast cells that appeared in *U. indica* is similar to those present in *Allium* species. But one difference is crystals are present in bulb scales of Allium but not in aerial leaves according to (Gregory, 1996). But in the present study crystals are present in all parts of the plant body in vegetative and reproductive parts of *Urginea.*

Raphides are present in all organs of *U. indica* examined. It is believed that the highest amount of polysaccharides, accumulates in the underground tissues and this is an adaptive strategy for the plant to survive during dormancy (Sharaf Al-Tar deh *et al.,* 2006). The idioblastic cells contain different phenotypes of crystalloid inclusions. The cell wall contains oil droplets and starch granules.

Initial orientation of calcium oxalate crystals has been improved by using Polarized Microscope and also in Bright Field Light Microscope.

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