Microscopic Features of Dominant Bladderworts of Northeast India

Kshetrimayum Raseshowri Devi, Nagulan Venugopal, Lal Bihari Singha

Abstract: ***Utricularia bifida Sm. and Utricularia pubescens Sm. are the most dominant and widely distributed bladderworts in Northeast India. The bladders of these species show double-layered walls. The antennae in U. bifida were unicellular and uniseriate, whereas, the antennae of U. pubescens were numerous, long and multicellular forming a fringe. The digestive glands were either bifid with two arms in U. bifida or quadrifid with four arms in the case of U. pubescens which bear short single-celled stalk. The stalk cells represent the basal portion of the arms or the terminal cells abutted from their respective sub-conical shaped pedestal cells. The wall partition between the pedestal and the basal portion of the stalk bear several finger-like projections of transfer cell type. The walls of pedestal, stalk and terminal arm cells were clearly differentiated into three layers. The outermost cuticle layer of pedestal cell was thick, which extended till the base of the terminal or arm cell. The middle layer was highly impregnated with opaque materials and fibrils. The innermost layer was not impregnated with variously shaped electron translucent numerous vacuoles filled with granules. The pedestal and basal cells were interconnected with plasmodesmata.***

Keywords: Utricularia, Ultrastructure, Digestive gland, Vacuole, Pedestal cell

I. INTRODUCTION

Utricularia commonly known as 'Bladderwort' with nearly 210 species is the largest and widely distributed genus among the insectivorous plants. Species under this genus are either annuals or perennials mostly growing in the marshy, rocky and aquatic habitats. They are widely distributed in tropical and subtropical regions and a few species are localized in temperate zones. Taylor (1989) has described 33 species of insectivorous plants of India belonging to the genus Utricularia in his taxonomic monograph. Recently, a total of 35 species of Utricularia has been reported from India, among which 13 species are endemic to Peninsular India and five species are endemic to Northeast (Janarthanam & Henry, 1992). Studies on various aspects of the genus Utricularia were carried out, such as, physiology (Sorenson & Jackson, 1968; Sasago & Sibaoka, 1985); seed biology (Farooq, 1964; Abraham & Subramanyam, 1965) and taxonomy (Taylor, 1964). Studies were also carried out on the ultrastructure and development of the external glands of Utricularia minor L. (Kristen, 1974).

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Vintejoux (1974) studied on the production of digestive enzymes by the internal glands and secretion of mucilage by the hairs in U. neglecta. Ultrastructure and organization of hairs and external glands in the traps of U. monanthos Hk. f. as well as development and histochemical localization of the major metabolites of the bladder of U. stellaris has been carried out by Fineran & Lee (1974a, b; 1980) and Cheema & co-workers (1992). Architecture of the transfer cell wall in the secretory hairs of U. intermedia and development of the wall labyrinth in the pavement epithelium hairs of U. volubilis, U. stygia and U. intermedia were studied by many workers (Plachno & Jankun, 2004; Plachno et. al. 2005 a, b). Due to suitable agroclimatic condition, diversity of Utricularia in Northeast India is very high, although, studies on the ultrastructure of the available Utricularia spp. are very meager. Therefore, the present study has made an attempt to understand the anatomy and ultrastructure of bladders of two selective species of Bladderworts such as, Utricularia bifida Sm. and Utricularia pubescens Sm. which are commonly distributed in the Northeast India.

II. MATERIALS AND METHODS

Bladders of *Utricularia bifida* Sm. and *Utricularia pubescens* Sm. were collected from Janiaw and adjoining areas of Shillong and North Eastern Hill University campus of East Khasi Hills District of Meghalaya, India. The bladders were fixed in FAA (Formalin 5cc: Acetic acid 5cc: 70% Ethanol 90cc), and glutaraldehyde (2-3% prepared in phosphate buffer at pH 7.2) in the field itself. Fixed bladders in FAA were used for microtomy following standard dehydration method using tertiary butyl alcohol series. Bladders fixed in glutaraldehyde were used for the studies on morphology and ultrastructure of digestive glands. Secondary fixation of the bladders was made by treating with 1% OsO_4 buffer for 4hr at 4^0C. The samples were dehydrated by double treatment in gradually increasing concentration of acetone, such as, 30%, 50%, 70%, 80%, 90%, 95% (at 15 min. interval) and three changes were made in absolute acetone (at 15 min. interval). For Transmission Electron Microscopy, the bladder samples were washed in propylene oxide after dehydration in acetone, and embedded in Araldite CY 212. Ultra-thin sections were made at 60-90nm (600Å - 900Å) through a Sorvall MT-2 ultramicrotome using a diamond knife, and then stained with 2% aqueous uranyl acetate and lead citrate. The bladder sections were observed under Zeiss EM-109 Transmission Electron Microscope (TEM).

III. RESULT

A. *Morphology of bladder*

The size of horseshoe shaped bladders of *Utricularia bifida* and *Utricularia pubescens* ranged from 0.3 mm to 1mm in

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diameter (Fig. 1a&b). Each bladder bears an orifice or mouth guarded by a valve-like door on the upper or dorsal end, extended from the dorsal surface of the bladder. The ventral surface of the orifice possess a few layers of cells-the collar or the ridge, which is attached to the thick radial wall known as pavement epithelium (Fig. 1c). In *U. bifida*, the antennae were bifid or forked, unbranched and slender on the upper orifice, whereas in *U. pubescens*, the antennae were long and multicellular forming a fringe (Fig. 1a&b). The main components of the bladder, which were actively involved in capturing preys, were the valve, ridge or collar, unicellular-uniseriate antennae and bristles bordering the orifice or mouth forming the threshold.

B. Anatomy of bladder

The bladders of *U. bifida* and *U. pubescens* bear two-layered walls (Fig. 1c). The mouth portion of the bladder was a complicated structure with different regions, where the upper cells of the mouth were differentiated into a valve and the lower region into a collar which were directed towards the inner side of the bladder. The collar was made up of three layers of cells: i) the outermost capital cell layer, ii) the middle layer with thick walled rectangular cells, and iii) the innermost layer with radially elongated basal cells. The valves as well as the pavement epithelium were directed towards the inner side of the bladder (Fig. 1c). In normal condition, the valve was tightly closed against the ridge or collar. In addition, the outer surface of the bladder bear many dome-shaped external glands.

C. Gland architecture

In *U. bifida*, the glands were observed to be bifid bearing two arms, whereas the glands present in *U. pubescens* were quadrifids with four arms. The bifid and quadrifid glands present in the inner walls of the bladders were ended at right angle to the walls of the bladders by a short single-celled stalk. The stalk cells represent the basal portion of the arms or terminal cells, which were abutted from pedestal cells (Fig. 1d&e). The pedestal cells were sub-conical in shape with circular base attached to the small-sized basal epidermal cells at the junction of the large-sized cells of the inner wall of the bladder (Fig. 1f). There were two pedestal cells present in *U. bifida* and four cells in *U. pubescens* which supported the terminal or the arm cells in both species. External morphology of the glands with the quadrifid arm and the stalk form an 'H-shaped' structure, which lie parallel to the inner surface of the trap. Thus, in surface view, four pedestal cells were arranged in tetrad form (Fig. 1e).

IV. ULTRASTRUCTURE OF DIGESTIVE GLAND

The arms of the digestive gland in *U. bifida* and *U. pubescens* were unicellular in nature in which organization of cell wall in different portions of the glands are uniform (Fig. 2a). The walls of pedestal, stalk and terminal arm cells were clearly differentiated into three layers. The cuticle layer at the basal portion of the terminal arm cells was thick, which become thin towards the proximal end of the arms (Fig. 2a). The middle layer was highly impregnated with opaque materials and fibrils, where presence of thin middle lamella was noticed. Although, innermost layer was unimpregnated but with multiple-shaped electron translucent numerous vacuoles filled with granules (Fig. 2b&c).

The transverse partition wall between the pedestal and basal portion of the stalk bear several finger-like projections occupying a large surface area in the protoplast (Fig. 2b&c). Ingrowths of the pedestal cell wall were mostly confined to the lateral side as well as to the junction of the stalk with pad-like appearance (Fig. 2d). The pedestal and basal epidermal cells were interconnected through plasmodesmata (Fig. 2d).

In terminal cells, wall ingrowths were present on the transverse walls of the stalk cells adjoining to the pedestal cells, which were totally absent on the lateral walls of the stalk cells (Fig. 2d). It reflects the organization of the wall that changes gradually towards the terminal portion of the arm with less opaque materials or impregnation. The cytoplasm of the terminal or arm cell was mostly concentrated towards the lower periphery of the arm cell with granular nucleus and nucleolus (Fig. 2e). The basal portion of the arm cells was observed to contain blue green algae, unicellular and filamentous green algae, uni and multicellular organisms and bacterial cells (Fig. 2f).

V. DISCUSSION AND CONCLUSION

This study has revealed that, *Utricularia bifida* grows in the marshy water logged habitats whereas; *Utricularia pubescens* was distributed on the moist rocky surfaces in the Northeast India. The inner cell layer of the bladders of *U. bifida* and *U. pubescens* were less flexible in nature and act as a resistant layer, whereas, the outer cell layers of the bladder were flexible and determine the shape of the bladder. Small pedestal cells attached to the basal epidermal cells of the inner wall of the bladders of both *Utricularia* spp. resembled to *U. monanthos*, from where bifid and quadrifid arms with respective stalk cells arose. The horseshoe-shape bladder with valve and collar and antennae bordering the mouth for capturing preys observed in *U. bifida* and *U. pubescens* was similar to that of the other *Utricularia* spp. (Drawin, 1875; Fineran & Lee, 1975). In *U. bifida*, the antennae of the bladder were bifid or forked, unbranched and slender, whereas in case of *U. pubescens*, the antennae were long and multicellular forming a fringe. Dome-shaped glands at the valve of bladders of both the spp. studied were consistent to the vestibule glands present at the entrance to the doorway of *U. monanthos* (Fineran & Lee, 1980). Uniform bilayered wall of the bladders of the two species also resembled *U. vulgaris* (Friday, 1991) and *U. stellaris* (Cheema *et. al.* 1992). The presence of digestive glands with two and four arms in *U. bifida* and *U. pubescens*, respectively differed from that of *U. monanthos* and *U. stellaris* bearing both type of glands in the same bladder (Fineran & Lee, 1975; Cheema *et. al.* 1992). Differentiation of a single terminal cell into an arm and a stalk without any wall partition is a highly specialized structure of the bladder where the arms play key roles in both absorption of nutrients and secretion of enzymes and the stalk for support and conduction of enzymes and nutrients. This specific feature of continuity was not being reported in any other plant trichomes, where the conduction

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from the terminal cell was usually carried out by separate stalk cells (Fahn, 1979). Vacuolated cell wall of the pedestal and proximal cell wall portions of the arm and the stalk, and the cell wall partition between the pedestal and the stalk portion was the peculiar characteristic feature present in *U. bifida* and *U. pubescens* which were not reported previously in any other insectivorous plants. Highly impregnated middle and innermost unimpregnated layers of wall in the pedestal and base of the stalk portion were also a common feature as in *U. monanthos* (Fineran & Lee, 1975). Wall ingrowths or protuberances in the pedestal and stalk portion of the glands of *U. bifida* and *U. pubescens* show functional differentiation of the cells particularly by increasing the surface area for absorption of nutrients and for a short distance transportation of enzymes and nutrients as in *U. monanthos* (Fineran and Lee, 1974 a), and as in transfer cells of certain aquatic plants (Pate & Gunning, 1972; Gunning, 1977).

Direct absorption of the nutrients from the lumen of the bladder may be probably through the thin fibrillar cuticle of the terminal arm portion, base of the stalk and the pedestal cell which were apoplastic in nature. The digested products were transported to the pedestal cell after digestion in the arm portion presumably by the finger-like projections present in the cell wall partition between the stalk and the pedestal cell. It may be assumed that a part of the digestion process might have taken place in the vacuolated cell wall between the two cells. The products were again transferred to the adjoining basal cells through the plasmodesmeta on the tangential cell wall through symplastic movement. Although, Fineran & Lee (1975) have the opinion that, the opaque impregnated wall of the pedestal and base of the stalk portion would not probably allow substances to pass directly to the wall labyrinth into the cells.

In the present study it may be concluded that, enzymes present in the vacuoles of the cell wall might have helped in the digestion of the trapped preys through the outer impregnated zone of the cell wall of pedestal cell, which does not allow substances to pass directly into the cells from the lumen of the bladder. Bonnett (1968) & Robards *et. al.* (1973) have compared the impregnated zone in the cell wall with the casparian strip of endodermal cells. Vacuolar nature of the cell wall is a peculiar feature present in these plant species, which might be an adaptational feature of carnivory. The confinement of the cell organelles especially nucleus at the base of the arm also suggested an advantage in adaptation of the whole plant system by controlling the metabolic activities of the cell e.g., in providing necessary energy for active uptake of materials or in transporting metabolites to the pedestal cells. Similar findings were also being reported by Fineran & Lee (1975).

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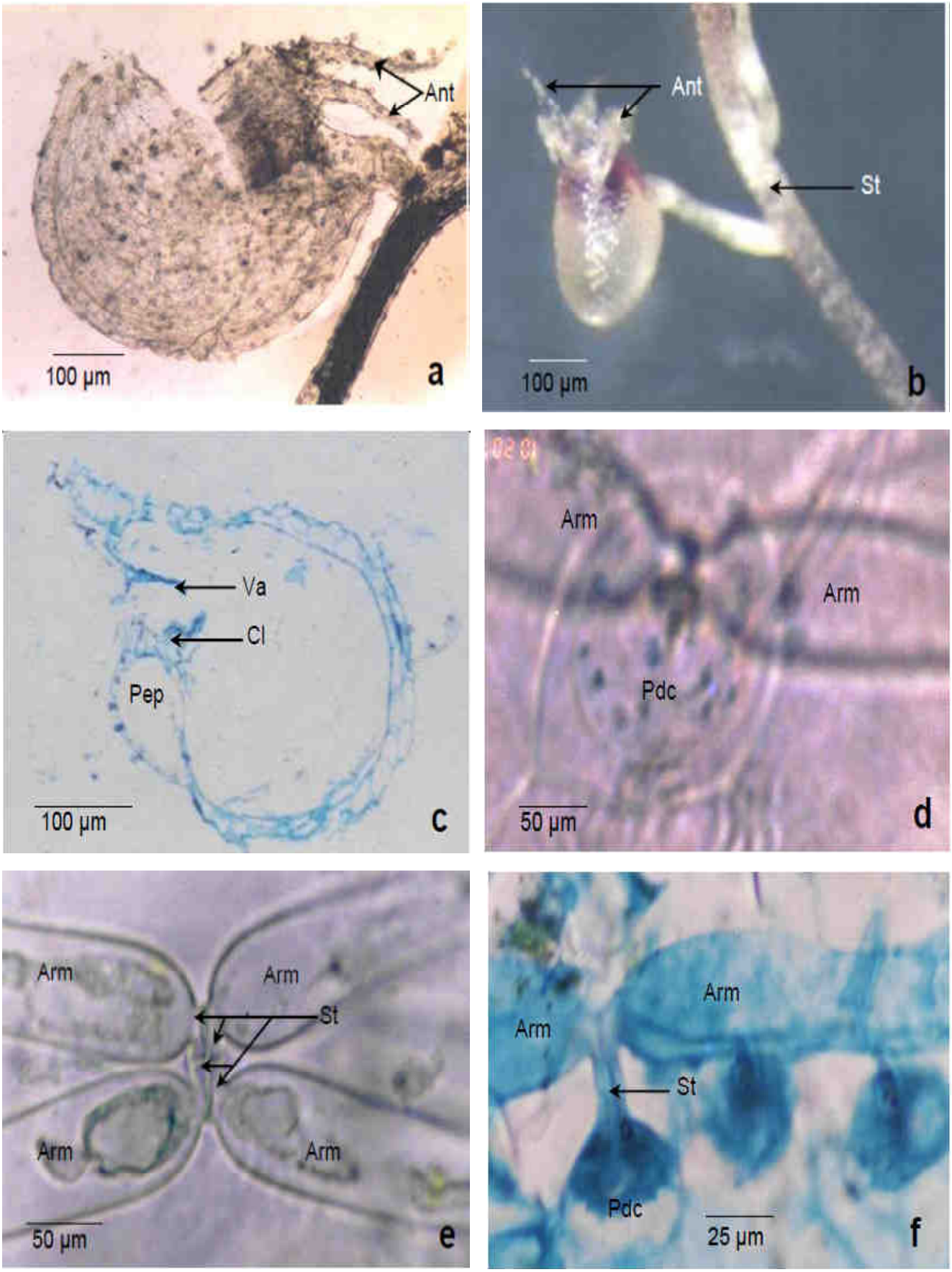

Figure 1. a) A matured bladder of *Utricularia bifida* with two antennae (Ant); b) a single bladder of *Utricularia pubescens* with numerous antennae (Ant); c) longitudinal section of the bladder showing valve (Va), collar (Cl) and pavement epithelium (Pep); d) a single gland of *Utricularia bifida* with two arms and pedestal cells (Pdc); e) a single gland of *Utricularia pubescens* with four arms in respective stalk cell (St); f) longitudinal section of the whole gland of *U. pubescens* showing the four arms, stalk cells (St) and the subconical shaped pedestal cell (Pdc).

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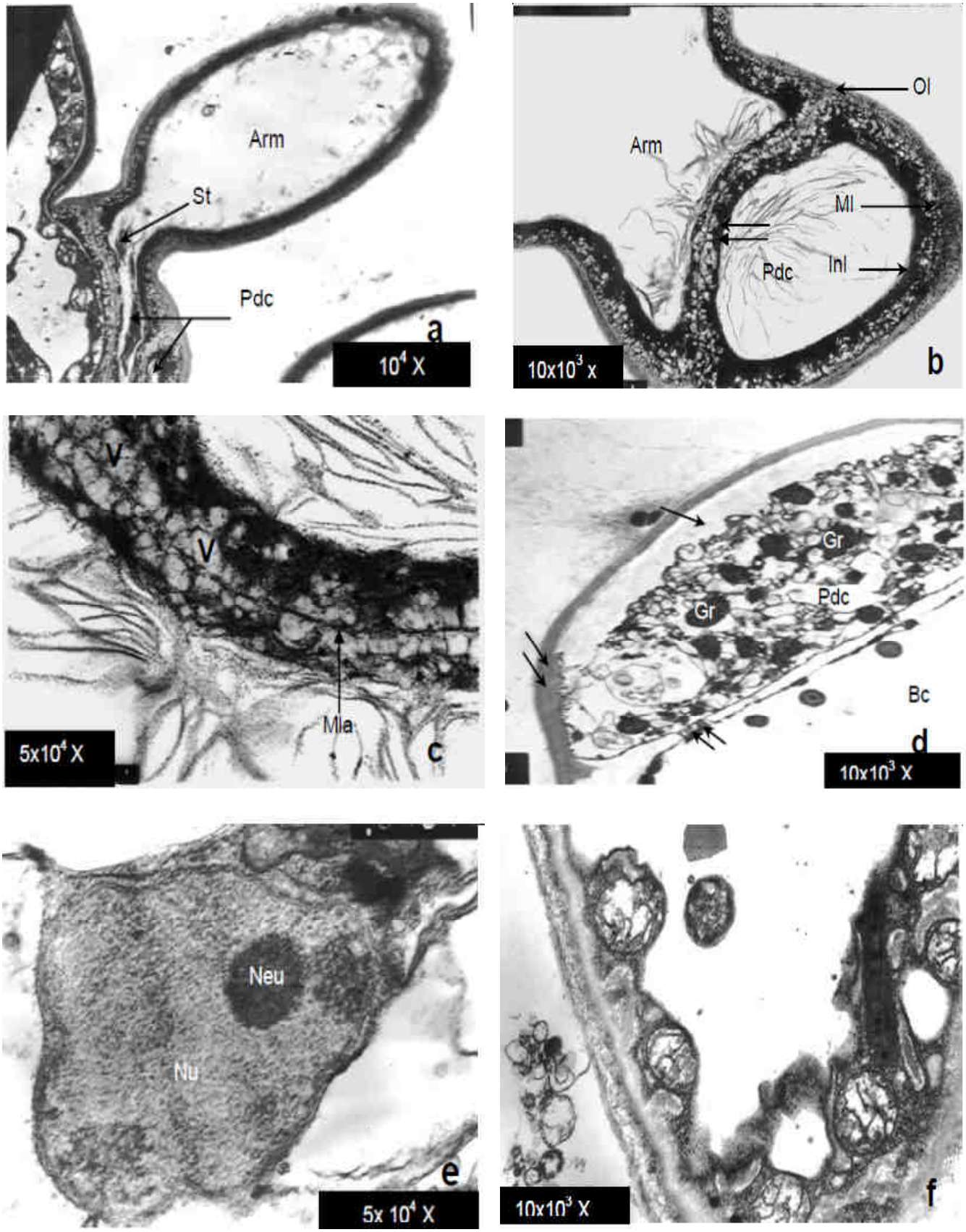

Figure 2. a) TEM of arm cell of *Utricularia bifida* showing stalk cell (St) and pedestal cell (Pdc). b) TEM of arm and Pdc showing the outer cuticle (Ol), middle layer (Ml) and the innermost layer (Inl). Double arrows show the extension of the cuticle c) TEM of the adjoining wall between pedestal and basal cells showing finger-like projections and the middle lamella (Mla). d) TEM of a pedestal cell showing pad-like wall ingrowths at the lateral side with numerous granules (Gr). Double arrows heads show pad-like wall ingrowths in the pedestal cell and plasmodesmetal connections. e) TEM of granular nucleus and nucleolus. f) TEM of protoplast of arm and enlarged view of arm protoplast.

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