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DEVELOPMENT OF THE WALL LABYRINTH IN PAVEMENT EPITHELIUM HAIRS OF SOME *UTRICULARIA* SPECIES

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Sodium hypochlorite-digested material and scanning electron microscopy was used to study the morphology of wall ingrowths in pavement epithelium hairs of *Utricularia* species from the primitive section Pleiochasia (*U. volubilis*) and the advanced section Utricularia (*U. stygia*, *U. intermedia*). Wall ingrowths were reticulate-type in all examined species. Wall ingrowth development started with the formation of small papillae, which later lengthened and eventually fused and branched, forming a network. The sequence of wall deposition in plant hairs is given for the first time with SEM. The wall labyrinth in transfer cells of pavement epithelium hairs was found to be far from static. Different stages of wall ingrowth development were observed within the same cell.

Key words: *Utricularia*, transfer cell, wall labyrinth, hairs, carnivorous plants, scanning electron microscopy.

INTRODUCTION

Transfer cells are plant or fungi cells having secondary wall ingrowths, in this case both an amplified area of wall and an area of plasma membrane. These cells play a key role in transport between the apoplast and symplast (Gunning and Pate, 1974; Offler et al., 2003). In the majority of cases, transmission electron microscopy (TEM) has been used to analyze the wall ingrowths, but this method does not enable the whole complexity of the cell wall labyrinth to be observed (Gunning and Pate, 1969; Chambers and Hamilton, 1973; Talbot et al., 2002). Moreover, TEM is not very useful for examining large surface areas (Chambers and Hamilton, 1973). Conventional fixation procedures for TEM can produce artefacts that influence the morphology of the transfer cell (Browning and Gunning, 1977). Since the 1970s, various methods have been used to visualize the 3D morphology of wall labyrinths by scanning electron microscopy (SEM). SEM has been used less frequently than TEM in studies of the transfer wall. The main problem is extraction of the cytoplasm to show the three-dimensional architecture of the wall labyrinth. Different authors have used different methods to accomplish this: enzyme digestion (Briarty, 1971), polyethylene glycol substitution (Idle, 1971), partial embedding in epoxy resin and later rinsing with acetone (Chambers and Hamilton, 1973), KOH treatment (Jones and Dropkin, 1976), and sodium hypochlorite digestion (Fineran and Calvin, 2000). Talbot et al. (2001, 2002) successfully used frozen material and Triton digestion to study the morphology and deposition of the cell wall labyrinth. The present study is a continuation of our work on transfer cell wall architecture in *Utricularia* hairs (Płachno and Jankun, 2004). Here, we improve our method to show better the morphology and development of the wall labyrinth in pavement epithelium hairs. We also use several species from different sections, both primitive and advanced, to find differences in wall labyrinth morphology between species and sections.

MATERIALS AND METHODS

Plants of three species *Utricularia* were examined: *U. volubilis* Brown (section Pleiochasia), *U. stygia* Thor, and *U. intermedia* Hayne (section Utricularia). *U. vo-*

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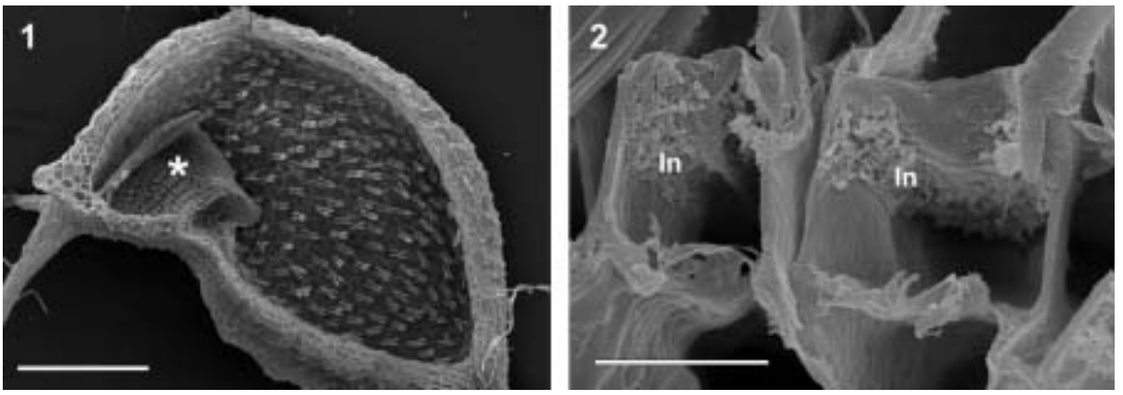


Fig. 1. Median section through trap of *Utricularia volubilis*: pavement epithelium (asterisk). Bar = 500 µm. **Fig. 2.** Longitudinal section of middle cells of pavement epithelium hairs, showing wall ingrowths (In). Bar = 5 µm.

lubilis and *U. stygia* were obtained from the collection of the Institute of Botany of the Academy of Sciences of the Czech Republic, Třeboń. *U. intermedia* plants were collected in the Jeleniak-Mikuliny Nature Reserve near Lubliniec, Poland. SEM was used to determine the three-dimensional morphology of the wall labyrinth. For SEM, traps were cut and fixed in 3% phosphate-buffered glutaraldehyde (GA) for 2 h at room temperature. To extract the cytoplasm after fixation, the tissue was placed in a 10% solution of commercial bleach (sodium hypochlorite). After 10 min the material was washed in water and later dehydrated in an ethanol series. The material was critical-point dried using liquid CO_2. The dried tissues were sputter-coated with gold and viewed with a HITACHI S–4700 SEM in the Scanning Microscopy Laboratory of Biological and Geological Sciences, Jagiellonian University.

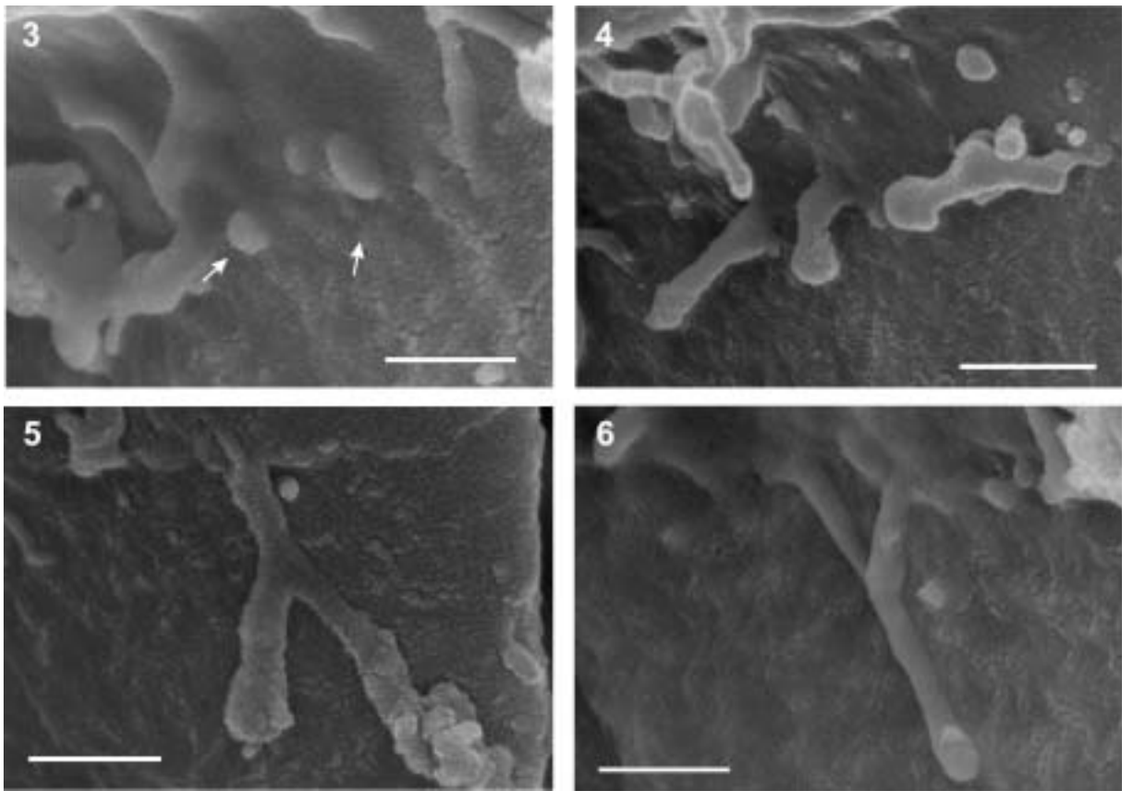
RESULTS

In this paper, using sodium hypochlorite-digested material according to Fineran and Calvin (2000), we show the three-dimensional morphology of the wall labyrinth. We shortened the digestion time because the bladder tissue is very soft. This method yielded results whose quality is comparable to those obtained by Talbot et al. (2001, 2002).

In the examined species, the pavement epithelium was situated on the top of the threshold at the entrance of the trap (Fig. 1), and was composed of sessile, closely packed glandular hairs. In *U. volubilis*, wall ingrowths were found only in the middle cell of the anterior hairs of the pavement epithelium. The wall labyrinth was formed on the distal transversal wall and the terminal part of distal lateral walls (Fig. 2). The wall ingrowths have reticulate-type morphology. In *U. intermedia*, wall ingrowths of the reticulate type were found in both the middle and basal cells of some hairs. In *U. stygia*, the reticulate wall labyrinth was observed in the middle and terminal cells of some hairs. The degree of development of wall ingrowths was not uniform in the latter two species. Development of the wall labyrinth began with the formation of small papillate wall ingrowths (Fig. 3), which lengthened (Fig. 4), branched (Fig. 5) and anastomosed (Fig. 6). In *U. intermedia*, small finger-like protrusions of wall material appeared on the wall ingrowths (Fig. 7). These appendages could connect neighboring wall ingrowths. Finally, wall ingrowths formed a complicated network (Fig. 8). The wall labyrinth could appear as a short network (Fig. 8) or form a lace-like wall network (Fig. 9). The differentiation sequence from papillate form to complex wall labyrinth was observed in cells of the same age (Fig. 9).

DISCUSSION

Hairs are a good model system for studying both the morphogenesis and the physiology of plant cells (Marks, 1997; Hülskamp et al., 1999; Schwab et al., 2000; Adlassnig et al., 2004). In particular, hairs possessing transfer cells may be used to study symplastic and apoplastic transport. The development of the wall labyrinth has been described in a variety of plant tissues (Wang et al., 1994; Talbot et al., 2001, 2002), but little is known about this process in hair cells. In *Utricularia*, transfer cells have been detected in different types of glandular hairs (Beltz, 1974; Fineran and Lee, 1974a,b, 1975; Ghirardelli Gambardella and Honsell, 1975; Fineran, 1980, 1985; Broussaud and Vintjoux, 1982; Płachno and Jankun, 2004). The reticulate-type morphology of the wall labyrinth was shown by Płachno and Jankun (2004) in *U. intermedia* internal trap hairs. Wall ingrowths described by Fineran and Lee (1974a, 1980) using TEM in *U. monanthos*



Figs. 3–6. The development of wall ingrowths in middle cells of pavement epithelium hairs of *Utricularia intermedia*. **Fig. 3.** First stage of deposition of wall ingrowths (arrows). Bar = 500 nm. **Fig. 4.** Lengthened papillate wall ingrowths. Bar = 1 µm. **Fig. 5.** Branched wall ingrowth. Bar = 500 nm. **Fig. 6.** Anastomosed wall ingrowths. Bar = 500 nm.

were classified by Offler et al. (2003) as reticulate. The wall labyrinth in two other species examined here (*U. volubilis* and *U. stygia*) should be considered of this type. Reticulate-type architecture of wall ingrowths occurred in species from both the primitive section Pleiochasia (*U. volubilis* and *U. monanthos*) and the advanced section Utricularia (*U. stygia, U. intermedia*), and probably also occurs in other species in this genus. Offler et al. (2003) showed that reticulate wall ingrowths occur in many taxonomic groups including plants, algae and even fungi. In contrast to ubiquitous transfer cells with reticulate wall ingrowths, transfer cells with flange wall ingrowths are rare and known mainly from monocotyledons. However, this type was described also in Dicotyledonae, for example in a node leaf trace of *Trollius europaeus* (Gunning et al., 1970; Gunning and Pate, 1974). Interestingly, both types of wall ingrowths could be observed in one plant and even in the same cell, as for example in *Hordeum vulgare* (Talbot et al., 2002). It should be added that the morphology of the wall labyrinth is so diverse that sometimes it is difficult to classify it as reticulate or flange (Talbot et al., 2002).

In this study we also present the development of the wall labyrinth in hairs of the pavement epithelium. To our knowledge, the sequence of wall deposition in plant hairs has not previously been described using SEM.

Talbot et al. (2001, 2002) reported detailed SEM observations of wall labyrinth deposition in other tissues. The first steps of wall ingrowth deposition in *U. intermedia* and *U. stygia* documented here resemble the development of reticulate wall ingrowths in other plants (Talbot et al., 2002), especially the sequential stages of wall ingrowth development in xylem parenchyma cells of both *Pisum sativum* and *Tradescantia virginiana*. We observed finger-like protrusions of wall material, which connected neighboring wall ingrowths. In *Vicia faba*, Talbot et al. (2002) described slender protrusions of wall material, which connected wall ingrowths. They also found discrete finger-like protrusions of the wall, and considered these structures to be localized points for wall deposition. The reticulate-type wall labyrinth can be said to have the same pattern of development regardless of the type of plant tissue (see also: Talbot et al., 2002; Offler et al., 2003).

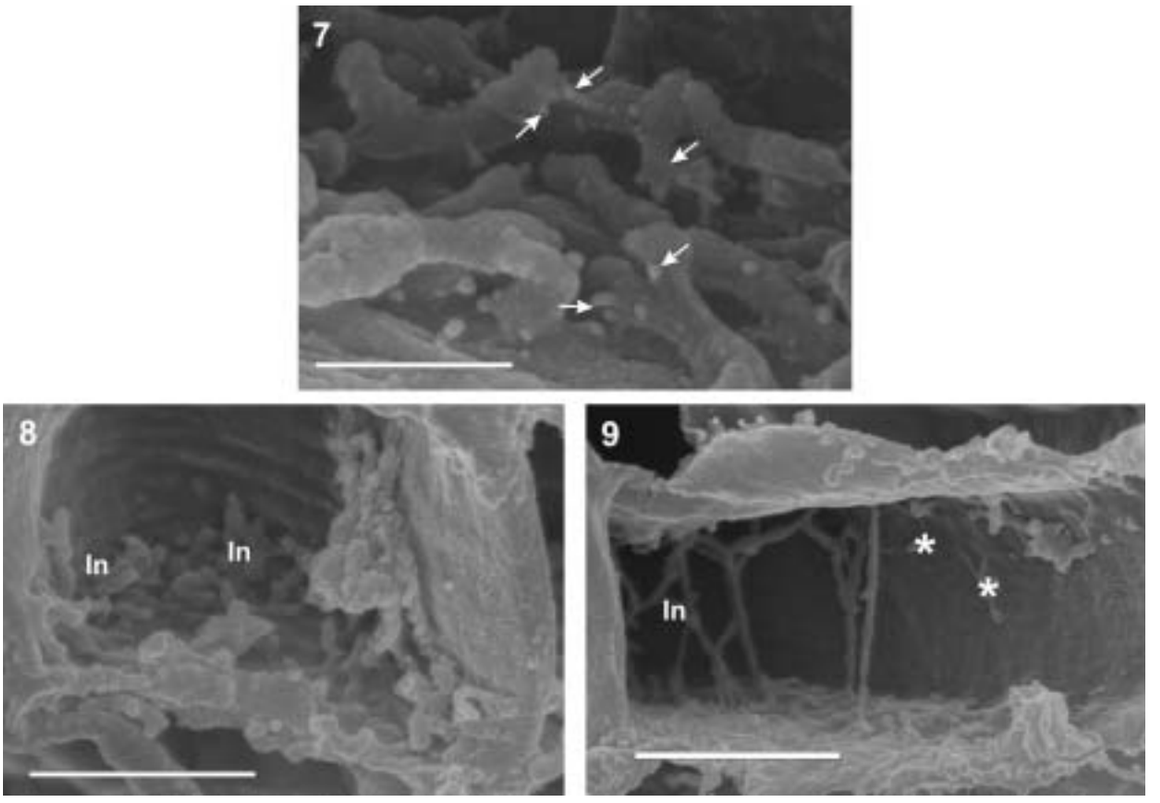


Fig. 7. Wall ingrowths which formed finger-like protrusions of wall material (arrows). Bar = 500 nm. **Fig. 8.** Short network of wall labyrinth in middle cell of pavement epithelium hair in *Utricularia intermedia*. Bar = 2 µm. **Fig. 9.** Different stages of wall ingrowth development in one cell: "juvenile" stages of wall ingrowths (asterisk); lace-like network of wall labyrinth (In). Bar = 3 µm.

Broussaud and Vintejoux (1982) showed a correlation between the degree of differentiation of wall ingrowths and the position of the hair in the pavement epithelium. We also found hairs having a middle cell without wall ingrowths. Many authors have noted that during cell ontogenesis the wall labyrinth is modified. For example, Wang et al. (1994) described a sequence of wall ingrowth differentiation in four stages of *Triticum* transfer cell development. In the first stages, wall ingrowths differentiated as a papillae form. Later they formed a complex wall labyrinth, which was compressed and infilled by wall material in the final stage. In this type of transfer cell, the final stage was accompanied with degeneration of the protoplast. In *Utricularia monanthos*, the terminal cell of external hairs was differentiated as a transfer cell at first. Later, secondary wall material was deposited over the wall ingrowths, and the function of the hairs was changed (Fineran, 1980). Moreover, wall ingrowths can be hydrolyzed, as in hair cells of carnivorous plants, for example in *Dionea* (Schwab et al., 1969) and *Pinguicula* (Heslop-Harrison, 1975). According to Offler et al. (2003), transfer cell differentiation is coordinated with organ development or is induced in response to external stresses such as nutrient deficiency or invasion by symbiotic or parasitic organisms. The differentiation of cells as transfer cells is especially interesting when it is the result of interactions between plants and other organisms, for example in a mycorrhiza (Lutz and Sjolund, 1973; Duddridge and Read, 1982).

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