J. Plant Biol. (2009) 52:355–363
DOI 10.1007/s12374-009-9046-6

ORIGINAL RESEARCH

Structure and Development of the Endophyte in the Parasitic Angiosperm *Cuscuta japonica*

Kyu Bae Lee

Received: 14 August 2008 / Revised: 10 April 2009 / Accepted: 14 April 2009 / Published online: 1 July 2009

Abstract The endophyte, that is, the haustorial part within the tissues of the host plant *Impatiens balsamina*, of the parasitic angiosperm *Cuscuta japonica* was studied with light and electron microscopy. The endophyte consisted mainly of vacuolated parenchymatous axial cells and elongate, superficial (epidermal) cells. Then the elongate, epidermal cells separated from each other and transformed into filamentous cells, called searching hyphae. The hyphae grew independently either intercellularly or intracellularly in the host parenchyma. The apical end of the hyphal cells was characterized by conspicuous, large nuclei with enlarged nucleoli and very dense cytoplasm with abundant organelles, suggesting that the hyphal cells penetrating host tissue were metabolically very active. Numerous osmiophilic particles and chloroplasts were noted in the hyphae. The osmiophilic particles were assumed to be associated with elongation of the growing hyphe. Plasmodemata connections between the searching hyphal cells of the parasite and the host parenchyma cells were not detected. Hyphal cells that reached the host xylem differentiated into water-conducting xylic hyphae by thickening of the secondary walls. A xylem bridge connecting the parasite and the host was confirmed from serial sections. Some hyphal cells that reached the host phloem differentiated into nutrient-conducting phloic hyphae. Phloic hyphae had a thin layer of peripheral cytoplasm with typical features of sieve-tube members in autotrophic angiosperms, i.e., parallel arrays of smooth endoplasmic reticulum, mitochondria, and plastids with starch granules. Interspecific open connections via the sieve pores of the host sieve elements and plasmodesmata of the parasite phloic hyphae were very rarely observed, indicating that the symplastic translocation of assimilate to the parasite from the host occurred.

Keywords Anatomy · *Cuscuta japonica* · Endophyte · Parasitic angiosperm · Ultrastructure

K. B. Lee (✉)
Department of Biological Science Education,
College of Education, Chosun University,
Gwangju 501-759, Republic of Korea
e-mail: leekb@chosun.ac.kr

The genus *Cuscuta* has been considered to be a holoparasitic angiosperm, obtaining both water and organic nutrients from its host plants. Some species have chlorophyll, thylakoids, and the enzyme Rubisco as in *C. europaea* (Machado and Zetsche 1990) or *C. grandiflora* and *C. odorata* (van der Kooij et al. 2000). However, several *Cuscuta* species do contain chlorophyll, especially in the tips of the seedlings (Panda and Choudhury 1992; Dawson et al. 1994; Lee et al. 2005), and various species are capable of photosynthesis. For example, photosynthetic pigments in *C. campestris* (Dinelli et al. 1993), many photosynthetic proteins as well as Rubisco in *C. reflexa* (Hibberd et al. 1998) and *C. pentagona* (Sherman et al. 1999), and photosynthetic plastid genes in parasitic *Cuscuta* stems (van der Kooij et al. 2000; Revill et al. 2005) have been reported. Several studies have demonstrated that water, photosynthate, and other macromolecules are transferred to the parasite via a specialized absorptive organ, the haustorium, from the host (Hibberd et al. 1998; Seel and Jeschke 1999; Hibberd and Jeschke 2001; Haupt et al. 2001; Birschwilks et al. 2006, 2007; Schwartz et al. 2008).

The tips of light-grown *Cuscuta japonica* seedlings have chloroplasts with thylakoids that are well organized into grana (Lee 2007a), which are converted from etioplasts with starch grains, prolamellar bodies, and crystalline structures (Lee 2007b). After the tips of the parasite seedlings

contact the host, the upper haustorium, lies external to the host, develops via three main successive stages—the haustorial initials, the meristem, the endophyte primordium (EP, a host-invading tissue)—from the middle layers of the cortex of the parasite stem (Lee 2007c). In the EP, the digitate cells also contain chloroplasts with well-developed grana and a number of other cell organelles. The structural development of *C. japonica* suggests that the parasite is capable of autotrophic photosynthesis during its period of seedling growth. As maturation of the *C. japonica* upper haustorium progresses, the hasustorial epidermal cells elongate and branch to form projections (Lee 2008). And then those projections have released dense particles into cell walls and secreted dense materials within the spaces between the projections. It suggests that the modification and secretion in the epidermal cells could play an important role in cementing the haustorium onto the surface of the host organ.

The endophyte primordium of the upper haustorium penetrates the host tissues, then the endophyte, lies internal to host tissues, differentiates. The endophyte of *C. australis* consists of axial parenchymatous cells and elongate tip cells with a dense cytoplasm including large, conspicuous nuclei (Lee and Lee 1989). The elongate tip cells independently grow and penetrate the host parenchyma tissue and transform into filamentous cells called searching hyphae (Dörr 1972; Vaughn 2003). The searching hyphal cells grow between and through the host parenchyma cells, then they reach the host vascular tissue and eventually differentiate into xylic or phloic conductive hyphae (Dörr 1972, 1987, 1990; Lee and Lee 1989; Vaughn 2006). The anatomical and ultrastructural features of the endophyte in *C. japonica*, however, have not yet been revealed in detailed. The purpose of this study was to describe the structural development of the endophyte in *C. japonica*. This report is the fourth part of a comprehensive study on the development of the embryo, seedling, upper haustorium, and endophyte of *C. japonica*.

Materials and Methods

Plant Materials

Mature, dormant seeds of dodder (*Cuscuta japonica* Choisy) were scarified with concentrated sulfuric acid for 45 min and then rinsed in tap water followed by distilled water. The seeds were placed on moist filter paper in petri dishes and germinated in an incubator in the dark at 30°C and allowed to develop into seedlings. The roots of 3-day-old dark-grown seedlings were wrapped with wet cotton, placed in 500 mL covered beakers, and placed near windows for exposure to sunlight for 3 days. The tip regions of these 6-day-old seedling shoots, when placed in contact with the stem of the host plant *Impatiens balsamina* L., entwined the host stem and produced upper haustoria on the side of the dodder stem in contact with the host. Portions of the endophytes that invaded the host tissue at subsequent developmental stages were examined with light and electron microscopy.

Transmission Electron Microscopy

For electron microscopy, segments of the endophytic tissue were fixed in a mixture of 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M sodium cacodylate buffer (pH 6.8) for 2–3 h at room temperature. Tissues were then exposed twice to microwave radiation for 10 and 20 s at 70% of the maximum in an 800-W Pelco Model 3450 Laboratory Microwave Processor (Ted Pella, Inc., Redding, California, USA) equipped with a thermistor copper temperature probe and an auxiliary Pelco 3420 Microwave Load Cooler (Ted Pella, Inc.). The tissues were washed in the same buffer (3 × 15 min), and postfixed in 1% osmium tetroxide in cacodylate buffer (pH 6.8), and microwaved three times for 40 s. The segments were washed in the buffer and dehydrated in a graded series of acetone in the microwave oven at a temperature of 37°C. The tissue pieces were then infiltrated with Spurr's resin (Spurr 1969). Thick sections were cut with an LKB-V ultramicrotome, then stained with 0.05% toluidine blue and examined with an Olympus BH2 light microscope. Thin sections were cut with a RMC MT-7000 ultramicrotome, mounted on grids and stained with uranyl acetate and lead citrate, then examined and photographed with a JEM l00 CXII or Hitachi H-7600 transmission electron microscope at 80 kV.

Results

Parasitic Hyphal Cells Growing within Host Parenchyma

The endophyte primordium (EP), a host-invading tissue, consisting of three types of cells, developed within the mature upper hasutorium of *C. japonica* (Lee 2007c). The primordium penetrated into the host stem and formed the endophyte (Fig. 1a). The endophyte consisted of highly vacuolated parenchymatous axial cells and elongated superficial epidermal cells (Fig. 1b). The parenchymatous axial cells divided anticlinally and periclinally. The tips of the elongated epidermal cells contained a dense cytoplasm and nuclei, and they separated from each other and grew independently. They then were transformed into the filamentous searching hyphae. These hyphal cells grew either intercellularly or intracellularly in the parenchyma of the host cortex (Figs. 1b–c and 2a). During tip growth of the hyphal cells, the host walls separated at the middle

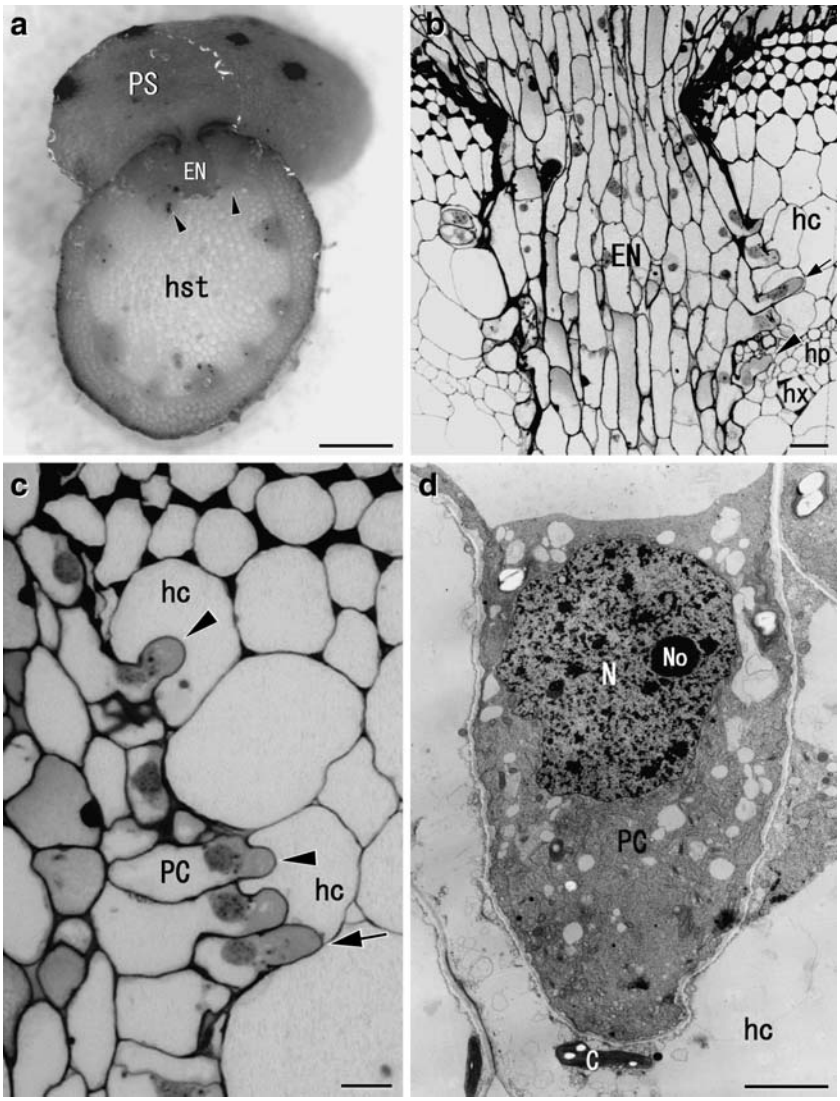

Fig. 1 Light (**a, b, c**) and electron micrographs (**d**) of endophyte of *Cuscuta japonica* with haustorial portion growing within the tissue of the host *Impatiens balsamina*. (**a**) Fresh section of endophyte (EN) that developed from upper haustorium in the parasite stem (PS). EN invaded the parenchyma between two vascular bundles (arrowheads) of host stem (hst). Bar = 25 μm. (**b**) EN, consisting of vacuolated parenchymatous axial cells and elongated epidermal filamentous hyphal cells. The hyphae have dense cytoplasm including nuclei at the tips and penetrated host parenchyma (arrow) and vascular tissues (arrowhead). hx, host xylem; hp, host phloem. Bar = 50 μm. (**c**) Parasitic hyphal cells (PC) in host parenchyma, sectioned transversely. Some PCs (arrowheads) grew through host parenchyma cells (hc), whereas others grew between host cells (arrow). Dense cytoplasm and nuclei of the PCs is limited at the apical region. Bar = 25 μm. (**d**) Apical end of a PC sectioned longitudinally shows conspicuous nucleus with enlarged nucleolus and dense cytoplasm including abundant other organelles. Chloroplast (arrow) of host parenchyma cell (hc) is visible near invading, apical end of the PC. Bar = 0.5 μm

lamella (Fig. 2a–c). The hyphal cells were highly vacuolated, and their apical regions had a dense cytoplasm characterized by prominent nuclei with enlarged nucleoli and a number of other organelles such as dictyosomes, mitochondria, small vacuoles, and plastids with starch grains (Figs. 1c–d, 2c–d and 3a). The hyphal cells especially contained a number of round to elliptic electron-opaque particles that were frequently visible near dictyosomes (Figs. 2b–d and 3a). Vesicles or small vacuoles containing osmiophilic contents seemed to fuse with the plasma membrane of the tip-growing hyphal cells (Fig. 2b), and the plasma membrane had a wavy appearance with parts somewhat darkly stained (Figs. 2b and 3a).

Some hyphal cells had several types of plastids, ie., chloroplasts with well-developed thylakoids and starch grains (Fig. 3b), and plastids with starch grains, a few thylakoids, phytoferritin, prolamellar body, and crystalline inclusions with a lattice substructure enclosed by a membrane (Fig. 3c). When the penetrating hyphal cells came in contact with the host cells, in certain area at the interface between the host

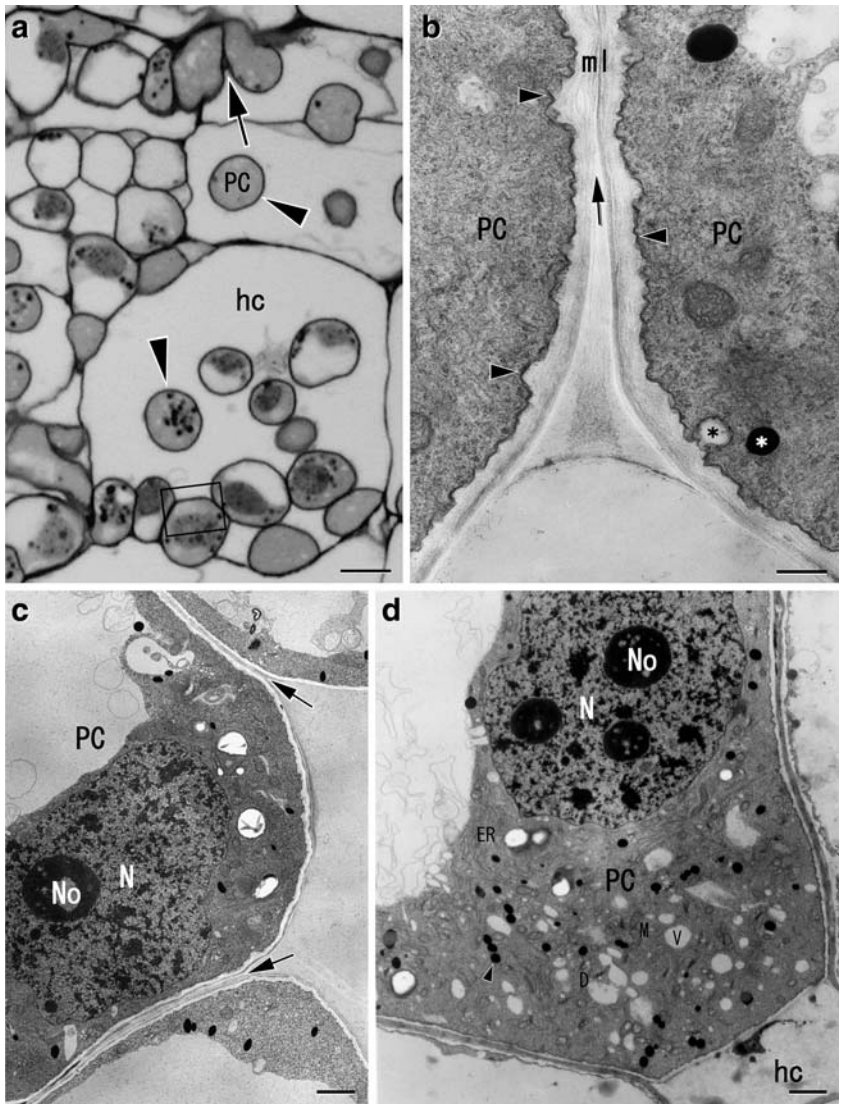

Fig. 2 Light (**a**) and electron micrographs (**b–d**) of parasitic hyphal cells of *Cuscuta japonica* endophyte within parenchyma of the host *Impatiens balsamina*. (**a**) Several parasitic hyphal cells (PC) are seen at the tip of a transversely sectioned endophyte. Some PCs are separating from each other (arrow) and growing independently (arrowheads) within host parenchyma cells (hc). PCs contain dense cytoplasm and large nuclei. Bar = 25 μm. (**b**) Middle lamella (ml) between two walls of PCs is separating. PC contains osmiophilic particles (white asterisk). Vesicle (black asterisk) with the particles appears to be fused with plasma membrane of hyphal cells. Wavy plasma membrane has more or less densely stained parts (arrowheads). Bar = 0.5 μm. (**c**) Rectangular area in Fig. 5 shows that the middle lamellae of three PCs within a host parenchyma cell have separated (arrows). PCs have prominent nuclei (N) with enlarged nucleolus (No) and dense cytoplasm including several osmiophilic particles. Bar = 2 μm. (**d**) PC in contacted with host cell (hc) has conspicuous nuclei (N) with enlarged nucleoli (No) and dense cytoplasm containing several other organelles including mitochondria (m), dictyosomes (d) , endoplasmic reticulum (er), small vacuoles (v), and numerous osmiophilic particles (arrow). Bar = 2 μm

and the parasite, both cell walls were intact and fused with each other (Fig. 3d). In other areas, however, the host walls appeared to be degraded, while the cell walls of the parasitic hyphae were perfectly preserved (Fig. 3e).

Conductive Hyphae Reached Host Vascular Tissues

The searching hyphae grew through the host cortex to the host xylem and phloem (Fig. 4a). The hyphal cells that reached the host xylem had differentiated into the xylic hyphae, that is, the water-conducting elements, by thickening of the secondary walls. In serial sections, a xylem bridge between the endophyte xylem of the parasite and the host xylem was seen (Fig. 4b–d). Some of the hyphal cells that reached the host phloem had features of immaure sieve elements: they still contained nuclei and plastids with a few of thylakoids (Fig. 5a–b). Eventually, these hyphae differentiated into the nutrient-conducting elements, phloic

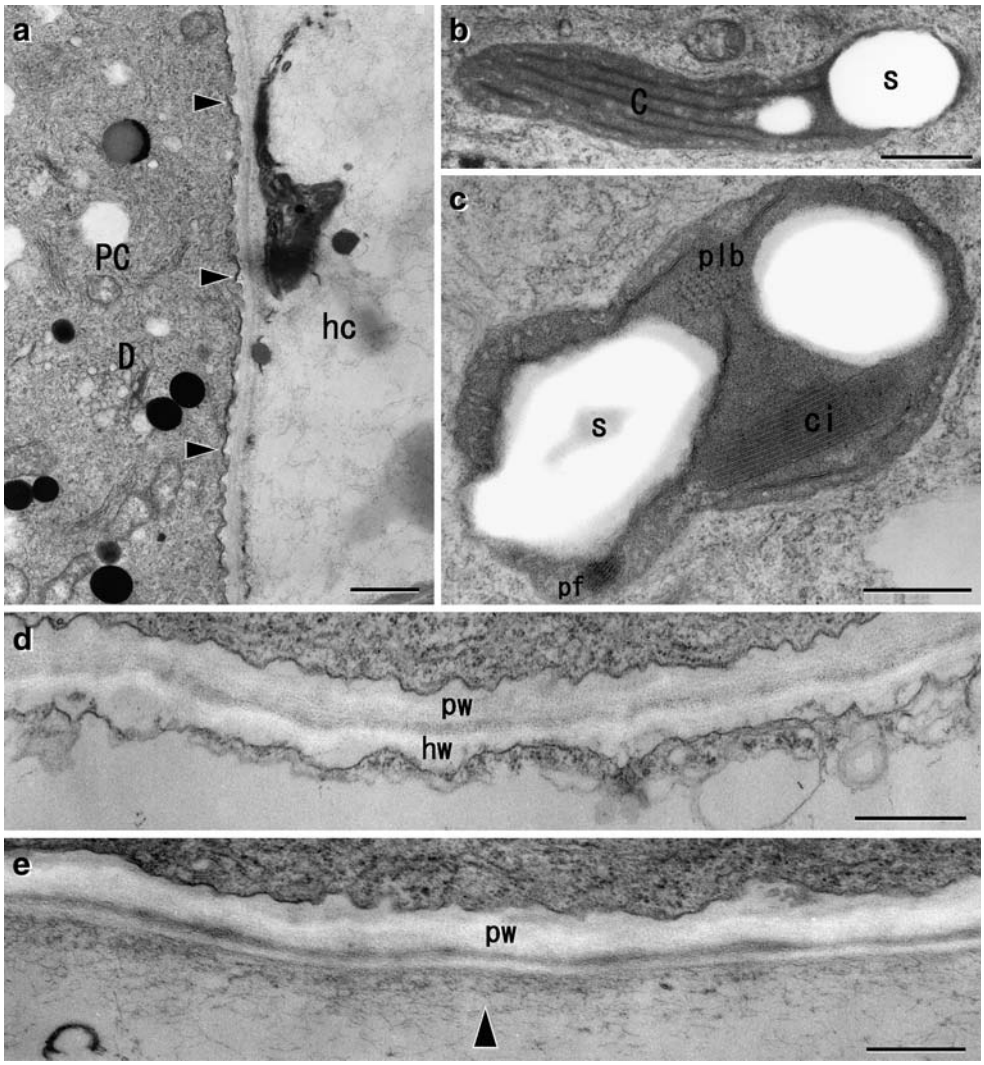

Fig. 3 Electron micrographs of parasitic hyphal cells (PC) of *Cuscuta japonica* endophyte. (**a**) Enlarged view of apical end of PC in contact with host cell (hc) in Fig. 2d shows osmiophilic particles near dictyosomes (D). Plasma membrane of PC is wavy with somewhat darkly stained portions (arrowheads). Bar = 1 μm. (**b**) Some PCs have chloroplasts (C) with somewhat developed thylakoid membranes and starch grains (s). Bar = 1 μm. (**c**) Plastids in PCs contain a few thylakoid membranes, crystalline inclusion (ci), phytoferritin (pf), prolamellar body (plb), and starch grains (s). Bar = 0.5 μm. (**d**) At host–parasite interface, cellulosic fusion between parasitic hyphal cell wall (pw) and host cell wall (hw) is clearly seen. Bar = 0.5 μm. (**e**) Host cell wall seems to be digested (arrows), while parasitic hyphal cell wall (pw) is intact. Bar = 0.25 μm

hyphae. The mature phloic hyphae contained a thin layer of peripheral cytoplasm, and had typical features of sieve-tube members of autotrophic higher plants: mitochondria, plastids with fine starch granules, and parallel arrays of smooth endoplasmic reticulum (Fig. 5c–d). At the interfaces the host sieve elements had sieve pores that were lined with small amounts of callose (Fig. 5c, e). The sieve pores of the host sieve elements connected with plasmodesmata of the parasite phloic hyphae (Fig. 5c, f). Such open connections between the host and the parasite were very scarcely detected.

Discussion

Origin of the Endophyte

The endophyte had two cell types. One was the highly vacuolated parenchymatous cells arranged in rows, forming the main axis of the endophyte. The other type, elongate epidermal cells (hyphae), with prominent nuclei and dense cytoplasm with many of other organelles, they located at the surface of the endophyte axis. The histological arrangement and cytological features of the axial and epidermal cells of the endophyte are similar to those of the file and digitate cells of the endophyte primordium (EP), a host-penetrating tissue, in the mature upper haustorium of *C. japonica* (Lee 2007c). In the EP, at the proximal region the file cells have prominent nuclei and are arranged in several rows, while at the distal region the digitate cells contain very dense cytoplasm and large nuclei with enlarged nucleoli. Based on such cytohisological structure of the EP, the cellular proliferation and enlargement after division of the file cells in the proximal zone of the EP may generate physical forces that enable the digitate cells to advance and penetrate the host (Lee 2007c). Eventually those two types of cells will form an endophyte. Therefore, it indicated that the axial and epidermal cells of the endophyte originated from the file and digitate cells of the EP, respectively. The structure of the endophyte in *C.*

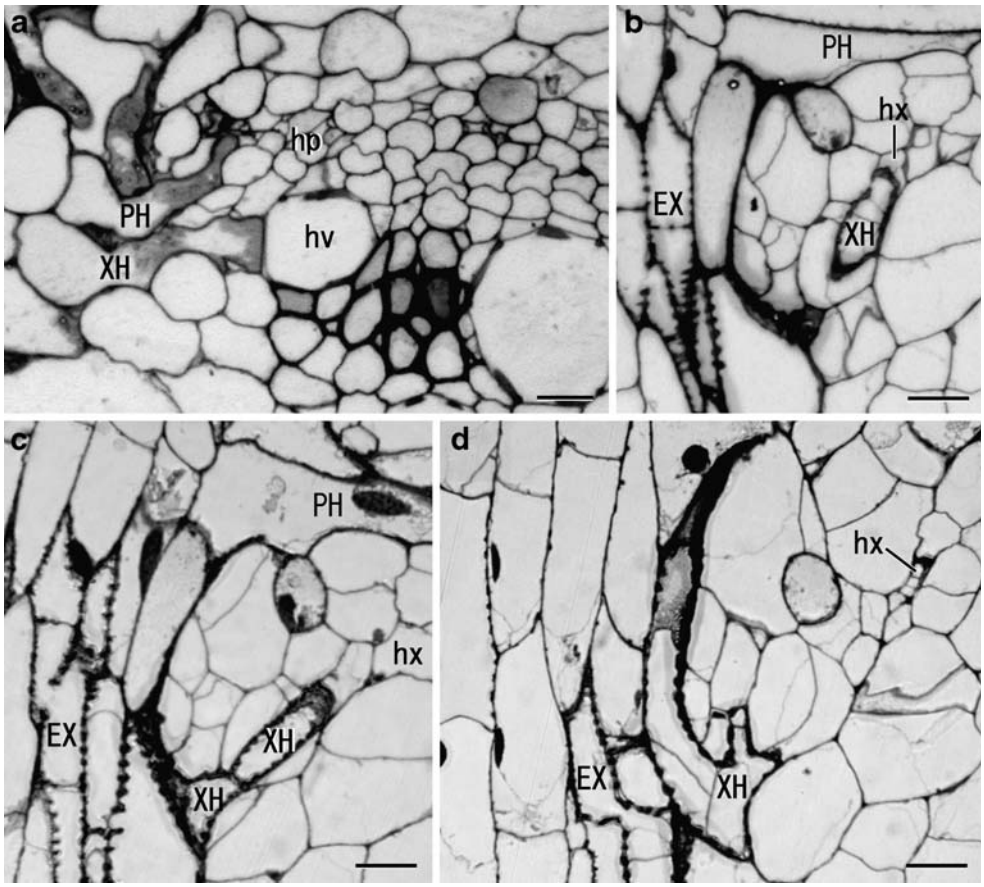

Fig. 4 Light micrographs of hyphal cells of *Cuscuta japonica* endophyte have reached host xylem and phloem, transversely sectioned. (**a**) Xylic hypha (XH) and phloic hypha (PH) reached the host vessel (hv) and phloem (hp), respectively. Bar = 25 μm. (**b–d**) Serial sections showing xylem bridge, formed by xylic hyphae (XH), between endophyte xylem (EN) and host xylem (hx). PH, phloic hyphae. Bars = 25 μm

japonica was very similar to that of C. *australis* (Lee and Lee 1989).

Cytological Features of Searching Hyphal Cells

The elongated epidermal cells known as searching hyphae grew from the endophyte between and through the host parenchyma cells as found in other *Cuscuta* species such as *C. odorada* (Dörr 1972), *C. australis* (Lee and Lee 1989), and *C. pentagona* (Vaughn 2003). The elongated hyphal cells seemed to be entirely vacuolated, but the apical end of the cells had large conspicuous nuclei with enlarged nucleoli and very dense cytoplasm including a number of other organelles, i.e., dictyosomes, r-ER, and mitochondria as in the hyphal cells of *C. reflexa* (Birschwilks et al. 2007). This cytological feature was very similar to that of the elongate digitate cells in the EP of the mature upper haustorium in *C. japonica* (Lee 2007c). Thus, the apical region of the hyphal cells may be metabolically very active, especially in the synthesis and secretion of enzymes such as acid phosphatase in *C. pentagona* (Tripodi 1970) and *Comandra umbellata* (Toth and Kuijt 1977) and wall-degrading enzymes in *Cuscuta reflexa* (Nagar et al. 1984) that can digest host tissue. This interpretation is supported by the degradation of walls of the host cells in contact with penetrating hyphal cells as in Fig. 3d–e.

In the growing hyphal cells, osmiophilic particles were frequently observed. The elongate digitate cells, consisting of an EP of the upper hautorium, also have numerous electron-dense particles within the small vacuoles near dictyosomes (see Fig. 16 in Lee 2007c). The small vacuoles appeared to be derived from dictyosomes. Considering that the hyphal cells originated from the digitate cells of the upper haustorium, the osmiophilic particles in the hyphal cells seemed to correspond to the electron-dense particles in the digitate cells. On the other hand, osmiophilic particles are frequently found in rapidly elongating parts of various plants, e.g., in coleoptiles of maize (Hoffmann-Benning et al. 1994; Edelmann et al. 1995; Robinson 1995; Edelmann and Volkmann 1996) and rye (Robinson 1995; Edelmann and Volkmann 1996), internodes of rice (Hoffmann-Benning et al. 1994), hypocotyls and epicotyls of some angiosperm and gymnosperm (Samajova et al. 1998), and epidermal cells of the upper haustorium in *Cuscuta japonica* (Lee 2008). The particles were demonstrated to be derived from secretory vesicles of dictyosomes and to be, at least in part, proteinaceous (Hoffmann-Benning et al. 1994) and containing protein expansin (Vaughn et al.

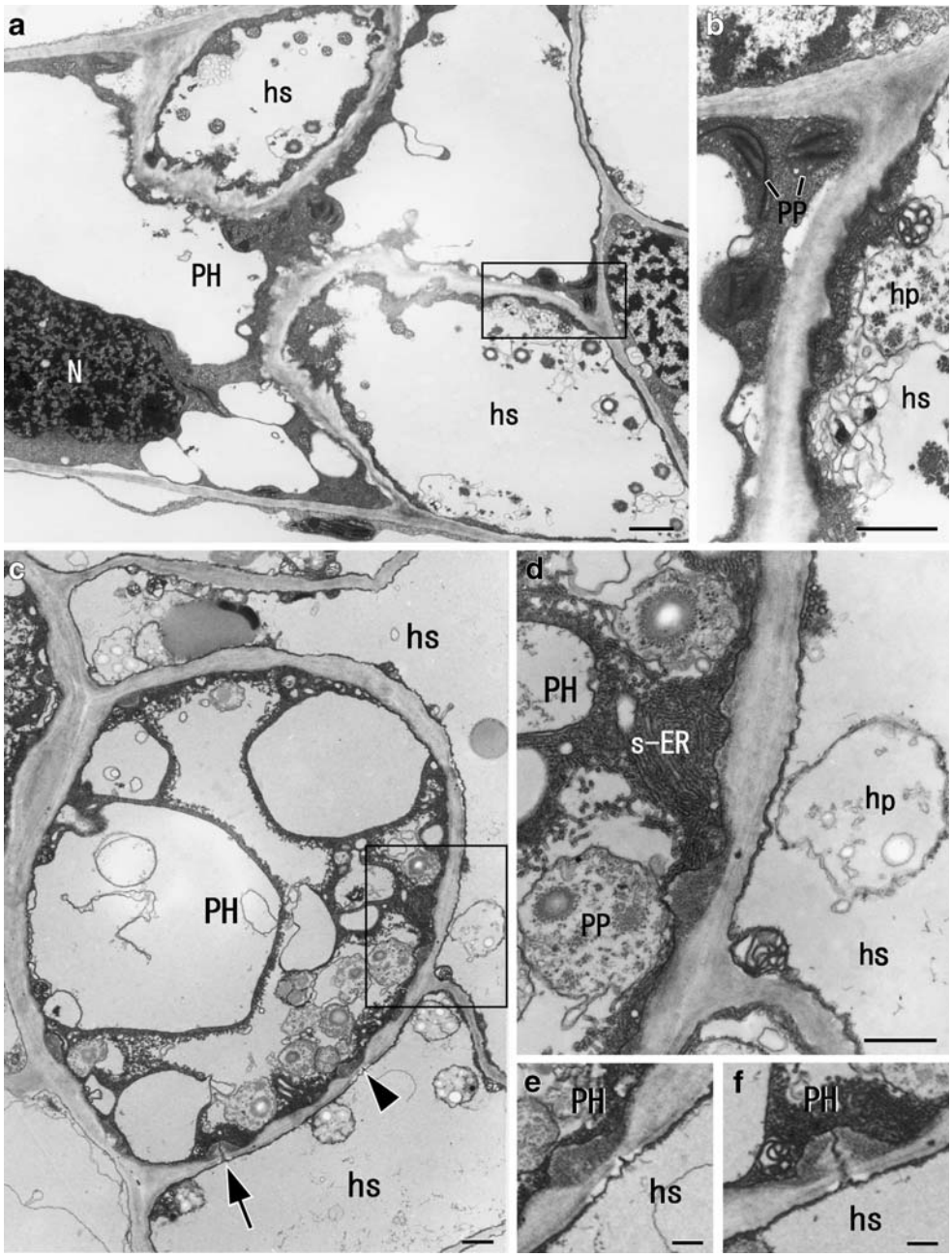

Fig. 5 Electron micrographs of phloic hyphae reaching the host phloem. (**a**) Immature phloic hypha (PH) longitudinally sectioned has penetrated between two host sieve elements (hs) transversely sectioned. Bar = 1 μm. (**b**) Enlarged view of a rectangular area in **a** shows that plastids (pp) of parasitic phloic hypha have thylakoids, whereas plastids (hp) of host sieve elements (hs) have fine starch granules. Bar = 1 μm. (**C**) Transversely sectioned, mature phloic hypha (PH) surrounded by host sieve elements (hs) contain vacuoles and plastids. Bar = 2 μm. (**d**) Enlarged view of a rectangular area in **c** exhibits typical features of sieve-tube member: parallel arrays of smooth endoplasmic reticulum (s-ER) and plastids (pp). hp, plastids of host; hs, host sieve element; pp, plastids of parasitic phloic hyphae. Bar = 1 μm. (**e**) Magnification of the part marked by an arrowhead in **c** shows a sieve pore the host sieve element (hs) abutting on the parasite phloic hyphae (PH). The sieve pore is lined with small amounts of callose. Bar = 0.25 μm. (**f**) Magnified view of a arrowed portion in **c** showing symplastic connection between a plasmodesma of parasite phloic hyphae (PH) and a sieve pore, lined with callose, of the host sieve element (hs). Bar = 0.25 μm

2001). Therefore, we assume that the small vacuoles or vesicles containing osmiophilic contents fused with the plasma membrane of the growing hyphal cells and that their contents were secreted into the walls. This assumption may be supported by the waviness and dark staining of plasma membrane of the growing hyphal cells as in Figs. 2b and 3a. The hyphae growing in the host parenchyma may extend up to 800 μm before reaching the host xylem and phloem (Dawson et al. 1994; Vaughn 2003). However, no osmiophilic particles are detected in the hyphal cells of the *Impatiens sultanii-Cuscuta pentagona* system (Vaughn 2003). Consequently, the osmiophilic particles are thought to be associated with the loosening and elongation of the hyphal cell walls as the hyphae grow (Edelmann et al. 1995; Samajova et al. 1998).

The chloroplasts with thylakoids and the etioplasts with a prolamellar body and crystalline inclusions in the hyphal cells in the host tissue suggest that the hyphal cells can photosynthesize. Such plastid types are also observed in the shoot subapical cells of light-grown seedling tips (Lee 2007a, b) and in digitate cells of an EP of the upper haustorium (Lee 2007c) during a free-living stage of *Cuscuta japonica* before it parasitizes its host. However, remarkably fewer chloroplasts were found in the hyphal

cells than in either the seedling cells (Lee 2007a, b) or the digitate cells of the upper haustorium (Lee 2007c). This result is coincident with the higher levels of cholophyll found in preparasitic stages of *Cuscuta* seedlings compared to the parasitic stems (Dinelli et al. 1993). Pattee et al. (1965) also reported that the photosynthetic efficiency of *C. indecora* is higher when seedlings are free-living rather than after they become parasitic. Furthermore, photosynthetic proteins, e.g., Rubisco, phosphoribonuclease, and plastocyanin, decrease greatly after *C. campestris* seedlings parasitize their host plants (Sherman et al. 1999).

In some host-*Cuscuta* systems, interspecific plasmodesmata at the interface between host parenchyma cells and *Cuscuta* hyphal cells have been observed in the *Pelargonium zonale-Cuscuta odorata* (Dörr 1987), *Nicotiana tabacum-Cuscuta reflexa* (Birschwilks et al. 2006), and *Arabidopsis thaliana-Cuscuta reflexa* (Birschwilks et al. 2007). Especially, in the *Impatiens sultanii-Cuscuta pentagona* system (Vaughn 2003) similar to *Impatiens balsamina -Cuscuta japonica* in the present study, plasmodemata are demonstrated between the host parenchyma cells and the *Cuscuta* hyphal cells. However, during the growth of the endophyte, no plasmodesmata connecting the cytoplasms between the cells of the host (*Impatiens balsamina*) parenchyma and the *Cuscuta japonica* hyphae were detected in this study. Symplastic continuity through plasmodesmata is not found even in the intraspecific or self-parasitism of *Cuscuta australis* haustoria, in which only half-plasmodemata occur on the cell walls of the parasitic hyphae (Lee 1993).

Xylic and Phloic Conductive Hyphae

The hyphae reached the host xylem and differentiated into xylic conductive hyphae. The xylic hyphae connected between the parasite xylem and host xylem, and thus a xylem bridge was formed (Fig. 4b–d) to maintain a flow of water and minerals between the parasite and its host. A lumen-to-lumen direct connection between parasite and host xylem is found in several host–parasite systems, i.e., *Zea mays-Striga hermonthica* (Dörr 1997), *Impatiens balsaminea–Cuscuta pentagona* (Vaughn 2006), and *Euphorbia dregenea–Hydnora triceps* (Tennakoon et al. 2006). However, such direct lumen-to-lumen links between host xylem and parasite xylem were not exactly observed in this study. Nor did we detect any hyphal penetration into the host vessels or differentiation into xylic hyphae within the host vessels as found for *Cuscuta australis* (Lee and Lee 1989).

The phloic conductive hyphae that reached the host xylem exhibited the features of sieve-tube members: the presence of a peripheral, thin cytoplasm including the parallel arrays of smooth endoplasmic reticulum, mitochondria, and plastids. The fine structure visible in autotrophic angiosperms could assist the absorption of organic nutrients from the host to the parasite (Vaughn 2006). Dörr (1972, 1987, 1990) reported wall ingrowths visible in transfer cells, a typical feature of the phloic or absorbing hyphae, in *Cuscuta odorata*, surrounding the sieve elements of the host (*Pelargonium zonale*). However, no wall ingrowths were found in *C. japonica* in this study or in *C. pentagona* (Vaughn 2006).

Ultrastucutral evidence showing the symplastic pathway via plasmosemata or sieve pore connections between the phloic hyphae of *Cuscuta* endophyte and its hosts has so far been not reported (Dörr 1987, 1990; Vaughn 2006). However, recent translocation studies suggest that there might be direct transfer of macromolecules via open sympalstic connections between the phloem of *Cuscuta* and its hosts, allowing absorption of green fluorescent protein (Haupt et al. 2001), the phloem-specific dye carboxyfluorescein (Birschwilks et al. 2006, 2007), and mRNA (Roney et al. 2007; Schwartz et al. 2008) from host plants to *Cuscuta*. Vaughn (2006) has stated that in *C. pentagona-Impatiens balsaminea* system the phloic hyphae with numerous smooth ER apoplastically transfers organic substances into the parasite. In the parasitic system, no open symplastic connections are found at the interface of the host phloem and parasite's phloic hyphae. In contrast to the previous reports, open symplastic pathway connecting the plasmodesmata of the parasite phloic hyphae with the sieve pores of the host sieve elements were detected in the present study. The open connections were very rarely observed as in *C. odorata-Hibiscus rosa* system, in which interconnections via the sieve pores between the sieve tubes of the host and the parasite are found (Schlenzka 1992). Such open direct connections between the sieve elements of the parasite and its host have been described well in the root paratsite *Orobanche* and its host *Vicia* system (Dörr and Kollmann 1995).

In summary, the elongate epidermal cells of *C. japonica* endophyte differentiated into filamentous hyphal cells, growing independently either intercellularly or intracellularly in the host parenchyma. The hyphal cells contained conspicuous, large nuclei with enlarged nucleoli and a dense cytoplasm, abundant osmiophilic particles, and various plastids. The osmiophilic particles were thought to be related to the elongation of the growing hyphal cells. No cytoplasmic pathway through plasmodesmata between the parasite's searching hyphae and the host cortical cells were observed. The hyphal cells reached the host xylem, and the phloem differentiated into xylic and phloic hyphae, respectively. The xylic and phloic conductive hyphae probably absorb water and nutrients from the host to the parasite. The presence of interspecific open connections between the plasmodesmata of the parasite phloic hyphae and the pores

of the host sieve elements suggested the symplastic movement of organic substances to the parasite from the host.

Acknowledgement This study was supported by a research fund from Chosun University in 2009.

References

Birschwilks M, Haupt S, Hofius D, Neumann S (2006) Transfer of phloem-mobile substances from the host plants to the holoparasite *Cuscuta* sp. J Exp Bot 57:911–921

Birschwilks M, Sauer N, Scheel D, Neumann S (2007) *Arabidopsis thaliana* is a susceptible host plant for the holoparasite *Cuscuta* spec. Planta 226:1231–1241

Dawson JH, Musselman LJ, Wolswinkel P, Dörr I (1994) Biology and control of *Cuscuta*. Rev Weed Sci 6:265–317

Dinelli G, Bonetti A, Tibiletti E (1993) Photosynthetic and accessory pigments in *Cuscuta campestris* Yuncker and some host species. Weed Res 33:253–260

Dörr I (1972) Der Anschluss der *Cuscuta*-Hyphen an die Siebrohren ihrer Wirtspflanzen. Protoplsma 75:167–184

Dörr I (1987) The haustorium of *Cuscuta*—new structural results. In: Weber HC, Forstreuter VW (eds) Proceedings of the 4th International Symposium on Parasitic Flowering Plants. Marbrug, FRG, pp 163–170

Dörr I (1990) Sieve elements in haustoria parasitic angiosperms. In: Behnke HD, Sjollund RD (eds) Sieve elements: comparative structure, induction and development. Springer, Berlin, FRG, pp 239–253

Dörr I (1997) How *Striga* parasitises its host: a TEM and SEM study. Ann Bot 79:463–472

Dörr I, Kollmann R (1995) Symplastic sieve elements continuity between Orobanche and its host. Bot Acta 108:47–55

Edelmann HG, Volkmann D (1996) The effect of brefeldin A on the redistribution of osmiophilic particles and the gravitropic response of rye coleoptiles. Protoplasma 190:1–7

Edelmann HG, Bergfeld R, Schopfer P (1995) Effect of inhibition of protein glycosylation on auxin-induced growth and the occurrence of osmiophilic particles in maizs (Zea mays L.) coleoptiles. J Exp Bot 46:1745–1752

Haupt S, Oparka KJ, Sauer N, Neumann S (2001) Macromolecular trafficking between *Nicotiana tabacum* and the holoparasite *Cuscuta reflexa*. J Exp Bot 52:173–177

Hibberd JM, Jeschke WD (2001) Solute flux into parasitic plants. J Exp Bot 52:2043–2049

Hibberd JM, Bungard RA, Press MC, Jeschke WD, Scholes JD, Quick WP (1998) Localization of photosynthetic metabolism in the parasitic angiosperm *Cuscuta reflexa*. Planta 205:506–513

Hoffmann-Benning S, Klomparens KL, Kende H (1994) Characaterization of growth-related osmiophilic particles in corn coleoptiles and deepwater rice internodes. Ann Bot 74:563–572

Lee KB (1993) Ultrastructural study on the cellular compatibility in self-parasiting *Cuscuta australis*. Kor J Bot 36(3):285–292

Lee KB (2007a) Ultrastructure and development of seedlings in the parasitic weed *Cuscuta japonica*. J Plant Biol 50:213–219

Lee KB (2007b) Ultrastructure of crystalline inclusions in the thylakoids of dodder (*Cuscuta japonica*) plastids. J Plant Biol 50:325–330

Lee KB (2007c) Structure and development of the upper haustorium in the parasitic flowering plant *Cuscuta japonica*. Am J Bot 97:737–745

Lee KB (2008) Anatomy and ultrastructure of the epidermal cells in the parasitic flowering plant *Cuscuta japonica* haustorium during attachment to the host. J Plant Biol 51:366–372

Lee KB, Lee CD (1989) The structure and development of the haustorium in *Cuscuta australis*. Can J Bot 67:2975–2982

Lee KB, Park J-B, Lee S (2005) Morphology and anatomy of mature embryos and seedlings in parasitic angiosperm *Cuscuta japonica*. J Plant Biol 43:22–27

Machado MA, Zetsche K (1990) A structural, functional and molecular analysis of plastids of the holoparasites *Cuscuta reflexa* and *Cuscuta europaea*. Planta 181:91–96

Nagar R, Singh M, Sanwal GG (1984) Cell wall degrading enzymes in *Cuscuta reflexa* and its hosts. J Exp Bot 35:1104–1112

Panda MM, Choudhury NK (1992) Effect of irradiance and nutrients on chlorophyll and carotenoid content and Hill reaction activity in *Cuscuta reflexa*. Photosynthtica 26:585–592

Pattee HE, Allred KR, Wiebe HH (1965) Photosynthesis in dodder. Weeds 13:193–195

Revill MJ, Staney S, Hibberd JM (2005) Plastid genome structure and loss of photosynthetic ability in the parasitic genus *Cuscuta*. J Exp Bot 56:2477–2486

Robinson DG (1995) Osmiophilic particles at the plasma membrane—what role do they play in extension growth. Bot Acta 109:81–83

Roney JK, Khatibi PA, Westwood JH (2007) Cross-species translocation of mRNA from host plants into the parasitic plant dodder. Plant Physiol 143:1037–1043

Samajova O, Volkmann D, Edelmann HG (1998) Occurrence of osmiophilic particles is correlated to elongation growth of higher plants. Protoplasma 202:185–191

Schlenzka B (1992) *Hibiscus rosa-sinensis/Cuscuta odorata*: Beispiel einer inkompatiblen Wirt-Parasiten-Beziehung. Christian-Albrechts-Universität Kiel, Germany, Thesis

Schwartz RD, Runo S, Townsley B, Machuka J, Sinha N (2008) Long-distance transport of mRNA via parenchyma cells and phloem across the host-parasite junction in *Cuscuta*. New Phytologist 179:1133–1141

Seel WE, Jeschke WD (1999) Simultaneous collection of xylem sap from *Rhinanthus m*inor and the host *Hordeum* and *Triticum*: hydraulic properties, xylem sap composition and effects of attachment. New Phytologist 143:281–298

Sherman TD, Pettigrew WT, Vaughn KC (1999) Structural and immunological characterization of the *Cuscuta Pentagona L.* chloroplast. Plant Cell Physiol 40:592–603

Spurr AA (1969) Low-viscosity epoxy resin embedding medium for electron micropscopy. J Ultrastr Res 26:31–43

Tennakoon KU, Bolin JF, Musselman LJ, Maass E (2006) Structural attributes of the hypogeous holoparasite *Hydnora triceps* Drege & Meyer (Hydnoraceae). Am J Bot 94:1439–1449

Toth R, Kuijt J (1977) Cytochemical localization of acid phosphatase in endophyte cells of the semiparasitic angiosperm *Comandra* umbellata (Santalaceae). Can J Bot 55:470–475

Tripodi G (1970) Localization of tryptophan rich protein and β-glycerophosphatase activity in Cuscuta haustorial cells. Protoplasma 71:191–196

van der Kooij TAW, Krause K, Dörr I, Krupinska K (2000) Molecular, functional and ultrastructural characterization of plastids from six species of the parasitic flowering plant genus *Cuscuta*. Planta 210:701–707

Vaughn KC (2003) Dodder hyphae invaded the host: a structural and immunocytochemical characterization. Protoplasma 220:189–200

Vaughn KC (2006) Conversion of the searching hyphae of dodder into xylic and phloic hyphae: a cytochemical and immunocytochemical investigation. Int J Plnat Sci 167:1099–1114

Vaughn KC, Barger W, Cosgrove D (2001) Dodders utilize expansin to attach to and invade the host. Plant Biol 2001:17–18