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Ultrastructural Study on the Haustorial Cells of *Cuscuta australis* R. Brown in the Region of the Host Parenchyma

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寄主植物의 柔組織 속에서 생장하는 실새삼(*Cuscuta australis* R. Brown) 吸器細胞의 미세구조

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ABSTRACT

Two cell types, tip cells and hyphal cells, were found at the front of *Cuscuta australis* endophyte growing into the stem parenchyma of the host plant, *Trifolium repens*. Each tip cell developed into an elongate, filamentous hypha. The cells of both types possessed a dense cytoplasm including abundant organelles and enlarged nuclei with the deeply lobed envelope. The unevenly thick walls were observed in certain tip cells. The wall penetrated through the middle lamellae of the host cells and engulfed the debris of broken host cells. Some front cells had the plasmalemma-wall invaginations, which increased the surface area and would facilitate material uptake from the host. No plasmodesmata between the host and parasite cells were found; instead, an apoplastic continuity was established by fused cell walls at the interface of the two partners. The apoplast was thought to be the main route for water and nutrients transport.

INTRODUCTION

In the advancing endophyte of *Cuscuta australis*, the tip cells grow separately from each other in a certain depth of the host tissue. They transform into filamentous hyphae. In the host, the hyphae reach vascular tissues through the cortical parenchyma, and differentiate into the xylary or phloic conductive elements (Lee and Lee, 1989). Eventually a continuous vascular system is formed between the host and parasite (Lee and Lee, 1991). The *Cuscuta* hyphae penetrating the cortical parenchyma of the host may selectively absorb nutrients from the host cells (MacLeod, 1961). The *Cuscuta*-hyphae growing into or between host parenchyma cells were observed (Dörr, 1968): such a hypha is called as a "searching hypha", which develops into a "contact hypha" reaching the host phloem. The cells of the intercellularly growing hypha contain rich protoplasts with numerous cell organelles and nucleus having highly lobed envelope (Dörr, 1969). In *Orobanche*, haustorial cells growing intercellularly contain dense protoplasts and abundant cell organelles (Dörr and Kollmann, 1974).

Since the conductive hyphae absorb water and nutrients from the host, those parasitic cells have been studied much in the parasitic angiosperms. On the contrary, the front cells of the endophyte growing within the host parenchyma have been described from a limited number of parasites. This study aims to describe the ultrastruc-

tural features in the front cells of advancing endophyte which grows at the region of the host parenchyma and interpret them on the functional aspects of the parasitic activity in *Cuscuta australis*.

MATERIALS AND METHODS

A parasitic plant *Cuscuta australis* R. Brown coiling on the host plant *Trifolium repens* L. (Fig. 1) was collected at the campus of the College of Natural Sciences, Sung Kyun Kwan University, Suwon. Methods followed our previous paper (Lee and Lee, 1989).

RESULTS

Some of the hyphal cells which originated from the tip cells, reached the host xylem and phloem through the host parenchyma, while others grew in the interfascicular parenchyma. Front cells of the endophyte growing in the host parenchyma regardless of their growing position, consisted of two cell types, tip cells and hyphal cells. They were distinguished from the axial cells, the rest of the front cells of the endophyte, by presence of densely stained cytoplasm and enlarged nuclei (Fig. 2).

Ultrastructurally, the tip cells had an electron-dense cytoplasm with abundant cell organelles. These cells contained multivesicular bodies (MVB) which were bounded by their own membrane (Fig. 3). The bounding membrane of MVB fused with plasmalemma and then released their vesicular and tubular contents into the cell wall (Fig. 4). Such a membrane fusion after releasing the contents seemed to play as a role to the invagination of the plasmalemma and cell wall. The invaginations contained fibrilar, vesicular, and tubular structures (Fig. 5). The latter two structures appeared to be derived from dictyosomes and endoplasmic reticulum which were closely associated with the invaginated plasmalemma. A fused appearance between both walls of the parasite and host cells was visible (Fig. 5), in which cellulosic fibrils were conspicuous.

Certain tip cells showed the unevenly thickened walls, seemingly consisting of an amorphous material (Fig. 6), and exhibiting unique behaviors such as penetrating the middle lamellae of the host parenchyma cells (arrow in Fig. 6) as well as engulfing the debris or remnants which were believed to be originated from the host cell. The origin from the broken host cell was obvious because of starch grain and thylakoid membranes (Figs. 7 and 8). The chloroplast of the host cells infected by the tip cells, was deformed in the structure, while the chloroplast in an uninfected host cell was intact (Fig. 9). The hyphal cells growing into or between the host cells also contained an electron-dense cytoplasm with numerous cell organelles (Figs. 10 and 11).

The intracellularly growing hyphal cells exhibited plasmalemma and wall invaginations (Figs. 11 and 12) similarly to those of the tip cell. Bundles of microfilament, each about 7.5 μm in diameter, sometimes appeared in the nucleus (Fig. 13) and cytoplasm (Fig. 14) of both tip and hyphal cells. In the cytoplasm, the microfilament bundles were closely associated with other organelles such as mitochondria, dictyosomes, multivesicular bodies, and vesicles. The deeply lobed nuclear envelope was another characteristic feature of the nuclei in the tip and hyphal cells (Fig. 15).

DISCUSSION

The endophyte tip and hyphal cells growing in the host parenchyma region were characterized by the presence of a dense cytoplasm having numerous cell organelles in *Cuscuta odorata* (Dörr, 1968, 1969), *Orobanche* (Dörr and Kollmann, 1974), and *Comandra* (Toth and Kuijt, 1977). The present study obtained the same result in *C. australis*. The cytological feature suggested that these tip and hyphal cells were metabolically active. Particularly, the enlarged nucleus with a deeply lobed envelope suggested a high degree of nuclear activity. The increase in the nuclear surface area and pores would possibly facilitate the material transport to and from cytoplasm (Jordan *et al.*, 1980).

Microfilament is likely to be actin in the chemical nature (Palevitz *et al.*, 1974) and is known to perform an active transport in the cytoplasmic streaming (Williamson, 1980). It also participates in the elongation of fungal mycelium (Hohl *et al.*, 1968). Microfilament bundles probably participate more actively in the movement of other organelles and in the nuclear cyclosis (von Stosch, 1972). Cresti *et al.* (1985) described microfilament bundles in pollen grains, but the bundles did not appear in the pollen tube. They interpreted that the microfilaments might be used as a source of protein for an elongation of the pollen tube. The presence of microfilament bundles in the endophyte front cells suggests their role for an elongation of the front cells.

The "callose cap" was reported to be accumulated on the wall of the *Cuscuta*-hyphae, and it was interpreted that the callose may be secreted from the host cell to

protect itself from the invasion of the parasite cell (Dörr, 1969). The fibrous "cap" that appears in the digitate cells of *Comandra* (Toth and Kuijt, 1977) causes to damage host cell. The heavily thickened-wall of some tip cells seemed to penetrate through the middle lamellae of the host parenchyma cells and to engulf the debris of the broken host cells. This feature has not been reported from the haustorial cells of parasitic angiosperms studied so far. The present observation suggests that the thickened-wall material would be amorphous and flexible rather than hard as in the *Comandra* (Toth and Kuijt, 1977), and that the wall might contain enzymes degrading the host cell wall (Nagar *et al.*, 1984).

It is expected that the front cells elongate toward the host vascular bundle during further growth. The cell wall materials are possibly derved from multivesicular bodies (Moore and McAlear, 1961; Halperin and Jensen, 1967; Fowke and Setterfield, 1969) or vesicles derived from ER and dictyosomes (Mollenhauer and Morre, 1980). It was suggested that membrane fusion between the plasmalemma and multivesicular bodies or vesicles, may result in forming the invaginations of the plasmalemma and wall of the endophyte front cells. Such invaginations could act to enlarge the absorptive surface area as in transfer cell, through which material translocation may be facilitated from the host to the parasite (Macleod, 1961; Gunning and Steer, 1975; Dell *et al.*, 1982; Alosi and Calvin, 1985).

The plasmodesmata between the walls of the host and the parasite cells have been proposed (Bennett, 1944; Dörr, 1969, 1987; Tainter, 1971, Dell *et al.*, 1982), however, that was not observed in this study and other parasites (Dobbins and Kuijt, 1973; Dörr and Kollmann, 1974; Toth and Kuijt, 1977; Alosi and Calvin, 1985; Kuijt *et al.*, 1985). The apoplastic continuity at the host-parasite interface would be responsible for the water and nutrients transport from the host to the parasite.

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적 요

기주식물(토끼풀, *Trifolium repens* L.)의 유조직에 침입하여 생장하는 실새삼(*Cuscuta australis* R. Brown)의 흡기세포들의 미세구조를 조사하였다. 기주세포들과 직접 접촉되어 있는 흡기의 정면부위는 아직 분지되지 않은 선단세포들 및 이들로부터 분지하여 신장된 세포들(hyphae)로 구성되었다. 이 두 유형의 세포들은 전자밀도가 높은 세포질을 지니며, 또한 핵막이 심하게 만입된 커다란 핵을 갖는 특징을 보였다. 어떤 선단세포에서는 비후된 세포벽 물질이 기주세포벽의 중엽(middle lamellae)으로 침입하고, 파괴된 기주세포의 잔유물을 내포하는 양상을 보였다. 두 유형의 세포들은 원형질막과 세포벽이 세포의 안쪽으로 돌출하는 구조를 갖는데, 이는 기주세포들로부터 물질흡수를 촉진하기 위한 표면적의 증가현상으로 해석된다. 기주와 흡기세포의 세포벽사이를 통과하는 원형질연락사는 관찰되지 않았다. 두 식물세포의 경계면에서는 융합된 세포벽을 관찰할 수 있는데, 이 구조는 기주로부터 흡기로의 수분 및 영양물질의 수송 경로로써 작용할 수 있을 것으로 사료된다.

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Explanation of Figures

Figs. 1 and 2. Light micrographs of *Cuscuta australis* and its host. Fig. 1. Parasite stem growing on the host stem. ×1.3. Fig. 2. Tip cells (TC) grow in the host's cortical parenchyma (CP) with chloroplast. Some hyphal cells (H) reach the host xylem (HX) and phloem (HP), while other grows in the interfascicular parenchyma (IP) of the host stem. ×250.

Figs. 3-6. Electron micrographs of the endophyte front cells. Fig. 3. Mutivesicular body (MVB) in the cytoplasm of the tip and hyphal cells. ×33,700. Fig. 4. Plasmalemma has somewhat invaginated portions (arrows). A MVB fuses (arrowheads) with the plasmalemma and then release the MVS's contents into the cell wall (CW). ×33,700. Fig. 5. Tip cell (TC) with many dictyosomes (D) and endoplasmic reticulum (ER) have the cell wall and plasmalemma ivaginations, which comprise of fibrilar, tubular, and vesicular structures. Note the fused appearance between the parasite (PW) and host cell walls (HW). ×24,200. Fig. 6. Thick wall material in a tip cell (TC) is separating the middle lamellae (arrows) of the host parenchyma cells (HC). ×6,200.

Figs. 7-11. Ultrastructures of the endophyte tip and hyphal cells. Fig. 7. The thick wall of the tip cell (TC) contains remnants (arrowheads) of the brocken host cells. A degenerating host parenchyma cell (HC) has a deformed chloroplast. ×25,800. Fig. 8. A debris derived from the broken host cells, marked by double arrowhead in Fig. 7, shows starch grain (Sg) and thylakoid membranes (arrow). ×131,600. Fig. 9. Normal structure of a chloroplast in an uninfected host cell. ×22,000. Fig. 10. Transectioned hyphal cell (H) growing intrusively through the intercellualr space of the host cells (HC) contains an electron-dense cytoplasm with several organelles. ×7,800. Fig. 11. Intracellular growing hyphal cell (H) has many projections of the cell wall and the plasmalemma (arrows). ×9,300.

Figs. 12-15. Fine structure of the front cells of endophyte. Fig. 12. Longitudinal view of a intracellular growing hyphal cell (H) with dense protoplast also exhibit irregular surface of the plasmalemma-cell wall (arrows). ×14,900. Fig. 13. Microfilament bundle (MFB) is seen near the nucleolus (NU). ×40,800. Fig. 14. MFB in the cytoplasm is closely associated with dictyosomes (D), mitochondrion (M), multivesicular body (MVB), and vesicles (V). × 39,000. Fig. 15. Enlarged nucleus (N) having highly lobed envelope. ×17,800.


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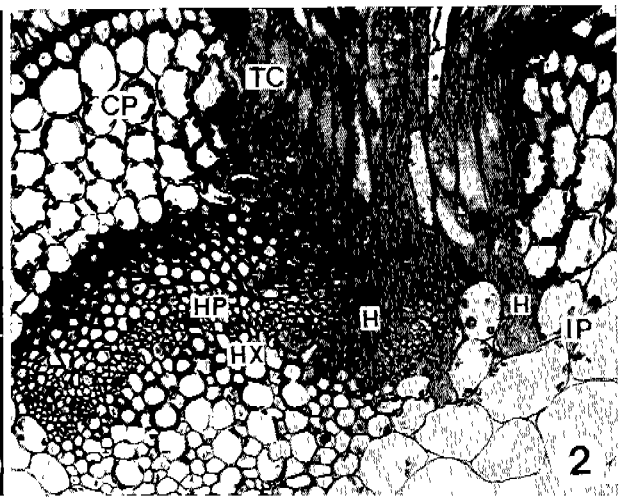
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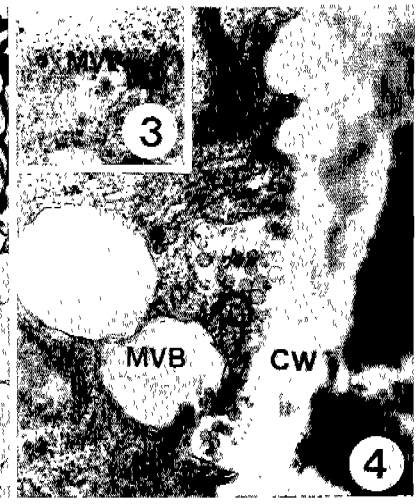
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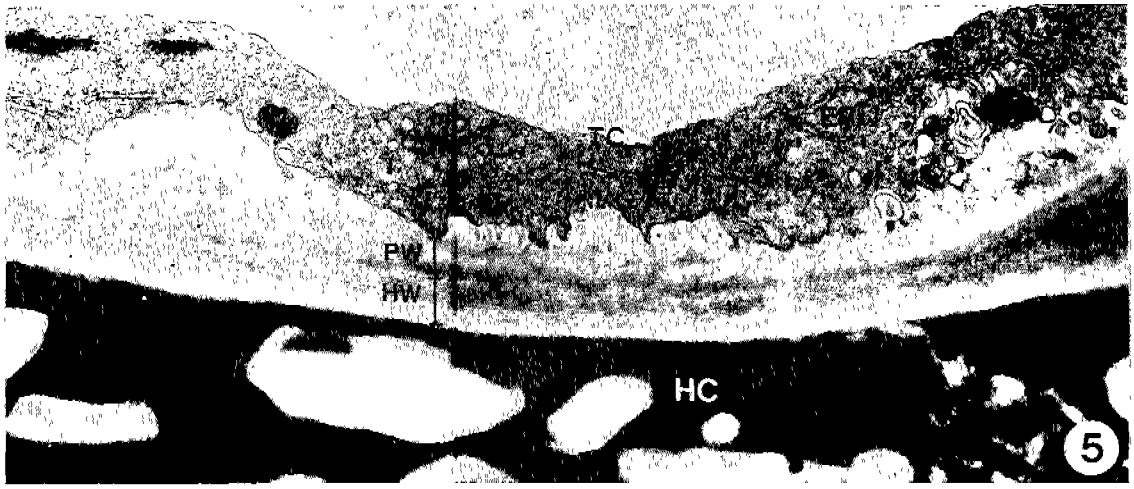
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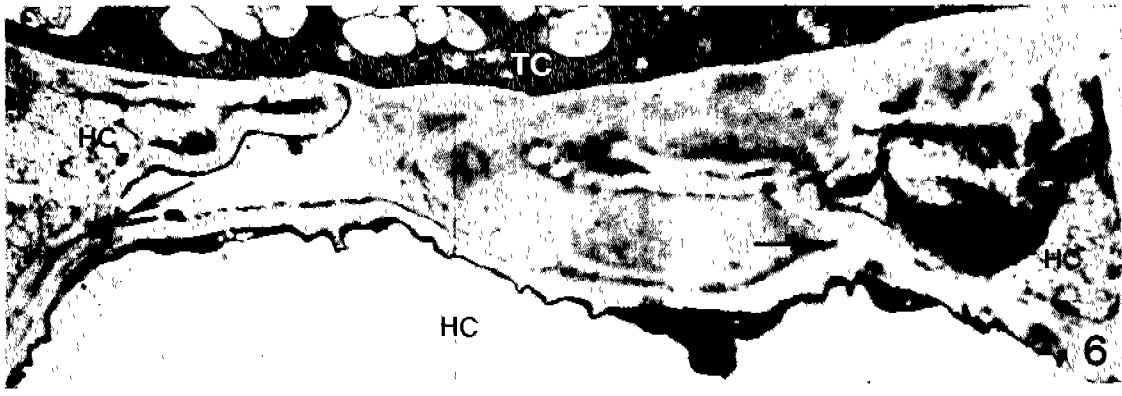
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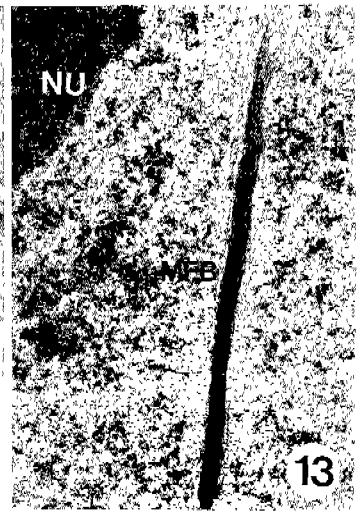

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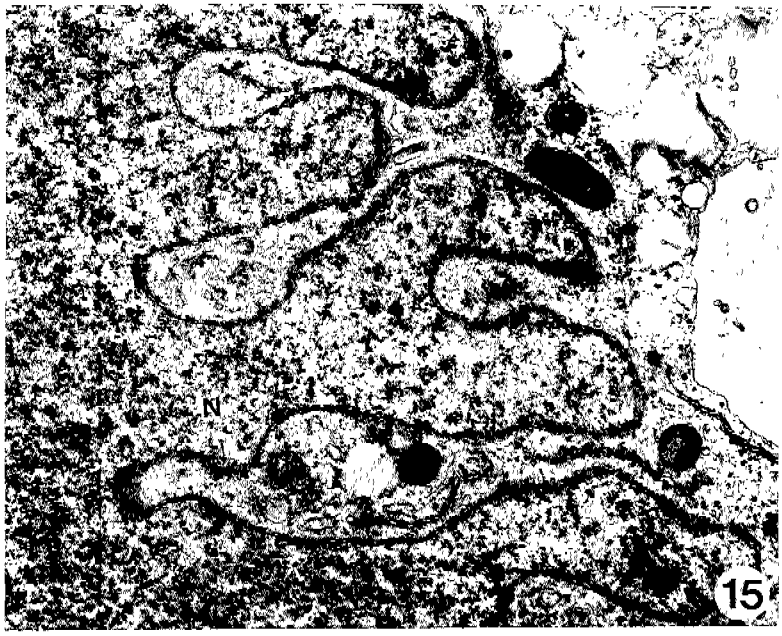

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