CYTOPATHOLOGICAL EVIDENCE FOR TRANSPORT OF PHYTOPLASMA IN INFECTED PLANTS

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ABSTRACT

Pleomorphic phytoplasmas were observed in sieve tubes, companion cells and in phloem parenchyma of *Tagetes patula* L., *Helichrysum bracteatum* Willd. and *Gladiolus* sp. L. plants with morphological changes typical for phytoplasma infection.

In the pores of the sieve plate phytoplasma cells were seen which suggests that the vertical transport of this pathogen goes in the sieve tubes of infected plants throughout the sieve tube pores.

The contact of the sieve tube with the neighbouring cells goes through the plasmodesmata, but no changes of the plasmodesmata were observed in the phloem of infected plants. The size and structure of unchanged plasmodesmata does not allow passing through such big structures like phytoplasma. Instead close contact between phytoplasma cells and vertical sieve tube walls takes place. Damages to the cell wall were observed forming cavities in which the phytoplasma cells were present. The damages of parenchyma and companion cells walls also were seen. In cells where the damages of the walls were observed phytoplasmas were present. The phytoplasma cells were sporadically seen also in the intercellular spaces of parenchyma. These data suggest that horizontal transport depends on damages to the infected plant cell walls caused by the phytoplasma itself.

KEY WORDS: phytoplasma, intercellular movement.

INTRODUCTION

The life cycle of intercellular plant pathogens is depending on the spread of the infecting agent from cell to cell of the host plant. The movement mechanism of such pathogens like plant viruses is well known. For plant viruses short distance movement goes by the plasmodesmata between adjacent cells and occurs by at least two mechanisms – as a whole particle (*comoviruses* and *nepoviruses*) or virus RNA (for other viruses) (Carrington et al. 1996; McLean et al. 1993). The virus encoded movement proteins are essential for both types of movement (Wolf and Lucas 1994). The long distance movement goes by sieve elements of phloem (Seron and Haenni 1996).

The pathogenic bacteria usually invade the plant by intercellular spaces and then invade cells by damaged cell walls and are released from cells the same way (Beattie and Lindow 1995). Xylem related bacteria are translocated through xylem elements (Nelson and Dickey 1970).

Phytoplasma (mycoplasma-like-organism MLOs) is a prokaryotic agent and only whole cells movement of it is effective. They live in infected plant cells, mostly sieve tubes but are sometimes observed also in other types of cells (Semancik and Peterson 1971; Esau et al. 1976; Siller et al. 1987).

Changes in the cell walls structure and damages in *Tagetes patula* L., *Helichrysum bracteatum* Willd. and *Gladiolus* sp. L. plants infected with phytoplasma were investigated.

MATERIAL AND METHODS

Tagetes patula L., *Helichrysum bracteatum* Willd. and *Gladiolus* sp. L. plants were naturally infected with phytoplasma. The symptoms included general chlorosis, leaf bronzing or reddening, abnormal production of secondary shoots, upright habit of growth and flower malformation. The PCR analysis suggests that phytoplasma found in these ornamental plants falls into the A-I phylogenetic cluster as American aster yellows (AAY) (Kamińska et al. 1996).

Specimens for electronmicroscopical examination were fixed in a mixture of 3% glutaraldehyde and 4% paraformaldehyde in cacodylate buffer pH = 7.2, for 4 h. Postfixation in 2% osmium tetroxide for 2 h. at 4°C was applied. The material was dehydrated in ethanol (10-70%), acetone (70-100%) and embedded in Epon 812. The ultrathin sections on copper grids were stained with uranyl acetate and lead citrate.

Fig. 1. Cross section of sieve tube of *Helichrysum bracteatum* Willd. phloem at the level of sieve plate. Framed part of sieve plate enlarged. 11 850 $\times$, bar represents 1 μ m.

Fig. 2. Magnification of sieve tube plate pores from fig. 1 with phytoplasma in them (star). Magn. 122 000 $\times$, bar represents 100 nm.

Fig. 3. Longitudinal section through the sieve plate with phytoplasma cells passing through the sieve pore. *Tagetes patula* L. plant. Magn. 70 000 $\times$, bar represents 250 nm.

Fig. 4. Longitudinal section through the sieve plate pore with fingerlike invaginations of phytoplasma cell. *Gladiolus* sp. L. phloem. Magn. 62 000 $\times$, bar represents 250 nm.

Fig. 5. Cross section of sieve tube. Damage to the cell wall (framed parts). *Helichrysum bracteatum* Willd. phloem. Magn. 23 000 $\times$, bar represents 500 nm.

Fig. 6. Magnification of frame 1 from Fig. 5. The cell wall between sieve tube and phloem parenchyma cell with damage. Phytoplasma in close contact with the cavity. Magn. 80 000 $\times$, bar represents 100 nm.

Fig. 7. Magnification of frame 2 from Fig. 5. Phytoplasma cells in the cavity. Magn. 124 000 $\times$, bar represents 100 nm.

Fig. 8. Cavity in the cell wall with phytoplasma inside. *Gladiolus* sp. L. phloem. Magn. 153 000 $\times$, bar represents 100 nm.

Fig. 9. Sieve tube cell wall with damage lined by callose (C). The phytoplasma is seen in close contact with the damage. *Helichrysum bracteatum* Willd. phloem. Magn. 60 000×, bar represents 250 nm.

Fig. 10. Cell walls with numerous damage. *Gladiolus* sp. L. phloem. Magn. 30 000×, bar represents 500 nm.

Fig. 11. Sieve tube cell wall with damage filled with callose (star). *Helichrysum bracteatum* Willd. phloem. Magn. 44 000×, bar represents 500 nm.

Fig. 12. Sieve tube cell wall with damage lined by callose. *Tagetes patula* L. phloem. Magn. 82 000×, bar represents 250 nm.

Observations were taken with the use of a JEM 1220 electron microscope in the Laboratory of Electron Microscopy Warsaw Academy of Agriculture.

RESULTS

The examined leaves and stems of *T. patula* L., *H. bracteatum* Willd. plants, leaves and roots of *Gladiolus* sp. L. plants showed the presence of phytoplasma cells in the phloem of these parts of plants. Pleomorphic phytoplasmas were observed in the sieve tubes and also in companion cells and the phloem parenchyma of examined plants. Most of the sieve tubes of the examined parts of plants had phytoplasmas in their lumen. The infection of companion cells was much less frequent. The infected parenchyma cells were rather rare in *T. patula* L., *H. bracteatum* Willd., plants, but were common in roots of *Gladiolus* sp. L.

In the vicinity of sieve plates phytoplasma cells were present as well as in pores of the sieve plates. The pore lumen was smaller than the diameter of the phytoplasma cell, but phytoplasma cells undergo fission and squeeze into the pore (Fig. 4). On the longitudinal transsection through the sieve plate fingerlike invaginations of phytoplasma cells were seen passing through the sieve pore (Fig. 3 and 4). In horizontally cut sieve plates phytoplasmas were also observed in the pores of the sieve plate (Fig. 1 and 2). In some of the sieve pores several phytoplasma cells were seen.

Contact of the sieve tube with the neighbouring cells takes place through plasmodesmata. The structure of these plasmodesmata was not changed compared to the structure of plasmodesmata of control plants of the same region of phloem. The phytoplasma cells were situated in close contact with the vertical sieve tube walls, but not with the plasmodesmata. In the place where phytoplasma were in close contact with vertical sieve tube walls there were observed damages to the cell wall forming cavities (Fig. 5, 6 and 7). In these cavities phytoplasmas were present (Fig. 8). In sieve tubes these cavities connected sieve tube with companion cells and parenchyma cells (Fig. 6). These pathological changes were observed not only in sieve tube walls but also in the cell walls of companion cells and phloem parenchyma cells in which phytoplasmas were present. The texture of the cell wall was loose in the places where damages take place (Fig. 12), and also phytoplasma cells were present in close contact with these damages of the cell wall (Fig. 7 and 10).

The presence of phytoplasmas in intact plant cells as well as damage to its walls does not lead to death of the cell. But plant cells line the edges of damages in walls by callose (Fig. 9, 11 and 12). Those deposits sometime are big and plug the hole completely (Fig. 11).

The phloem tissue has no intercellular spaces between the cells, but on the border of conducting tissue in *Gladiolus* sp. L. leaves the phytoplasma cells were sporadically seen in intercellular spaces of parenchyma.

DISCUSSION

The phytoplasma are the pathogens living in the sieve elements of phloem, so movement in sieve tubes is the natural way in which they can spread through the plant body. As phloem is widely distributed in the plant body as a discreet system of conducting tissue it is an easy way of movement for the phytoplasmas. Phytoplasmas have been shown to pass through sieve plate pores (Jacoli 1974; Parthasarathy M.V.

1974; Worley J.F. 1973) which was also observed by us. In a single bundle of vascular tissue only sieve elements of a single sieve tube and neighbouring sieve tubes are connected by sieve plates (Knoblauch and van Bel 1998). The distribution of phytoplasmas in numerous sieve tubes and the fact that they were seen also in the companion cells and phloem parenchyma cells indicates that it is rather difficult to believe it is the only way of movement of this infecting agent through the plant body. The connections between sieve tubes and companion cells as well as with phloem parenchyma cells take place by plasmodesmata (Van Bel and Gamelei 1992; Evert 1977). The size of the plasmodesmata is so small that it is difficult to believe the phytoplasma cells could pass through them (Lucas and Wolf 1993). Compared to viruses (for example tomato spotted wilt virus 80-100 nm) phytoplasmas are big (observed in thin sections of phloem cells are 200-1100 nm in diameter). Even plant viruses, much smaller in size rarely pass through plasmodesmata as a whole particle (Carrington et al. 1996; McLean et al. 1993), moreover for the procaryotic pathogens like phytoplasmas only transfer of a whole cell is possible. The presence of phytoplasmas in intact plant cells as well as the damage to its walls do not lead to the death of the cell, so the damage to the cell walls observed in the cells of infected plants suggests that this damage is the initial step towards the phytoplasmas spread from cell to cell. As the damage to cell walls was observed in three examined hosts it seems that the process of damaging of the infected plant cell walls caused by the phytoplasma itself is often way for horizontal transport of this pathogen and could be treated like such.

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CYTOLOGICZNE DOWODY NA TRANSPORT FITOPLAZM W ROŚLINACH PORAZONYCH

STRESZCZENIE

W roślinach *Tagetes patula* L., *Helichrysum bracteatum* Willd. and *Gladiolus* sp. L. wykazujących typowe morfologiczne objawy porażenia fitoplazmami stwierdzono obecność fitoplazm w rurek sitowych, komórkach towarzyszących i miękiszu floemowym.

Obserwowano komórki fitoplazm przeciskające się przez pory płytek sitowych co sugeruje, że pionowy transport tego patogena przebiega właśnie przez rurki sitowe.

Połączenia międzykomórkowe rurek sitowych z komórkami sąsiednimi, innymi niż człony rurek sitowych przebiegają dzięki plazmodesmom. Wielkość fitoplazm wyklucza transport tego patogena przez niezmodyfikowane plazmodesmy. Jednocześnie w badanych tkankach nie zaobserwowano zmian w strukturze plazmodesm. Natomiast obserwowano bliski kontakt fitoplazm i podłużnych ścian rurki sitowej. W ścianach tych powstawały nadżerki. W takich kanałach obserwowano komórki fitoplazm. Również obserwowano rozerwane ściany komórek miękiszowych i komórek towarzyszących, w których były obecne fitoplazmy. Sporadycznie obserwowano fitoplazmy w przestrzeniach międzykomórkowych komórek miękiszu. Obserwacje te wskazują, że poziomy transport fitoplazm jest związany z uszkodzeniami ściany komórkowej powodowanymi przez fitoplazmy.

SŁOWA KLUCZOWE: Fitoplazma, transport międzykomórkowy.