Ultrastructure in Collenchyma Cells of Cortex in Squash Hypocotyls Treated with Sodium Diethyldithiocarbamate

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Ultrastructure in Collenchyma Cells of Cortex in Squash Hypocotyls Treated with Sodium Diethyldithiocarbamate

Takio ICHITANI and Minoru TAKAHASHI *

Laboratory of Plant Pathology, College of Agriculture

Introduction

Browning spots are produced on the hypocotyls of the solution-grown Utsugi seedlings in a plant growth chamber following invasion by *Pythium ultimum.*[2,4,5,8] Spreading water-soaked lesions are, however, formed on the hypocotyls when the roots are pre-treated with sodium diethyldithiocarbamate (NaDEDC).[2,4,5,8] Ultrastructure in the collenchyma cells of the cortex of the hypocotyls was observed when NaDEDC was applied to the roots at the concentrations sufficient to produce the spreading water-soaked lesions.[6] Results of a more detailed study are presented in this paper.

Materials and Methods

Plant materials The plant and its cultivation as well as NaDEDC treatment were basically the same as those described in the previous papers.[2,4,5,8] The seedlings were rinsed in distilled water after treating with NaDEDC and then put back to a half-strength Hoagland's solution. Ten seedlings of the uniform size were used for each treatment. Half of them was inoculated and the rest remained as uninoculated. Inoculation test was designed to examine the possibility of producing the spreading water-soaked lesion on the uninoculated hypocotyls used for an electron microscopy. The same pathogen was maintained as described in the previous papers.[3,4] The inoculation was made by the method explained before[4] with an exception of supplementing the inoculum medium with 30 ppm streptomycin to reduce bacterial contamination.[12] Inoculated seedlings were placed in a dark moist chamber at 24 C for 24 hours. After placing in the plant growth chamber for another 24 hours, the inoculated seedlings were observed for external symptom development and categorized according to more simplified types than previously reported.[4,5] Uninoculated seedlings were sampled for electron microscopy immediately after treating with NaDEDC. Three trials were conducted.

Electron microscope study The routine electron microscopy method in this laboratory[1,11] with a slight modification was followed. Five cylinders of about 15 mm in length were cut near the center of each treated uninoculated hypocotyl. Specimens covering the epidermis and the collenchyma of the cortex were obtained from the cylinder. They were fixed in 6 % glutaraldehyde (in 0.1 M phosphate buffer at pH 7.0, v/v) for 2 hours, washed for 1 hour with phosphate buffer

* Present address : Estação Experimental De Videira Caixa Postal 3, 89560 Videira, Santa Catarina, Brasil

used in pre-fixation, postfixed in 1 % osmiun tetroxide (in 0.1 M phosphate buffer at pH 7.4, w/v) for 3 hours, and washed for 40 minutes with phosphate buffer used in postfixation. All procedures together with a part of dehydration mentioned below were done at about 4 C. Fixed material was dehydrated in ethanol within two and a half hours. After displacing ethanol with propylene oxide, the material was embedded in Epon (7 parts of Epon 812, 6 parts of DDSA and 4 parts of MNA). During polymerization, the material was oriented[10] to obtain the transverse section and sectioned on a "Reichert Om U_2" ultramicrotome. Sections were mounted on a formvar-filmed, carbon-coated copper grids (150-B mesh), stained with saturated uranyl acetate in 50 % ethanol at about 33 C for 50 minutes and with lead citrate for 6 minutes at room temperature, and examined by a JEM-100 U electron microscope.

Results and Discussion

Susceptibility of the hypocotyls used Five uninoculated seedlings per treatment were used as samples for electron microscopy. Another five seedlings were simultaneously inoculated with *P. ultimum* to examine the possibility whether the uninoculated hypocotyls became susceptible (Table 1). Typical data obtained together with those as reported previously[4,5] may indicate that the hypocotyls sampled for electron microscopy were considered to be susceptible to the invasion of *P. ultimum.*

Table 1. Spreading water-soaked lesions on inoculated hypocotyls pre-treated with sodium diethyldithiocarbamate

Concentration (M)	No. of seedlings exhibiting:				Phytotoxicity[a]
	Symptomless	Browning spot	Spreading water-soaked lesion	Damping-off	
10^{-3}	0	0	3	2	+
10^{-4}	0	1	4	0	−
10^{-5}	0	5	0	0	−
10^{-6}	0	5	0	0	−
10^{-7}	0	4	1	0	−
Distilled water	0	5	0	0	−

a); (+) denotes the phytotoxic symptoms such as scanty roots, stunted lateral roots, and slightly stunted top were observed; and (−) denotes the phytotoxic symptoms were not observed.

Ultrastructure of collenchyma cells of cortex in NaDEDC-treated seedlings Collenchyma cells of the cortex from the untreated seedlings were first examined with an electron microscopy (Figs. 1—3). The structural organization in the plant cells that are commonly recognized[9] was observed. Membrane systems, a double membrane of mitochondria as well as the cytoplasmic matrix were well preserved. With 10^{-4}M NaDEDC treatment, however, ultrastructural changes frequently

recognized were as follows: a fragmentation or a decrease in the amount of rER, and a decrease in electron density or an increase in vacuolation in the cytoplasmic matrix. Since these changes became noticeable when treated with 10^{-3}M NaDEDC (Figs. 4, 5), the chemicals might have induced these manifestations. On the other hand, the changes induced by 10^{-5}—10^{-7}M treatments were not distinct from those in untreated water control. These ultrastructural changes may indicate that NaDEDC at the concentrations of 10^{-3}M or 10^{-4}M inhibits various metabolic activities in the collenchyma cells of the cortex.

The results obtained tended to show that the collenchma cells used for electron microscopy were susceptible to the invasion of *P. ultimum* and low in various metabolic activities. Attempts to find in detail the ultrastructural changes induced by 10^{-4}M NaDEDC at the corresponding processes of infection and to observe the ultrastructures of NaDEDC-treated, inoculated collenchyma cells of the cortex are in progress.[7] The ultrastructural changes in relation to spreading and water-soaked phenomena of the lesions must then be properly evaluated.

Summary

1. Hypocotyls of squash Utsugi seedlings sampled for electron microscope study were revealed the susceptibility to the invasion of *P. ultimum*.
2. Ultrastructural changes in 10^{-3}—10^{-4}M NaDEDC treated collenchyma cells were as follows: a fragmentation or a decrease in the amount of rER, and a decrease in electron density or an increase in vacuolation in the cytoplasmic matrix. By 10^{-5}—10^{-7}M treatments, no distinct ultrastructural changes were observed.

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Explanation of Plates

Table of Abbreviations

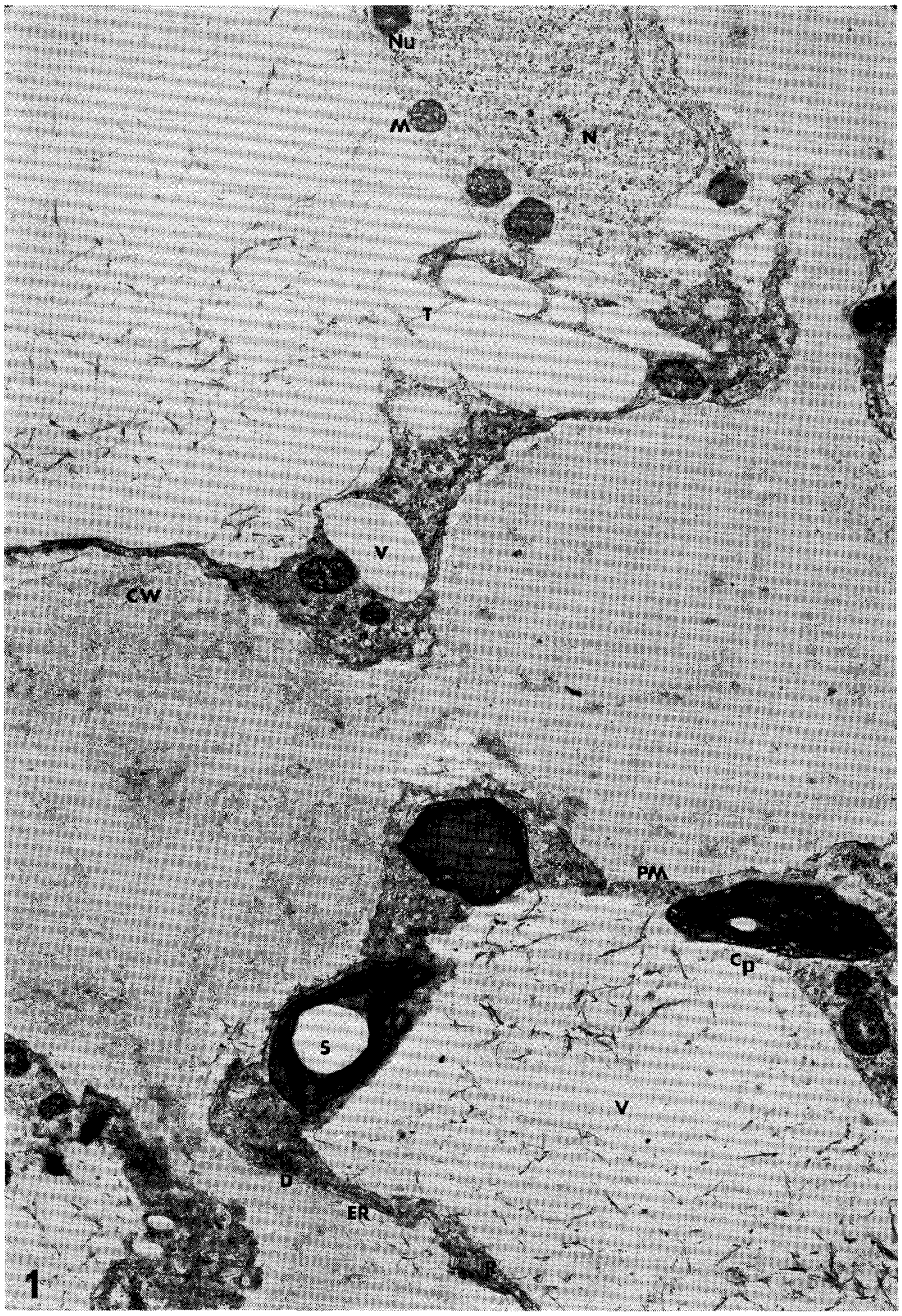
CP	Chloroplast	PM	Plasma Membrane
CW	Cell Wall	R	Ribosome
D	Dictyosome	RER	Rough Endoplasmic Reticulum
ER	Endoplasmic Reticulum	S	Starch
M	Mitochondrion	T	Tonoplast
N	Nucleus	V	Vacuole
Nu	Nucleolus		

Fig. 1 Ultrastructure of collenchyma cells of the cortex in untreated Utsugi squash seedlings (x 19, 000)

Figs. 2-3 Cytoplasm of collenchyma cells of the cortex in untreated Utsugi squash seedlings (x 47, 000 ; x 35, 000)

Fig. 4 Fragmentation or decrease in the amount of rER in collenchyma cells of the cortex in 10^{-3}M NaDEDC-treated Utsugi squash seedlings (x 40, 000)

Fig. 5 Decrease in electron density or increase in vacuolation in the cytoplasmic matrix in collenchyma cells of the cortex in 10^{-3}M NaDEDC-treated Utsugi squash seedlings (x 30, 000)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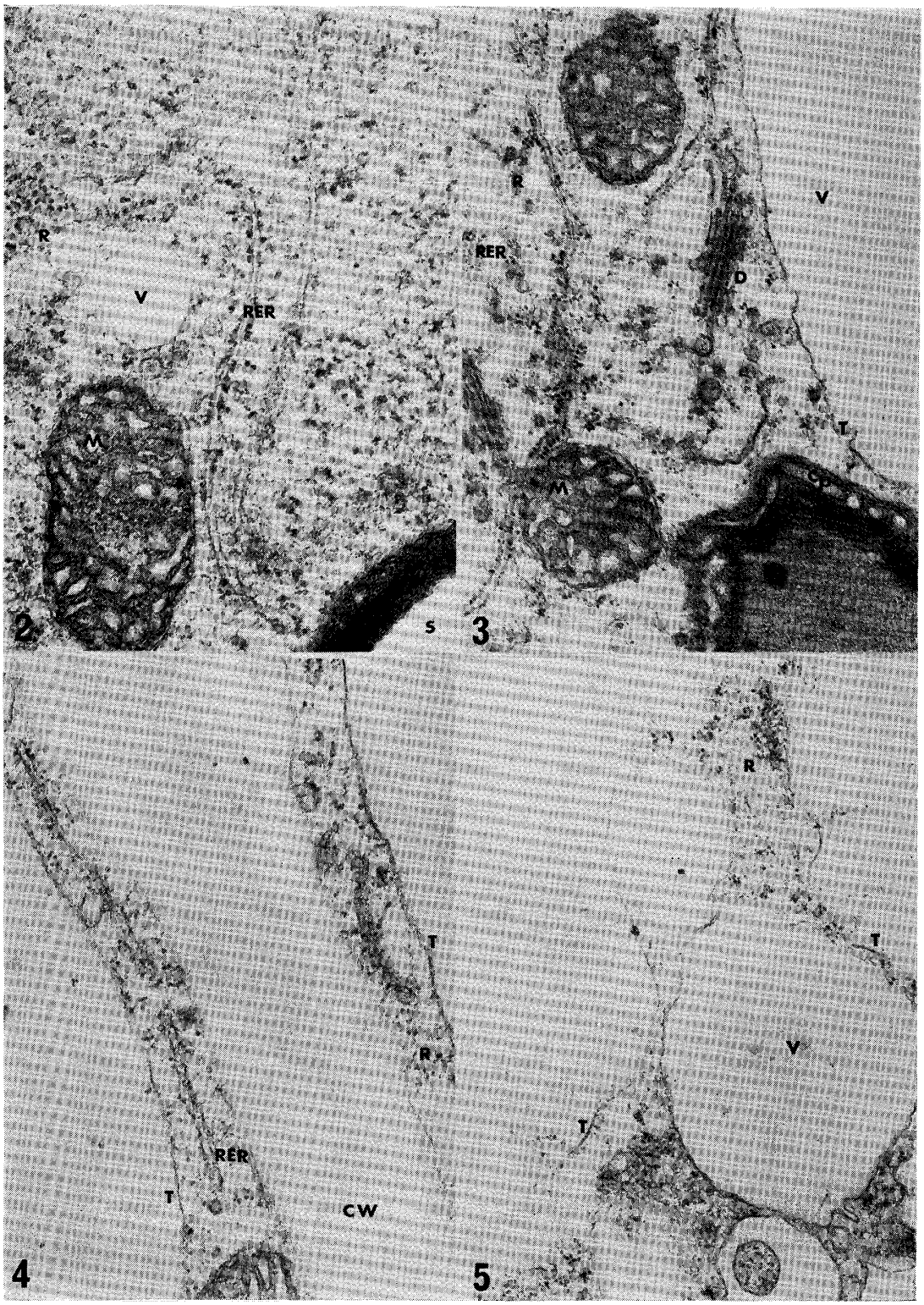
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