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# *Verticillium dahliae* toxin induced alterations of cytoskeletons and nucleoli in *Arabidopsis thaliana* suspension cells

**Hai-Yong Yuan, Lin-Lin Yao, Zhi-Qi Jia, Yun Li,** and **Ying-Zhang Li***

State Key Laboratory of Plant Physiology and Biochemistry, College of Biological Sciences, China Agricultural University, Beijing

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**Summary.** In plant cells, cytoskeletons play important roles in response to biotic and abiotic stresses. However, little is known about the dynamics of cytoskeletons when cells are attacked by unphysical stress factors such as elicitors and toxins. We report here that the toxin of *Verticillium dahliae* (VD toxin) induced changes of microfilaments (MFs) and microtubules (MTs) in *Arabidopsis thaliana* suspension-cultured cells. When cells were treated with a low concentration of VD toxin, MFs were disrupted ordinally from the cortex to the perinuclear region, and then recovered spontaneously; but the MTs persisted. The MFs in the perinuclear region showed more resistance to VD toxin than the cortical ones. In contrast, when cells were treated with a high concentration of VD toxin, MFs and MTs were disrupted sooner and more severely and did not recover spontaneously. Treatments with high concentrations of VD toxin also induced changes of nucleoli. At the early stages of treatment, a nucleus had a single ring-shaped nucleolus. At the later stages, multiple smaller and more brightly fluorescing nucleoli emerged in a single nucleus. Disrupted MFs could be recovered by removing the VD toxin before the ring-shaped nucleoli appeared. All these results showed that MFs and MTs play important roles in the early defense responses against VD toxin in Arabidopsis suspension cells. The cytoskeletons may be used as sensors and effectors monitoring the defense reactions. The changes of nucleoli induced by VD toxin should be important characteristics of cell death.

**Keywords:** *Arabidopsis thaliana*; Suspension cell culture; Microfilament; Microtubule; Nucleolus; Toxin of *Verticillium dahliae*.

## Introduction

Plant cytoskeletons play important roles in plant cell growth and development and other cellular processes (Staiger 2000, Wasteneys and Galway 2003). Studies have implicated that plant cytoskeletons are also involved in biotic- and abiotic-stress responses (Vantard and Blanchoin 2002, Smith 2003, Kwok and Hanson 2004, Wada and Suetsugu 2004). Microfilaments (MFs) and microtubules (MTs) are necessary for plants to block fungal penetration (Kobayashi et al. 1992, 1997; Genre and Bonfante 2002; Kobayashi and Hakuno 2003). However, there are only a few data that show cytoskeleton dynamics in plant cells induced by pathogen elicitors or toxins (Takemoto and Hardham 2004; Takemoto et al. 1997, 1999; Sandovsky-Losica et al. 2002).

*Verticillium dahliae* Kleb. is a fungal pathogen that can induce verticillium wilt and causes large damages to many woody and herbaceous plants, such as cotton, elm, potato, pepper, watermelon, mint, and lettuce (Subbarao et al. 1995, Veronese et al. 2003). The infection by the pathogens can induce many reactions in the host cells, such as changes of the concentration of some organic solutes including proline, total proteins, total soluble sugars, and starch in leaves, and of the concentration of abscisic acid in the xylem (Goicoechea et al. 2000). The pathogen infection also induced active expression of some disease response genes (Hill et al. 1999, McFadden et al. 2001) and production of some antifungal compounds, such as terpenoids, PR (pathogenesis-related) proteins, and lignin (Resende et al. 1996, Daayf et al. 1997, Dubery and Slater 1997, Smit and Dubery 1997, Li et al. 2003, Zhen and Li 2004). However, how the cytoskeletons of plant cells respond to *V. dahliae* pathogen or VD toxin is yet to be studied. We studied the dynamics of the cytoskeleton in *Arabidopsis thaliana* suspension cells treated with VD toxin. In addition, we observed some alterations in nucleoli.

Our results showed that the microfilament and microtubular cytoskeletons are involved in the basic and primitive defense of *A. thaliana* suspension cells against VD toxin. The major functions of microtubules (MTs) and microfilaments (MFs) in resistance reactions against VD toxin

* Correspondence and reprints: Department of Plant Sciences, College of Biological Sciences, China Agricultural University, Beijing 100094, People's Republic of China.
E-mail: liyingzhang@cau.edu.cn

may be related to signal transduction. The cytoskeletons, especially MTs, may be used as sensors and effectors monitoring the defense reactions against VD toxin. The observed alterations of nucleolar shapes are important characteristics that are accompanied by resistance-related cell death which may be programmed cell death.

## Material and methods

*Suspension cell culture*

To induce calluses, seeds of *Arabidopsis thaliana* ecotype Columbia were sown on callus induction medium and cultured at a photoperiod of 16 h light (21 °C) and 8 h dark (24 °C). The callus induction medium contained B5 inorganic salts, 2× B5 vitamins (Gamborg et al. 1968), 20 g of glucose per liter, 4.52 μM 2,4-dichlorophenoxyacetic acid (2,4-D), 0.23 μM kinetin, 3 μM morpholineethanesulfonic acid (MES), and 8 g of agar per liter, pH 5.8. After four weeks, the formed calluses were transferred into liquid suspension culture medium containing MS (Murashige and Skoog 1962) inorganic salts, 4× B5 vitamins, 30 g of sucrose per liter, and 2.26 μM 2,4-D, pH 5.8. One week later, 10 ml of cell suspension was subcultured into 100 ml of fresh cell culture medium. 3-day-old cell cultures were used for further experiments. To maintain the callus and suspension cell cultures, the cultured callus and suspension cells were subcultured monthly and weekly, respectively.

*Preparation of crude VD toxin*

A highly infectious and nondefoliating strain of *Verticillium dahliae* Kleb. (V229) that was originally isolated from diseased cotton tissues was a gift from Prof. Guiliang Jian, Institute of Plant Protection, Chinese Academy of Agricultural Sciences. The fungus was cultured in Czapek medium (Meyer et al. 1994) on a shaker at 120 rpm at 25 °C for 14 days. The fungal cultures were filtered through filter paper; the filtrates were centrifuged at 10,000 **g** for 30 min to remove the spores. The supernatants were then filtered through a 0.45 μm pore size Millipore filter. The filtrates were used as VD toxin for further experiments (Dubery and Smit 1994, Chu et al. 1999, Zhen and Li 2004). Protein contents in the crude extracts were used to represent concentrations of VD toxin and determined according to Bradford (1976). Bovine serum albumin (Sigma) was used as a standard.

*VD toxin treatments*

To examine the effects of VD toxin on MFs and MTs, the cultured cells were treated with different concentrations of VD toxin (represented by total protein contents) in a range from 1.7 μg/ml to 17 μg/ml and 20 μg/ml at different time points. The cells in VD toxin-containing medium were incubated for 15 min to 3 h or more. Two controls were used, one were cells treated with Czapek medium only and the other were cells treated with Arabidopsis cell suspension medium only. More than 200 cells were examined in each treatment.

The cell death assay was performed as described by de Pinto et al. (1999).

*Staining and investigation of MFs and MTs*

MFs were stained as described by Ou and Yuan (2002) with some modifications. 1.5 ml of the disposed *Arabidopsis* suspension-cultured cells was transferred into an Eppendorf tube (volume, 1.5 ml) for precipitation. The supernatant was removed as much as possible. The cells were treated for 30 min with 100 μM 3-maleimidobenzoyl-N-hydroxysuccinimide ester solution in PME buffer (50 mM piperazine-N,N′-bis(2-ethanesulfonic acid), 5 mM EGTA, 5 mM $MgSO_4$, pH 6.9) containing 0.2% (v/v) Triton X-100, and 0.3 mM phenylmethylsulfonyl fluoride (PMSF) to stabilize actin cytoskeleton. The cells were fixed with 4% paraformaldehyde solution (4% paraformaldehyde, 0.2% [v/v] Triton X-100, 1% [v/v] dimethyl sulfoxide, and 0.3 mM PMSF in PME buffer) for 20 min; then, the fixed cells were rinsed twice with PME buffer and once with 10 mM MES buffer (pH 5.7). The cells were further incubated in enzyme solution (1% [w/v] cellulase R10 and 0.3% macerozyme R10, 0.4 mM PMSF in MES buffer) for 10 min and then washed with MES buffer twice and once with phosphate-buffered saline (PBS), pH 7.4. Finally, the cells were stained with 8 nM tetramethylrhodamine (TRITC)-phalloidin (Sigma) in PBS buffer for 60 min.

MTs were visualized by indirect immunofluorescene. Briefly, fixation of cells was done as for MF staining. And after three washes in PME buffer, the cells were stuck to poly-L-lysine-coated slides and extracted in PME buffer containing 0.5% (w/v) Onozuka R-10 cellulase (Yakult, Tokyo, Japan), 0.05% (w/v) pectolyase Y-23 (Yakult), and 0.05% PMSF. Cells were labeled with mouse anti-β-tubulin (Sigma) diluted 1 : 800. Primary antibody binding was then detected after 3 washes in PBS (pH 7.4) by applying anti-mouse TRITC-conjugated immunoglobulin G (heavy plus light chains) (Sigma) diluted 1 : 100.

The stained cells were viewed and the images were recorded with a Zeiss LSM-5 META confocal laser scanning microscope with a 63× oil immersion objective. The 543 nm wavelength excitation line of the laser was used for TRITC-phalloidin and TRITC-immunoglobulin G (heavy plus light chains). About 10–30 optical sections in 0.7 μm steps were collected and projected with an imaging software package. Images were further processed by Adobe Photoshop 5.5 (Adobe, San Jose, Calif., U.S.A.).

## Results

### *Effects of different concentrations of VD toxin on MFs and MTs*

To determine the effects of different concentrations of VD toxin on the cytoskeletons of Arabidopsis suspension cells, 3-day-old cell aliquots were treated separately with a series of concentrations of VD toxin. The actin MFs began to be disrupted at 30 min after a high concentration of VD toxin (20 μg/ml) was added to the culture, while the disruption of the MFs was delayed significantly when the cells were treated with a lower concentration of VD toxin (1.7 μg/ml). It appeared after at least 2 h treatment. Cortical MTs were disrupted at 15 min of a treatment with high concentration of VD toxin (20 μg/ml), and no disruption of the MTs was found when the cells were treated with a lower concentration of VD toxin (1.7 μg/ml).

When the cells were treated with a high concentration of VD toxin (20 μg/ml) for 30 min, most of the cortical MFs in mature cells were totally collapsed, whereas diffuse MFs appeared in perinuclear region (Fig. 1C). After a 45 min treatment, no cortical actin filaments persisted and the short and thin perinuclear MFs disappeared (Fig. 1D). Furthermore, the treatment also changed the status of the nuclei (Fig. 1D–F). Ring-shaped nucleoli and multiple small ones were often observed in the treated cells. The dye, TRITC,

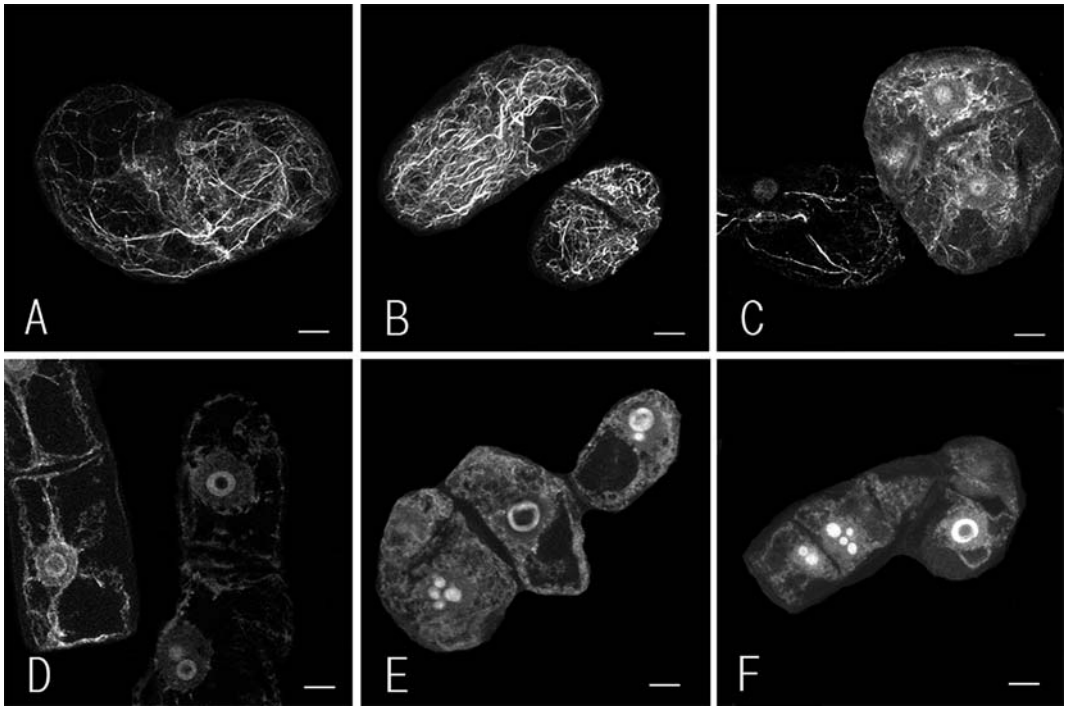


**Fig. 1 A–F.** High concentration of VD toxin (20 μg/ml) alters the actin cytoskeleton. **A** and **B** Controls showing cells treated with Czapek medium for 5 h and Arabidopsis cell suspension medium, respectively. **C** Cells treated with VD toxin for 30 min, showing that most of the cortical actin filaments disappeared. The diffuse actin fluorescence indicates short and thin actin filament bundles located in perinuclear regions. **D** The short and thin actin bundles around nuclei were disrupted when the cells were treated with VD toxin for 45 min. **E** All actin cytoskeletons in cells were disrupted when the cells were treated for 75 min. Ring-shaped nucleoli appeared and multiple small nucleoli were ubiquitous. **F** Fluorescence of actin staining accumulated in the nucleoli when the cells were treated with VD toxin for 105 min and it increased as the disruption of actin filaments proceeded. Bars: 10 μm

was accumulated in the nucleoli and increased as the disruption of MFs proceeded (Fig. 1F).

In normal cells, MTs were aligned perpendicular to the longitudinal cell axis (Fig. 2A, B). MTs were disrupted more quickly than MFs with a high concentration of VD toxin (20 μg/ml). 8 min treatment could make MTs in some cells partially disrupted (Fig. 2C). 15 min was enough to make the cortical MTs disappear (Fig. 2D). And the changes of nucleoli were the same as in the MF case above (Fig. 2E–G).

Alterations of actin cytoskeleton proceeded more slowly in the cells treated with low concentration of VD toxin (1.7 μg/ml) than with high concentrations of VD toxin (3.4–20 μg/ml). The cortical MFs began to disappear after the cells were treated with a low concentration VD toxin for 2 h (Fig. 3B). However, when the treatment was prolonged, short and thin MFs around the nuclei began to disappear (Fig. 3C). All MFs in the treated cells were broken down when the cells were treated with VD toxin for 3 h (Fig. 3D). After longer time of treatment with VD toxin (about 4 h), some nuclei were stained by TRITC. The fluorescence mainly appeared in the nuclei (Fig. 3E). Interestingly, the cortical F-actin arrays spontaneously recovered gradually in most of the cells after they were treated for 5–6 h (Fig. 3F).

There was no disruption of MTs by treatment with low concentration of VD toxin (Fig. 4A, B). Every 15 min cells were checked for MT status, and no disruption of MTs was found during the whole duration (5 h) of treatment (Fig. 4C).

Besides, cortical F-actin arrays in Arabidopsis suspension cells were related to the age of cells. Cortical actin filaments were arranged in parallel with the longitudinal cell axis in mature cells (Fig. 1B, upper cell) and in randomly arranged bundles in the cortical region in young cells (Fig. 1B, lower one). The effects of VD toxin on cortical MFs were similar in mature and young Arabidopsis suspension cells. That is, the changes of the cortical actin filaments were dependent on the concentrations of VD toxin but not related to the arrays of the cortical MFs.

### *Recovery of disrupted actin cytoskeleton*

As shown above, the alternations of MFs caused by low concentration of VD toxin could be recovered spontaneously, possibly because cells have certain mechanisms against the VD toxin (Fig. 3). In contrast, high concentrations of VD toxin caused the unrecoverable disruption of MFs (Fig. 1C–F). However, when cells were washed to

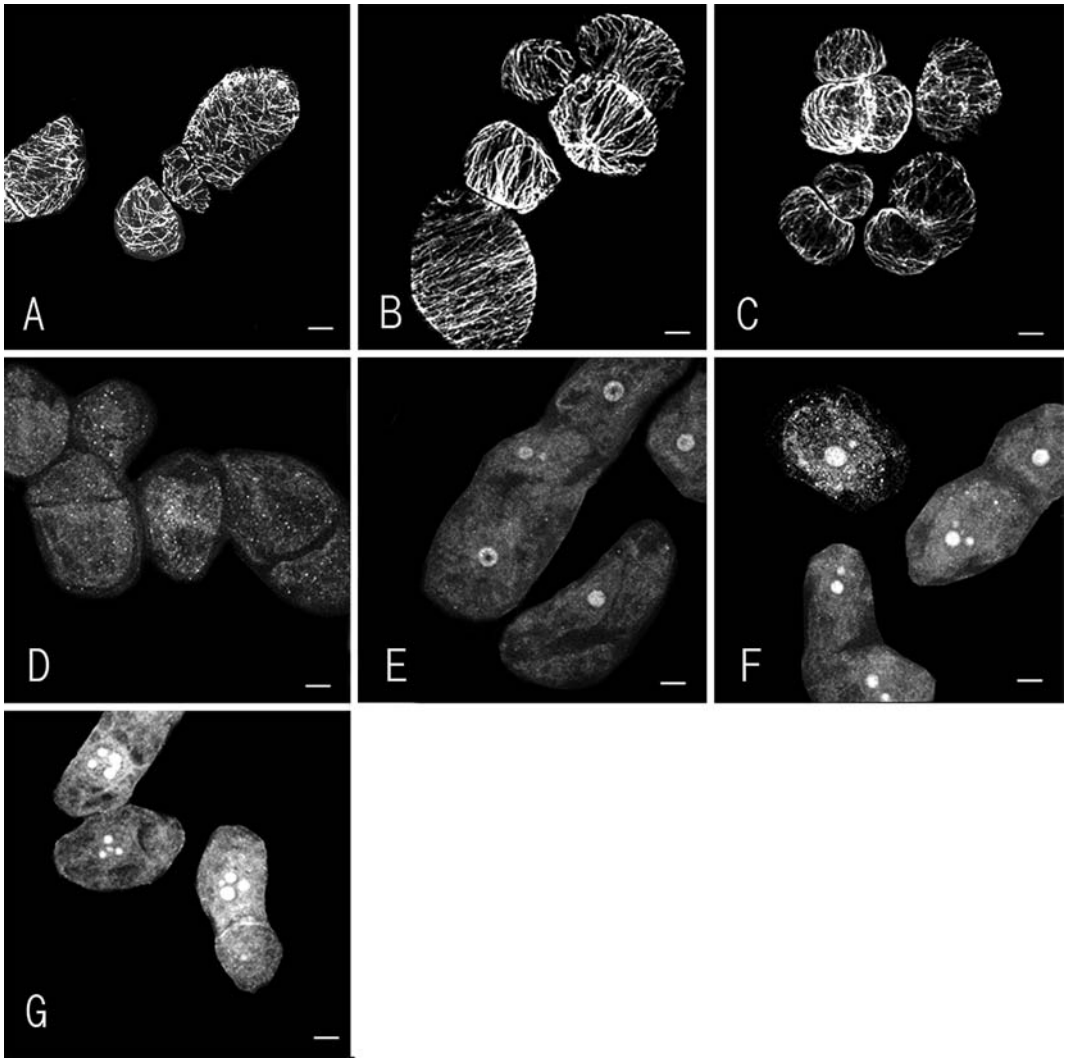


**Fig. 2 A–G.** High concentration of VD toxin (20 μg/ml) alters MTs and nucleoli. **A** and **B** Controls showing cells treated with Czapek medium for 5 h and Arabidopsis cell suspension medium, respectively. **C** Cells treated for about 8 min, showing that MTs in some cells were partially disrupted. **D** Cells treated for 15 min, showing that most of the cortical MTs disappeared. **E** Some nucleoli were dyed and ring-shaped after 30 min of treatment. **F** Multiple small nucleoli were ubiquitous after treatment for more than 60 min. **G** Fluorescence in the fragmented nucleoli became increasingly stronger as the treatment time increased. Treatment time was 180 h. Bars: 10 μm

remove the VD toxin, the MFs still could be recovered after high-concentration treatment. The process of recovery was much slower than the process of disruption. MFs in most cells re-formed gradually in cytoplasmic compartments after washing out the VD toxin when cells had been treated for 30 min (Fig. 5C). But when the treatment was prolonged, fewer cells could recover their MFs. It could be observed in very few cells that the actin filaments were recovered when the time of treatment was 45 min. When the treatment was longer than 60 min, there was no recovery of MFs in any cells. Interestingly, when the treatment time was more than 45 min, the ring-shaped nucleoli could be seen clearly in cells in which no recovery of MFs occurred. Finally, most of the cells died.

*Alterations of nucleoli in treated cells*

As cortical MFs and MTs were disappearing in the VD toxin-treated cells, some changes happened in nuclei. When treated with a high concentration of VD toxin (20 μg/ml), one hollowed or ring-shaped nucleolus with weak fluorescence began to appear in a single nucleus in most of the cells treated for 30–45 min (Figs. 1C, D and 2E). After 45–75 min, the fluorescence in nucleoli became brighter and multiple small nucleoli could be observed in one single nucleus in some cells (Figs. 1E and 2F). When the cells were treated for 75–105 min or more, even more nucleoli with much brighter fluorescence were observed in a single nucleus in the cells (Fig. 1F and 2G). For treatments with

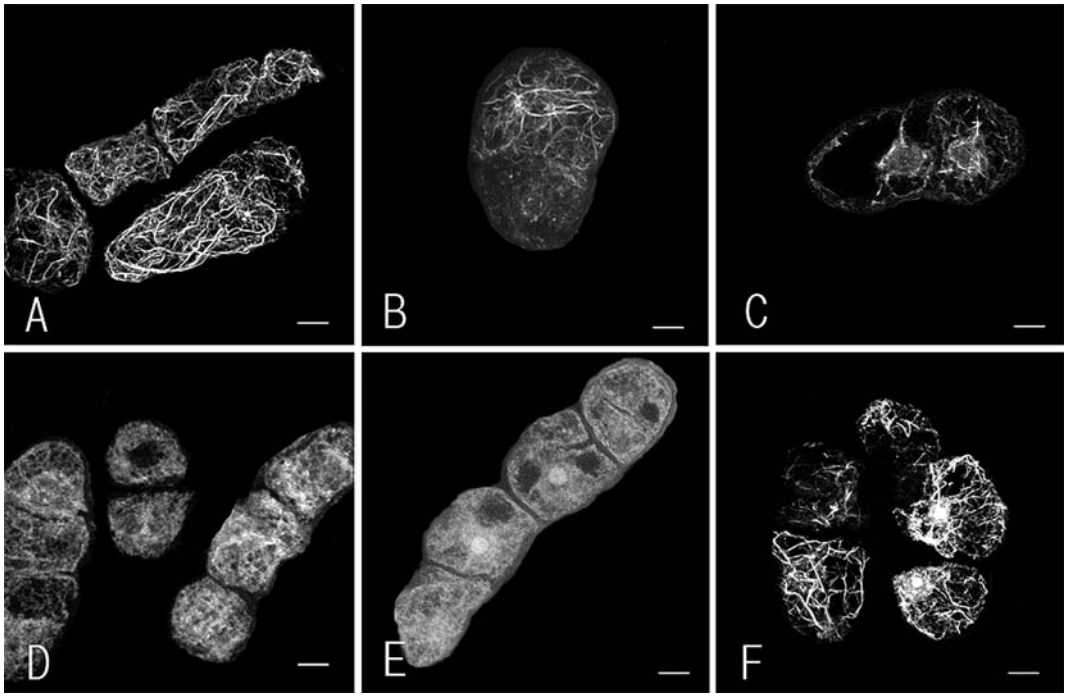


**Fig. 3 A–F.** Alterations of actin cytoskeleton in cells treated with low concentration of VD toxin (1.7 μg/ml). **A** Controls showing cells treated with Arabidopsis cell suspension medium. **B** When cells were treated with VD toxin for 2 h, the cortical actin filaments began to disappear, whereas actin filaments around nuclei still persisted. **C** Actin filaments around nuclei were disrupted after 2.5 h of treatment with VD toxin. **D** All actin filaments in cells broke down when cells were treated for 3 h. **E** Fluorescence of actin staining accumulated in nuclei after 4 h of treatment with VD toxin. **F** Cortical actin array was reestablished in most of the cells after 5–6 h treatment with VD toxin. Bars: 10 μm

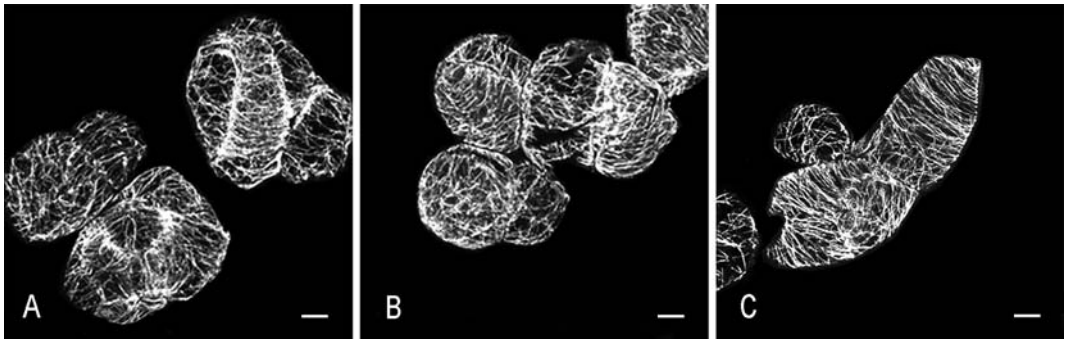


**Fig. 4 A–C.** No alterations of MTs in cells treated with low concentration of VD toxin (1.7 μg/ml). **A** Control. **B** Cells treated with low concentration of VD toxin (1.7 μg/ml) for 1 h, showing that MTs were intact. **C** After 5 h of treatment, MTs remained perfectly organized. Samples were taken after every 15 min of treatment and no disruption of MTs was found. Bars: 10 μm

moderate concentration (e.g., 17 μg/ml), the changes were similar to those caused by high concentration (20 μg/ml) of VD toxin. Though, the treatments differed for the time points at which the changes appeared.

Once abnormal nucleoli were formed, no recovery of MFs could be achieved even if the VD toxin was removed, and the cells died eventually. But if no abnormal nucleoli appeared, the disrupted MFs could be recovered after VD toxin was removed (Fig. 5), and the cells survived.

## Discussion

Fungal culture filtrates (CFs) have been widely used to study the early defense mechanisms in plant–pathogen interactions (Trillas and Araus 1993, Davis et al. 1998, Zemanek et al. 2002). CFs are presumed to contain toxic metabolites and/or toxins that are capable of inducing plant defense responses. CFs of *V. dahliae* (VD toxin) can cause cotton-wilting syndrome, and the glycoproteins of

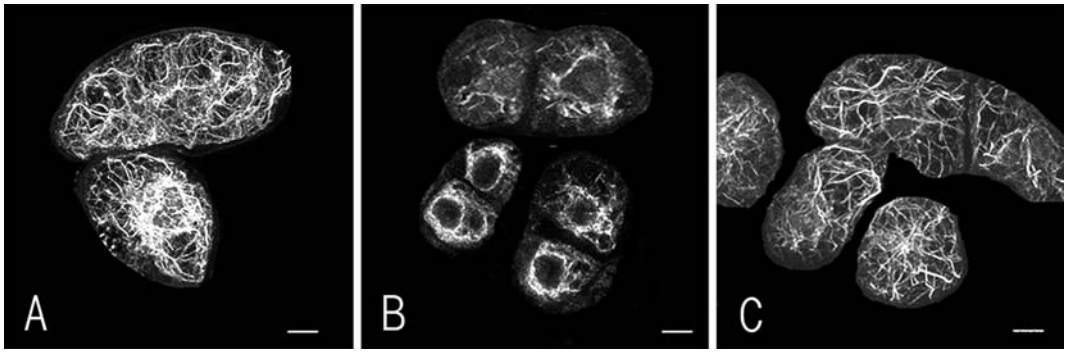


**Fig. 5 A–C.** Recovery of actin MFs after washout of VD toxin. **A** Control cells treated without VD toxin. **B** Cells treated with high concentration of VD toxin (20 μg/ml) for 30 min. **C** About 2 h after washout of VD toxin, actin MFs recovered in cells that had been treated with a high concentration of VD toxin for 30 min. Bars: 10 μm

CFs have been found to play an important role (Keen and Long 1972, Meyer et al. 1994, Zhang et al. 1989, Dubery and Smit 1994, Chu et al. 1999). It was also demonstrated by our recent research data (Zhen and Li 2004). In this study, we used CFs of *V. dahliae* as VD toxin to study their effects on the actin microfilament and microtubular cytoskeletons in *A. thaliana* suspension cells.

Plant cytoskeletons are very active in many cellular processes. And many studies have shown that the actin cytoskeleton takes important roles in plant defense reactions against biotic and abiotic stresses (Kobayashi et al. 1997, Komis et al. 2002). But little has been known about the resistance mechanisms of plant hosts against *V. dahliae*. To our knowledge, the responses of the plant cell cytoskeleton to *V. dahliae* or VD toxin still remain unknown. By studying the direct or indirect effects of VD toxin on MFs and MTs, we reported here how MFs and MTs responded to *V. dahliae*. This may be helpful to understand multiple factors involved in defense mechanisms of plants.

### *Roles for MFs and MTs in defense responses against VD toxin*

As we know, the microfilament and microtubular cytoskeletons in plant cells respond sensitively to many environmental cues, such as fungal penetration and hyperosmotic stress (Komis et al. 2002), and may be involved in some hypersensitive reactions (Furuse et al. 1999, Takemoto et al. 1999). And also, MF and MT arrays in plant cells participate in signaling cascades (Staiger 2000, Kobayashi et al. 1997, Sivaguru et al. 2003, Wasteneys and Galway 2003, Wasteneys and Yang 2004, Takemoto and Hardham 2004). In the present study, we demonstrated that when a low concentration of VD toxin was applied to cells, the cortical and perinuclear MFs disappeared in succession. It has been reported that cortical actin filaments were more dynamic than actin filaments deeper in the cytoplasm, since the dynamic actin filaments seem to be more easily disrupted than the stable actin filaments (Ou and Yuan 2002). Furthermore, a recovery process occurred in actin MFs. Cortical MTs were not disrupted by low-concentration VD toxin. The results suggested that there were some mechanisms in *A. thaliana* cells to resist the disrupting effects of low-concentration VD toxin on the cytoskeleton.

By high concentration of VD toxin, the MTs were disrupted quickly and disappeared at 15 min of treatment. The cortical MFs were disrupted at 30 min of treatment and did not spontaneously recover. The partial depolymerization of MTs seen here within 10 min of treatment with high-concentration VD toxin reflected early events in signal transduction. That is, the onset of cortical MT disruption is earlier than MF disruption. The results indicated that MTs were more sensitive than MFs in signal transduction of the defense responses against VD toxin in Arabidopsis cells.

When treatment with high-concentration VD toxin was prolonged, most of the cells died. It has been suggested that this type of cell death was resistance-dependent cell death, it might be considered as programmed cell death (data not shown). These results indicated that intact MTs and MFs were necessary for cell survival and normal cellular processes. The disruption of MTs and MFs might be an important and early sign of programmed cell death. It has been reported that the MF changes might be a general feature of the HR (hypersensitive response) cell death which is one type of plant programmed cell death, a rapid cell death in plants associated with disease resistance (Škalamera and Heath 1998).

Taking all together, these results demonstrated that MTs and MFs play important roles in the basic and primitive defense reactions in Arabidopsis suspension cells against VD toxin. MFs and MTs are instrumental in mediating Arabidopsis cell responses to VD toxin. It is plausible that

one of the major functions of MTs and MFs in expression of resistance reactions against VD toxin may be related with signal transduction. Consequently, molecular components regulating signaling mediated via the MTs and MFs have evolved in Arabidopsis suspension cells in order to adapt to stress of VD toxin. The results support our inference that the stressed cells use the cytoskeleton, especially the MTs, as sensor and effector to monitor the defense reactions against VD toxin. It has been proposed that the actin filaments are involved in transmitting the fungal contact signal to the nucleus for changes in gene expression (Kobayashi et al. 1992, Furuse et al. 1999, Jarosch et al. 2005). To our knowledge the present work is the first study in which the defense responses against VD toxin have been investigated with respect to MTs and MFs.

### *Nucleoli implicated in resistance responses against VD toxin*

The nucleolus is a plurifunctional nuclear organelle. In addition to its roles in rDNA transcription and ribosome subunit assembly, the nucleolus is also implicated in many other processes that are still poorly understood. So far, most of our knowledge about it was achieved from the studies on animal and human nucleoli (Jeong et al. 1998, Leung et al. 2003, Lam et al. 2004). In the present study, the nucleoli were stained with TRITC, a kind of cationic dye. The shape of nucleoli was changed when the cells were treated with high concentrations of VD toxin. Two types of nucleoli could be observed in the cells as the treatment proceeded. At the early stages of the treatment, one single nucleus had one nucleolus in most cells, and the nucleolus was ring-shaped. At the later stages, a single nucleus had smaller, multiple and brighter nucleoli. Once the ring-shaped nucleoli were formed in the treated cells, MFs would fail to recover and cell death resulted eventually. Studies have shown that the ring-shaped nucleoli appeared in human lymphocytes when cells entered the resting state (Smetana et al. 1968, 2004; Vandelaer et al. 1993). Therefore, the ring-shaped nucleoli induced by the VD toxin treatment may indicate that cells entered the resting state that was accompanied by resistance-related programmed cell death. Our study suggests that in addition to their normal roles, the nucleoli may also play some roles in the resistance response of Arabidopsis suspension cells against VD toxin. It is possible that the changes of nucleolar shape are important or even essential to switch on the process of resistance-related cell death, and this kind of cell death should be considered as a positive and active response to stress. And taking MFs and MTs into account, there should be some links between MFs, MTs, and nucleoli, and may be the unrecoverable disruption of MFs and MTs initiated some special cellular processes that resulted in the emergence of abnormal nucleoli and cell death.

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## References

Bradford MN (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein using the principle of protein-dye binding. Anal Biochem 72: 248–254

Chu Z-Q, Jia J-W, Zhou X-J, Chen X-Y (1999) Isolation of glycoproteins from *Verticillium dahliae* and their phytotoxicity. Acta Bot Sin 41: 972–976

Daayf F, Nicole M, Geiger JP (1995) Differentiation of *Verticillium dahliae* populations on the basis of vegetative compatibility and pathogenicity on cotton. Eur J Plant Pathol 101: 69–79

Daayf F, Nicole M, Boher B, Pando A, Geiger JP (1997) Early vascular defense reactions of cotton roots infected with a defoliating mutant strain of *Verticillium dahliae*. Eur J Plant Pathol 103: 125–136

Davis DA, Low PS, Heinstein P (1998) Purification of a glycoprotein elicitor of phytoalexin formation from *Verticilium dahliae*. Physiol Mol Plant Pathol 52: 259–273

de Pinto M-C, Francis D, De Gara L (1999) The redox state of the ascorbate-dehydroascorbate pair as a specific sensor of cell division in tobacco BY-2 cells. Protoplasma 209: 90–97

Dubery IA, Slater V (1997) Induced defence responses in cotton leaf disks by elicitors from *Verticillium dahliae*. Phytochemistry 44: 1429–1434

Dubery IA, Smit F (1994) Phenylalanine ammonia-lyase from cotton (*Gossypium hirsutum*) hypocotyls: properties of the enzyme induced by a *Verticillium dahliae* phytotoxin. Biochim Biophys Acta 1207: 24–30

Furuse K, Takemoto D, Doke N, Kawakita K (1999) Involvement of actin filament association in hypersensitive reactions in potato cells. Physiol Mol Plant Pathol 54: 51–61

Gamborg OL, Miller RA, Okima K (1968) Nutrient requirements of suspension cultures of soybean root cells. Exp Cell Res 50: 151–158

Genre A, Bonfante P (2002) Epidermal cells of a symbiosis-defective mutant of *Lotus japonicus* show altered cytoskeleton organisation in the presence of a mycorrhizal fungus. Protoplasma 219: 43–50

Goicoechea N, Aguirreolea J, Cenoz S, Garcia-Mina JM (2000) *Verticillium dahliae* modifies the concentrations of proline, soluble sugars, starch, soluble protein and abscisic acid in pepper plants. Eur J Plant Pathol 106: 19–25

Hill MK, Lyon KJ, Lyon BR (1999) Identification of disease response genes expressed in *Gossypium hirsutum* upon infection with the wilt pathogen *Verticilliium dahliae*. Plant Mol Biol 40: 289–296

Jarosch B, Collins NC, Zellerhoff N, Schaffrath U (2005) RAR1, ROR1, and the actin cytoskeleton contribute to basal resistance to *Magnaporthe grisea* in barley. Mol Plant Microbe Interact 18: 397–404

Jeong J-S, Kim I-H, Lee H-J, Choi Y-C (1998) Nucleolus contains signal molecules that constitute membrane-nucleolus linked pathway. Exp Mol Med 30: 205–213

Keen NT, Long M (1972) Isolation of a protein-lipopolysaccharide complex from *Verticillium albo-altrum.* Physiol Plant Pathol 2: 307–315

Kobayashi I, Hakuno H (2003) Actin-related defense mechanism to reject penetration attempt by a non-pathogen is maintained in tobacco BY-2 cells. Planta 217: 340–345

Kobayashi I, Kobayashi Y, Yamaoka N, Kunoh H (1992) Recognition of a pathogen and a nonpathogen by barley coleoptile cells. III. Responses of microtubules and actin filaments in barley coleoptile cells to penetration attempts. Can J Bot 70: 1815–1823

Kobayashi I, Kobayashi Y, Hardham AR (1994) Dynamics reorganization of microtubules and microfilaments in flax cells during the resistance response to flax rust infection. Planta 195: 237–247

Kobayashi Y, Yamada M, Kobayashi I, Kunoh H (1997) Actin microfilaments are required for the expression of nonhost resistance in higher plants. Plant Cell Physiol 38: 725–733

Komis G, Apostolakos P, Galatis B (2002) Hyperosmotic stress-induced actin filament reorganization in leaf cells of *Chlorophytom comosum*. J Exp Bot 53: 1699–1710

Kwok EY, Hanson MR (2004) In vivo analysis of interactions between GFP-labeled microfilaments and plastid stomules. BMC Plant Biol 4: 2–10

Lam YW, Andersen J, Mann M, Lamond A (2004) Quantitative proteomics of the human nucleolus. Eur J Cell Biol 83: 54

Leung AKL, Andersen JS, Mann M, Lamond AI (2003) Bioinformatic analysis of the nucleolus. Biochem J 376: 553–569

Li Y-Z, Zheng X-H, Tang H-L, Zhu J-W, Yang J-M (2003) Increase of β-1,3-glucanase and chitinase activities in cotton callus cells treated by salicylic acid and toxin of *Verticillium dahliae* Kleb. Acta Bot Sin 45: 802–808

McFadden HG, Chapple R, Fayter R de, Dennis E (2001) Expression of pathogenesis-related genes in cotton stems in response to infection by *Verticillium dahliae*. Physiol Mol Plant Pathol 58: 119–132

Meyer R, Slater V, Dubery IA (1994) A phytotoxic protein-lipopolysaccharide complex produced by *Verticillium dahliae*. Phytochemistry 35: 1449–1453

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15: 473–479

Ou GS, Chen ZL, Yuan M (2002) Jasplakinolide reversibly disrupts actin filaments in suspension-cultured tobacco BY-2 cells. Protoplasma 219: 168–175

Resende MLV, Flood J Ramsden JD, Rowen MG, Beale MH, Cooper RM (1996) Novel phytoalexins including elemental sulphur in the resistance of cocoa (*Theobroma cacao* L.) to Verticillium wilt (*Verticillium dahliae* Kleb.). Physiol Mol Pathol 48: 347–359

Sandovsky-Losica H, Berdicevsky I, Tsarfaty I, Segal E (2002) Effect of *Candida albicans* metabolite(s) on cellular actin. FEMS Microbiol Lett 215: 57–62

Sivaguru M, Pike S, Gassmann W, Baskin T (2003) Aluminum rapidly depolymerizes cortical microtubules and depolarizes the plasma membrane: evidence that these responses are mediated by a glutamate receptor. Plant Cell Physiol 44: 667–675

Škalamera D, Heath MC (1998) Changes in the cytoskeleton accompanying infection-induced nuclear movements and the hypersensitive response in plant cells invaded by rust fungi. Plant J 16: 191–200

Smetana K, Freireich E-J, Busch H (1968) Chromatin structures in ring-shaped nucleoli of human lymphocytes. Exp Cell Res 52: 112–128

Smetana K, Pluskalová M, Marinov Y, Hrka Z (2004) The effect of 5-aminolevulinic acid-based photodynamic treatment (PDT) on nucleoli of leukemic granulocytic precursors represented by K562 blastic cells in vitro. Med Sci Monit 10: BR405–409

Smit F, Dubery IA (1997) Cell wall reinforcement in cotton hypocotyls in response to a *Verticillium dahliae* elicitor. Phytochemistry 44: 811–815

Smith LG (2003) Cytoskeleton control of plant cell shape: getting the fine points. Curr Opin Plant Biol 6: 63–73

Staiger CJ (2000) Signaling to the actin cytoskeleton in plants. Annu Rev Plant Physiol Plant Mol Biol 51: 257–288

Subbarao KV, Chassot A, Gordon TR, Hubbard JC, Bonello P, Mullin R, Okamoto D, Davis RM, Koike ST (1995) Genetic relationship and cross pathogenicities of *Verticillium dahliae* isolates from cauliflower and other crops. Phytopathology 85: 1105–1112

Takemoto D, Hardham A (2004) The cytoskeleton as a regulator and target of biotic interactions in plants. Plant Physiol 136: 3864–3876

Takemoto D, Furuse K, Doke N, Kawakita K (1997) Identification of chitinase and osmotin-like protein as actin-binding proteins in suspension-cultured potato cells. Plant Cell Physiol 38: 441–448

Takemoto D, Maeda H, Yoshioka H, Doke N, Kawakita K (1999) Effect of cytochalasin D on defense responses of potato tuber discs treated with hyphal wall components of *Phytophthora infestans*. Plant Sci 141: 219–226

Takemoto D, Jones DA, Hardham AR (2003) GFP-tagging of cell components reveals the dynamics of subcellular re-organization in response to infection of *Arabidopsis* by oomycete pathogens. Plant J 33: 775–792

Trillas MI, Araus JL (1993) Effects of *Fusarium oxysporum* culture filtrates on carnation callus cell ultrastructure and cytoplasmic calcium distribution. Physiol Mol Plant Pathol 43: 231–241

Vandelaer M, Thiry M, Goessens G (1993) Ultrastructural distribution of DNA within the ring-shaped nucleolus of human resting T lymphocytes. Exp Cell Res 205: 430–432

Vantard M, Blanchoin L (2002) Actin polymerization process in plant cells. Curr Opin Plant Biol 5: 502–506

Veronese P, Narasimhan ML, Stevenson RA, Zhu J-K, Weller SC, Subbarao KV, Bressan RA (2003) Identification of a locus controlling Verticillium disease symptom response in *Arabidopsis thaliana*. Plant J 35: 574–587

Wada M, Suetsugu N (2004) Plant organelle positioning. Curr Opin Plant Biol 7: 626–631

Wasteneys GO, Galway ME (2003) Remodeling the cytoskeleton for growth and form: an overview with some new views. Annu Rev Biol 54: 691–722

Wasteneys GO, Yang Z (2004) The cytoskeleton becomes multidisciplinary. Plant Physiol 136: 3853–3854

Zemanek AB, Ko T-S, Thimmapuram J, Hammerschlag FA, Korban S (2002) Changes in β-1,3-glucanase mRNA levels in peach in response to treatment with pathogen culture filtrates, wounding, and other elicitors. J Plant Physiol 159: 877–889

Zhang Y-S, Wang J-X, Liu J-F, Fang Z-D (1989) Studies on the isolation, purification and bioassay of toxin from *Verticillium dahliae* Kleb. Acta Mycol Sin 8: 140–147

Zhen X-H, Li Y-Z (2004) Ultrastructural changes and location of β-1,3-glucanase in resistant and susceptible cotton callus cells in response to treatment with toxin of *Verticillium dahliae* and salicylic acid. J Plant Physiol 161: 1367–1377