Extracts of Killed *Penicillium chrysogenum* Induce Resistance Against Fusarium Wilt of Melon

Hezhong Dong[1,2] and Yigal Cohen[1,*]

Dry fungal biomass of *Penicillium chrysogenum* (dry mycelium), a waste product of the pharmaceutical industry, was extracted with water and applied to the roots of melon plants before or after inoculation with *Fusarium oxysporum* f.sp. *melonis* (*Fom*). Seedlings (4-6 days after emergence) treated with either acidic dry mycelium extract (DME) or neutralized dry mycelium extract (NDME) were protected against challenge infection with *Fom*. A single drench with 2–5% DME applied 12–72 h before inoculation provided significant control of the disease compared with water-drenched, challenged seedlings. No protection was seen in plants treated 0–6 h before inoculation or 0–48 h after inoculation. Neither DME nor NDME (0.5–5%) had any effect on fungal growth *in vitro*, which implied that disease control *in vivo* was mediated by induced resistance. The resistance induced by DME protected melon plants not only against race 1,2, but also against the three other races of the pathogen, indicating a race-non-specific resistance against *Fom*. Both DME and NDME significantly increased peroxidase activity and free L-proline content in seedlings 12 h and 48 h after soil drench, respectively. Resistance to Fusarium wilt was significantly associated with elevated levels of peroxidase activity but not with free L-proline content. Thus, peroxidase might be involved in the defense mechanisms activated by DME or NDME.

KEY WORDS: *Penicillium chrysogenum*; Fusarium wilt; *Fusarium oxysporum* f.sp. *melonis*; induced resistance; peroxidase; L-proline.

INTRODUCTION

Fusarium wilt caused by *Fusarium oxysporum* f.sp. *melonis* (*Fom*) is a devastating disease of melon. Current measures for control are based mostly on soil disinfection, resistant cultivars or fungicides (3). The necessity of reducing pesticide levels in the environment has enhanced the need for development of alternative methods of disease control. Among these, the technology for disease control based on activation of the plant's own defense system by certain chemical compounds has attracted attention in recent years (7,20). In contrast to genetic resistance, acquired or induced resistance may be activated in plants that are susceptible to a pathogen's attack and is normally achieved after an induction period necessary for the activation of the plant's defense mechanisms (20). Induced resistance is often characterized by a broad spectrum of protection and relatively long duration of expression. Induced resistance is based on multiple defense mechanisms, which makes it less likely to be overcome by the pathogens (20).

Received March 25, 2001; received in final form July 9, 2001; http://www.phytoparasitica.org posting Aug. 31, 2001. *Author for correspondence [Fax: +972-3-5354133; e-mail: coheny@mail.biu.ac.il].
[1]Faculty of Life Sciences, Bar-Ilan University, Ramat Gan 52900, Israel.
[2]On sabbatical leave from Cotton Research Center, Shandong Academy of Agricultural Sciences, Jinan 250100, China.

Induced resistance can be achieved by using biotic inducing agents or through application of chemicals (2,4-6,11,14). Various chemicals, such as salicylic acid (SA), 2,6-dichlorisonicotic acid (INA) and benzo[1,2,3]thiadiazole-7-carbothionic acid-S-methyl ester (BTH), are able to induce resistance in a number of plants against a wide range of pathogens without possessing any direct antimicrobial activity *in vitro* or *in vivo* (9,11,14). Recently, a new class of compounds, belonging to 3-aminobutyric acids (BABA), has been reported by Cohen and co-workers to be effective in inducing local and systemic resistance against fungal diseases (including *Fom*) and nematodes (4,5,7,21). Zimmerli *et al.* (31) and Siegrist *et al.* (26) reported that 3-aminobutyric acid protects against bacteria and tomato mosaic virus, respectively. Induced resistance is associated with increased transcription of a group of mRNAs encoding PR-1, chitinases, β-1,3-glucanases and peroxidase (16). SA, produced naturally in plants, is involved in signaling for induced resistance (20). Synthetic inducers such as INA, BTH and BABA have been reported to induce resistance downstream of SA (4,9,29). Some plant extracts (8,15) were also reported to protect plants against certain pathogens. Dry mycelium (DM) used in this study is made from the dry killed fungal biomass of *Penicillium chrysogenum* after extraction of penicillin. It was reported that live *P. chrysogenum* is a potential biological control agent against *Botrytis fabae* in faba beans (17). When added to the soil, DM enhances plant growth by supplying nutrients and improving soil fertility (13). Recently Gao *et al.* (10) reported that DM applied to the soil protected corn plants against *Fusarium moniliforme*.

Reuveni *et al.* (22) noted that foliar application of phosphates could control powdery mildew of wine grapes and result in a remarkable increase in soluble peroxidase activity. Peroxidase catalyzes the final polymerization step of lignin synthesis, and may therefore be directly associated with the increased ability of protected tissue to lignify (12). Peroxidase activity could also be a biochemical marker for genetic resistance (22-24). Like peroxidase, L-proline accumulation has also been correlated in many plants with resistance to environmental stress (30). However, very little is known about the effect of dry fungal biomass on peroxidase activity and L-proline accumulation, or on their relationship to induced resistance. In this paper we describe how extracts of DM can induce resistance against *F.o.* f.sp. *melonis* in melon, with particular reference to the relationship between resistance and peroxidase activity or L-proline content.

MATERIALS AND METHODS

Preparation of extracts Penicillin-free DM (powder) of *P. chrysogenum* was obtained from Biochemie Ltd., Kundl, Austria. This fungal biomass was dried by the manufacturer for 4 h at 110°C. Dry mycelium extract (DME) was prepared by the following procedure: 100 g of DM was suspended in 1000 ml distilled water (= 10% DME, w/v), shaken for 2 h at 100 rpm, and then stored for 22 h at room temperature. It was then briefly agitated and filtered through Whatman No. 1 filter paper. The filtrate was autoclaved for 30 min at 110°C and the pH was measured after cooling (10% DME, pH=2.6–2.8). Neutralized DME (NDME, pH=6.8) was obtained by adding 0.1 M KOH. 10% DME and 10% NDME were stored as stock solutions at 4°C.

Plants and DME (and NDME) treatment Twenty seeds of melon (*Cucumis melo* L.) cv. 'En-Dor', which is susceptible to *Fom*, were sown in 0.5-*l* pots containing a mixture of perlite and peat (1:1, v/v) and allowed to grow in the greenhouse (18–28°C). After emergence (usually 8 days after sowing), ten seedlings of uniform size were left in each pot.

Each pot was drenched with DME or NDME (20 ml per pot) of various concentrations(0.5–10%) using a pipette. Potted plants drenched with 20 ml distilled water served as controls unless indicated otherwise. Plants were watered every 2 days with an equal volume of water per pot, starting 3 days after treatment.

Fungal inoculum The four known races of *Fom*, race 0, race 1, race 2 and race 1,2, were grown in 9-cm petri dishes on PDA at 25°C in the dark. Conidia were removed from 10–15-day-old cultures into distilled water and their concentration was adjusted to 10^6–10^7 conidia per ml with a cytometer before inoculation.

Inoculation and disease assessment Plants were inoculated with one of the four races of *Fom* 2–3 days after application of DME or NDME, unless indicated otherwise. To evaluate the effect of time of DME-treatment on disease control, plants were also inoculated with race 1,2 of *Fom* 48 h before DME-treatment or 0–72 h after DME-treatment. For inoculation, seedlings were carefully removed from the soil, thoroughly washed with water, root tips were cut off, the root system dipped for 1 min in the conidial suspension, and then transplanted into fresh potting mixture. Inoculated plants were allowed to grow in the greenhouse under the conditions described above. Inoculated seedlings usually exhibited wilt symptoms 3–4 days after inoculation. The number of wilted seedlings was counted at 6–8 days after inoculation, and the percentage of diseased and protected plants was calculated. Each experiment was carried out with five replicates of ten plants each, and was repeated at least twice.

Fungitoxicity tests Czapek Dox agar supplemented with 0.5–5% (v/v) DME or NDME was autoclaved for 30 min at 110°C, poured into 9-cm petri dishes, and inoculated with three mycelial plugs (2×4 mm) of race 1,2 of *Fom* per petri dish (three plates / extract / concentration). The inoculated plates were kept at 25°C in the dark and colony diameters were measured at 2 and 4 days after inoculation.

Peroxidase activity and L-proline content Non-inoculated or *Fom*-infected plants, either treated or untreated with DME or NDME, were used for assessing peroxidase activity and free L-proline content. Plants were removed from soil after DME (or NDME) treatment or inoculation, thoroughly washed with water, blotted dry and weighed. One whole plant (~ 0.5 g) was ground in 10 ml of cold 15 mM sodium phosphate buffer (pH 6), and the suspension was centrifuged (10,000 *g* for 10 min at 4°C). Peroxidase activity of the supernatant was measured and expressed as the change in absorbance per minute per gram fresh weight (22). Four replicate plants per treatment were used in each experiment.

Plant samples for L-proline assessment were obtained as described above. L-proline was extracted and its concentration was determined according to the method of Bates *et al.* (1). Briefly, samples (~ 0.5 g each) were homogenized with 3% sulfosalicylic acid and the homogenate was centrifuged (10,000 *g* for 5 min) The supernatant was treated with acetic acid and ninhydrin solution, boiled for 1 h, and the absorbance at 520 nm was determined. Amounts of proline were expressed as ng/g fresh weight.

Data analysis Means and standard deviations (SD) of diseased plants percentage, peroxidase activity and L-proline content were calculated using Excel (Microsoft, Bothell, WA, USA). Analysis of variance was performed by Duncan's Multiple Range Test to establish significant difference (P=0.05). To test if peroxidase activity and L-proline had some bearing on plant protection, Spearman's rank correlation coefficient (r) test (P=0.01) was also performed, based on data in Figures 4, 5 and 6.

RESULTS

***In vitro* studies** Various concentrations (0–5%) of DME or NDME were added to Czapek Dox agar media to study the possible fungitoxic activity of DME against race 1,2 of the wilt pathogen. Colony diameter (range) did not differ significantly between DME- or NDME-free (control) and DME- or NDME-amended plates (data not shown), indicating that DME or NDME at up to 5% had no fungicidal activity against *Fom*.

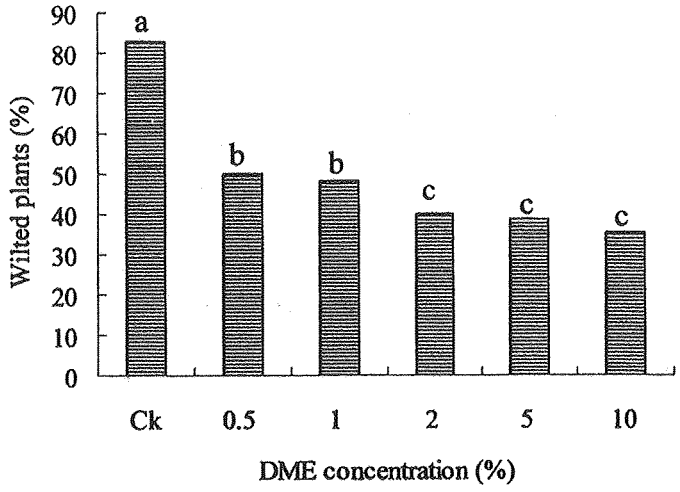

Fig. 1. Protection obtained in melon plants against *Fusarium oxysporum* f.sp. *melonis* (race 1,2) by dry mycelium extract (DME). Plants were treated with various concentrations of acidic DME by soil drench 2 days before inoculation. Plants drenched with water were used as control. Data were recorded 8 days after inoculation. Columns with the same letter do not differ significantly (P=0.05) according to Duncan's multiple range test.

Disease control by DME Data in Figure 1 show that melon plants exposed 2 days before inoculation to various concentrations of DME (0.5–5%) were significantly protected against wilt 8 days after inoculation. A significantly higher level of protection (52%) was obtained with 2% DME than with 0.5–1% DME (40%). At 5–10% no further enhancement of protection was observed, and phytotoxic symptoms were seen in treated but non-inoculated plants (data not shown). Protected plants started to wilt 15 days after inoculation. Control and DME-treated plants (2%) were wilted at 10 and 21 days after inoculation, respectively

Protection induced by DME is race-non-specific Figure 2 presents data showing that DME protected melon plants against *Fom* of race 0, 1, 2 or 1,2. Among the four races, race 1,2 was the most aggressive, with 85% of the untreated plants evincing wilt 7 days after inoculation. Plants treated with 2% DME were 85% protected against this race. Percentage protection against races 0, 1 and 2 was 74%, 55% and 71%, respectively.

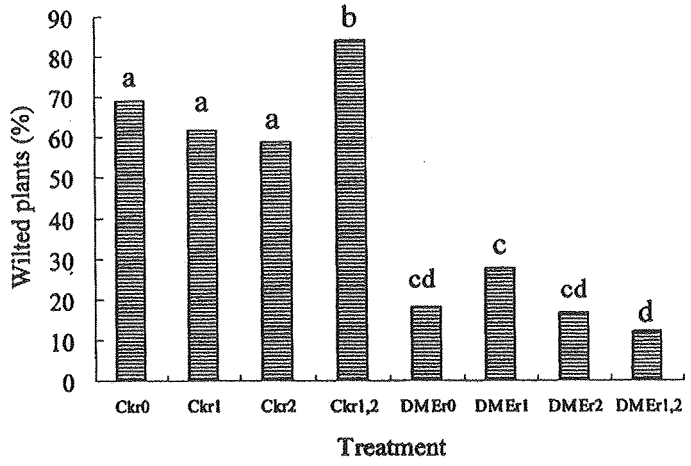

Fig. 2. Protection obtained in melon plants by 2% dry mycelium extract (DME) against four races of *Fusarium oxysporum* f.sp. *melonis* (r0, r1, r2, and r1,2). 2% DME was applied by soil drench 3 days before inoculation. Data were recorded 7 days after inoculation. Columns with the same letter do not differ significantly (P=0.05) according to Duncan's multiple range test. Ck = control inoculated.

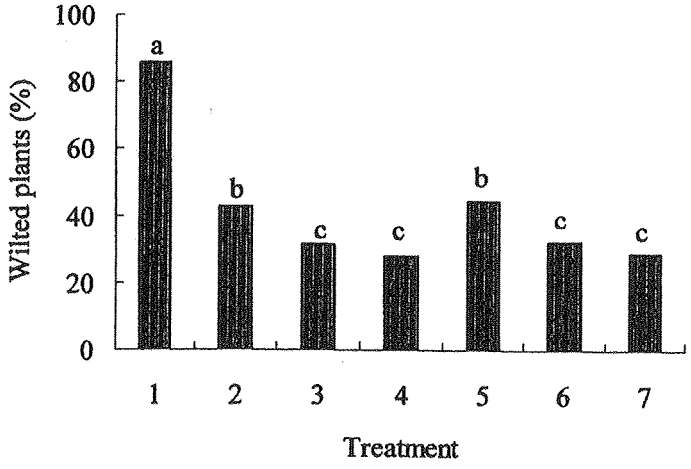

Fig. 3. Protection obtained in melon plants by various concentrations of dry mycelium extract (DME) and neutralized DME (NDME) against *Fusarium oxysporum* f.sp. *melonis* (race 1,2). Plants were treated by soil drench of 0.5–2% DME or 0.5–2% NDME 2 days before inoculation, or with water as the control. Data were recorded 8 days after inoculation. 1, control; 2, 0.5% DME; 3, 2% DME; 4, 5% DME; 5, 0.5% NDME; 6, 2% NDME; 7, 5% NDME. Columns with the same letter do not differ significantly (P=0.05) according to Duncan's multiple range test.

Effect of NDME on induced protection Data presented in Figure 3 show that 8 days after inoculation both DME and NDME significantly reduced the percentage of wilted plants relative to the control. No significant difference was found in the percentage of wilted plants between the two extracts of equal concentrations. DME at 0.5%, 2% and 5% provided 50%, 63% and 67% protection, and NDME at those concentrations gave 48%, 62% and 66% protection, respectively.

Effect of time of DME application on induced protection Data in Figure 4 were recorded at 6 days after inoculation. A soil drench with 2% DME applied either 0–48 h after inoculation or 0–6 h before inoculation did not suppress Fusarium wilt, whereas a

significant decrease in the percentage of wilted plants occurred when the soil drench was done 12–72 h before inoculation. It seems that a root exposure period of at least 12 h was required for DME to control *Fom* effectively.

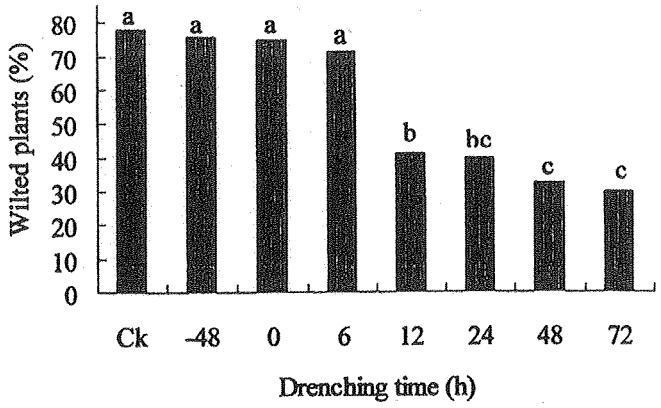

Fig. 4. Time-dependent efficacy of dry mycelium extract (DME) application on protection obtained in melon against *Fusarium oxysporum* f.sp. *melonis*. Soil drench with water (Ck) or 2% DME was done either before (-) or after inoculation. 0 indicates that 2% DME was applied soon after inoculation. Data were recorded 6 days after inoculation. Columns with the same letter do not differ significantly (P=0.05) according to Duncan's multiple range test.

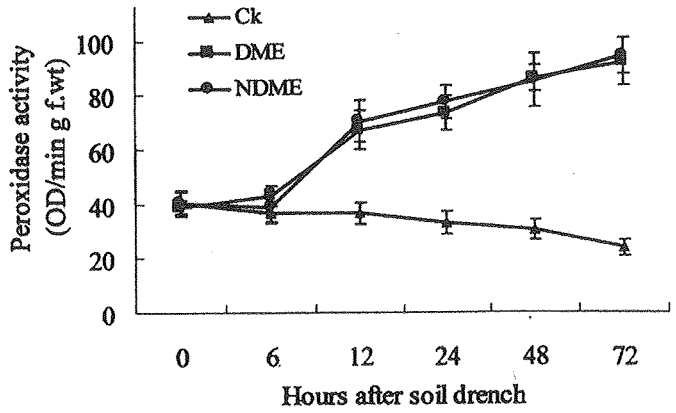

Fig. 5. Changes in peroxidase activity in healthy melon plants (not inoculated) after soil drench with 2% dry mycelium extract (DME) or 2% neutralized DME (NDME). Ck = soil drench with water. Plants were treated 3 days after emergence. Bars represent ±SD.

Effect of DME on peroxidase activity and L-proline content Data in Figure 5 show that peroxidase activity in water-treated, DME-treated and NDME-treated plants was equally low at 0–6 h after treatment. Significant enhancements (up to fourfold) in peroxidase activity in both DME-treated and NDME-treated plants, relative to control plants, occurred at 12–72 h after treatment. Data in Figure 6 show that L-proline in DME- or NDME-treated plants did not accumulate significantly until 48–72 h after treatment. Spearman's rank correlation coefficient test on the data in Figures 4, 5 and 6 showed a significant (P = 0.01) negative correlation between peroxidase activity and percentage of wilted plants (r = -0.98).

Other data presented in Figure 7 show that DME significantly increased peroxidase activity in both non-inoculated and *Fom*-inoculated plants. Three days after DME application, a fourfold increase in peroxidase activity was measured in DME-treated plants compared with water-treated plants. Four and 6 days after soil drench, significantly higher peroxidase activity was seen in DME-treated inoculated plants as compared with DME-treated healthy plants (not inoculated), whereas only at 6 days (3 days after inoculation) did water-treated inoculated plants exhibit a significantly higher peroxidase activity than healthy plants. L-proline content was not measured in *Fom*-inoculated plants.

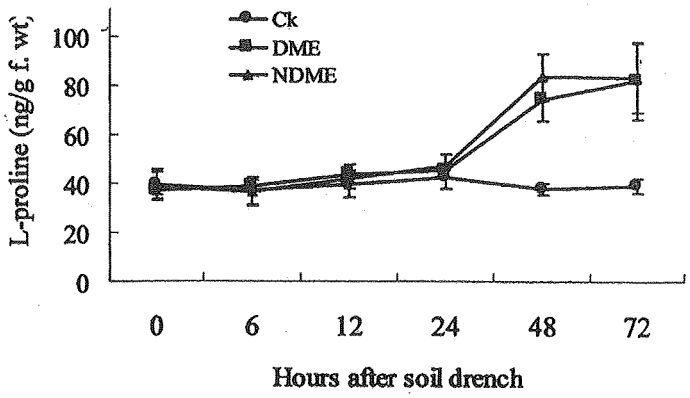

Fig. 6. L-proline content in melon plants after application of 2% dry mycelium extract (DME) or 2% neutralized DME (NDME). Ck = soil drench with water. Plants were treated 3 days after emergence. Bars represent ±SD.

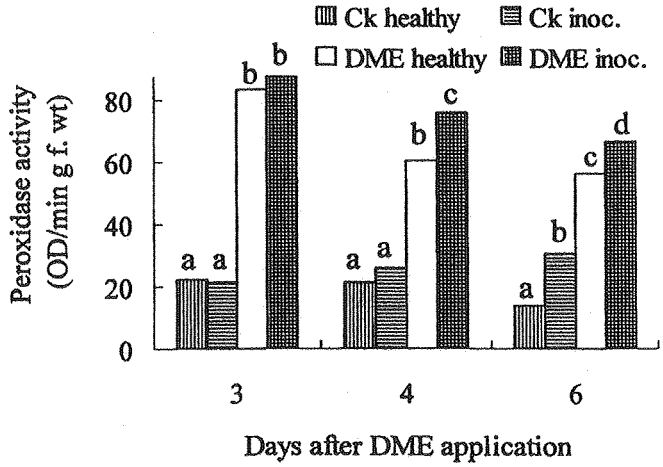

Fig. 7. Peroxidase activity in *Fusarium oxysporum* f.sp. *melonis*-inoculated (race 1,2) and non-inoculated melon plants after either water (Ck) or 2% dry mycelium extract (DME) treatment. For each time interval, columns with the same letter do not differ significantly (P=0.05) according to Duncan's multiple range test.

DISCUSSION

We report herein that melon plants growing in pots drenched with a water extract of DM made from *P. chrysogenum* were protected against wilt caused by *Fom.* Data from several

experiments revealed that DME may provide up to 85% protection against Fusarium wilt relative to water-treated control. In general, as the concentration of DME rose, the control effect increased. However, at concentrations of 5% or higher, no further increase in disease control was observed. Water extract of DM is quite acidic (pH 2.6–2.8). We found that neutralized DME (NDME, pH 6.8) was as effective as acidic DME against the disease. DME had no negative effect on growth of the pathogen *in vitro*. Thus, it is inferred that the disease control effected by DME or NDME was probably based on induced resistance mechanisms. The resistance induced by DME was effective not only against race 1,2, but also against the three other races of the pathogen, indicating a race-non-specific resistance against *Fom*.

Induced resistance is characterized by a broad spectrum of protection and relatively long duration of expression (20), but its expression is usually dependent on activators, plant species and pathogens (18,20). Expression of induced resistance is also associated with concentration and application mode of activators and infection pressure. High infection pressure often causes insufficient protection against the pathogen (19). In this study, we found that DME provided significant protection against *Fom* for 15 days after inoculation, relative to water-treated control. However, the surviving plants wilted 21 days after inoculation, implying that induced resistance by a single drench of DME could supply only a limited duration of protection under high infection pressure (10^7 spores per ml). Additional treatment with DME might be needed to boost protection. DME had to be applied 12 h or more before challenge inoculation with *Fom*, indicating that an interval of time was necessary for DME uptake by the root and/or activation of defense mechanisms by the plant. Unlike other inducers such as BABA (4,5), DME spray on melon leaves did not protect against *Fom* (data not shown), indicating that DME was either not taken up by leaves or not translocated to the root system.

During the last decade a number of studies concerning the mechanisms responsible for induced resistance were conducted with various plant species. In general, induced defense responses include processes of oxygen burst (26), lignification of host cell walls (25), the formation of cell wall apposition at sites of attempted penetration by fungal pathogens, the accumulation of PR-proteins and other biochemical and physiological changes in the host plants (18). The role of oxidative enzymes, such as peroxidase, and of their metabolic products in the defense mechanisms of infected plants, has also been studied. It has been reported (22-24,28) that infection with pathogens or application of inorganic chemicals enhances peroxidase activity, and this is often associated with resistance. Among different genotypes of muskmelon, those with high peroxidase activity often had higher levels of resistance (23,24). The data presented in this paper demonstrate that DME increased peroxidase activity. Peroxidase activity was raised only after a lag period following DME treatment and/or inoculation with *Fom*. It was found that induced resistance by DME was closely associated with enhanced levels of peroxidase activity. We assume that the enhanced peroxidase activity may contribute to induction of resistance. A time interval of 12 h between DME application and the onset of resistance is required for this phenomenon to take place.

Environmental stress can increase accumulation of free proline in many plants. Proline accumulation has been correlated in many organisms with adaptation to osmotic stress (30). In addition to its role in osmoprotection, proline accumulation in plants under stress may function in the storage of energy, amino nitrogen and reducing power (27). We have

found that BABA significantly increases L-proline content in tomato leaves 2 days after foliar application (unpublished data). However, the direct relationship between proline accumulation and induced resistance is still unclear. We found that 48 h after application, DME significantly increased L-proline content in melon plants. This increase occurred $\sim$ 24 h later than that of peroxidase activity and no significant correlation with induced resistance to *Fom* was found. It seems, therefore, that L-proline accumulation is an incidental physiological response to DME application rather than a factor in the induced resistance. Nevertheless, further investigation into its relationship with induced resistance is needed before final conclusions are drawn.

In conclusion, DM of *P. chrysogenum* is not only an organic fertilizer providing nutrients to crop plants and improving humus in the soil, but also a good inducer of resistance against root diseases such as *Fom.* Its application may have multiple effects for both crop nutrition and crop protection. Future research should examine the efficacy of DME and DM powder against diseases under field conditions.

ACKNOWLEDGMENTS

This research was supported in part by a Fred and Barbara Kort Sino-Israel Postdoctoral Fellowship to Dr. Hezhong Dong. The authors wish to thank Dr. Alexander Baider and Dr. Marjana Galperin for technical assistance throughout this research.

REFERENCES

1. Bates, L.S., Waldren, R.P. and Teare, I.D. (1973) Rapid determination of free proline for water stress studies. *Plant Soil* 39:205-207.
2. Christ, U. and Mosinger, E. (1989) Pathogenesis-related proteins of tomato: 1. Induction by *Phytophthora infestans* and other biotic and abiotic inducers and correlations with resistance. *Physiol. Mol. Plant Pathol.* 35:53-65.
3. Cohen, R., Yarden, O., Katan, J., Riov, J. and Lisker, N. (1987) Paclobutrazol and other plant growth-retarding chemicals increase resistance of melon seedlings to fusarium wilt. *Plant Pathol.* 36:558-564.
4. Cohen, Y. (1994) 3-Aminobutyric acid induces systemic resistance against *Peronospora tabacina. Physiol. Mol. Plant Pathol.* 44:273-288.
5. Cohen, Y. (1994) Local and systemic control of *Phytophthora infestans* in tomato plants by DL-3-amino-*n*-butanoic acids. *Phytopathology* 84:55-59.
6. Cohen, Y. and Gisi, U. (1994) Systemic translocation of ^{14}C-DL-3-aminobutyric acid in tomato plants in relation to induced resistance against *Phytophthora infestans. Physiol. Mol. Plant Pathol.* 45:441-456.
7. Cohen, Y., Reuveni, M. and Baider, A. (1999) Local and systemic activity of BABA (DL-3-aminobutyric acid) against *Plasmopara viticola* in grapevines. *Eur. J. Plant Pathol.* 105:351-361.
8. Daayf, F., Ongena, M., Boulanger, R., El Hadrami, I. and Belanger, R.R. (2000) Induction of phenolic compounds in two cultivars of cucumber by treatment of healthy and powdery mildew-infected plants with extracts of *Reynoutria sachalinensis. J. Chem. Ecol.* 26:1579-1593.
9. Friedrich, L., Lawton, K., Ruess, W., Masner, P., Specker, N., Gut Rella, M. *et al.* (1996) A benzothiadiazole derivative induces systemic acquired resistance in tobacco. *Plant J.* 10:61-70.
10. Gao, J.P., Ben-Daniel, B.H. and Cohen, Y. (2001) Organic fertilizers of microbial origin enhance growth and reduce infection of sweet corn by *Fusarium moniliforme. Phytoparasitica* 29:268 (abstr.).
11. Graham, T.L. and Graham, M.Y. (1999) Role of hypersensitive cell death in conditioning elicitation competency and defense potentiation. *Physiol. Mol. Plant Pathol.* 55:13-20.
12. Gross, G.G. (1979) Recent advances in the chemistry and biochemistry of lignin. *Recent Adv. Phytochem.* 12:177-220.
13. Haselwandter, K. (1997) Soil micro-organisms, mycorrhiza, and restoration ecology. pp. 65-80. *in:* Urbanska, K.M., Webb, N.R., and Edwards, P.J. [Eds.] Restoration Ecology and Sustainable Development. Cambridge University Press, Cambridge, UK.
14. Heller, W.E. and Gessler, C. (1986) Induced resistance in tomato plants against *Phytophthora infestans. J. Phytopathol.* 116:323-328.

15. Herger, G., Klingauf, F., Mangold, D., Pommer, E.H. and Sherer, M. (1988) Efficacy of extracts of *Reynoutria sachalinensis* against fungal disease, especially powdery mildews. *Nachrbl. Dtsch. Pflanzenschutzd.* 40:56-60.
16. Hunt, M.D., Neuenschwander, U.H., Delaney, T.P., Weymann, K.B., Friedrich, L.B., Lawton, K.A. *et al.* (1996) Recent advances in systemic acquired resistance research - A review. *Gene* 179:89-95.
17. Jackson, A.J., Walters, D.R. and Marshall, G. (1994) Evaluation of *Penicillium chrysogenum* and its antifungal extracts as potential biological control agents against *Botrytis fabae* on faba beans. *Mycol. Res.* 98:1117-1126.
18. Jeun, Y.C., Siegrist, J. and Buchenauer, H. (2000) Biochemical and cytological studies on mechanisms of systemically induced resistance to *Phytophthora infestans* in tomato plants. *J. Phytopathol.* 148:129-140.
19. Kalix, S., Anfoka, G., Li, Y., Stadnik, M. and Buchenauer, H. (1995) Induced resistance in some selected crops – prospects and limitations. pp. 451-460. *in:* Lyr, H., Russel, P.E., and Sisler, H.D. [Eds.] Modern Fungicides and Antifungal Compounds. Athenaeum Press, Gateshead, UK.
20. Kessmann, H., Staub, T., Hofmann, C., Maetzke, T., Herzog, J., Ward, E. *et al.* (1994) Induction of systemic acquired resistance in plants by chemicals. *Annu. Rev. Phytopathol.* 32:439-459.
21. Oka, Y., Cohen, Y. and Spiegel, Y. (1999) Local and systemic induced resistance to the root-knot nematode in tomato by DL-β-amino-*n*-butyric acid. *Phytopathology* 89:1138-1143.
22. Reuveni, M. and Reuveni, R. (1995) Efficacy of foliar application of phosphates in controlling powdery mildew fungus on field-grown winegrapes: effects on cluster yield and peroxidase activity in berries. *J. Phytopathol.* 143:21-25.
23. Reuveni, R., Shimoni, M. and Karchi, Z. (1990) A rapid assay for monitoring peroxidase activity in melon as a marker for resistance to *Pseudoperonospora cubensis*. *J. Phytopathol.* 129:333-338.
24. Reuveni, R., Shimoni, M., Karchi, Z. and Kuç, J. (1992) Peroxidase activity as a biochemical marker for resistance of muskmelon (*Cucumis melo*) to *Pseudoperonospora cubensis*. *Phytopathology* 82:749-753.
25. Siegrist, J., Glenewinkel, D., Kolle, C. and Schmidtke, M. (1997) Chemically induced resistance in green bean against bacterial and fungal pathogens. *J. Plant Dis. Prot.* 104:599-610.
26. Siegrist, J., Orober, M. and Buchenauer, H. (2000) Beta-aminobutyric acid-mediated enhancement of resistance in tobacco to tobacco mosaic virus depends on the accumulation of salicylic acid. *Physiol. Mol. Plant Pathol.* 56:95-106.
27. Singh, B.K. (1999) Plant Amino Acids. Marcel Dekker Inc., New York, NY.
28. Smith, J.A. and Hammerschmidt, R. (1988) Comparative study of acidic peroxidase associated with induced resistance in cucumber, muskmelon and watermelon. *Physiol. Mol. Plant Pathol.* 33:255-261.
29. Ward, E.R., Uknes, S.J., Williams, S.C., Dincher, S.S., Wiederhold, D.L., Alexander, D.C. *et al.* (1991) Coordinate gene activity in response to agents that induce systemic acquired resistance. *Plant Cell* 3:1085-1094.
30. Yang, C.W. and Kao, C.H. (2000) Ammonium in relation to proline accumulation in detached rice leaves. *Plant Growth Regul.* 30:139-144.
31. Zimmerli, L., Jakab, C., Metraux, J.P. and Mauch-Mani, B. (2000) Potentiation of pathogen-specific defense mechanisms in Arabidopsis by beta-aminobutyric acid. *Proc. Nat. Acad. Sci. USA* 97:12920-12925.