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ORIGINAL PAPER

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Effect of vesicular-arbuscular mycorrhizal fungi on verticillium wilt of cotton

Abstract The development of vesicular-arbuscular mycorrhizal fungi (VAMF): *Glomus mosseae* (Nicol and Gerd.) Gerdemann and Trappe, *Glomus versiforme* (Karsten) Berch, *Sclerocystis sinuosa* Gerdemann and Bakhi and *Verticillium dahliae* and the effects of the VAMF on the verticillium wilt of cotton (*Gossypium hirsutum* L. and *Gossypium barbadense* L.) were studied with paper pots, black plastic tubes and clay pots under natural growth conditions. All of the tested VAMF were able to infect all the cotton varieties used in the present experiment and typical vesicles and arbuscules were formed in the cortical cells of the cotton roots after inoculation. The cap cells, meristem, differentiating and elongating zones of the root tip were found to be colonized by the VAMF. In the case of most *V. dahliae* infection, the colonization occurred mostly from the root tip up to 2 cm. VAMF and *V. dahliae* mutually reduced their percentage of infection when inoculated simultaneously. VAMF inoculation reduced the numbers of germinable microsclerotia in the soil of the mycorrhizosphere, while the quantity of VAM fungal spores in the soil was not influenced by infection of with *V. dahliae.* The % of arbuscule colonization in roots was negatively correlated with the disease grades, while the numbers of vesicles in roots were not. These results suggest that certain vital competition and antagonistic reactions exist between VAMF and *V. dahliae.* VAMF reduced the incidence and disease indices of verticillium wilt of cotton during the whole growth phase. It is evident that cotton seedling growth was promoted, flowering was advanced, the numbers of flowers and bolls were increased, and this resulted in an increase in the yield of seed cotton. Among the VAMF species, *Glomus versiforme* was the most effective, and *Sclerocystis sinuosa* was inferior. So far as the author is aware, such an effect of VAMF on the increase of cotton wilt tolerance/resistance is reported here far the first time.

Key words Vesicular-arbuscular mycorrhizal fungi Verticillium wilt · Cotton infection · *Verticillium dahliae*

Introduction

Studies on the effects of VAMF on plant disease began in the 1960s (Safir 1968). Since then there have been many reports about the ability of VAMF to alleviate soil-borne disease of plants (Schonbeck and Dehne 1977; Chakravarty and Mishra 1986; Jalali et al. 1990). Hwang et al. (1992) showed that VAMF can reduce the verticillium and fusarium wilt incidence of alfalfa and the pathogen propagule numbers in the soil. However, some reports show that the plant disease were not influenced by infection with VAMF (Ross 1972; Davis 1980; McGraw and Schenck 1981; Reddy et al. 1989). Davis et al. (1979) found that the propagules of *Verticillium dahliae* in petioles of cotton plants and vascular discoloration indices were increased by inoculation with *Glomus fasciculatum.* They considered that the verticillium wilt of cotton was aggravated by the VAMF. In contrast, the present author found previously that some cotton plants with propagules of *V. dahliae* did not show any leaf symptoms of the wilt disease (unpublished work). This phenomenon led the author to study the effect of VAMF on verticillium wilt of cotton plants in various ways.

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Materials and methods

Preparation of the cotton seeds, VAMF and *V. dahliae* inocula and soil

Seeds of wilt-susceptible cultivar Litai 8 and 86-1 and wilt-tolerant cultivar Zhong 12 (*Gossypium hirsutum*) and wilt-tolerant cultivar Xinhai 3 (*Gossypium barbadense*) were surface sterilized (1 min in 1% NaOCl) and germinated. Strains of *V. dahliae*, Jing-Yang (high virulent type) and An-Yang (intermediate virulent type) were inoculated on potato-dextrose-agar (PDA) medium and cultured for 1–2 weeks under temperature 23° C. Both conidia and microsclerotia were harvested and used, and root segments of *Trifolium repens* infected with *Glomus mosseae, Glomus hoi, Glomus versiforme* and *Sclerocytis sinuosa* were used as inocula. Sterilized sandy loam soil (121° C, 2 h), which contained 2.36% organic matter, 0.17 μg/g total nitrogen, 78 μg/g available potassium, pH 8.1 was used for pot cultures.

Experimental design

Experiment I

Twelve treatments were designed with each cultivar grown in paper pots (0.2 l); noninoculated pots were left as controls (CK), and the remaining pots were treated as follows: inoculation of the tested cotton cultivars separately with fungus strains Jing-Yang (Vd_1), An-Yang (Vd_2), *Glomus mosseae* (G. m), *Glomus versiforme* (G. v), *Sclerocystis sinuosa* (S. s), G. m + Vd_1, G. v + Vd_1, S. s + Vd_1, G. m + Vd_2, G. v + Vd_2 and S. s + Vd_2.

Experiment II

Two treatments were set up with cultivar Litai 8 grown in clay pots (2.5 l) and inoculated with An-Yang (Vd_2), and Vd_2 + G. v.

Experiment III

Twelve treatments were set up with cultivars 86-1, Zhong 12 and Xinhai 3 grown in paper pots (0.2 l). Pots without inoculation were left as controls (CK), the remaining pots were inoculated with strains Vd_1, Vd_2, *Glomus hoi* (G. h), G. m, G. v, G. h + Vd_1, G. h + Vd_2, G. m + Vd_1, G. m + Vd_2, G. v + Vd_1 or G. v + Vd_2.

Experiment IV

The treatments in this experiment were as in experiment III.

Experiment V

Twelve treatments were set up with cotton cultivars 86-1, Zhong 12, and Xinhai 3 grown in clay pots (2.5 l). Noninoculated pots were left as controls and the remaining pots were inoculated with Vd_1, Vd_2, G. m + Vd_1, G. v + Vd_1, S. s + Vd_1, G. m + Vd_2, G. v + VD_2, or S. s + Vd_2. All the treatments were arranged randomly and replicated five times.

Methods of inoculation, sowing and management

The germinated seeds of the tested cotton were sown in pots containing sterilized sandy loam mixed with 4000 inoculum potential units (IPU) (Liu and Luo 1994) per paper pot, 12000 IPU per clay pot and with no inoculum as controls. The inoculum of *V. dahliae* was 3%. (v/v) of the cotton seed culture in clay pots and none in the controls. In experiment IV, either no or 100 microsclerotia (100–200 μm in diameter) of *V. dahliae* were added to the paper pot soil. The roots of seedlings with one leaf were soaked in conidial suspensions of *V. dahliae* (10^7 conidia/ml) in experiments I and III. Five cotton seedlings were maintained in each pot. All of the clay pot plants were kept in the open, watered, fertilized and pest controlled according to need.

Table 1 The growth status of Litai 8 grown in paper pots for 50 days. Values in one column followed by different letters are significantly different ($P<0.05$). An aliquot of 4000 inoculum potential units of VA mycorrhizal fungi was inoculated in each pot at sowing, and 10 ml (10^7 conidia/ml) of a conidial suspension of *Verticillium dahliae* was used to soak the cotton roots when the seedlings had one leaf. Leaf areas was measured on the second leaf from the top (Gm *Glomus mossea, Gv Glomus versiforme, Ss Sclerocystis sinvesa, Vd_1* Jin-Yang strain of *Verticillium dahliae, Vd_2* An-Yang strain of *V. dahliae, CK* control)

Treatments	Plant height (cm)	Leaf area (cm^2)	Stem diameter (mm)	Dry wt. of leaf and stem (mg/plant)	Root dry wet. (mg/plant)
CK	12.3 bc	10.2 bc	2.0 c	132 bc	47.0 a
Vd_1	10.5 a	6.7 d	1.4 d	117 a	41.0 ab
Vd_2	12.0 bc	8.3 cd	1.6 d	113 a	35.4 b
Gm	14.6 f	14.7 a	2.7 a	212 f	43.3 ab
Gv	14.5 df	14.7 a	2.7 a	222 f	50.5 a
Ss	13.4 cdf	12.3 ab	2.5 ab	196 ef	53.2 a
Gm + Vd_1	13.1 cdf	12.9 a	2.6 ab	176 cd	46.0 a
Gv + Vd_1	13.2 cdf	14.9 a	2.6 ab	234 f	36.3 b
Ss + Vd_1	11.2 ab	12.6 ab	2.3 b	184 de	48.8 a
Gm + Vd_2	13.3 cdf	13.4 a	2.7 a	142 ab	34.0 b
Gv + Vd_2	12.5 bc	13.0 a	2.7 a	217 ab	43.0 ab
Ss + Vd_2	11.3 ab	12.8 a	2.4 ab	181 de	50.0 a

Table 2 The growth and the disease status of 86-1 seedlings (*Gh Glomus hoi*; other abbreviations and symbols as in Table 1)

Treatments	Plant height (cm)	Dry wt. (mg/plant)	Disease incidence (%)	Disease index
CK	10.5 bc	169 c	0	0
Vd_1	9.7 cb	145 d	43.3 a	24.2 a
Vd_2	8.3 d	151 d	35.5 b	14.5 b
Gh	12.8 ab	186 ab	0	0
Gm	13.1 a	183 ab	0	0
Gv	13.1 a	193 a	0	0
Gh + Vd_1	11.1 bc	172 b	23.3 c	10.0 cd
Gm + Vd_1	11.7 bc	178 b	23.3 c	13.0 bc
Gv + Vd_1	12.3 ab	189 a	20.0 cb	8.3 cd
Gh + Vd_2	8.9 cd	180 b	23.3 c	10.8 c
Gm + Vd_2	9.9 cd	181 b	23.3 c	10.0 cd
Gv + Vd_2	12.5 ab	190 a	16.7 d	6.7 d

Parameters and methods of measurement

When differences in seedling growth between various treatments appeared, seedling height, numbers of leaves, leaf area, and stem diameter were measured. The yield of seed cotton was obtained by calculating the boll weight and the numbers of bolls. The fibre length was determined by the method of carding. The incidence and disease indices of verticillium wilt of cotton were determined on the basis of leaf symptoms as 0 = no symptoms to 4 = 100% leaf area with symptoms or dead. The infection percentage of the VAMF, arbuscules, and the number of vesicles were counted ac-

Table 3 Effects of *Glomus versiforme* on the growth and yield of Litai 8 grown in clay pots. An aliquot of 12000 inoculum potential units of Gv and 3% (v/v) of the cotton seed culture of *V. dahliae* (An-Yang strain) were mixed in the soil before the cotton seed were sown. Leaf area was measured on the 5th leaf from the top. Abbreviations and symbols as in Table 1

Treatments	Plant height (cm)	Leaf area (cm^2)	Stem diameter (mm)	Dry wt. (g)	Flower bud (no./plant)	Boll (no./plant)	Seed cotton (g/plant)	Fibre length (mm)
Vd_2(CK)	49.2 b	11.8 b	4.2 b	468 b	4.3 b	1.6 b	9.0 b	24.2 b
$Gv+Vd_2$	64.4 a	30.6 a	5.8 a	637 a	7.0 a	3.0 a	20.0 a	26.5 a

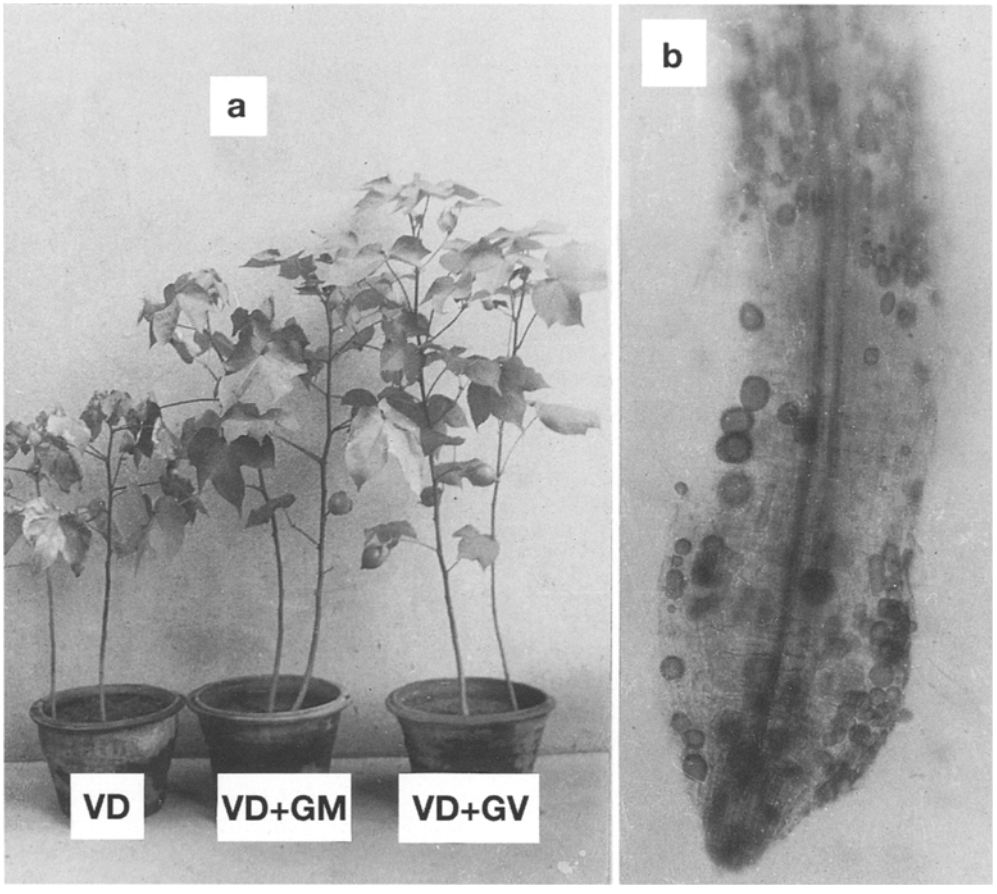

Fig. 1 **a** The status of verticillium wilt of 86-1 cotton in different treatments: *VD* germinated seeds inoculated with the Jing-Yang strain of *Verticillium dahliae*; *VD+GM* germinated seeds inoculated with the Jing-Yang strain of *V. dahliae* and with *Glomus mosseae*; *Vd+GV* germinated seeds inoculated with the Jing-Yang strain of *V. dahliae* and with *G. versiforme*. **b** The infection status of the root tip of Litai cotton infected by *G. versiforme*

cording to the procedure described by Biermann and Linderman (1981). All the data obtained were analysed using softare from SAS.

Results

Effect of VAMF and *V. dahliae* on cotton plants

The growth differences between treatments appeared in seedlings with 1–3 leaves. Leaf area, plant height, and dry weight of the seedlings in VAMF inoculation treatments were greater than those of the controls. The *V. dahliae* significantly reduced the leaf area, plant height, and dry weight of the plants compared to the control seedlings (Tables 1, 2). The leaf colour of the seedlings was a darker green in the VAMF inoculation pots than in any other treatments. The plants inoculated with *Glomus versiforme* flowered 2–3 days in advance of any other plants. The numbers of bolls, seed cotton per plant, and fibre lengths of the plants inoculated with *Glomus mosseae* or *Glomus versiforme* were significantly greater than those of other plants. The plants inoculated with *V. dahliae* developed yellow spots, with lower seed cotton and shorter fibre lengths than control plants in other treatments (Table 3).

Effects of VAMF on verticillium wilt of cotton

The seedlings inoculated with the microsclerotia of *V. dahliae* did not show any symptoms on the leaves. The disease incidence and the disease indices of the plants in the treatments with VAMF inoculation were significantly lower than those of the control plants (Table 2; Fig. 1a). The tested VAMF reduced verticillium wilt of cotton at different growth stages. The diseased plants in the inoculation treatments with only Vd_2 defoliated severely and hardly recovered, while the diseased plants

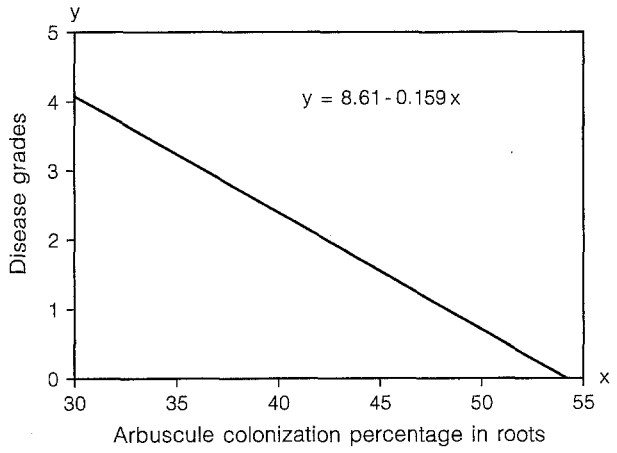

Fig. 2 The relationship between arbuscule colonization percentage in roots of Litai 8 and disease grades of verticillium wilt

inoculated with *Glomus versiforme* and *V. dahliae* were able to recover to some extent. *Glomus versiforme* was the most effective VAMF species in inducing disease tolerance/resistance while *S. sinuosa* was inferior. The Litai 8 showed the greatest benefit from inoculation with VAMF, especially in induction of disease tolerance/resistance. The multiple regressions of VAMF infection percentage, arbuscule percentage, arbuscule/VAMF, vesicle and entry points per unit root length and disease indices were analysed. There was no relationship between the numbers of vesicles in roots and the disease grades. The percentage of arbuscules in the root and the arbuscules/VAMF ratio were negatively correlated with the disease grade on cultivars Litai 8 and 86-1 (Fig. 2). This showed that the amount of arbuscules is closely related to the development of the disease.

The interaction between *V. dahliae* and VAMF development

All of the tested VAMF were able to infect all the cotton varieties and cultivars in the present experiments by formation of typical vesicles and arbuscules. The mycelium of VAMF was observed after seedling growth for 3–7 days; the cap cells, meristem, and differentiating and elongating zones of the root tip were all colonized by the VAMF (Fig. 1b). In prior inoculations with VAMF prior to *V. dahliae,* the VAMF infection percentage was not influenced by the *V. dahliae* inoculation (Table 4), but when both were inoculated simultaneously, the VAMF and *V. dahliae* mutually reduced infection percentages. VAMF inoculation reduced the numbers of germinable microsclerotia in the mycorrhizosphere soil, while the quantity of VAM fungal spores in the soil was not influenced by the infection of *V. dahliae.*

Discussion

The cotton seedlings of certain varieties or cultivars inoculated with microsclerotia of *V. dahliae* did not show any symptoms on leaves. This is similar to the result earlier obtained by Davis et al. (1979). However, in the present work the VAMF infection limited the increase in the number of *V. dahliae* propagules. The effect of VAMF infection in reducing the incidence and disease indices of verticillium wilt of cotton during the whole growth period is important, in that is provides evidence of active competition between VAMF and a pathogenic fungus. Although many researchers have noticed that plant diseases are reduced by VAMF, few have conducted further research on the relationship between the development of the arbuscules or vesicles and plant disease tolerance/resistance. For example, O'Bannon et al. (1979) observed arbuscule development but no vesicles around the site of nematode infection, while we observed that the colonization percentage of arbuscules in the roots was positively correlated with increase in wilt tolerance/resistance. Therefore, certain stains of VAMF might be used for biological control of plant diseases.

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Table 4 The infection status of the VAMF on Litai 8 grown in paper pots for 50 days. Treatments, abbreviations and symbols as in Table 1

Treatment	VAMF infection (%)	Arbuscules in roots (%)	Vesicles (no./mm root)	Entry points (no./mm root)	Arbuscule/ VAMF
CK	0	0	0	0	0
Gm	25.5 a	21.8 a	3.5 a	3.3 b	0.85 ab
Gv	21.0 ab	19.6 ab	1.2 b	2.8 bc	0.93 a
Ss	16.3 b	11.8 c	3.7 a	2.4 c	0.72 b
Gm + Vd_1	20.3 ab	15.1 bc	4.7 a	4.5 a	0.74 b
Gv + Vd_1	22.0 ab	19.8 ab	1.8 b	3.6 b	0.90 a
Ss + Vd_1	16.8 b	9.1 c	4.9 a	3.1 bc	0.54 c
Gm + Vd_2	21.9 ab	16.9 b	4.1 a	3.6 b	0.77 b
Gv + Vd_2	23.0 a	21.3 a	1.5 b	4.2 a	0.93 a
Ss + Vd_2	19.7 ab	13.6 c	4.7 a	5.3 a	0.69 b

References

Biermann B, Linderman RG (1981) Quantifying vesicular-arbuscular mycorrhizas: a proposed method towards standarization. New Phytol 87:63–67

Chakravarty P, Mishra RR (1986) The influence of VA mycorrhizal fungi on the wilting of *Albizia procera* and *Dalbergia sissoo*. Eur J For Pathol 16:91–97

Davis RM (1980) Influence of *Glomus fasciculatum* on *Thielaviopsis basicala* root rot of citrus. Plant Dis 64:839–840

Davis RM, Menge JA, Erwin DC (1979) Influence of *Glomus fasciculatum* and soil phosphorus on verticillium wilt of cotton. Phytopathology 69:453–456

Hwang SF, Chang KF, Chakravarty P (1992) Effects of vesicular-arbuscular mycorrhizal fungi on the development of verticillium and fusarium wilts of alfalfa. Plant Dis 76:239–243

Jalali BL, Chhabra ML, Singh RP (1990) Interaction between vesicular-arbuscular mycorrhizal endophytes and *Macrophomina phaseolina* in mungbean. Ind Phytopathol 43:527–530

Liu RJ, Luo XS (1994) A new method to quantify the inoculum potential of arbuscular mycorrhizal fungi. New Phytol 128:89–92

McGraw AC, Schenck NC (1981) Effects of two species of vesicular-arbuscular mycorrhizal fungi on the development of fusarium wilt of tomato (abstr.). Phytopathology 71:894

O'Bannon JH, Inserra RN, Nemec S, Voilas N (1979) The influence of *Glomus mosseae* on *Tylenchulus semipenetrans*-infected and uninfected *Citrus limon* seedlings. J Nematol 11:247–250

Reddy MV, Rao JN, Krishna KR (1989) Influence of vesicular-arbuscular mycorrhizae on fusarium wilt in pigeonpea. Int Pig Newsl 9:23

Ross JP (1972) Influence of Endogene mycorrhiza on phytophthora rot of soybean. Phytopathology 62:896–897

Safir G (1968) The influence of vesicular-arbuscular mycorrhiza on the resistance of onion to *Pyrenochaeta terrestris*. MSc thesis, University of Illinois, Urbana

Schönbeck F, Dehne HW (1977) Damage to mycorrhizal and nonmycorrhizal cotton seedligns by *Thielaviopsis basicola*. Plant Dis 61:266–267