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Effects of grafting on rhizosphere microorganisms of eggplants

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ABSTRACT

We studied the changes in rhizosphere microbial community of grafted eggplants at different stages and the effect of grafting on disease resistance of eggplant. Antagonistic microbes against *Verticillium dahiae* from rhizosphere soil of grafted and non-grafted eggplants were screened. Compared with non-grafted eggplant, grafting changed the rhizosphere microbial community structure of eggplants, the increase of bacteria (B) and actinomycetes (A), decreased the fungi (F), increased ratios of B to F and A to F, also decreased incidence of Verticillium wilt. Of the 352 strains isolated from rhizosphere soil of grafted and non-grafted eggplants, 7 strains were inhibitory against *Verticillium dahliae* and their inhibition zone was > 13 mm. In greenhouse experiment, two antagonistic strains 'B631' and 'F111' showed significant inhibitory effects against *Verticillium dahliae*. The prevention and curative effects of strain 'B631' and strain 'F111' on *Verticillium* wilt were 62.50%, 53.33%, 56.25% and 46.67%, respectively. Using the taxonomic classification and Bergey's Manual of Determinative bacteriology, 'B631' was identified as *Serratia Bizio* and 'F111' was identified as *Trichoderma*. The results from this study indicated that the rhizosphere microbial community from grafting is favourable to eggplant's disease resistance.

Key words: Actinomycete bacteria, antagonistic microbes, eggplants, grafting, microbial community structure, *Verticillium* wilt

INTRODUCTION

Verticillium wilt, caused by *Verticillium dahliae* is major constraint to eggplant production in most countries. Continuous cropping of eggplant enhances the occurrence of Verticillium wilt and causes drastic yield reduction (30%～40%, or even no harvest). Grafting is effective technique for the successful eggplant cultivation in continuous cropping and is widely used in China. It significantly reduces the disease incidence upto 90% (14). Zhou *et al.* (13) and Wang *et al*. (10) studied many aspects of the mechanisms of disease resistance and yield increase of grafted eggplants viz., histological structure, physiology and biochemistry and resistance induction. The plant resistance to soil-borne disease is closely related to its rhizosphere microorganisms (6). However, few studies reported the relationship between rhizosphere microorganisms of grafted eggplants and resistance to soil-borne diseases. Zhang *et al.* (12) studied the allelopathic inhibitory effects of root exudates of grafted eggplants on *Verticillium dahliae*. This study aimed to determined the changes in the population and community structure of microorganisms

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(bacteria, actinomycetes and fungi) of grafted eggplants rhizosphere and the induction antagonistic microbes against *Verticillium dahliae* and to understand the relationship between rhizosphere microbial community structure and resistance to soil-borne diseases.

MATERIALS AND METHODS

Verticillium dahliae was isolated from the pathogenic plants of continuous eggplant cropping field in our University. Bacteria and actinomycetes were isolated from the grafted eggplants rhizosphere and incubated on BPA medium and Modified Gause1 medium respectively. Fungi were isolated from grafted eggplants rhizosphere on Martin agar medium and incubated on PDA culture medium. *Verticillium dahliae* was incubated on PDA culture medium. The fermentation culture of fungi, bacterium and actinomycetes was conducted on NA, Czapek's and Non-agar Modified Gause1 medium, respectively. The commonly grown eggplant (*Solanum melongena* L) cultivar Xi'anlu was used as scion. It was grafted with a wild eggplant rootstock (*Solanum torvum*,). Eggplant cultivar Xi'anlu was used as control.

Soil sampling

The grafted and non-grafted eggplants were transplanted in a greenhouse in which eggplants were successively cultivated in May 2005. In 1 month transplanted eggplants, root washing method was used for sampling at 45~60-days intervals. The sampling was done on June 19, July 30 and September 30, respectively. Soil dilution was made to separate bacteria, actinomycetes and fungal community on their individual cultural medium in 90-mm dia sterilized plates (11). Then the plates were incubated at 25^{o} C. Each treatment had 3 replications (3 plates). The population of microbes were counted, when the colonies reached 10~100 per plate. Rhizosphere microbial quantity per g dry soil. Oven drying method was used to determine the weight of rhizosphere soil per ml of soil suspension**,** 20 ml diluted soil suspension was put into the evaporative dish for drying up. The dried soil was weighed. Then the soil weight per ml of soil suspension was calculated.

Disease investigation in greenhouse

The resistance level (0-4 scale) of eggplants against *Verticillium* wilt was evaluated 5 times at 12 d intervals, beginning from the first appearance of typical wilt symptoms in non-grafted eggplants after transplanting. Ratings of disease index of *Verticillium* wilt was done as per Zhou *et al.* (15). Disease index and disease incidence were calculated as under:

Disease index = [$\sum$(Rating number× number of plants with the rating)] ×100%/ (Total number of plants × highest rating)

Disease incidence (%) = (number of disease plants/ Total number of plants) ×100

Screening of antagonistic strains from rhizosphere soil

The method to isolate the rhizosphere microorganisms was as mentioned above. Each treatment has 3 replications (3 plates). Single colonies were separated from the plates

incubated at 28~30° C, transferred to culture medium of coded test-tubes. They were used for the experiment.

Verticillium dahliae conidial suspension and zymotic fluid of antagonistic microbes

Verticillium dahliae incubated in 250 ml flask containing 100 ml PD culture broth on a rotary shaker at 110 rpm, 23° C for 7 days. Then the fluid was filtered through 4-layer sterile gauze and the filtrate containing 1×10^7 cfu·ml^{-1} spores were determined with haemacytometer. This filtrate was *Verticillium dahliae* conidial suspension. Antagonistic microbes grown in test-tubes for 3-days were drenched with sterile distilled water and the suspension containing 1×10^8 cfu·ml^{-1} spores was obtained. Then 1 ml suspension was put into 250 ml flask containing 100 ml ferment culture medium incubated on a rotary shaker at 120 rpm, 25°C for a day. This suspension was the zymotic fluid of antagonistic microbes.

Screening bacteria against *Verticillium dahliae* in vitro

The dual culture, spore germination and cylinder-plate methods were used to screen antagonistic microbes (5). Each treatment was replicated three times. The plates were incubated at 23° C for 3 days, then inhibition distance and diameter were measured.

Antagonistic microbes assay

Non-grafted eggplants at 3 leaf stage were treated with zymotic fluid of 7 antagonistic microbes in greenhouse on September, 2006. There designed the disease prevention and curative studies. The experiments were conducted in randomized complete block design.

Disease prevention study

Fifty ml zymotic fluid of each antagonistic microbe was applied into a pot. Next day, eggplants were slowly uprooted from the pots and the roots were immersed in conidial suspension of *Verticillium dahliae* (1×10^7 cfu·ml^{-1}spores) for 30 min. Six plants were treated for each kind of antagonistic microbes treatment. Each treatment had 3 replications.

Disease curative study

Eggplants were slowly uprooted from the pots and the roots were immersed in spore suspension of *Verticillium dahliae* for 30 min. Next day, zymotic fluid for each kind of antagonistic microbes was added into the corresponding treated pots. There were six plants for each treatment. Each treatment had 3 replications. Another two treatments were treated with fungicide and sterilized distilled water (as control). After disease investigation in all treatments, the disease index and % efficacy were calculated. Rating method on disease index of *Verticillium* wilt was same as mentioned above. Per cent efficacy was calculated as under.

$$\text{Efficacy (\%)} = [(DI_w - DI_t)/DI_w]\times100$$

Where, DI_t is the DI of the treatment and DI_w is the DI of control.

RESULTS

Change in rhizosphere microbial community

The population of total microorganisms and each microbial community in rhizosphere were maximum on July 30 and minimum on September 30 in all treatments (Table 1). The ratios of B to F and A to F decreased gradually with passage of time with less variations in grafted eggplants compared with non-grafted eggplants. On June 19, the total population of microorganisms and bacteria and the ratio of B to F in grafted eggplants were lower, while the population of actinomycetes and fungi and the ratio of A to F of grafted eggplants were higher. On July 30, the population of rhizosphere microorganisms, bacteria, actinomycetes and the ratio of B to F and A to F in grafted eggplants were higher and that of fungi was lower. The results on September 30 were similar to July 30. In general, the population of microorganisms (bacterium and actinomycetes) in rhizosphere in grafted eggplants increased, the population of fungi decreased and the ratios of B to F and A to F increased.

Resistance to *Verticillium* wilt of grafted eggplants

The grafted plants showed significantly higher disease resistance than non-grafted plants, with lower disease index and disease incidence (Table 2). The results showed that the resistance of grafted eggplants was mainly due to lighter disease infection as well as lower disease incidence.

Table 2. Effects of grafted eggplants on resistance to *Verticillium* wilt

Date	Disease Index		Disease Incidence(%)	
	Grafted	Non-grafted	Grafted	Non-grafted
June 14	0.00 aA	0.56 bB	0.00 aA	2.22 bB
June 26	0.30 aA	4.76 bB	1.19 aA	9.52 bB
July 7	0.60 aA	11.31 bB	2.38 aA	26.19 bB
July 20	2.08 aA	36.16 bB	5.95 aA	53.57 bB
August 01	2.38 aA	50.89 bB	8.33 aA	67.85 bB

Screening rhizosphere antagonistic microbes

Dual culture and spore germination studies

112 strains of fungi, 142 strains of bacteria and 98 strains of actinomycetes were isolated from the rhizosphere of grafted and non-grafted eggplants. In dual culture and spore germination trials, 32 strains significantly inhibited the *Verticillium dahliae* (Table 3). In dual culture trial, the inhibitory distances of all strains were > 8.0 mm, except for B362 (5.0 mm) and some fungi being parasitic. The B631 caused maximum inhibition upto 19.0 mm. In spore germination trial, all strains inhibitory diameters were > 8.0 mm, and B118 proved most inhibitory 34.5 mm diameter. Most of antagonistic microbes were from the rhizosphere soil of grafted eggplants, but only B716 and F721 were from the rhizosphere soil of non-grafted eggplants.

Table1. Changes in microbial community structure in rhizosphere soil of grafted eggplant ($\times 10^6$cfu·g^{-1}DW)

Date	Treatment	Total microbes	Bacteria (B)		Actinomycetes (A)		Fungi (F)		Ratio	
			Number	(%)	Number	(%)	Number	(%)	B/F	A/F
June 19	Grafted	13251	13063 aA	98.58	185 aA	1.40	3.05 aA	0.02	4285	60.76
	Non-grafted	14292	14247 bB	99.69	42 bB	0.29	2.89 bB	0.02	4932	14.5
July 30	Grafted	25157	23378 aA	92.93	829 aA	6.73	84.72 aA	0.34	276	20
	Non-grafted	16960	16000 bB	94.34	790 bB	4.66	170.27 bB	1.00	94	4.64
September 30	Grafted	8465	7995 aA	94.44	444 aA	5.25	26.04 aA	0.31	307	17.06
	Non-grafted	2903	2659 bB	91.57	158 bB	5.44	86.82 bB	2.99	31	1.82

Percentage of total microorganisms.

Table 3. Screening of antagonistic microbes to pathogen *V. dahliae*

Bacteria strain			Actinomycetes strains			Fungal strains		
Strain number	Inhibition distance (mm)	Inhibition diameter (mm)	Strain number	Inhibition distance (mm)	Inhibition diameter (mm)	Strain number	Inhibition diameter (mm)	Inhibition diameter (mm)
B118	8.5	34.5	A134	17.5	26.0	F111	-	16.0
B131	15.0	12.5	A148	13.0	20.5	F135	9.0	8.0
B140	9.0	10.5	A231	18.0	22.0	F213	8.0	17.0
B221	10.0	10.0	A321	18.0	12.5	F314	-	29.0
B225	8.0	8.0	A411	9.5	15.5	F332	12.0	13.0
B259	11.0	20.0	A443	15.5	21.0	F442	17.5	14.5
B321	8.5	13.0	A454	16.0	18.0	F513	-	17.0
B322	9.0	32.0	A543	10.0	8.3	F631	9.0	13.0
B341	10.0	15.5	A658	8.5	21.0	F721	-	10.0
B362	5.0	23.5						
B433	13.0	18.0						
B542	9.5	12.0						
B631	19.0	19.0						
B652	8.0	25.0						
B716	8.5	16.5						

“B”= bacterium, “A” = actinomycetes, “F” = fungi, “-” = occurrence autoeciousness once again fungi.

Cylinder-plate method

Of the 32 antagonistic strains, the zymotic fluids of 7 strains (B221, B631, B131, B140, A134, A231 and F111) significantly inhibited the *Verticillium dahliae* in diameter > 13.0 mm. The inhibitory diameters of B631, A134 and F111 were ≥ 20.0 mm (Table 4).

Table 4. Inhibitory zone of antagonistic microbe to *V. dahliae*

Strain number	Inhibition zone (mm)	Strain number	Inhibition zone (mm)
B221	13.0	A134	25.0
B631	20.0	A231	17.5
B131	17.0	F111	27.0
B140	18.0		

Greenhouse study

All antagonistic strains had some inhibitory effect on the *Verticillium* wilt of eggplants. The disease indexes of eggplants treated with B221, B131 and B140 were significantly lower than control but were unable to control than fungicide (Table 5). However B631 and F111 strains provided disease control similar to fungicide. The prevention and curative effects of B631 and F111 were 62.50, 53.33, 56.25 and 46.67%, respectively, reached the level of fungicide treatment (56.25% and 46.67%). The prevention effect was better than curative effect. As per taxonomic classification and Bergey's Manual of Determinative bacteriology (3, 4), B631 was identified as *Serratia Bizio* and through microscope observation, F111 was identified as *Trichoderma.*

Table 5. Preventive and curative efficacy of antagonistic microbe to eggplant *Verticillium* wilt

Strain	Prevention		Curative effect	
number	Disease index	Efficacy (%)	Disease index	Efficacy (%)
B221	58.33abA	12.50	54.17abAB	13.33
B131	54.17abA	18.75	50.00abABC	20.00
B140	54.17abA	18.75	50.00abABC	20.00
B631	25.00cB	62.50	29.17dCD	53.33
A134	50.00bA	25.00	45.83bcABCD	26.67
A231	50.00bA	25.00	50.00abABC	20.00
F111	29.17cB	56.25	33.33cdBCD	46.67
Fungicide	29.17cB	56.25	33.33cdBCD	46.67
Control	66.67aA	--	62.50aA	--

Lower cases mean differences were at 0.05 levels respectively in same sampling, statistical analysis for Data by Duncan's of DPS software.

DISCUSSION

It is known that plant resistance to soil-borne diseases is related to rhizosphere microorganisms. Not only the quantity but also the microbial composition, dominant group and fungistatic action were closely related to the resistance. Plant genotype may also affect the functional versatility of their community. This study showed that grafting changed the rhizosphere microbial community structure of eggplants, with increase of bacteria (B) and

actinomycetes (A), decrease in fungi (F) and increased the number of antagonist microbe against pathogens of eggplants. The ratios of B to F and A to F were more in grafted eggplants, i.e. population of bacteria or actinomycetes increased more than fungi in rhizosphere soil of grafted eggplants, compared to non-grafted eggplants. Grafting changed the root system of eggplant and also soil microecology. The fungi dominant soil types changed to bacteria dominant soil, a sign of healthy soil. Sturz and Matheson (9) showed that the population densities of bacterial endophytes with antibiosis to the Erwinia soft rot pathogen were more prevalent inside the tubers of resistant than susceptible potato cultivars. Grafted eggplants have significant disease resistance. There were more antagonistic microbes in rhizosphere of grafted eggplants than non-grafted eggplants. Similar studies (1,2) showed that the population of pathogen antagonistic and nitrogen-fixing bacteria was higher in rhizospheres of resistant cultivars. The antagonistic microflora from rhizosphere of susceptible plants had lower diversity and more antagonistic strains were isolated from the rhizosphere of susceptible plants with light incidence and resistant plants (16). We found two antagonistic strains of B631 and F111 from rhizosphere soil of grafted eggplants, inhibitory to *Verticillium dahiae.* In greenhouse study, the disease indexes of eggplants treated with two antagonistic strains were significantly lower than control, perhaps due to systemic resistance induced by the antagonistic strains. This study indicated that grafted eggplants not only reduced disease development, but also changed the microbial community structure in rhizosphere soil to induce the formation of antagonistic stains against *Verticillium dahliae,* which may be one of the main reasons why grafted eggplant is highly resistant to disease and more productive.

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