
Correlation between Resistance of Eggplant and Microorganisms, Enzyme Activities of Soil

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ABSTRACT

Aims: To determine the relationship between Microbial quantity and enzyme activities of rhizosphere soil from different resistant eggplant cultivars, and resistance of eggplant to *Verticillium* wilt.

Study design: Analyse the changes of microorganisms and enzyme activities of the rhizosphere soil from different resistant eggplants after inoculated treatment.

Place and Duration of Study: The plant materials were planted in plastic greenhouse of the Vegetable Crops Experimental Station, and the laboratory experiments were taken at Horticulture College, Shenyang Agricultural University during August to November, 2008.

Methodology: 14 eggplant cultivars were selected and inoculated with *Verticillium dahliae* to screen their resistance against *Verticillium* wilt, and classified by the final disease index. The quantity of main microorganisms and some functional bacteria were investigated by the method of dilution cultivation. Activities of oxidoreductase and hydrolase enzymes of rhizosphere soil were determined by the methods of incubating and colourimetry or titration.

Results: The correlation analysis of resistance with microorganisms and enzyme activities showed that, the number of actinomyces, the ratios of bacterium to fungi and actinomyces to fungi, and the activities of catalase, polyphenol oxidase, protease and urease, were significant positively related with the resistance of eggplant to *Verticillium* wilt.

Keywords: *Verticillium* wilt; disease resistance; rhizosphere soil; microbial quantity; soil enzyme activity

1. INTRODUCTION

Verticillium wilt is a destructive disease in eggplant production, which is mainly caused by the infection of *Verticillium dahliae* through root surface to vascular system (Garibaldi et al, 2005; Wang et al., 2005). The *V. dahliae* can survive in soil for more than 6 years, and infect many plant varieties, while chemical fungicides is hard to effect directly to the infected part, all these cause the disease is hard to control in many areas of the world (Pegg and Brady 2002; Ligoxigakis et al., 2002; Korolev et al., 2008; Berbegal et al., 2010). Therefore, it is necessary to consider the relation among plant, soil and pathogen synthetically, to manage this disease (Han et al, 2006). Plant can affect the pathogen directly through root exudation, or reconcile the soil condition

indirectly, such as alter the quantity or quality of microorganism and the activities of soil enzyme. All these regulatory actions are determined by the resistances of different varieties (Bertin et al, 2003; Wu et al, 2010). But the regulatory mechanism of plant to soil microorganisms and enzyme activities, and their relationship with plant resistance, are not quite clear now. This paper has taken different resistant eggplant cultivars to define the relative soil characters for disease resistance identification, provided theoretical basis for soil environment management, and defined the direction for integrated management of V. wilt of eggplant.

2. MATERIAL AND METHODS

2.1 Plant material

The research group had selected eggplant sources, especially the main cultivars planted in North China. According to previous experiments, 14 different resistant eggplant cultivars were selected to assess their resistance to *V. dahliae*. They were: Bu Lang (BL for short), Liao Qie 6 (L6), HeiYouliang (HL), Hei Mei (HM), Xin Wujin (XW), Bang Lv (BV), Xi'an Lv (XL), Lv Baoshi (LB), Liao Qie 3 (L3), Liao Qie 5 (L5), Li Yuan (LY), Tianjin Kuai Yuan (TY), *Solanum torvum* and *Solanum tovu*.

2.2 Pathogen

V. dahliae isolated from pathogenic eggplant was separated, inoculated and separated again, according to Koch's postulate, and identified by the Mycology Laboratory of College of Plant Protection, Shenyang Agricultural University. After 20 days growing at 27 °C, on Potato Dextrose Agar (PDA) culture medium, the colonies were put into sterile distilled water in 250 mL triangular flask shaking for a night. The filter liquid was filtrated through two layer sterile gauze, and adjusted to 1×10^7 spores/mL with sterile distilled water, using hemocytometer.

2.3 Experimental design

The plant materials were planted in a plastic greenhouse of the Vegetable Crops Experimental Station at Shenyang Agricultural University during August to November, 2008. The eggplants seeds were sterilized with 10 % H₂O₂, and sprouted separately according to their germinating time, to make all cultivars at the same growth stage. The nursery substrates were sterilized at 121.6 °C for 1.5 h, trays and other tools were disinfected with KMnO₄ solution. The seedlings were transplanted into plastic pots (13×13 cm) containing with soil, peat and horse manure (3:2:1), at the two leaves growth stage. Each plant was injured and inoculated with 10 mL of the *V. dahliae* spores suspension, at the 4 leaves growth stage. Each treatment had fifteen plants and was repeated three times. To accelerate the incidence of V. wilt, eggplants were cultured under

suitable cultivation, and investigated when the disease appeared.

2.4 Disease assessment

The disease was assessed on leaf symptoms by a wilt index 0 to 4, according to Xiao et al (1995). Health incidence, disease incidence and disease index were evaluated every 5 days since the first appearance of the typical wilt, using the following calculations:

Health incidence (%) = (Number of Health Plants/Total Number of Plants) × 100 %

Disease incidence (%) = (Number of Infected Plants/Total Number of Plants) × 100 %

Disease Severity Index = $\sum (\text{Rating number} \times \text{number of plants with the rating}) /$
 $(\text{Total number of plants} \times \text{highest rating}) \times 100$

Classification method of resistance type: Resistant type (R), $DI \leq 15$; moderate resistant type (MR), $15 < DI \leq 30$; tolerant type (T), $30 < DI \leq 50$; moderate susceptible (MS), $50 < DI \leq 70$; susceptible type (S), $DI > 70$.

2.5 Determination of microbial quantity

The eggplant was taken out from pot carefully, and shaken to remove the needless soil, only rhizosphere soil was retained for further determination. For each cultivar, 3 plants were selected to take rhizosphere soil, and the soil was mixed well. 10 g rhizosphere soil was weighted, put into a 250 mL triangular flask contained 100 mL sterile distilled water, and shaken for 15 min, then after 5min diluted with sterile distilled water to different concentration. The initial soil suspensions were dried to work out the microbial quantity of every gram dry soil. The mediums and methods for microorganism cultivation were showed in Table 1. All these mediums were sterilized at 121 °C for 30 min. The liquid medium was put into test-tubes, for 5mL per tube, and counted using the method of most probable number (MPN). The dilution method of plate counting was used to count the numbers of others cultivated on the solid medium. Each concentration was inoculated three plates or tubes, which were three replications. All these plates and tubes were incubated at 27 °C in darkness and counted according to the growth (Xv et al, 1986; Yan, 1988).

Table 1. The formulas and method of cultivation mediums.

Microorganism	Name of medium	Components	Amount	Comments
Bacterium	Beef extract-peptone medium	Beef Extract	3.0 g	pH 7.0-7.2. Incubated 2-3d.
		Peptone	10.0 g	
		NaCl	5.0 g	
		Agar	20 g	
		Distilled Water	1000 ml	
Actinomyces	Gause's synthetic No.1 medium, modified	Soluble Starch	20.0 g	pH 7.2-7.4;
		KNO ₃	1.0 g	
		K ₂ HPO ₄	0.5 g	1mL 3%K ₂ Cr ₂ O ₇
		MgSO ₄ ·7H ₂ O	0.5 g	solution per 300mL
		NaCl	0.5 g	
		FeSO ₄ ·7H ₂ O	0.01 g	medium.
		Agar	20 g	Incubated 10d
		Distilled Water	1000 ml	
Fungi	Martin's medium	Dextrose	10.0 g	3mL 1% streptomycin
		Peptone	5.0 g	
		KH ₂ PO ₄	1.0 g	
		MgSO ₄ ·7H ₂ O	0.5 g	per 1000mL medium.
		Rose Bengal	0.034 g	Incubated 2-3d.
		Agar	20 g	
		Distilled Water	1000 ml	
Azotobacteria	Ashby medium	Dextrose	10 g	Incubated 7d.
		K ₂ SO ₄	0.2 g	

		MgSO ₄ ·7 H ₂ O	0.2 g	
		K ₂ HPO ₄	0.2 g	
		NaCl	0.2 g	
		CaCO ₃	5 g	
		Agar	20 g	
		Distilled Water	1000 mL	
Amonifying	Peptone	Beef Extract	3 g	pH 7.2-7.4.
	ammonification	Peptone	5 g	
		Agar	20 g	Incubated 2-3d.
	medium	Distilled Water	1000 mL	
		(NH ₄) ₂ SO ₂	2.0 g	
		NaH ₂ PO ₄	0.25 g	
Nitrite bacteria	Stephenson medium,	MnSO ₄ ·H ₂ O	0.01 g	Incubated 15d.
	modified	K ₂ HPO ₄	0.75 g	
		MgSO ₄ ·7 H ₂ O	0.03 g	
		CaCO ₃	5 g	
		Distilled Water	1000 mL	
		K ₂ HPO ₄	1g	
		FeCl ₃ ·5H ₂ O	0.1g	
Cellulose	Имшецкий's	MgSO ₄ ·7H ₂ O	0.3g	pH 7.2- 7.4.
decomposing		CaCl ₂	0.1g	Incubated 15d.
bacteria	medium	NaCl	0.1g	
		NaNO ₃	2.5g	
		Distilled Water	1000 mL	

2.6 Determination of soil enzyme activities

The soils selected above were air-dried, passed through a 1 mm screen, and mixed for the determination of enzyme activities. The catalase activity was determined by KMnO₄ titration method, and defined by milliliters of 0.01 mol·L⁻¹ KMnO₄ per gram dry soil. The polyphenol oxidase and peroxidase activities were determined by means of pyrogallol method. The protease activity was analyzed by the ninhydrin colorimetric method, and expressed in terms of NH₂-N per gram dry soil for 24 h. The cellulase and invertase activities were measured

by the colorimetric method, and estimated through the production of glucose. The urease activity was determined by the sodium phenate-sodium hypochlorite colorimetric method, and expressed in terms of $\text{NH}_4^+\text{-N}$ per 100 gram dry soil for 24 h. The phosphatase activity was measured by the disodium phenyl phosphate colorimetric method, and the activity unit was phenol per gram dry soil in 24 h at 37 °C (Xv and Zheng, 1986; Yan, 1988; Li et al, 2008).

2.7 Statistical analysis

The data were processed by Excel. Analysis of variance was performed using the Data Processing System software (DPS). The correlation was analyzed by Statistics Package for Social Science software (SPSS).

3. RESULTS

3.1 Resistance to Verticillium wilt

According to the final disease index (Table 2), *S. torvum* and *S. tovu* were resistant type, the disease incidences were significantly lower than other cultivars; LY was the only moderate resistant (MR) type cultivar; XL was susceptible type (S). The remaining cultivars were categorized as tolerant (T) and moderate susceptible type (MS), with the disease index ranged from 35.00 to 58.75.

3.2 Relationship between microorganisms and resistance to V.wilt

The larger numbers of microbes were observed in rhizosphere soil than those in non-planting (CK). The bacterium and actinomyces quantities at rhizosphere soils of resistant cultivars were generally higher than susceptible types, while the situation of fungi was opposite (Table 3). The changes of B/F and A/F showed a similar trend with the resistance. The amount of functional bacterium showed different among cultivars, but no rule was found.

The disease index of different resistant cultivars showed significant negative correlation with the amount of actinomyces, but no correlation with the bacterium and fungi quantity. B/F and A/F were significant negatively correlated with the disease index, with the correlation coefficients of -0.560 and -0.576. The number of the functional bacterium, such as azotobacteria, ammonifying bacteria, nitrite bacteria and cellulose decomposition bacteria, were uncorrelated with disease index.

Table 2. Resistance of different eggplant cultivars to Verticillium wilt.

Cultivar	2008-10-17			2008-10-22			2008-10-27			Resistant types
	Health	Disease	Disease index	Health	Disease	Disease index	Health	Disease	Disease index	
	incidence	incidence		incidence	incidence		incidence	incidence		
	%	%	%	%	%	%				
BL	95.00	5.00	5.00	50.00	50.00	23.75	35.00	65.00	46.25	T
L6	65.00	35.00	35.00	30.00	70.00	41.25	15.00	85.00	57.50	MS
HL	95.00	5.00	5.00	65.00	35.00	18.75	50.00	50.00	38.75	T
HM	80.00	20.00	17.50	50.00	50.00	31.25	15.00	85.00	57.50	MS
XW	95.00	5.00	5.00	45.00	55.00	33.25	25.00	75.00	53.75	MS
BV	80.00	20.00	12.50	40.00	60.00	41.75	20.00	80.00	55.00	MS
XL	35.00	65.00	45.00	5.00	95.00	48.75	0.00	100.00	71.75	S
LB	95.00	5.00	5.00	65.00	35.00	16.25	40.00	60.00	36.25	T
L3	75.00	25.00	25.00	50.00	50.00	38.25	40.00	60.00	35.00	T
L5	55.00	45.00	30.00	15.00	85.00	40.00	25.00	75.00	58.75	MS
LY	100.00	0.00	0.00	85.00	15.00	15.00	50.00	50.00	28.75	MR
TY	90.00	10.00	10.00	35.00	65.00	48.00	35.00	65.00	50.00	T
S. torvum	100.00	0.00	0.00	100.00	0.00	0.00	90.00	10.00	10.00	R
S. tovu	100.00	0.00	0.00	95.00	5.00	5.00	85.00	15.00	12.50	R

Note: The “Resistant types “ were classified according the final “Disease index”.

BL- Bu Lang; L6- Liao Qie 6; HL -HeiYouliang; HM- Hei Mei; XW- Xin Wujin; BV -Bang Lv ; XL-Xi'an Lv;

LB-Lv Baoshi; L3-Liao Qie 3; L5-Liao Qie 5; LY- Li Yuan; TY -Tianjin Kuai Yuan.

R —resistant type; MR—moderate resistant type; T—tolerant type; MS— moderate susceptible;

S— susceptible type. The same below.

Table 3. Variation analyses of rhizosphere microorganisms of different eggplants cultivars and their correlation with resistance. (10^6 cfu·g⁻¹DM)

Cultivar	B/F	A/F	Bacterium	Actinomyces	Fungi	Azotobacteria	Ammonifying	Nitrite	Cellulose
CK	338 jH	27.57 cdCD	198 iH	16.12 fF	0.58 gF	38.40 iG	332 iH	49 gE	0.14kK
BL	964 hFG	18.99 efE	1459 ghG	28.72 cCD	1.51 bB	28.13 jH	2813 bcBC	688 cB	4.95 cC
L6	1803 fE	18.25 fEF	2615 efE	26.48 cdD	1.45 bcB	76.33 cBC	1012 ghFG	139 fE	1.39 hH
HL	2287 eD	21.77 eE	2929 eDE	27.89 cdCD	1.28 cdBCD	72.52 cdCD	5698 aA	570 dC	2.33 fF
HM	3310 dC	25.17 dD	4460 bcB	33.91 bB	1.35 bcBC	83.21 bAB	1059 ghFG	832 aA	0.59 jJ
XW	3828 cB	30.04 cC	4129 cBC	32.41 bBC	1.08 DE	90.14 aA	966 hFG	161 fE	0.53 jJ
BV	1880 fE	19.42 efE	2383 fEF	24.62 deDE	1.27 cdBCD	69.01 deCDE	828 hG	138 fE	1.38 hH
XL	1147 ghF	19.32 efE	1585 ghG	26.69 cdD	1.38 bcB	50.58 ghF	945 hFG	742 bcAB	0.20 kK
LB	1269 gF	25.35 dD	1423 ghG	28.44 cCD	1.12 deCDE	52.00 gF	1875 fE	520 dCD	1.65 gG
L3	662 iGH	15.36 gF	1252 hG	29.05 cCD	1.89 aA	25.79 jH	1344 gF	115 fgE	6.30 aA
L5	1697 fE	19.31 efE	1890 gFG	21.50 eE	1.11 deCDE	63.38 efDE	2093 efE	807 abA	6.34 aA
LY	3908 bcB	43.46 aA	3522 dCD	39.18 aA	0.90 fE	49.49 ghF	3044 bB	517 dCD	3.40 eE
TY	1147 ghF	19.84 efE	1541 ghG	26.67 cdD	1.34 bcBC	44.04 hiFG	841 hG	80 fgE	4.40 dD
<i>S. torvum</i>	4898 aA	35.51 bB	5399 aA	39.14 aA	1.10 deCDE	68.13 defCDE	2568 cdCD	535 dC	5.35 bB
<i>S. tovu</i>	4139 bB	28.95 cC	4686 bB	32.78 bBC	1.13 deCDE	61.25 fE	2283 deDE	418 eD	1.03 iI
Correlation	-0.562*	-0.579*	-0.504	-0.653*	0.261	0.157	-0.449	0.084	-0.294

Note: Different small and capital letters mean significant differences from control at 0.05 and 0.01 levels respectively.

CK: non-planting soil. ***- Significant at 0.01 probability level; *- Significant at 0.05 probability level. The same below.

Correlation coefficients- The correlation coefficients between final disease index and rhizosphere microorganisms.

3.3 Relationship between soil enzyme activities and resistance to V. wilt

Table 4 showed that the enzyme activities of rhizosphere were higher than non-planting soil. Meanwhile, the enzyme activities mainly increased with the improvement of resistance. But the significant difference only

showed among few cultivars, which had greater different resistances, while resistant differences among most cultivars were not significant. The enzyme activities of *S. torvum* and LY were extremely significant or significant higher than XL.

The soil enzyme activities showed effect on the resistance of eggplant against V. wilt. The activities of catalase and polyphenol oxidase were significant negatively correlated with disease index, and the correlation coefficients were -0.650 and -0.601. Meanwhile, the activities of protease and urease were significant negatively correlated, and the correlation coefficients were -0.593 and -0.546. Other enzyme activities showed no correlation with disease index. The relationship between resistance and disease incidence was almost the same as disease index, but opposite with health incidence.

Table 4. Variation analyses of soil enzyme activities of different eggplants cultivars and their correlation with resistance.

Cultivar	Catalase	Polyphenol oxidase /mg·100g ⁻¹ ·3h ⁻¹ mL·g ⁻¹	Peroxidase /mg·g ⁻¹ ·3h ⁻¹	Protease /(NH ₂ -N mg·g ⁻¹ ·24h ⁻¹)	Cellulase /mg·g ⁻¹ ·72h ⁻¹	Urease /NH ₄ ⁺ -Nmg· 100g ⁻¹ ·24h ⁻¹	Invertase	Phosphatase / phenol mg·g ⁻¹ ·24h ⁻¹
	/0.01mol						/ glucose	
	·L ⁻¹ KMnO ₄						mg·g ⁻¹ ·24	
							h ⁻¹)	
	mL·g ⁻¹							
CK	1.38 kI	39.09 gG	0.96 jJ	0.108 fE	1.29 fI	23.87 iF	2.03 eF	1.59 gF
BL	1.82 hiFG	80.76 bcABC	1.89 fgF	0.197 cdeCD	1.91 eFGH	34.07 hE	4.56 bBC	3.40 bcdeBCD
L6	1.67 jH	67.80 defDEF	2.37 eE	0.190 deD	2.21 dDE	38.81 abAB	4.32 cCD	3.42 bcdeBCD
HL	1.92 fgEF	71.87 cdCDE	1.19 iHI	0.202 cC	1.90 eGH	35.67 efgCDE	4.55 bBCD	3.42 bcdeBCD
HM	2.05 deD	84.83 abAB	2.89 dCD	0.214 bB	2.25 dDE	34.79 ghDE	4.35 cCD	3.04 defCDE
XW	1.99 efDE	80.01 bcABCD	3.22 cBC	0.212 bB	2.44 cCD	36.08 defCD	4.95 aA	4.03 aA
BV	2.09 dD	79.64 bcABCD	1.18 iHI	0.201 cdCD	2.19 dE	37.23 cdBC	4.69 bB	3.52 bABC
XL	1.87 ghEF	68.35 deDEF	2.10 efEF	0.195 deCD	2.17 dE	35.41 efgDE	4.53 bBCD	2.83 fDE
LB	1.80 hiFG	75.94 bcdBCD	2.04 fgEF	0.198 cdeCD	2.11 dEFG	36.20 deCD	4.30 cD	4.00 aAB
L3	1.75 ijGH	75.57 cdBCD	1.49 hGH	0.195 deCD	2.15 dEF	34.83 fghDE	4.53 bBCD	3.51 bcaBC
L5	1.89 ghEF	59.28 fF	1.77 gFG	0.217 bB	2.08 dEFGH	35.75 efgCDE	4.53 bBCD	2.71 fE
LY	2.21 cC	73.35 cdBCD	3.52 bB	0.215 bB	3.55 aA	38.34 bcAB	4.64 bB	3.71 abAB
TY	1.86 ghEF	60.95 efEF	2.76 dD	0.190 deD	1.86 eH	37.25 cdBC	3.89 dE	3.07 cdefCDE
<i>S. torvum</i>	2.47 aA	91.30 aA	4.04 aA	0.232 aA	2.54 cC	39.80 aA	4.99 aA	3.45 bcdABC

<i>S. tovu</i>	2.34 bB	90.38 aA	3.25 bcBC	0.227 aA	2.86 bB	38.98 abA	4.66 bB	2.98 efCDE
Correlation	-0.658*	-0.602*	-0.472	-0.595*	-0.500	-0.549*	-0.358	-0.260

4. DISCUSSION

Rhizosphere is a microenvironment made up of root-soil-microorganism. Plant can affect the soil microorganism and enzyme activities through root exudates, the effects vary with variety and relate to resistance of plant (Kong et al, 2008a, 2008b; Bonkowski et al, 2009; Raaijmakers et al, 2009). But there is no unifying understanding of the correlation between resistance and soil microorganism quantity and enzyme activity (Miao et al, 2004; Li et al, 2007; Yin et al, 2009; Gu et al, 2009; Zhou et al, 2011). Study about the population of rhizosphere microorganism of 6 cotton cultivars that have different resistance to *V. dahliae* has showed that, the fungi and actinomyces population of resistance cotton cultivars is positively correlated with cotton resistance, but rhizosphere bacteria population is not close correlated with resistance (Li et al, 1998). This experiment analyzed the changes of rhizosphere microbial quantity and soil enzyme activity under infection of *V. dahliae*, which was completely different with Li (1998), whose experiment was taken under nature condition without pathogens. In this test, the soil was sampled when disease incidences among cultivars were varied. When the roots of susceptible cultivars were browned and rotted, the root activities were decrease sharply, which leaded to the reduction of regulation and control to rhizosphere microorganism and soil enzyme activity. Conversely, the resistant cultivars showed greater defense to prevent damage to root activity, and then some specific exudates released by root increased the quantity of rhizosphere microorganisms and soil enzyme activities. Especially, the increase of A/F and B/F, and the improvement of catalase, polyphenol oxidase, protease and urease activities, inhibited the spread of *V. dahliae*.

In this paper, the relationship between the resistances of different eggplant cultivars to *V. wilt*, and rhizosphere microorganism and soil enzyme characters, were systematically analyzed. The identified factors for resistance were defined, which could be used as complementary indexes for soil environment manage in biological control of *V. wilt*. Further studies are needed on relevant physiological and biological process, to reveal the mechanism of root exudation.

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