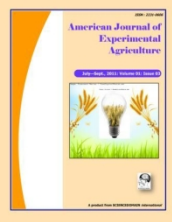

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Resistance of Eggplant (*Solanum melongena* L.) to Verticillium Wilt Correlates to Microbial Abundance and Soil Enzyme Activities

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Authors' contributions

This work was carried out in collaboration between all authors. BLZ designed the study, supervised the experiment and revised both the protocol and manuscript. ZXC performed the experiment and statistical analysis, wrote the protocol and manuscript, and revised the manuscript. LD, XLY and YFL helped to manage the analyses of the study. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: To determine the relationship between microbial abundance and enzyme activities of rhizosphere soil from different resistant eggplant cultivars and resistance of eggplant to Verticillium wilt.
Study Design: The changes of microbial and enzymatic activities of the rhizosphere soil from different resistant eggplants after inoculation of *Verticillium dahliae* were analysed.
Place and Duration of Study: The plants were grown in a plastic greenhouse of the Vegetable Crops Experimental Station, and the laboratory experiments were conducted at the Horticulture College, Shenyang Agricultural University from August to November, 2008.
Methodology: 14 eggplant cultivars were selected and inoculated with *Verticillium dahliae* to screen their resistance against Verticillium wilt, and classified according the final disease index. The quantities of main cultivable microorganisms and some functional bacteria were investigated by the serial dilution method. Activities of oxidoreductase and hydrolase enzymes of rhizosphere soil were determined by spectrophotometry or colorimetric titrations.
Results: The correlation analysis among resistance of eggplant to Verticillium wilt,

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microorganisms and enzyme activities showed that, the abundance of actinomyces, the ratios of bacteria to fungi and actinomyces to fungi, and the activities of catalase, polyphenol oxidase, protease and urease, were significantly positively related with the resistance.

Keywords: Verticillium wilt; disease resistance; rhizosphere soil; microbial abundance; 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). *V. dahliae* can survive in soil for more than 6 years, and infect many plant varieties, while chemical fungicides have no direct effects on infected plants, so the disease is hard to control all over 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 relationship between plant, soil and pathogen synthetically, to manage this disease (Park, 1963; Han et al., 2006). Plant can affect the pathogen directly through root exudation, or modulate the soil conditions (such as the quantity of microorganisms and the activities of soil enzymes) indirectly (Bertin, 2003; Brusetti et al., 2004). Antagonistic rhizosphere bacteria, actinomycetes and fungi contribute to the induction of remarkable soil suppressiveness against *V. dahliae* (Marois et al., 1982; Berg et al., 1994; Tjamos et al., 2004). Meanwhile, beneficial rhizosphere microorganisms may increase plant growth and development indirectly, through the biocontrol of phytopathogens in the root zone (Weller, 1988; Chet, et al., 1990; Garbeva et al., 2004) and the enhanced availability of minerals (Davison, 1988; Murty and Ladha, 1988), as well as directly through the production of phytohormones (Patten and Glick, 1996). Soil enzymes are important for improving and maintaining soil fertility to ensure productivity, as they take part in organic matter decomposition and nutrient cycling (Verstraete and Voest, 1977; Sinsabaugh et al., 1991; Bohme et al., 2005; Mandal et al., 2007). Oxidoreductases (such as catalase polyphenol oxidase and peroxidase) act in the oxidation reduction in soil (Wang et al., 2011; Finkenbein et al., 2012) and hydrolases are involved in the N (e.g. protease, urease), C (e.g. cellulase, invertase), P (e.g. acid phosphatase) cycling and decomposition of cellulose, lignin, carbohydrate polymers and other biomacromolecules (Verstraete and Voest, 1977; Prieto et al., 2011; Wang et al., 2011). Several studies show that enzyme activities can be used as early indicators of changes in soil properties and microbial activity (Skujins, 1973; Ajwa et al., 1999; Kandeler et al., 2006; Makoi and Ndakidemi, 2008). The regulatory of pathogens by the plants is determined by the resistances of different varieties (Bertin et al., 2003; Wu et al., 2010). But the regulatory mechanisms of plant to soil microorganisms and enzyme activities, and their relationship with plant resistance, are not quite clear now. This paper has studied different resistant eggplant cultivars to define the relative soil parameters for disease resistance determination, to provide theoretical basis of soil environment management, and to define the direction for integrated management of Verticillium wilt of eggplant.

2. MATERIAL AND METHODS

2.1 Plant Material

The research group has selected the main eggplant cultivars grown in North China. According to previous experiments, 14 different eggplant cultivars were selected to assess their resistances to Verticillium wilt. 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

The *V. dahliae* was isolated from tap roots and stems of diseased eggplant plants, inoculated to healthy eggplants and isolated again from newly formed symptoms of Verticillium wilt, according to Koch's postulate. The identity of isolate was confirmed on the basis of colony morphology, conidiophore formation, conidial production, and presence of microsclerotia, by the Mycology Laboratory of the Shenyang Agricultural University. The colonies were grown at 27ºC in dark for 20 days on Potato Dextrose Agar (PDA) medium. Then, the pure culture of *V. dahliae* was put into sterile distilled water in 250 mL triangular flasks and shaken (100 $r \cdot min^{-1}$) for a night. The liquid culture was filtrated through two layers of sterile gauze, and adjusted to 1×10^7 $spores \cdot mL^{-1}$ with sterile distilled water, using hemocytometer.

2.3 Experimental Design

The plants were grown in a plastic greenhouse of the Vegetable Crops Experimental Station at Shenyang Agricultural University from August to November, 2008. The eggplant seeds were sterilized with 10% H_2O_2 and accelerated germination separately according to their germinating time, to make all cultivars at the same growth stage. In order to improve the seedling quality, seeds were sprouted under changed temperature, 20ºC for 8h and 30ºC for 16h. The nursery substrates were sterilized at 121.6ºC for 1.5h, trays and other tools were disinfected with $KMnO_4$ solution. The seedlings were transplanted into plastic pots (13×13 cm) containing farmland soil, peat and horse manure (3:2:1), at the two-leaf growth stage. Each plant was injured through the soil surface with a sterile knife, and inoculated with 10mL of the *V. dahliae* spore (1×10^7 $spores \cdot mL^{-1}$) suspension into the injured place, at the 4-leaf growth stage. Each treatment had fifteen plants and was repeated three times. To accelerate the incidence of Verticillium wilt, eggplants were cultured under suitable cultivation (under 18-28ºC and high humidity) and observed daily until the disease appeared.

2.4 Disease Assessment

The disease was assessed on leaf symptoms by a wilt index from 0 to 4, according to Emmanouil and Wood (1981) and 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 Index =Σ (Rating number× number of plants with the rating)/
(Total number of plants × highest rating) ×100

Classification method of resistance type: The eggplant cultivars were classified to different types according to the final DI (Disease Index). Resistant type (R), DI≤15; moderate resistant type (MR), 15<DI≤30; tolerant type (T), 30<DI≤50; moderate susceptible (MS), 50<DI≤70; susceptible type (S), DI>70.

2.5 Determination of Microbial Abundance

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, the rhizosphere soil of 3 plants was taken and well mixed. Ten grams of rhizosphere soil was weighted, put into a 250mL triangular flask containing 100mL of sterile distilled water, and shaken for 15min (100rpm). Then after 5 min standing, 1mL of supernatant was diluted into 9mL of sterile distilled water (10^{-1}), and then diluted the same way to different concentrations (from 10^{-2} to 10^{-9}). The initial soil suspensions were oven dried to calculate the microbial abundance in each sampled soil. Count of cultivable bacteria, actinobacteria, fungi, azotobacteria, ammonifying bacteria, nitrifying bacteria and cellulose decomposing bacteria was done with the soil serial dilution method. 200μl of each dilution were plated in Petri dishes containing selective media, all sterilized at 121ºC for 30 min. Beef extract-peptone medium for bacteria, Gause's synthetic No.1 medium, modified for actinomyces, Martin's medium for fungi, Ashby medium for azotobacteria and Peptone ammonification medium for ammonifying bacteria. All these plates were incubated at 27ºC in darkness, for 2d for bacteria, 10d for actinomyces, 3 d for fungi and ammonifying bacteria and 5d for nitrifying bacteria. The microflora growing on solid media was assessed by plating each serial soil dilution on agar media and counting the number of colony forming units on each plate. Nitrifying bacteria and cellulose decomposing bacteria were counted by putting 1mL of each dilution into test-tubes containing 5mL of adapted liquid medium, Stephenson medium, modified for nitrifying bacteria and Имшенецкий's modified for cellulose decomposing bacteria. Enumeration of nitrifying bacteria (using sulfanilic acid and α-naphthylamine as chromogenic agent) and cellulose decomposing bacteria (adding filter paper without cellulose to exam the exits of cellulose decomposing bacteria) grown in liquid medium was done with the method of most probable number (MPN, using the 3-tube MPN Table), after 15 days' incubation at 27ºC in dark. Each concentration was inoculated on three plates or tubes, which were three replications (Xu and Zheng, 1986; Yan, 1988; Yin et al., 2009).

2.6 Determination of Soil Enzyme Activities

The soils (from each cultivar) selected above were air-dried for 7 days, passed through a 1mm sieve and mixed for the determination of enzymatic activities (Xu and Zheng, 1986; Yan, 1988; Li et al., 2008; Gu et al., 2009; Zhao et al., 2012). The experiments were repeated three times.

The catalase activity was determined by $KMnO_4$ titration method. Two grams of soil samples wetted with 0.5ml of methylbenzene were incubated for 0.5h at 4ºC. After that, 40 mL of distilled water and 5mL of 0.3% H_2O_2 (as substrate) were added, and shaken for 30min (120 $r \cdot min^{-1}$), then 5mL of 3 $mol \cdot L^{-1}$ H_2SO_4 were added immediately to terminate the reaction. The remaining H_2O_2 was titrated by 0.01 $mol \cdot L^{-1}$ $KMnO_4$ and the enzyme activity was defined by

milliliters of 0.01 mol·L^{-1} $KMnO_4$ per gram of dry soil. Samples without soil were used as control.

The polyphenol oxidase and peroxidase activities were determined by the pyrogallol method, and expressed by the production of gallocatechin for 3h. For determination of polyphenol oxidase activity, 10mL of 1% pyrogallol and 2mL of 0.5% H_2O_2 were added to 1g of soil in a 50mL volumetric flask, and incubated at 30ºC for 3h. The optical density of gallocatechin extracted by diethyl ether at 430nm was measured to express the polyphenol oxidase activity. Samples without soil and without reaction substrate were used as control. The activity of peroxidase was measured the same way without H_2O_2 as substrate.

The protease activity was analyzed by the ninhydrin colorimetric method. For determination of protease activity, 2g of soil samples were wetted by 0.5 ml of methylbenzene and incubated for 24h at 30ºC with 10 ml 1% of gelatin solution. After incubation, 0.5ml of 0.1 mol·L^{-1} H_2SO_4 and 3ml of 20% Na_2SO_4 were used to precipitate the proteins. The solutions of soil sample were centrifuged for 15min (4000 rpm) and then 1mL of 2% ninhydrin solution was added. The mixture was boiled to develop color, and diluted with distilled water to 50mL, then measured optical density at 500nm. Samples without soil were used as control. Finally, protease activity was expressed in terms of NH_2-N per gram of dry soil for 24h at 30ºC.

The cellulase activity was measured by the UV spectrophotometry method and estimated through the production of glucose. For the determination of cellulase activity, 10g of soil samples were wetted with 2mL of methylbenzene, then 5mL of acetate buffer (pH 5.5) and 5mL of 1% carboxyl methyl cellulose (CMC) were added. The mixture was incubated at 37ºC for 72h, then boiled to stop the reaction. 0.3mg of $KAl(SO_4)_2 \cdot 12H_2O$ was added to precipitate residual cellulose. The filtrate was diluted with distilled water to 50mL, then 2 mL of diluent and 5 mL of 0.1% anthrone were mixed and boiled for 10 min to develop color. The color intensity was measured at 551nm. Blanks were incubated without substrate or soils.

The invertase activity was measured using sucrose as substrate, the soils (10 g) were mixed with 10 mL of 20% sucrose solution and 10 mL of phosphate buffer (pH 5.5) and incubated at 37ºC. After 24 h, the mixture was diluted with distilled water to a final volume of 50 mL. 20 mL of diluent were mixed with 10 mL of Fehling reagent and 20 mL of distilled water, then 3 mL of 33% KI and H_2SO_4 (v/v 1:3) solutions were added. 0.1 mol·L^{-1} hyposulphite solution was used to titrate the mixture from blue to white, using starch as the indicator. Blanks were incubated without substrate or soils.

The urease activity was determined by the sodium phenate-sodium hypochlorite colorimetric method. As substrate, 10mL of 10% urea solution and 20mL of citrate buffer (pH 6.7) were added to 10 g of soil samples before being incubated at 37ºC for 24 h. After incubation, the soil samples were shaken for 30 min and filtrated. 1mL of filtrate was poured into a 50mL volumetric flask, and then 4mL of sodium phenate and 3mL of sodium hypochlorite were added. After coloration, optical density at 578nm was measured for the extractions within 60 min. Urease activity was expressed in terms of NH_4^+-N per 100 grams of dry soil for 24h. Samples without soil and without reaction substrate were used as control.

The acid phosphatase activity was measured by the disodium phenyl phosphate colorimetric method and the activity unit was phenol per gram of dry soil in 24 h at 37ºC. A sample of 10g of soils and 1.5mL of methylbenzene were mixed and 10mL of disodium phenyl phosphate ($C_6H_5PO_4Na_2 \cdot 2H_2O$) and 10mL of acetate buffer (pH 5.0, because the pH values of soil samples were less than 7.0) were added. After 24 h incubation at 37ºC, 1mL of filtrate was

transferred into a 100mL volumetric flask, 5mL of borate buffer (pH 9.6) and 1mL of Gibbs reagent were added to develop color. After dilution to 100mL volume, the color intensity was measured at 578nm. Blanks were incubated without substrate or soils.

2.7 Statistical Analysis

The data were statistically analyzed with Excel software. Analysis of variance was performed using the Data Processing System software (DPS). The correlation coefficients and Standard Deviation (SD) were calculated by Statistics Package for Social Science software (SPSS).

3. RESULTS

3.1 Resistance to Verticillium Wilt

According to the final disease index (Table 1), *S. torvum* and *S. tovu* were of the resistant types, the disease incidence and resistant index were significantly lower than other cultivars. LY was the only moderate resistant (MR) type cultivar, with a final disease index of 28.75. XL was susceptible type (S) and the only cultivar with a disease incidence of 100%. The remaining cultivars were categorized as tolerant (T) and moderate susceptible types (MS), with the disease index ranging from 35.00 to 58.75.

3.2 Relationship between Microflora Abundance and Resistance to Verticillium Wilt

The abundance of microorganisms was higher in rhizosphere soils than in non-planting soil (CK). The abundance of bacteria and actinomyces in rhizosphere soils of resistant cultivars were generally higher than in rhizosphere soil of other types, while the abundance of fungi in soils of resistant types were lower than the average level (about $1.28x10^6$ $cfu \cdot g^{-1}$) (Fig.1). The disease index of different resistant cultivars was significantly negatively correlated with the amount of actinomyces (r=-0.653), but not correlated with the quantities of bacteria and fungi (Table 2). Meanwhile, the disease incidence was significantly negatively correlated with the abundance of bacteria (r=-0.541) and actinomyces (r=-0.586), but not correlated with the abundance of fungi. The ratios of B/F (ratio of bacteria to fungi) and A/F (ratio of actinomyces to fungi) of resistant and moderate resistant cultivars were higher than others. B/F and A/F were significantly negatively correlated with the disease index, with the correlation coefficients of -0.560 and -0.576.

The abundance of functional bacteria showed differences among cultivars, but were uncorrelated with disease incidence and disease index.

3.3 Relationship between Soil Enzyme Activities and Resistance to Verticillium Wilt

As Table 3 and Table 4 show, the enzyme activities in rhizosphere soil were higher than in non-planting soil. Meanwhile, the enzyme activities mainly increased with the improvement of resistance. But significant differences were only showed among few cultivars, which had greater difference in resistances levels, such as *S. torvum* (R), LY (MR) and XL (S). The enzyme activities of *S. torvum* and LY were extremely significant or significantly higher than XL.

Table 1. Resistance of different eggplant cultivars to verticillium wilt

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

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

XL- Xi'an Lv; L5- Liao Qie 5; L6- Liao Qie 6; HM- Hei Mei; BV- Bang Lv; XW- Xin Wujin; TY- Tianjin Kuai Yuan; BL- Bu Lang; HL- Hei Youliang; LB- Lv Baoshi; L3- Liao Qie 3; LY- Li Yuan;

S— susceptible type; MS— moderate susceptible; T—tolerant type; MR—moderate resistant type; R —resistant type. The same below.

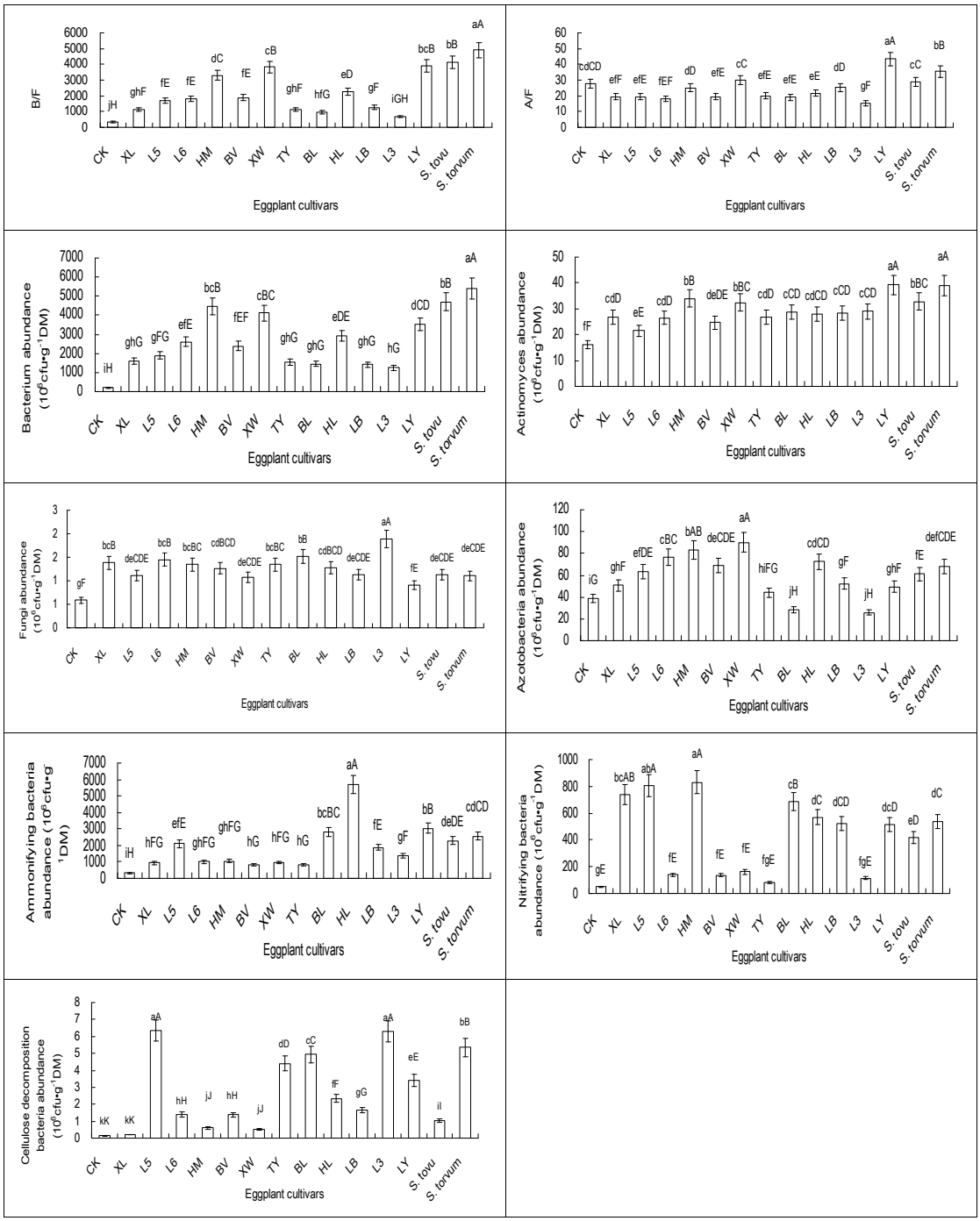

Fig. 1. Comparison of microbial abundance in rhizosphere soil of different eggplant cultivars

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

CK: non-planting soil. The unit of microbial abundance is 10^6 cfu·g^{-1}DM.

Table 2. Correlations between disease incidence, disease index of eggplant to Verticillium wilt and microbial abundance in rhizosphere soil of different eggplant cultivars

Correlation coefficients	B/F	A/F	Bacteria (B)	Actinomyces (A)	Fungi (F)	Azotobacteria	Ammonifying bacteria	Nitrifying bacteria	Cellulose decomposition bacteria
Disease incidence	-0.591*	-0.543*	-0.541*	-0.586*	0.315	0.093	-0.493	0.033	-0.321
Disease index	-0.562*	-0.579*	-0.504	-0.653*	0.261	0.157	-0.449	0.084	-0.294

*Note: Correlation coefficients- The correlation coefficients between final disease incidence, disease index (2008-10-27) and microorganisms of the rhizosphere. * Significant at 0.05 probability level.*

Table 3. Oxidoreductases activity in rhizosphere soil of different eggplant cultivars

Cultivar	Catalase ($0.01mol \cdot L^{-1} KMnO_4 mL \cdot g^{-1}$)	Polyphenol oxidase ($mg \cdot 100g^{-1} \cdot 3h^{-1}$)	Peroxidase ($mg \cdot g^{-1} \cdot 3h^{-1}$)
CK	1.38 (0.012) lL	39.09 (7.753) fF	0.96 (0.141) hH
XL	1.87 (0.012) ghGH	68.35 (5.797) deCDE	2.10 (0.228) efDEF
L5	1.89 (0.012) ghG	59.28 (5.249) eE	1.77 (0.020) fgEFGH
L6	1.67 (0.012) kK	67.80 (3.699) deCDE	2.37 (0.185) deCDE
HM	2.05 (0.024) eDE	84.83 (3.254) abAB	2.89 (0.071) cdBC
BV	2.09 (0.024) dD	79.64 (6.959) bcABC	1.18 (0.043) hGH
XW	1.99 (0.012) fEF	80.01 (5.936) bcABC	3.22 (0.027) bcB
TY	1.86 (0.012) hGHI	60.95 (6.117) eDE	2.76 (0.181) cdBCD
BL	1.82 (0.012) iHIJ	80.76 (7.283) bcABC	1.89 (0.200) efgEFG
HL	1.92 (0.000) gFG	71.87 (2.546) cdBCDE	1.19 (0.200) hGH
LB	1.80 (0.000) iIJ	75.94 (4.852) bcdBC	2.04 (0.184) hGH
L3	1.75 (0.074) jJ	75.57 (5.602) bcdBC	1.49 (0.063) ghFGH
LY	2.21 (0.023) cC	73.35 (0.848) cdBCD	3.52 (0.181) bAB
S. torvum	2.47 (0.360) aA	91.30 (5.298) aA	4.04 (0.369) aA
S. tovu	2.34 (0.048) bB	90.38 (1.697) aA	3.25 (0.562) bcB

Note: Different small and capital letters mean significant differences from control at 0.05 and 0.01 levels respectively. CK: non-planting soil. Numbers in parentheses are Standard Deviation (SD). The same below.

Table 4. Hydrolases activity in rhizosphere soil of different eggplant cultivars

Cultivar	Protease (NH_2-Nmg·g^{-1}·$24h^{-1}$)	Cellulase (mg·g^{-1}·$72h^{-1}$)	Urease (NH_4^+-Nmg·$100g^{-1}$·$24h^{-1}$)	Invertase (glucose mg·g^{-1}·$24\ h^{-1}$)	Phosphatase (phenol g·g^{-1}·$24h^{-1}$)
CK	0.108 (0.0045) fG	1.29 (0.044) hF	23.87 (2.124) fE	2.03 (0.026) fE	1.59 (0.238) eE
XL	0.195 (0.0053) eF	2.17 (0.098) defCDE	35.41 (0.333) deCD	4.53 (0.014) bcBC	2.83 (0.166) dD
L5	0.217 (0.0017) bcABC	2.08 (0.160) efgDE	35.75 (1.525) cdCD	4.53 (0.115) bcBC	2.71 (0.209) dD
L6	0.190 (0.0131) eF	2.21 (0.150) deCDE	38.81 (0.386) aAB	4.32 (0.113) dC	3.42 (0.225) bcBC
HM	0.214 (0.0041) cBCD	2.25 (0.072) deCDE	34.79 (0.610) deD	4.35 (0.025) cdC	3.04 (0.255) cdCD
BV	0.201 (0.0062) deCDEF	2.19 (0.105) defCDE	37.23 (1.226) bcBC	4.69 (0.063) bB	3.52 (0.137) bABC
XW	0.212 (0.0045)cdBCDE	2.44 (0.124) cdCD	36.08 (0.872) cdCD	4.95 (0.090) aA	4.03 (0.207) aA
TY	0.190 (0.0070) eF	1.86 (0.148) gE	37.25 (0.220) bcBC	3.89 (0.029) eD	3.07 (0.121) cdCD
BL	0.197 (0.0078) eEF	1.91 (0.214) fgE	34.07 (0.174) eD	4.56 (0.202) bBC	3.40 (0.255) bcBC
HL	0.202 (0.0030) deCDEF	1.90 (0.115) fgE	35.67 (0.586) cdCD	4.55 (0.090) bBC	3.42 (0.358) bcBC
LB	0.198 (0.0078) eDEF	2.11 (0.100) efgDE	36.20 (0.462) cdCD	4.30 (0.152) dC	4.00 (0.336) aA
L3	0.195 (0.0072) eF	2.15 (0.275) efDE	34.83 (0.113) deD	4.53 (0.152) bcBC	3.51 (0.137) bABC
LY	0.215 (0.0078) cBC	3.55 (0.214) aA	38.34 (0.301) abAB	4.64 (0.104) bB	3.71 (0.079) abAB
S. tovu	0.227 (0.0062) abAB	2.86 (0.195) bB	38.98 (0.313) aAB	4.66 (0.142) bB	2.98 (0.129) dCD
S. torvum	0.232 (0.0043) aA	2.54 (0.091) cBC	39.80 (0.586) aA	4.99 (0.080) aA	3.45 (0.264) bcBC

The results of Table 5 showed that, activities of catalase, polyhenol oxidase, protease and urease were significantly negatively correlated with disease incidence (with the correlation coefficients of -0.702, -0.559, -0.654 and -0.568 respectively) and disease index (the correlation coefficients were -0.650, -0.601, -0.593 and -0.546 respectively). Other enzyme activities showed no significant correlation with disease incidence and disease index.

3.4 Correlation between Soil Enzymic Activities and Microbial Abundance

Table 6 shows the relationship between activities of soil enzymes (including oxidoreductases and hydrolases) and abundance of microorganisms in rhizosphere soil. The activities of catalase, protease and cellulase were significantly positively correlated with the ratios of B/F and A/F and the abundance of bacteria, actinomyces and fungi. Peroxidase showed extremely significant correlation with the ratios of B/F and A/F, and abundances of bacteria and actinomyces, with the correlation coefficients of 0.793, 0.790, 0.738 and 0.807. The ratio of B/F and bacteria abundance were correlated with the activities of catalase, polyphenol oxidase, peroxidase, protease, cellulase, urease, and invertase, but not related to acid phosophatase activity. There was no significant correlation between acid phosophatase activity and the abundance of the rhizosphere microorganisms.

4. DISCUSSION

Rhizosphere is a microenvironment made up of root，soil and microorganisms (Lambers et al., 2009), could be more accurately defined as the volume of soil influenced by root activity (Hinsinger, 1998). Plants can affect the soil microorganisms and enzyme activities through root exudates (Landi, et al., 2006; Broeckling et al., 2008; Zhou B.L. et al., 2011; Gao et al., 2012). The effects vary with cultivars and are related to resistance of plant (Kong et al., 2008a, 2008b; Bonkowski et al., 2009; Raaijmakers et al., 2009). Since rhizosphere microbial communities are strongly influenced by root exudates (Brant et al.., 2006), it has been hypothesised that plants select for beneficial microbial communities in their rhizosphere (Singh et al.., 2007). Furthermore, plants may also play an important role in determining soil enzyme activities, as a mainly energy source of extracellular enzymes in soil (Martens et al., 1992; Gramss et al., 1999). But there is no unifying understanding of the correlation between resistance of plants, soil microorganisms abundance and enzyme activities (Harper, 1950; Larkin, 1993; Li et al., 2007; Yin et al., 2009; Gu et al., 2009; Zhou et al., 2011). Study about the population of rhizosphere microorganisms of 6 cotton cultivars that have different resistance to *V. dahliae* has showed that, the diversity of populations of rhizosphere fungi and actinomyces are positively correlated with cotton resistance, but the diversity of rhizosphere bacteria population is not closely correlated with resistance (Li et al., 1998). Our experiment analyzed the changes of rhizosphere microbial abundance and soil enzyme activity under infection of *V. dahliae*, which was completely different from Li et al. (1998), whose experiment was taken under natural conditions without pathogens.

In our test, the soils were sampled when disease incidences among cultivars varied. When the roots of susceptible cultivars turned brown and rotted, the root activities (including the excretive, absorptive and enzymatic activities et al.) decreased sharply, which leaded to the reduction of regulation and control to rhizosphere microorganisms and soil enzyme activities. Conversely, the resistant cultivars showed greater defense to prevent damages and maintain root activity, so the regulation and control to rhizosphere soil remained at a high level. Especially, the increase of B/F and A/F, linked with the improvement of catalase, polyphenol oxidase, protease and urease activities, is correlated to the inhibition or the slowing down of the spread of *V. dahliae*.

Table 5. Correlations between disease incidence, disease index of eggplant to Verticillium wilt and enzymatic activities of rhizosphere soil of different eggplant cultivars.

Correlation coefficients	Catalase	Poly phenol oxidase	Peroxidase	Protease	Cellulase	Urease	Invertase	Acid phosphatase
Disease incidence	-0.702**	-0.559*	-0.483	-0.654*	-0.419	-0.568*	-0.370	-0.129
Disease index	-0.658*	-0.602*	-0.472	-0.595*	-0.500	-0.549*	-0.358	-0.260

*Note: **: Significant at 0.01 probability level; *: Significant at 0.05 probability level. The same below.*

Table 6. Correlations between soil enzymatic activities and the abundance of rhizosphere microorganisms of different eggplants cultivars

Correlation coefficients	B/F	A/F	Bacteria (B)	Actinomyces (A)	Fungi (F)	Azotobacteria	Ammonifying bacteria	Nitrifying bacteria	Cellulose decomposition bacteria
Catalase	0.869**	0.748**	0.821**	0.703**	-0.596*	0.284	0.199	0.160	-0.043
Polyphenol oxidase	0.631*	0.434	0.691**	0.663**	-0.114	0.191	0.084	0.070	-0.223
Peroxidase	0.793**	0.790**	0.738**	0.807**	-0.528	0.259	-0.124	0.030	-0.077
Protease	0.868**	0.689**	0.827**	0.610*	-0.611*	0.382	0.238	0.358	0.072
Cellulase	0.703**	0.858**	0.567*	0.732**	0.567*	0.105	0.087	0.020	-0.128
Urease	0.604*	0.543*	0.546*	0.415	-0.497	0.286	-0.006	-0.341	-0.068
Invertase	0.613*	0.466	0.566*	0.447	-0.314	0.302	0.219	0.100	-0.023
Acid phosphatase	0.149	0.357	0.068	0.346	-0.160	0.085	0.084	-0.428	-0.135

Further studies are needed on relevant physiological and biological process, to determine the possible composition of root exudates which might be involved in the soil management, and reveal the mechanism of root exudation. And with the progress in molecular biology techniques (Söederberg et al., 2004; Wei et al., 2006; Broeckling et al., 2008), the isolation and identification of microorganisms should be more accurate to avoid the error caused by using traditional serial dilution method (only 1-4 % of the microorganisms in soil could be cultured in medium, Amann et al., 1995).

5. CONCLUSION

In this paper, the relationships between the resistance levels of different eggplant cultivars to Verticillium wilt, the rhizosphere microbial population and the soil enzyme activities, were systematically analyzed. The ratios of B/F and A/F, the abundance of actinomyces and the activities of catalase, polyphenol oxidase, protease and urease in rhizosphere soil, showed positive correlation with the resistance level of eggplant to Verticillium wilt, so they could be useful indicators to assess soil health level and manage soil environment.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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