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

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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, microorganisms and enzyme activities showed that, the abundance of actinomycetes, the ratios of bacteria to fungi and actinomycetes 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

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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 can‘t effectively 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 among between plant, soil and pathogen synthetically, to manage this disease (Park et al., 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; Chen 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). All these regulatory actions The regulation of pathogens by the plants are 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 taken 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
Correlation between resistance and soil factors

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 our the Shenyang Agricultural University. The colonies were grown at 27°C in dark for 20 days on Potato Dextrose Agar (PDA) medium. Then, put the pure culture of V. dahliae was put into sterile distilled water in 250 mL triangular flasks and shook shaken (100 r·min⁻¹) for a night. The liquid culture was filtrated through two layers of sterile gauze, and adjusted to 1×10⁷ spores 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 % H₂O₂, and accelerated germination 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 farmland soil (in farmland), 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 10 mL of the V. dahliae spore (1×10⁷ spores 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, and observed daily/weekly until investigated when the disease appeared.

2.4 Disease assessment

Comment [MF2]: Explain the way you do this.

Comment [MF3]: What do you mean with “suitable cultivation”?
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 = \[\sum \text{(Rating number} \times \text{number of plants with the rating)}/\left(\text{Total number of plants} \times \text{highest rating}\right) \times 100\]

Classification method of resistance type: The eggplant cultivars were classified to different types according to the final DI (Disease Index). Resistant type (R), DI \(\leq 15\); 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, the rhizosphere soil of 3 plants was taken and well mixed. Ten grams of rhizosphere soil was weighted, put into a 250 mL triangular flask containing 100 mL of sterile distilled water, and shaken for 15 min (100rpm·min⁻¹). Then after 5 min standing, 1 mL of supernatant were was decanted into 9 mL of sterile distilled water (10⁻¹), and then 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. Enumeration Count of cultivable bacteria, actinobacteria, fungi, azotobacteria, ammonifying bacteria, nitrifying bacteria and cellulose decomposing bacteria was done with the soil serial dilution method. 200 \(\mu\)l of each dilution were plated in Petri dishes containing selective media, all sterilized at 121 °C 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). 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. All these plates were incubated at 27 °C in darkness, for 2 d for bacteria, 10 d for actinomyces, 3 d for fungi and ammonifying bacteria and 5 d for nitrifying bacteria, after which colonies were counted. 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. Put Nitrifying bacteria and cellulose decomposing bacteria were counted by putting 1 mL of each dilution into test-tubes.
Correlation between resistance and soil factors

containing 5 mL of adapted liquid medium, per tube containing Stephenson medium, modified (for nitrifying bacteria) and Имшелецкий’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 examine the exist 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 27 °C in dark. All these mediums were sterilized at 121 °C for 30 min. Each concentration was inoculated on three plates or tubes, which were three replications (Xue and Zheng, 1988; 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 1 mm screen, and mixed for the determination of enzymatic activities (Xue 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 0.5 ml of methylbenzene, was were incubated for 0.5 h at 4 °C. After that, 40 mL of distilled water and 5 mL of 0.3% H$_2$O$_2$ (as substrate) were added, and oscillated-shaken for 30 min (120 r·min$^{-1}$), then added 5 mL of 3 mol·L$^{-1}$ H$_2$SO$_4$ were added immediately to terminate the reaction. The remaining H$_2$O$_2$ was titrated by 0.01 mol·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 background control.

The polyphenol oxidase and peroxidase activities were determined by means of the pyrogallol method, and expressed by the production of gallocatechin for 3 h. For determination of polyphenol oxidase activity, 10 mL of 1% pyrogallol and 2 mL of 0.5% H$_2$O$_2$ were added to 1 g of soil in a 50 mL volumetric flask, and incubated at 30 °C for 3 h. The optical density of gallocatechin extracted by diethyl ether at 430 nm was measured to express the polyphenol oxidase activity. Samples without soil and without reaction substrate were used as background control. The activity of peroxidase was measured the same way without H$_2$O$_2$ as substrate.

The protease activity was analyzed by the ninhydrin colorimetric method. For determination of protease activity, 2 g of soil samples was were wetted by 0.5 ml of methylbenzene and incubated for 24 h at 30 °C with 10 ml 1% of gelatin solution. After incubation, 0.5 ml of 0.1 mol·L$^{-1}$ H$_2$SO$_4$ and 3 ml of 20% Na$_2$SO$_4$ were used to precipitate the proteins. The solutions of soil sample were centrifuged for 15 min (4000 rpm·min), then added 1 mL of 2% ninhydrin solution was added. The mixture was extracted by boiled water for the color.
Correlation between resistance and soil factors

development, and diluted to 50 mL, then the optical density at 500 nm was measured. Samples without soil were used as background control. Finally, protease activity was expressed in terms of NH$_2$-N per gram of dry soil for 24 h at 30 °C.

The cellulase activity was measured by the UV spectrophotometry method, and estimated through the production of glucose. For the determination of cellulase activity, 10 g of soil samples were wetted with 2 mL of methylbenzene, then 5 mL of acetate buffer (pH 5.5) and 5 mL of 1% carboxyl methyl cellulose (CMC) were added. The mixture was incubated at 37 °C for 72 h, then boiled to stop the reaction. Add 0.3 mg of KAl(SO$_4$)$_2$·12H$_2$O to precipitate residual cellulose. The filtrate was diluted with distilled water to 50 mL, 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 551 nm. Blanks were incubated without substrate or soils.

The invertase activity was measured using sucrose as substrate, the soils (10g) were mixed with 10 mL of 20% sucrose solution and 10 mL of phosphate buffer (pH 5.5) and incubated at 37 °C. After 24 h, the mixture was diluted with distilled water to a final volume of 50 mL. Mixed 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$_2$SO$_4$ (v/v 1:3) solutions were added. Used 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, 10 mL of 10% urea solution and 20 mL of citrate buffer (pH 6.7) were added to 10 g of soil samples before being incubated at 37°C for 24 h. After incubation, the soil samples were shaken for 30 min and filtrated. 1 mL of filtrate was poured into a 50 mL volumetric flask, then 4 mL of sodium phenate and 3 mL of sodium hypochlorite were added. After coloration, optical density at 578 nm 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 24 h. Samples without soil and without reaction substrate were used as background 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 37 °C. A sample of 10 g of soils and 1.5 mL of methylbenzene were mixed, and 10 mL of disodium phenyl phosphate (C$_6$H$_5$PO$_4$Na$_2$·2H$_2$O) and 10 mL 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 37 °C, 1 mL of filtrate was transferred into a 100 mL volumetric flask, 5 mL of borate buffer (pH 9.6) and 1 mL of Gibbs reagent were added to develop color. After diluting to 50 mL volume,
the color intensity was measured at 578 nm. 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 the final disease index of 28.75. XL was of the 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.

Table 1. Resistance of different eggplant cultivars to Verticillium wilt.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>2008-10-17</th>
<th>2008-10-22</th>
<th>2008-10-27</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Health</td>
<td>Disease</td>
<td>Health</td>
</tr>
<tr>
<td></td>
<td>incidence</td>
<td>incidence</td>
<td>disease</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>BL</td>
<td>95.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>L6</td>
<td>65.0</td>
<td>35.0</td>
<td>35.0</td>
</tr>
<tr>
<td>HL</td>
<td>95.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>HM</td>
<td>80.0</td>
<td>20.0</td>
<td>17.50</td>
</tr>
<tr>
<td>XW</td>
<td>95.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>BV</td>
<td>80.0</td>
<td>20.0</td>
<td>12.50</td>
</tr>
<tr>
<td>XL</td>
<td>35.00</td>
<td>65.00</td>
<td>45.00</td>
</tr>
<tr>
<td>LB</td>
<td>95.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>L3</td>
<td>75.0</td>
<td>25.00</td>
<td>25.00</td>
</tr>
<tr>
<td>L5</td>
<td>55.0</td>
<td>45.00</td>
<td>30.00</td>
</tr>
<tr>
<td>LY</td>
<td>100.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>
Correlation between resistance and soil factors

<table>
<thead>
<tr>
<th></th>
<th>TY</th>
<th>S. torvum</th>
<th>S. torvum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>90.00</td>
<td>100.00</td>
<td>100.00</td>
</tr>
<tr>
<td></td>
<td>10.00</td>
<td>0.00</td>
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<td></td>
<td>10.00</td>
<td>0.00</td>
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<tr>
<td></td>
<td>35.00</td>
<td>100.00</td>
<td>95.00</td>
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<td></td>
<td>65.00</td>
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<td></td>
<td>50.00</td>
<td>65.00</td>
<td>85.00</td>
</tr>
<tr>
<td></td>
<td>48.00</td>
<td>35.00</td>
<td>15.00</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>R</td>
<td>R</td>
</tr>
</tbody>
</table>

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.

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 quantities abundance of bacteria and actinomyces in rhizosphere soils of resistant cultivars were generally higher than in rhizosphere soil of other types, while the quantities abundance of fungi in soils of resistant types were lower than the average level (about 1.28x10⁶ cfu·g⁻¹) (Fig.1). The disease index of different resistant cultivars was significantly negatively correlated with the amount of actinomyces, 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 and actinomyces, 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 significantly 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 Table3 and Table 4 showed, 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 the significant differences were only showed among few cultivars, which had greater difference in resistance 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.

The results of Table 5 showed that, activities of catalase, polyphenol 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.
Correlation between resistance and soil factors

Figure 1 Comparison of microbial abundance in rhizosphere soil of different resistant 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 The correlations between disease incidence, and disease index of eggplant to Verticillium wilt and microbial abundance in rhizosphere soil of different resistant eggplant cultivars.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Catalase (0.01 mol·L^-1 KMnO₄·mL^-1·g^-1)</th>
<th>Polyphenol oxidase (mg 100g^-1·3h^-1)</th>
<th>Peroxidase (mg g^-1·3h^-1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK</td>
<td>1.38 (0.012) IL</td>
<td>39.09 (7.753) EF</td>
<td>0.96 (0.141) HH</td>
</tr>
<tr>
<td>BL</td>
<td>1.82 (0.012) IHJ</td>
<td>80.76 (7.283) bAABC</td>
<td>1.89 (0.200) eFEG</td>
</tr>
<tr>
<td>L6</td>
<td>1.67 (0.012) kK</td>
<td>67.60 (3.699) deCDE</td>
<td>2.37 (0.185) deCDE</td>
</tr>
</tbody>
</table>

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

* Significant at 0.05 probability level.
### Table 4 Variation analyses of hydrolyases activity in rhizosphere soil of different eggplant cultivars.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Protease</th>
<th>Cellulase</th>
<th>Urease</th>
<th>Invertase</th>
<th>Phosphatase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(NH$_4^+$-N mg·g$^{-1}$·24h$^{-1}$)</td>
<td>(mg·g$^{-1}$·72h$^{-1}$)</td>
<td>(NH$_4^+$-N mg·100g$^{-1}$·24h$^{-1}$)</td>
<td>(glucose mg·g$^{-1}$·24h$^{-1}$)</td>
<td>(phenol g·g$^{-1}$·24h$^{-1}$)</td>
</tr>
<tr>
<td>CK</td>
<td>0.108 (0.0045) fG</td>
<td>1.29 (0.044) fG</td>
<td>23.87 (2.124) fE</td>
<td>2.03 (0.026) fE</td>
<td>1.59 (0.238) eE</td>
</tr>
<tr>
<td>BL</td>
<td>0.197 (0.0078) eEF</td>
<td>1.91 (0.214) fG</td>
<td>44.07 (0.174) eD</td>
<td>4.56 (0.202) bBC</td>
<td>3.40 (0.255) bBC</td>
</tr>
<tr>
<td>L4</td>
<td>0.190 (0.0131) eF</td>
<td>2.21 (0.150) decD</td>
<td>38.81 (0.386) aAB</td>
<td>4.32 (0.113) dC</td>
<td>3.42 (0.225) bBC</td>
</tr>
<tr>
<td>HL</td>
<td>0.202 (0.0030) decDEF</td>
<td>1.90 (0.115) fG</td>
<td>35.67 (0.586) cdCD</td>
<td>4.55 (0.090) bBC</td>
<td>3.42 (0.358) bBC</td>
</tr>
<tr>
<td>HM</td>
<td>0.214 (0.0041) cdCD</td>
<td>2.25 (0.072) decD</td>
<td>44.79 (0.610) deD</td>
<td>4.35 (0.025) cdC</td>
<td>3.04 (0.255) cdCD</td>
</tr>
<tr>
<td>XW</td>
<td>0.212 (0.0045) cdCD</td>
<td>2.44 (0.124) cdCD</td>
<td>36.08 (0.872) cdCD</td>
<td>4.95 (0.090) aA</td>
<td>4.03 (0.207) aA</td>
</tr>
<tr>
<td>BV</td>
<td>0.201 (0.0062) dDEF</td>
<td>2.19 (0.105) dDEF</td>
<td>37.23 (1.226) bcBC</td>
<td>4.69 (0.063) bB</td>
<td>3.52 (0.137) bABC</td>
</tr>
<tr>
<td>XL</td>
<td>0.195 (0.0053) eF</td>
<td>2.17 (0.098) dDEF</td>
<td>35.41 (0.333) cdCD</td>
<td>4.53 (0.014) bBC</td>
<td>2.83 (0.166) dD</td>
</tr>
<tr>
<td>LB</td>
<td>0.198 (0.0078) eDEF</td>
<td>2.11 (0.100) dDEF</td>
<td>36.20 (0.462) cdD</td>
<td>4.30 (0.152) dC</td>
<td>4.00 (0.336) aA</td>
</tr>
<tr>
<td>L3</td>
<td>0.195 (0.0072) eF</td>
<td>2.15 (0.275) dDEF</td>
<td>34.83 (0.113) deD</td>
<td>4.53 (0.152) bBC</td>
<td>3.51 (0.137) bABC</td>
</tr>
<tr>
<td>L5</td>
<td>0.217 (0.0017) bcABC</td>
<td>2.06 (0.160) dDEF</td>
<td>35.75 (1.525) cD</td>
<td>4.53 (0.115) bBC</td>
<td>2.71 (0.209) dD</td>
</tr>
<tr>
<td>LY</td>
<td>0.215 (0.0078) eBC</td>
<td>3.55 (0.214) aA</td>
<td>38.34 (0.301) abAB</td>
<td>4.64 (0.104) bB</td>
<td>3.71 (0.079) abAB</td>
</tr>
<tr>
<td>TY</td>
<td>0.190 (0.0070) eF</td>
<td>1.86 (0.148) gE</td>
<td>37.25 (0.220) bcBC</td>
<td>3.89 (0.029) eD</td>
<td>3.07 (0.121) dCD</td>
</tr>
<tr>
<td>S. torvum</td>
<td>0.232 (0.0043) aA</td>
<td>2.54 (0.091) eBC</td>
<td>39.80 (0.586) aA</td>
<td>4.99 (0.080) aA</td>
<td>3.45 (0.264) bBC</td>
</tr>
<tr>
<td>S. tovu</td>
<td>0.227 (0.0062) abAB</td>
<td>2.86 (0.195) bB</td>
<td>38.98 (0.313) sAB</td>
<td>4.66 (0.142) bB</td>
<td>2.98 (0.129) dCD</td>
</tr>
</tbody>
</table>

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 5 The correlations between disease incidence, and disease index of eggplant to Verticillium wilt and enzymatic activities of rhizosphere soil of different resistant eggplant cultivars.
Correlation between resistance and soil factors

<table>
<thead>
<tr>
<th>Correlation coefficients</th>
<th>Catalase</th>
<th>Polyphenol oxidase</th>
<th>Peroxidase</th>
<th>Protease</th>
<th>Cellulase</th>
<th>Urease</th>
<th>Invertase</th>
<th>Acid phosphatase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disease incidence</td>
<td>0.702**</td>
<td>0.559</td>
<td>-0.483</td>
<td>-0.654</td>
<td>0.419</td>
<td>0.568</td>
<td>-0.370</td>
<td>0.129</td>
</tr>
<tr>
<td>Disease index</td>
<td>0.658*</td>
<td>0.602</td>
<td>-0.472</td>
<td>-0.596</td>
<td>-0.500</td>
<td>0.549</td>
<td>-0.358</td>
<td>0.260</td>
</tr>
</tbody>
</table>

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

3.4 Correlation between soil enzymic activities and microbial abundance

Table 6 showed 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 quantity abundance of bacteria, actinomycyes and fungi. Peroxidase showed extremely significant correlation with the ratios of B/F and A/F, and abundances of bacteria and actinomycyes, 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 phosphatase activity. There was no significant correlation between acid phosphatase activity and the abundance of the rhizosphere microorganisms.

<p>| Table 6 Correlations between soil enzymatic activities and the abundance of rhizosphere microorganisms of different eggplants cultivars. |
|---------------------------------------------------|----------|--------|----------|------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Correlation coefficients</th>
<th>B/F</th>
<th>A/F</th>
<th>Bacteria (B)</th>
<th>Actinomycyes (A)</th>
<th>Fungi (F)</th>
<th>Azotobacteria</th>
<th>Ammonifying bacteria</th>
<th>Nitrifying bacteria</th>
<th>Cellulose decomposition bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalase</td>
<td>0.869**</td>
<td>0.748**</td>
<td>0.921**</td>
<td>0.703**</td>
<td>0.596</td>
<td>0.284</td>
<td>0.199</td>
<td>0.160</td>
<td>0.043</td>
</tr>
<tr>
<td>Polyphenol oxidase</td>
<td>0.631</td>
<td>0.434</td>
<td>0.691**</td>
<td>0.663**</td>
<td>0.114</td>
<td>0.191</td>
<td>0.084</td>
<td>0.070</td>
<td>0.223</td>
</tr>
<tr>
<td>Peroxidase</td>
<td>0.793**</td>
<td>0.790**</td>
<td>0.738**</td>
<td>0.607**</td>
<td>0.528</td>
<td>0.258</td>
<td>0.124</td>
<td>0.030</td>
<td>0.077</td>
</tr>
<tr>
<td>Protease</td>
<td>0.868**</td>
<td>0.689**</td>
<td>0.827**</td>
<td>0.610**</td>
<td>0.611</td>
<td>0.362</td>
<td>0.238</td>
<td>0.358</td>
<td>0.072</td>
</tr>
<tr>
<td>Cellulase</td>
<td>0.703**</td>
<td>0.858**</td>
<td>0.567**</td>
<td>0.732**</td>
<td>0.567</td>
<td>0.105</td>
<td>0.087</td>
<td>0.020</td>
<td>0.128</td>
</tr>
<tr>
<td>Urease</td>
<td>0.604</td>
<td>0.543</td>
<td>0.546</td>
<td>0.415</td>
<td>0.497</td>
<td>0.296</td>
<td>0.006</td>
<td>-0.341</td>
<td>0.096</td>
</tr>
</tbody>
</table>

Comment [MF16]: Already mentioned above
Comment [MF17]: Already mentioned above
Correlation between resistance and soil factors

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>0.613</th>
<th>0.466</th>
<th>0.566</th>
<th>0.447</th>
<th>0.314</th>
<th>0.302</th>
<th>0.199</th>
<th>0.100</th>
<th>0.029</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid phosphatase</td>
<td>0.149</td>
<td>0.357</td>
<td>0.066</td>
<td>0.346</td>
<td>0.160</td>
<td>0.085</td>
<td>0.084</td>
<td>0.429</td>
<td>0.135</td>
</tr>
</tbody>
</table>

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 variety-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 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 and soil microorganisms quantity-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 quantity-abundance and soil enzyme activity under infection of V. dahliae, which was completely different with 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 were browned and rotted, the root activities (including the excretive, absorptive and enzymatic activities) were decrease sharply, which led 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 to root activity, so the regulation and control to rhizosphere soil remained at a high level. Especially, with the increase of B/F and A/F, and 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 was inhibited or slowed.

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.

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 (Söderberg 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 microbiological methods (only 1-4% of the microorganisms in soil could be cultured in medium, Amann et al., 1995).

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Correlation between resistance and soil factors


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Comment [MF22]: As there is two references called Zhou et al., 2001. Find a way to distinguish them in the text.
Note: Anonymous Reviewer