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Correlation between resistance of eggplant and defense-related enzymes and biochemical substances of leaves

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14 eggplant cultivars were inoculated by *Verticillium dahliae* to screen their resistance against verticillium wilt. The resistances were shown as the disease incidence and disease index, and eggplant cultivars were classified into resistant type (R), moderate resistant type (MR), tolerant type (T), moderate susceptible (MS) and susceptible type (S), according to the final disease index. To find out the correlated physiological and biochemical indexes for evaluating the resistance of eggplant to verticillium wilt, the activities of defense-related enzymes, and the contents of some biochemical substances of leaves were investigated. The results show that the activities of polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PAL) were significantly positively correlated with resistance (P < 0.01) and the resistance was significantly positively correlated with the activity of peroxidase (POD) and the content of total chlorophyll (P < 0.05), but significantly negatively correlated with the relative electric conductivity and the content of malondialdehyde (MDA) (P < 0.05). The correlations between resistance and catalase (CAT) activity, the contents of sucrose, soluble protein and proline, were not detected.

Key words: Verticillium wilt, eggplant, disease resistance, defense-related enzyme, biochemical substance.

INTRODUCTION

Verticillium wilt is a destructive disease of eggplant production, which is mainly caused by *Verticillium dahliae* Kleb. (Wang et al., 2005). The *V. dahliae* can survive in soil for more than 6 years, and infect many plant varieties, which made the disease hard to control all over the world (Ligoxigakis et al., 2002; Korolev et al., 2008; Berbegal et al., 2010). At present, the control of verticillium wilt mainly focus on grafting (Garibaldi et al., 2005), chemical fungicides (Rekanovic et al., 2007), biological control (Paplomatas et al., 2005; Elmer and Ferrandino

2009; Lang et al., 2011) and so on. Researches on the pathogenesis of verticillium wilt and plant-resistant mechanism of eggplant have no clear conclusion. Studies have shown that the plant-resistant mechanisms of eggplant against Verticillium wilt, similar to cotton, can be mainly divided into blocking theory and toxin theory (Zhang et al., 1990; Chen et al., 1996; Smit and Dubery, 1997). In allusion to these, the resistant mechanism of eggplant can be studied from the structure and biochemical resistance (Benhamon, 1995; Castoria et al., 1995). There are so many studies about the biochemical resistant index. Tang (2003) takes polyphenol oxidase (PPO), peroxidase (POD), phenylalanine ammonia lyase (PAL) and superoxide dismutase (SOD) as important factors of systemic acquired resistance of eggplant, which play important roles in induced resistance to verticillium wilt. Zhao et al. (2003) and Liu et al. (2003) have studied about the influence of V. dahliae on the POD, PAL and PPO activities of eggplant, and detected that the activities

Abbreviations: R, Resistant type; MR, moderate resistant type; T, tolerant type; MS, moderate susceptible; S, susceptible type; PPO, polyphenol oxidase; PAL, phenylalanine ammonia lyase; POD, peroxidase; CAT, catalase; MDA, malondialdehyde.

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are positively correlated with the resistances of eggplant cultivars to verticillium wilt. The decrease of the relative electric conductivity, the soluble sugar infiltration, the malondialdehyde (MDA) contain, and the increase of catalase (CAT), POD, PPO are recognized as the reason of resistance to verticillium wilt in grafted eggplant (Zheng et al., 2005). Researches about the defense-related enzymes and biochemical substances all focus on their changes in response to *V. dahliae*, instead of systematic and comprehensive analyses, so there is no unanimous specific indicator to differ resistance of different eggplant cultivars to verticillium wilt.

This paper took different resistant eggplant cultivars to measure the physiological and biochemical characters comprehensively and systematically, and define the relative parameters for disease resistance determination of eggplants. The conclusions can establish physiological and biochemical characters for selecting resistant eggplant sources, provide theoretical basis for disease control and resistant breeding, and indicate the research direction of the mechanism of resistance to verticillium wilt in eggplant.

MATERIALS AND METHODS

Plant material

The research group has selected eggplant sources, especially the main cultivars planted in North China. According to the results of previous experiment, 14 different resistant 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 (S. torvum) and Solanum tovu (S. tovu).

Pathogen

According to Koch's postulate, *V. dahliae* was isolated from roots and stems of diseased eggplants, and then inoculated to healthy eggplants after 20 days growth in potato dextrose agar (PDA) medium at 27°C; after the appearance of classic symptoms, the pathogens were separated again. The isolates were identified by the Mycology Laboratory of College of Plant Protection, Shenyang Agricultural University. After 20 days of growing at 27°C, in 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 1×10⁷ spores/ml with sterile distilled water, using hemocytometer.

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 germination was accelerated separately according to their germinating time; to make all cultivars be 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 soil, peat and horse manure (3:2:1), at the

2-leaf growth stage. The pots had been disinfected with $KMnO_4$ solution, while the complex substrates was mixed with carbendazim and sealed for 15 days. Each plant was inoculated with 10 ml of the V. dahliae spore (1×10 7 spores/ml) suspension at the 4-leaf growth stage. Each treatment had 15 plants and was repeated three times. The eggplants were cultured under conventional general management, and sampled 15 days after inoculation. The experiments were repeated three times.

Disease assessment

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

Disease incidence (%) = (Number of infected plants/Total number of plants) \times 100%

Disease index = Σ (Rating number × number of plants with the rating)/(Total number of plants × highest rating) ×100

Classification method of resistance type were: Resistant type (R), disease index (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.

Assay of defense-related enzyme and biochemical substance

One gram of leaf samples were homogenized in 4 ml of 0.1 mol/L sodium borate buffer (pH 8.8) with ice bath. The homogenates were centrifuged at 12,000 rpm for 20 min, and then the supernatants were used to analyze the enzyme activities of PPO, PAL, POD and CAT, and the content of MDA.

PPO activity was measured using the pyrogallol method. For determination of PPO activity, 100 µl of crude enzyme fluid and 4.9 mL of 0.02 mol/L catechol solution (using 0.1 mol/L sodium phosphate buffer, pH 6.8) were put into test tube. The mixture was bath at 35°C for 5 min, and then optical density at 398 nm was measured. Samples with buffer instead of crude enzyme fluid were used as control. One unit of PPO activity was presented as the change in absorbance of 0.01 at 398 nm for 1 g fresh weight per minute.

PAL activity was determined by the cinnamic acid colorimetric method. For determination of PAL activity, 0.5 ml of enzyme fluid, 0.5 ml of phenylalanine solution and 4 ml of distilled water were mixed, then water was bath at 38°C for 30 min. The reaction was stopped with ice bath, and the optical density was analyzed at 290 nm. One unit of PAL activity was represented as the change in absorbance of 0.01 for 1 g fresh weight per hour, equal to 1 μg cinnamic acid produced in 1 ml of reaction liquid. Samples with buffer instead of crude enzyme fluid were used as blank control.

POD activity was assayed by guaiac-based phenol method. The reaction mixtures contained 50 µl of supernatant and 4.8 ml of 18 mmol/L guaiacol (mixed with 0.1 mol/L sodium phosphate buffer, pH 5.8); after bathing at 35°C for 1 min, 150 µl of hydrogen peroxide (2.5%) was added to start the action. The optical density at 470 nm was measured for 5 min, and one unit of enzyme activity was defined by the change in absorbance of 0.1 for 1 g fresh weight per minute. Control experiments were performed in the absence of enzyme fluid.

CAT activity was analyzed using the ultraviolet spectrophotometry method. 0.6 ml of 30% hydrogen peroxide was made up to 100 ml as substrate, with 0.1 mol/L of sodium phosphate buffer (pH 7.0). 1 ml of substrate and 3.9 ml of distilled water were mixed, water bath at 25°C for 1 min, then 0.1 ml of enzyme fluid was added

to start the reaction. When the change of optical density at 240 nm was steady, it was measured for 5 min; a unit of CAT activity was defined by the decrease in absorbance of 0.0436 for 1 g fresh weight per minute.

Relative electric conductivity was measured by conductivity meter. 10 ml of distilled water was added into a test tube, and the electric conductivity was measured as S_0 . Then, 0.2 g of leaves were sampled by a hole punch and put into the tube. After shaking (120 rpm) for 3 h, the electric conductivity was measured again, as S_1 . Then, test tube was boiled for 20 min, and the final electric conductivity was analyzed, as S_2 .

Relative electric conductivity = $(S_1-S_0)/(S_2-S_0)$

Total chlorophyll content was measured as follows. For each treatment, 0.1 g fresh leaf was immersed in 10 ml mixture of acetone and ethanol (1:1) for 12 h in dark. The absorbance was read at 663 and 645 nm.

The content of total chlorophyll = $20.3 \times OD_{645} + 8.04OD_{663}$.

MDA level was assayed by thiobarbituric acid (TBA) method. 1.5 ml of enzyme fluid was mixed with 2.5 ml of 0.5% barbituric acid (prepared with 5% trichloroacetic acid), boiled for 15 min, then ice-bathed to room temperature. The mixture was centrifuged for 10 min (4000 rpm), measured the optical density of supernate at 450, 532 and 600 nm. Samples with buffer instead of crude enzyme fluid were used as blank control.

MDA (μ mol/L) = 6.45(A₅₃₂-A₆₀₀) - 0.56A₄₅₀

where, A is the difference in optical density between treatment and control.

The sucrose content was measured by the anthrone colorimetry method. The sucrose was extracted by distilled water and boiled for 30 min. The reaction mixture contained 0.5 ml of extract and 1.5 ml of distilled water, then 0.5 ml of anthrone (prepared using ethyl acetate) and 5 ml of 98% H_2SO_4 were added and boiled for 5 min. When the mixture was cooled, the optical density was measured at 630 nm. The content of sucrose was estimated by standard curve, and expressed as μ mol/g.

The soluble protein was detected by the Coomassie brilliant blue G250 method. The soluble protein was extracted by distilled water, 0.5 g leaf was homogenized in 5 ml of distilled water and centrifuged at 3000 rpm for 10 min. 0.1 ml of supernatants and 5 ml of Coomassie brilliant blue solution were mixed and the optical density was measured at 595 nm after 2 min standing. The protein content was expressed as mg per gram leaf.

The proline content was analyzed according to the method of ninhydrin in the acid condition. The leaves were sampled at same positions, 0.5 g leaf was homogenized in 5 ml of 3% sulfosalicylic acid, and extracted at 100°C for 10 min. After filtering, 2 ml of the extract, 2 ml of glacial acetic acid and 2 ml ninhydrin were mixed together, and boiled for 30 min to develop color. The mixture was extracted by toluene, and the optical density of supernatant was measured at 520 nm. The proline content was expressed as microgram per gram leaf. All these methods were according to Zhang (1992), Li (1999) and Hao and Liu (2001).

Statistical analysis

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

RESULTS

Resistance to verticillium wilt

As shown in Table 1, disease reaction varied with eggplant cultivars. According to the final disease index, *S. torvum* and *S. tovu* were resistant type; the disease incidence were 10 and 15% respectively, while the disease indexes were 10 and 12.5, which were significantly lower than for other cultivars; LY was the only moderate resistant (MR) type cultivar, with the disease index of 28.75; XL was susceptible type, the disease incidence and disease index were 100% and 71.75, which were the most serious of all cultivars. The disease indexes of the remaining cultivars ranged from 35.00 to 58.75, and were categorized as moderate susceptible (MS) and susceptible type (S).

Relationship between defense enzyme activities and resistance to verticillium wilt

Defense-related enzyme activities of leaves from different eggplant cultivars significantly varied with their resistances to verticillium wilt, and totally enhanced with the increased of resistance (Table 2). All the four kinds of defense enzyme activities of *S. torvum* and *S. tovu* were significantly higher than for other cultivars. The PPO and PAL activities of resistant type (*S. torvum* and *S. tovu*), moderate resistant type (LY) and susceptible type (XL) showed extremely significant differences, and POD activity among them was significant different but the differences of PPO, PAL and POD activities among other cultivars were not so clear. The CAT activity among cultivars showed differences, but no rule was found.

Relationship between biochemical substances and resistance to verticillium wilt

The relative electric conductivity and the content of MDA and proline decreased with the increase of resistance (Table 3). The content of MDA and proline, and the relative electric conductivity of leaves showed as: XL>LY> S. torvum, besides, extremely significant differences were found between the resistance of eggplant and the relative electric conductivity and content of MDA. The content of total chlorophyll showed as S. torvum >LY> XL, and the chlorophyll content of S. torvum was extremely higher than others but there was no significant difference between resistance and the total chlorophyll content of other cultivars. No significant variation was detected between resistance and contents of sucrose and soluble protein.

Correlation between resistance and physiological and biochemical characters

The resistance of different eggplants was correlated with

Table 1. Resistances of different eggplant cultivars to verticillium wilt.

| Cultivar | 2008-10-17 | | 2008-10 | 0-22 | 2008-10 | | |
|-----------|-----------------------|------------------|-----------------------|------------------|-----------------------|------------------|--------------------|
| | Disease incidence (%) | Disease index | Disease incidence (%) | Disease index | Disease incidence (%) | Disease index | Resistant types |
| BL | 5.00 | 5.00 | 50.00 | 23.75 | 65.00 | 46.25 | Т |
| L6 | 35.00 | 35.00 | 70.00 | 41.25 | 85.00 | 57.50 | MS |
| HL | 5.00 | 5.00 | 35.00 | 18.75 | 50.00 | 38.75 | Т |
| HM | 20.00 | 17.50 | 50.00 | 31.25 | 85.00 | 57.50 | MS |
| XW | 5.00 | 5.00 | 55.00 | 33.25 | 75.00 | 53.75 | MS |
| BV | 20.00 | 12.50 | 60.00 | 41.75 | 80.00 | 55.00 | MS |
| XL | 65.00 | 45.00 | 95.00 | 48.75 | 100.00 | 71.75 | S |
| LB | 5.00 | 5.00 | 35.00 | 16.25 | 60.00 | 36.25 | Т |
| L3 | 25.00 | 25.00 | 50.00 | 38.25 | 60.00 | 35.00 | Т |
| L5 | 45.00 | 30.00 | 65.00 | 40.00 | 75.00 | 58.75 | MS |
| LY | 0.00 | 0.00 | 15.00 | 15.00 | 50.00 | 28.75 | MR |
| TY | 10.00 | 10.00 | 65.00 | 48.00 | 65.00 | 50.00 | Т |
| S. torvum | 0.00 | 0.00 | 0.00 | 0.00 | 10.00 | 10.00 | R |
| S. tovu | 0.00 | 0.00 | 5.00 | 5.00 | 15.00 | 12.50 | R |

The "Resistant types" were classified according to 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.

Table 2. Changes of defense-related enzyme activity in different resistant eggplants after inoculation of V. dahliae.

| Cultivar | PPO activity (U/g-min) | PAL activity (U/g·h) | POD activity (U/g-min) | CAT activity (U/g-min) | |
|-----------|-------------------------|-----------------------|------------------------|------------------------|--|
| BL | 38.89 ^{bC} | 844.40 ^{dD} | 655.11 ^{gF} | 42.89 ^{deC} | |
| L6 | 25.56 ^{cdeDEF} | 851.73 ^{dD} | 665.83 ^{gEF} | 44.27 ^{dC} | |
| HL | 22.22 ^{efgEFG} | 842.53 ^{dD} | 702.61 ^{efD} | 55.96 ^{bAB} | |
| НМ | 23.33 ^{deDEF} | 694.53 ^{fF} | 586.11 ^{iHI} | 52.06 ^{cB} | |
| XW | 30.00 ^{cD} | 561.60 ^{hG} | 792.33 ^{aA} | 33.72 ^{gF} | |
| BV | 21.11 efgEFGH | 689.07 ^{fF} | 640.61 gFG | 59.63 ^{aA} | |
| XL | 14.44 ^{hH} | 400.53 ^{iH} | 557.00 ^{jl} | 27.75 ^{hG} | |
| LB | 17.22 ^{ghGH} | 896.40 ^{cC} | 697.83 ^{fDE} | 41.28 ^{deCD} | |
| L3 | 20.00 ^{efgFGH} | 667.47 ^{gF} | 727.83 ^{deCD} | 33.95 ^{gF} | |
| L5 | 25.56 ^{cdeDEF} | 418.40 ^{iH} | 614.17 ^{hGH} | 35.78 ^{gEF} | |
| LY | 43.33 ^{bC} | 915.60 ^{cC} | 731.22 ^{cdBC} | 36.47 ^{gDEF} | |
| TY | 28.33 ^{cdDE} | 749.73 ^{eE} | 469.89 ^{kJ} | 37.16 ^{fgDEF} | |
| S. torvum | 53.89 ^{aA} | 1024.80 ^{aA} | 774.78 ^{abAB} | 40.14 ^{efCDE} | |
| S. tovu | 50.00 ^{aAB} | 951.20 ^{bB} | 754.72 ^{bcBC} | 51.38 ^{cB} | |

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

the activities of defense-related enzymes and contents of chlorophyll (Table 4). The activities of PPO and PAL were significantly (P <0.01) negatively correlated with the disease index; the correlation coefficients were -0.753 and -0.795. While the POD activity was significantly (P <0.05) negatively correlated with the disease index, the correlation coefficient was -0.646. The disease index showed significant (P <0.05) positive correlation with the relative electric conductivity (r=0.637) and content of

biochemical substances, such as, MDA and total MDA (r=0.749). There was significant (P <0.01) negative correlation between the content of total chlorophyll and disease index (r=-0.657). The contents of sucrose, soluble protein and proline showed no correlation with disease index but the proline content was significantly (P <0.05) correlated with the disease incidence (r=0.534). The correlations between disease incidence and the physiological and biochemical characters were almost the

Table 3. Changes of biochemical substance content in different resistant eggplants after inoculation of *V. dahliae*.

| Cultivar | Relative electric conductivity (%) | Total chlorophyll (mg/g) | MDA (nmol/g) | Sucrose (µmol/g) | Soluble protein (mg/g) | Proline (ug/g) |
|-----------|------------------------------------|-----------------------------|-----------------------|---------------------------|--------------------------|------------------------|
| BL | 14.60 ^{aA} | 1.832 ^{cdBCD} | 3.838 ^{deCD} | 0.304 ^{efEFG} | 9.911 ^{dD} | 125.42 ^{hiGH} |
| L6 | 10.03 ^{eE} | 1.268 ^{fF} | 3.674 ^{efDE} | 0.430 ^{bcdABCDE} | 10.370 ^{cdD} | 328.80 ^{bB} |
| HL | 11.97 ^{dD} | 1.755 ^{cdCDE} | 4.322 ^{bB} | 0.506 ^{abAB} | 10.885 ^{cCD} | 139.32ghFGH |
| HM | 12.13 ^{dD} | 1.702 ^{cdeCDE} | 2.911 ^{jH} | 0.341 defDEFG | 10.565 ^{cdD} | 535.54 ^{aA} |
| XW | 13.55 ^{bABC} | 1.903 ^{cBC} | 3.211 ^{iG} | 0.376 ^{cdeCDEFG} | 11.135 ^{bcABCD} | 274.69 ^{dC} |
| BV | 11.83 ^{dD} | 1.646 ^{cdeCDEF} | 4.031 ^{cC} | 0.546 ^{aA} | 10.718 ^{cdCD} | 156.41 ^{fgEF} |
| XL | 14.72 ^{aA} | 1.626 ^{cdeCDEF} | 4.663 ^{aA} | 0.472 ^{abcABC} | 11.983 ^{abABC} | 307.19 ^{cB} |
| LB | 12.63 ^{cdBCD} | 1.669 ^{cdeCDEF} | 3.419 ^{ghFG} | 0.400 ^{cdBCDEF} | 10.523 ^{cdD} | 210.02 ^{eD} |
| L3 | 11.77 ^{dD} | 1.419 ^{efEF} | 3.273 ^{hiG} | 0.297 ^{efFG} | 10.592 ^{cdD} | 118.21 ^{iH} |
| L5 | 13.34 ^{bcBC} | 1.586 ^{deCDEF} | 3.901 ^{cdCD} | 0.457 ^{abcABCD} | 11.135 ^{bcABCD} | 224.76 ^{eD} |
| LY | 12.41 ^{dCD} | 1.664 ^{cdeCDEF} | 2.593 ^{kl} | 0.257 ^{fG} | 11.010 ^{cBCD} | 148.70 ^{gEFG} |
| TY | 13.76 ^{bAB} | 1.433 ^{efDEF} | 3.513 ^{fgEF} | 0.411 cdBCDEF | 10.968 ^{cBCD} | 127.59 ^{hiGH} |
| S. torvum | 10.00 ^{efE} | 2.374 ^{aA} | 2.194 ^{IJ} | 0.383 ^{cdeBCDEF} | 12.303 ^{aA} | 149.04 ^{gEFG} |
| S. tovu | 9.16 ^{gE} | 2.187 ^{bB} | 2.534 ^{kl} | 0.449 ^{bcABCD} | 12.164 ^{aAB} | 168.14 ^{fE} |

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

same as disease index.

DISCUSSION

Verticillium wilt is a major factor in eggplant yield loss, and researchers have paid attention to verticillium wilt resistance in eggplant. According to previous reports, there exist few eggplant varieties that exhibit resistance to verticillium wilt, thus to screen the resistant varieties for breeding and find out the resistant mechanism, we should study the correlate biochemical indicators. For this study, we chose to focus on the activities of defense-related enzymes and content of some biochemical substances.

Plants defend themselves against pathogen challenges by the activation of defence response pathways, and also develop complex antioxidant defence systems that respond to pathogen (Kawaoka et al., 2003; Lee and Hwang, 2005). The activities of defense-related enzyme system in plant are generally enhanced, with the aggravation of verticillium wilt, to defense the damage of active oxygen or oxyradical and strengthen the resistance (Caruso et al., 2001; Umesha, 2006). According to Kavitha and Umesha. (2008), in highly resistant tomato cultivars, the enzyme levels of POD and PPO are increased in comparison with highly susceptible tomato cultivars, and a significant (P < 0.05) correlation is observed between the degree of host resistance and the enzyme levels. However, although a clear-cut correlation between defense-related enzymes activities and plant resistance has been established, it is not yet clear about the rule, characteristic and mechanism of defense reaction and their relationship with different plant disease (Modafar et al., 2006; Armas et al., 2007; Santiago et al., 2009; Liu et al., 2009). This paper analyzed the PAL,

PPO, POD and CAT activities in leaves of different resistant eggplant cultivars induced by V. dahliae, and observed that both PAL and PPO activities were significantly positively correlated with the resistance (P<0.01), POD activity was significantly positively correlated (P<0.05), but the CAT was not related to resistance.

The osmoregulation of cell membrane is injured with the infection of V. dahliae, and the cell permeability increases, which leads to the exosmosis of cytoplasm. The injured level of cell can be judged by the relative electric conductivity (Zhou et al., 1998). MDA is a final production of the lipid peroxidation, and considered a general indicator of lipid peroxidation, so the content of MDA has direct correlation with the damage of cell (Chaoui et al., 1997; Radwana et al., 2006). Proline has been reported to be a response to stresses in a large number of species, which is thought to protect cells against damage, so the content of proline can be used to reflect the resistance of plant (Trovato et al., 2008; Chaum and Kirdmanee 2010). In this study, the relative electric conductivity and MDA content of leaves was significantly negatively related to the resistance of cultivar, so they can be taken as indicators for resistant identification of eggplant to verticillium wilt.

As early as 1935, Fleischer had put forth that there was a linear relationship between the rate of photosynthesis and the chlorophyll content in each case, and chlorophyll content could be used in initial screening of progenies in a breeding program for high photosynthetic rate (Fleischer, 1935; Buttery and Buzzell, 1977). The total chlorophyll contents of different non-heading Chinese cabbages are positively correlative with resistance to downy mildew, and the disease resistance can be judged by the color of cabbage (Shen and Huo 2009). In this paper, the content of total chlorophyll was significantly

Table 4. Correlation between the incidence of verticillium wilt and the physiological and biochemical characteristics of eggplants.

| Correlation coefficient | PPO | PAL | POD | CAT | Relative electric conductivity | Total chlorophyll | MDA | Sucrose | Soluble protein | Proline |
|-------------------------|----------|----------|---------|--------|--------------------------------|-------------------|---------|---------|-----------------|---------|
| Disease incidence | -0.801** | -0.742** | -0.582* | -0.185 | 0.608* | -0.732** | 0.694** | 0.150 | -0.485 | 0.534* |
| Disease index | -0.755** | -0.796** | -0.647* | -0.173 | 0.637* | -0.657** | 0.749** | 0.274 | -0.387 | 0.515 |

^{**,} Significant at 0.01 probability level; *, significant at 0.05 probability level.

positively correlated to the resistance of different eggplants to verticillium wilt.

Some biochemical substances are the energy materials of self-defense reaction, or products of injury, which have some correlation with the plant resistance (Deng 2006). There are some researches about the correlations between the resistance of plant and the content of sucrose and soluble protein, but no unified conclusion has been gotten (Zhang, 2002; Zhang et al., 2002). Our study shows no relationship between resistance of eggplant and the contents of sucrose and soluble protein. That maybe caused by the variant severity of verticillium wilt for different cultivars, at the moment of sampling.

In this paper, the relationship between the resistances of different eggplant cultivars to verticillium wilt, and physiological and biochemical characters, were systematically analyzed, and the identified indicators for resistance were defined. It is possible to use these indicators for the screening of verticillium wilt resistance levels of different eggplant cultivars, and it would help breeding programs to characterize promising varieties. Further studies are needed on relevant physiological and biological process, to reveal the mechanism of resistance to verticillium wilt of eggplant.

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