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Full Length Research Paper

Biological characteristics of grafted eggplant on tomato rootstocks

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Grafting eggplant (*Solanum melongena* L.) on tomato rootstock is an effective method to control Verticillium wilt (caused by *Verticillium dahliae*), a soil-borne disease. This study investigated the fruit quality and biological characteristics associated with disease resistance of eggplants grafted onto tomato rootstocks 'Lydl'. The plant height, stem diameter, leaf area and root volume of grafted eggplant were higher than those of non-grafted eggplant. And the increase rates were up as the date prolonged. The root active-absorbing area, chlorophyll content and peroxidase (POX) and phenylalanine ammonialyase (PAL) activities of grafted eggplants were higher than those of non-grafted eggplants, while the comparative conductivity and the free proline content decreased by 42.42 and 44.08%. Obviously, grafting on tomato rootstock improved the growth and development of eggplant. The soluble sugar, vitamin C and protein content in fruit of grafted plants were not lower than those in non-grafted ones. Fruit qualities were not significantly affected by grafting. At the all three sampling stages, grafted plants showed higher amounts of bacteria and actinomyces, total microbial population, and the ratios of actinomyces to fungi (A/F), bacteria to fungi (B/F) and microbial biomass (MBC, MBN, MBP, MQ), but lower fungi population. The activities of urease and catalase in rhizosphere were enhanced by grafting. Grafting could modify the plant growth and microbial characteristics which may enhance the resistance to Verticillium wilt.

Key words: Verticillium wilt, grafted eggplant, microbial property, microbial biomass.

INTRODUCTION

Verticillium wilt is one of the most destructive soil-borne diseases of eggplant (*Solanum melongena* L.). The pathogenic fungus *Verticillium dahliae* Kleb cause yellow-bronze leaf spots, vascular discoloration, yield losses, reduction of growth, fruit quality and plant death in eggplant (O'Brien, 1983; Bletsos et al., 1999). Continuous cultivation of sensitive plant in the same field increase soil-borne inoculum of this pathogen (Karagiannidis et al., 2002). About 30 to 40% decrease of the yield in Chinese eggplant production was ascribed to verticillium wilt (Wang et al., 2005). *V. dahliae* infects eggplants through the roots in the form of microsclerotia in the soil, and thus the control of verticillium wilt by chemical fungicides is difficult especially on infected plants. Preventive cultural practices are often recommended, including crop rotation, solarization, resistant cultivars, and grafting eggplant on resistant rootstocks. Among the methods used in controlling the soil-borne disease, grafting on resistant rootstocks has been highly effective (Lee, 1994; Oda, 1995). Numerous reports have suggested that grafting can successfully prevent the eggplant Verticillium wilt. While wild species eggplant have been demonstrated as potential rootstocks with high resistance to verticillium wilt (Alconero et al., 1988; Bletsos et al., 2003; Zhou et al., 1997), using resistant tomato (*Solanum lycopersicum lycoperscium*) rootstocks was also reported to be effective (Lockwood et al., 1970; Wang et al., 2003; Zhou

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and Wang, 2002).

We have studied means of preventing Verticillium wilt by using grafting for nearly twenty years. In our previous study, we found that grafting eggplant on wild eggplant rootstocks [*Solanum torvum* (*S. torvum*), *Solanum integrifolium* (*S. integrifolium*), CRP, *Solanum sisymbriiflium* (*S. sisymbriiflium*)] increased the disease resistance to Verticillium wilt. The total yield and fruit quality of eggplant were not affected by grafting, or even higher than those of non-grafted eggplant (Zhou et al., 1997). These conclusions were consistent with others (Lockwood, 1970; Yamakawa, 1982; Bletsos et al., 2003). The activities of phenylalanine ammonialyase (PAL), superoxide dismutase (SOD) and peroxidase (POX) which are closely related to plant disease resistance were enhanced in grafted eggplant onto wild eggplant rootstocks (Zhou et al., 1998a, 1998b); and the biological characteristics in rhizosphere such as the quantities and proportions of microbial populations and the soil enzyme activities showed a dynamic change (Li et al., 2007; Yin et al., 2008). In the beginning, the levels of microbial populations and the soil enzyme activities were lower than those of non-grafted eggplant, but as the growth prolong, both values in grafted plants were getting higher than those of non-grafted. Grafting on wild eggplant rootstocks is likely to regulate the plant growth and micro-environment, which are beneficial to increase resistance to verticillium wilt. Therefore, we supposed that the modification of scion growth and soil biological characteristics in rhizosphere by grafting are the main resistance mechanisms.

Compared to wild eggplant, tomato rootstock has some advantages that may be preferred in grafted eggplant production. In production, wild eggplant rootstock seedlings have some obstacles such as low germination rate, poor emergence and retarded early growth. Therefore, scheduling the optimum time for successfully grafted seedling production can be very challenging. Such problems may be overcomed by using selected tomato rootstocks. Until now, there are some reports on biological characteristics of grafted eggplant on wild eggplant rootstocks (Zhou et al., 1998a, 1998b; Li et al., 2007; Yin et al., 2008). The study on grafting onto tomato rootstock is mostly concerned with the survival rate and the effect of disease resistance. However, little is known about how grafting eggplant on tomato rootstock regulates the plant growth and soil biological characteristics in rhizosphere. After nearly ten years of study, we have selected ideal tomato rootstocks possessing good grafting compatibility and high disease resistance to Verticillium wilt. In former study, we have reported that eggplant grafted onto tomato rootstock exhibited markedly higher disease resistance and yield than non-grafted eggplant when challenged with *V. dahliae* (Liu et al., 2009). This work was undertaken to study the performance of plant growth, fruit quality and rhizosphere soil biological characteristics of grafted eggplant on tomato rootstock.

The results will provide biological base for disease resistance mechanisms, and conduce to extended application of grafting.

MATERIALS AND METHODS

Experimental design and plant materials

The commercial eggplant cultivar 'Xi'anlu' was used as scion, and a local tomato cultivar 'Lydl', with identified resistance to Verticillium wilt, was used as the rootstock. Non-grafted eggplant and tomato rootstock were used as control. The seed are supplied by Horticulture College, Shengyang Agricultural University. The greenhouse experiment was carried out in the Vegetable Crops Experimental Station at Shenyang Agricultural University. In the greenhouse, the temperatures were between 25 to 28°C in the daytime and about 18°C by night. Rootstock and scion seeds were sown into sterilized medium consisting of 3 perlite: 2 turf soil: 1 vermiculite (by volume) on March 17 and February 13, 2009, respectively. When the rootstock seedlings reached 4 to 5 leaf stage (April 23), grafting was carried out using the cleft method (Wang et al., 2003). After 17 days healing, the grafted eggplant seedlings were transplanted into 20.5 cm-diameter containers (about 4800 cm^3 growth media for each plant) with the same sterilized substrate mixture as used for seedling germination, and cultured in the greenhouse. Each plant was supplied with 500 ml Hoagland nutrient solution twice a week. Plants and soils were sampled at seedling stage (May 6), flowering (May 28) and fruit set stages (June 19) for the measurements of plant growth, microorganisms and soil parameters. The fruit quality parameters were measured on June 9 after harvesting.

The experimental design was randomized complete block. Each treatment was replicated 3 times with 10 plants.

Plant growth and physiological measurements

Plant height (cm), stem diameter (cm), total leaf area (dm^2) and root volume (ml) were measured at all three investigation stages. Root active-absorbing area (m^2), leaf chlorophyll content ($mg \cdot dm^{-2}$), leaf cell membrane permeability (%), leaf free proline content ($\mu g \cdot g^{-1}$), peroxidase (POX) activity ($\triangle A465 \cdot g^{-1} \cdot min^{-1}$ protein) for root and leaf and phenylalanine ammonialyase (PAL) activity ($\triangle A290 \cdot g^{-1} \cdot min^{-1}$ protein) for root and leaf were determined at the stage of flowering (May 28). For stem diameter, the plants were measured 5 cm above the soil surface. For leaf area and root volume, three plants randomly selected from each treatment were removed from the pots. The leaf area was measured by Portable Area Meter (Model L1-3000). And the roots were washed carefully in tap water followed by distilled water to remove substrate, put into the graduated flask contained with 100 ml of distilled water, and then the scale was recorded. The dispersion was considered as the root volume. Root active-absorbing area was measured by methylene blue colorimetry. Briefly, 0, 0.01, 0.02, 0.03, 0.04, 0.05 and 0.06 $mg \cdot ml^{-1}$ of methylene blue solution was measured; the absorbance in spectrophotometer at 660 nm to draw the standard curve of the absorbance against the concentration of methylene blue. Prepared three beakers contained with 100 ml 0.075 $mg \cdot ml^{-1}$ of methylene blue solution in each.

The root was placed into the first beaker for 1.5 min and then taken out, making sure that the residual water flows back to the first beaker. Afterwards, the root was put into the second breaker and this action was repeated for the third beaker. 1 ml from the third beaker was mixed with 9 ml distilled water. The absorbance was read at 660 nm, and the standard curve was checked to get the corresponding concentration of the methylene blue solution. Then,

the root active-absorbing area (m^2) was calculated as follows:

Root active-absorbing area (m^2) = [(0.075-C) × V] × 1.1

Where, C is the concentration of methylene blue solution getting from the standard curve; V is the volume of the solution in the third beaker.

Chlorophyll content was measured as follows. In brief, 0.1 g of fresh leaf was immersed in 10 ml of the mixture of acetone and ethanol (1:1, by ratio) for 12 h in the dark. The absorbance was then read at 652 nm. Result was reported as milligram of chlorophyll formed per liter. Cell membrane permeability was measured by relative electrical conductivity method (Hao and Liu, 2001). Briefly, 1 g of fresh leaf sample was minced and immerged into 20 ml of distilled water for 5 h at room temperature. Electrical conductivity values were measured by Conductmeter (DDS-11A). Then, the samples were boiling for 15 min, the electrical conductivity values were measured again. The relative electrical conductivity values were calculated by the ratio of pre-boiling to after boiling the samples. Free proline content was measured as indicated by Bates (1973). In brief, samples were homogenized with 10 ml of 3% sulphossalicilic acid as solvent. After being filtered to a tube, 2.5% ninhidrine solution and glacial acetic acid were added to the extract. The reaction mixtures were kept in a water bath at 100°C for 1 h to develop the colours. Soon after removal from the water bath, the tubes were cooled in ice bath and toluene was added to separate chromophore. The absorbance was read in spectrophotometer at 520 nm. Proline content was calculated by comparing the sample absorbance value with the standard proline curve in a concentration range from 0 to 25 $mg \cdot L^{-1}$.

For protein and enzyme extractions, 0.5 g samples were homogenized with 50 mM sodium phosphate buffer (pH 7.8) containing 1 mM EDTA Na_2 and 2% (w/v) polyvinylpolypyrrolidone (PVPP) at 4°C. The homogenates were centrifuged at 14,000 revolutions per minute for 40 min, and supernatants were used for determination of protein content and enzyme activity assays. Total soluble protein content of enzyme extracts was determined according to Bradford (1976) using bovine serum albumin as standard protein solution. Briefly, imbibed 1.0 ml of sample solution to a test tube, added 5 ml of coomassie bright blue G-250 reagent, and also kept standing at room temperature for 2 min; used blank tube as zero, and then made color matching at 595 nm. Record the absorbance and looked up concentration of sample solution on calibration curve. POX activity was determined spectrophotometrically by following the increase in absorbance at 470 nm. The reaction mixture contained 3.9 ml of guaiacol (20 mM), 1 ml of K-phosphate buffer (10 mM, pH 7.0) and 0.1 ml of enzyme extract in a total volume of 5 ml. The reaction was initiated by adding 1 ml of H_2O_2 (40 mM). POX activity was determined by measuring the oxidation of guaiacol in the presence of H_2O_2 (extinction coefficient, 26.6 mM^{-1} cm^{-1}) at 470 nm over a 2 min interval. For PAL activity, the reaction mixture (4 ml) is consisted of crude enzyme, 20 mM L-phenylalanine and 0.1 M Na-borate buffer (pH 8.8). Reaction was carried out in tubes, incubated at 30°C for 60 min and stopped by the addition of 35% (w/v) trifluoroacetic acid.

PAL activity was measured by monitoring the yield of cinnamic acid at 290 nm and the enzyme activity expressed as △A290 min^{-1} g^{-1} protein.

Fruit quality evaluation

Five fruits from different plants of each treatment were randomly sampled, and crushed using a mortar and pestle and juice extracted squeezing through cheesecloth to determine soluble sugar content (%), vitamin C (VC) content ($mg \cdot 100g^{-1}$) and protein content ($mg \cdot g^{-1}$). As coomassie brilliant blue G-250 when combined with protein shows a shift in its absorption maximum from 465 to 595 nm. The absorption at 595 nm is directly proportional to protein concentration (Hao and Liu, 2001). Soluble sugar content was measured by anthrone method (Takahashi et al., 1990). In brief, 0.2 g sample was added to 50 ml water and extracted in water at 20°C by shaking for 1 h. The homogenates were centrifuged at 4,000 revolutions per minute for 15 min. For reaction, 200 µL of the supernatant was added to 10 ml anthrone reagent, mixed and heated in a boiling water bath for 10 min, then cooled. The samples were measured using a photoelectric colorimeter with a 620 nm. The anthrone reagent was made by dissolving 0.2 g of anthrone in 100 ml of H_2SO_4. Vitamin C content was determined by spectrophotometeric method according to Hao and Liu (2001). In brief, 100 mg of pure VC was added to a 100 ml measuring flask and complete to the mark with $C_2H_2O_4$-EDTA to make the standard solution of VC (1 $mg \cdot mL^{-1}$). Then, 0, 0.1, 0.2, 0.4, 0.6 and 0.8 ml of this solution were transferred into 25 ml test tubes, mixed with 5 ml of the $C_2H_2O_4$-EDTA, 0.5 ml of HPO_3-$C_2H_4O_2$, 2 ml of 5% $(NH_4)_6Mo_7$ (w/v) and 1 ml of 0.05% H_2SO_4 (v/v), and made up to the mark with distilled water.

The reaction mixtures were kept in a water bath at 30ºC for 15 min, then the absorbance was measured in spectrophotometer at 760 nm. A graph of the absorbance against the concentration of the VC solutions as the standard curve was draw. Next, 5 g of the fruit sample was minced and blended with about 3 ml of $C_2H_2O_4$-EDTA, and $C_2H_2O_4$-EDTA was added to the mark of 50 ml, churned for 20 min, and then left for 30 min. The homogenates were centrifuged at 4,000 revolutions per minute for 15 min, the supernatant were fixed to 25 ml with $C_2H_2O_4$-EDTA. Then, the same procedure was repeated as drawing the standard curve. And measure the colour absorbance at 760 nm, compared with the standard calibration graph to calculate the concentration of VC. For each treatment, three replications were designed.

Rhizosphere microorganism

Surface growth media and growth media far from root system of the plants from each treatment were carefully removed. And the roots attached with little soil were sampled for preparing the soil suspensions. Three roots in each treatment were cut and put into conical flask with 90 ml distilled water in it. The roots were shaken for 30 min and were then taken out and washed 3 times with sterilized water to get the soil suspensions. Fungi, bacteria and actinomyces were then diluted and separated on Martin's medium, beef extract-peptone medium and ament Gao-surname 1 mark medium, respectively. Three species can be separated at the same time with 4 replications. After diluting, the microorganisms were incubated at 25°C and the growth of the colonies was observed and calculated. Soil mass was measured by drying method. A total of 50 ml uniform soil suspension was dried in evaporating dish to calculate the mass of soil in rhizosphere per ml and total amount of soil microorganisms in 1 g dry soil was calculated.

Rhizosphere soil microbial biomass evaluation

Growth media samples were collected with a shovel to a depth of 5 cm, within 5 cm from the base of the stem. Soil microbial biomass C (MBC), microbial biomass N (MBN) and microbial biomass P (MBP) of the plants were measured using the chloroform fumigation-extraction method (Brookes et al., 1982; Vance et al., 1987). Moist soil samples (20 g) from each treatment were fumigated with alcohol-free chloroform for 24 h in an evacuated desiccator. Fumigated and control (unfumigated) samples were extracted with 80 ml 0.5 M K_2SO_4 for MBC and MBN, 80 ml 0.5 M $NaHCO_3$ for MBP and shaken for 1 h on a reciprocating shaker. Extracts were filtered using Whatman No.42 filter papers and kept frozen at -18°C

Table 1. Effects of tomato rootstocks on plant growth of grafted eggplant.

Treatment	Plant height (cm)			Stem diameter (cm)			Leaf area (dm^2)			Root volume (ml)		
	May 5	May 28	June 19	May 5	May 28	June 19	May 5	May 28	June 19	May 5	May 28	June 19
Grafted	27.37	45.93[a]	63.75[a]	1.16	1.26[a]	1.55[a]	3.65[a]	18.99[a]	44.64[a]	12.30[a]	27.00[a]	30.00[a]
Non-grafted	26.27	42.50[b]	45.75[b]	0.98	1.05[b]	1.30[b]	2.47[b]	12.32[b]	17.66[b]	9.33[b]	11.00[b]	22.50[b]
Percent increase by grafting (%)	4.19	8.07	39.34	18.37	20.00	19.23	47.77	54.14	15.28	31.83	145.45	33.33

Means values in the same column followed by the same letter are not significantly different (P<0.05) according to DMRT.

until further analysis. Extractable C and N were analyzed using the TOC-V analyzer (Shimadzu Corporation, Kyoto, Japan) connected with a TN module. To determine total extractable P in the fumigated and unfumigated soils, 10 ml extracts were digested for about 1 h after adding 0.5 ml saturated $MgCl_2$ and 2 ml $HClO_4$ (70%, v/v) (Brookes et al., 1982). After digestion, the residue was boiled for 2 to 3 min with 10 ml 0.6 M HCl, cooled down to room temperature, and then diluted to 50 ml before colorimetric analysis. Inorganic P was analyzed by the ammonium molybdate ascorbic acid method described by Kalra and Maynard (1991).

Soil MBC, MBN, and MBP was calculated as the difference in extractable C, N, and P contents between the fumigated and control samples divided by a K_{EC} factor of 0.38 for MBC (Vance et al., 1987), a K_{EN} factor of 0.45 (Jenkinson, 1988) for MBN, and a K_{EP} factor of 0.40 for MBP (Brookes et al., 1982), respectively. The K_{EC}, K_{EN}, and K_{EP} factors were used to account for the efficiency of extraction for MBC, MBN and MBP, respectively. The microbial quotient (MQ) was calculated as the ratio of MBC to total organic carbon (TOC) (Anderson and Domsch, 1989).

Rhizosphere soil enzyme measurement

The activities of soil urease and soil catalase (CAT) for grafted eggplant, non-grafted eggplant and tomato rootstock were surveyed. Soil urease activity was determined by the method of Wang (2007). Briefly, 5 g of air-dried soil was mixed with 1.5 ml methylbenzene, 10 ml buffer with pH 6.7 and 5 ml 10% (w/v) urea solution in a reaction flask and incubated at 37°C for 24 h. This method is based on the determination of the NH_3^+ released and expressed as $mg \cdot NH_3\text{-}N \cdot g^{-1} \cdot h^{-1}$. Soil catalase (CAT) activity was determined by back-titrating residual H_2O_2 with $KMnO_4$ (Ya, 1988). 5 g of soil samples were added to 40 ml distilled water and 5 ml 0.3% hydrogen peroxide solution. The mixture was incubated at 37°C for 20 min. After incubation, the reaction was stopped by adding 5 ml of 1.5 M H_2SO_4. The mixture was filtered and titrated using 0.1 M $KMnO_4$. Consumed amount of 0.1 M $KMnO_4$ per gram of dried soil was used to express the CAT. The results were expressed as an average of three parallel determinations of the mixture of three duplicate soil samples.

Statistical analysis

Data was subjected to variance analysis using the Data Processing System software (Refine Information Tech. Co., Ltd., Beijing, China) and significant differences among treatments were determined by Duncan's Multiple Range Test (DMRT) at P<0.05.

RESULTS

Plant growth after grafting with tomato rootstock

The growth parameters of grafted eggplant such as plant height, stem diameter, leaf area and root volume were higher than those of non-grafted eggplant during the survey period (Table 1). The increase rates compared to control of plant height were increasing as the plant growth stages advanced, and reached its peak value (39.34%) on June 19. The leaf area and root volume of grafted treatment were significantly different than those of non-grafted eggplant in three samplings. And the increase rates of other indices in eggplant were increased from May 5 to 28, but none thereafter. Obviously, grafting with tomato rootstock improved the plant growth. Physiological characteristics were modified by grafting (Table 2). Compared with non-grafted eggplant, the root active-absorbing area and the chlorophyll content in grafted eggplant were significantly increased by 149.93 and 69.59%, respectively, while decreased in the cell membrane permeability and the free proline content by 42.42 and 44.08%, respectively. The activities of POX and PAL in root and leaf of grafted eggplant were higher than those of non-grafted eggplant.

Compared with the non-grafted eggplant treatment, the POX activities in root and leaf of grafted eggplant were higher up by 65.75 and 3.94%, respectively; and the PAL activities in root and leaf increased by 33.68 and 40.94%, respectively.

Performance of grafted eggplant on fruit quality

Fruit quality of eggplant was improved by grafting on tomato rootstock (Table 3). Compared with the non-grafted treatment, the contents of VC in

Table 2. Effects of tomato rootstocks on some physiological characteristics of eggplant plant compared with non-grafted.

Treatment	Root active-absorbing area (m^2)	Chlorophyll content ($mg \cdot dm^{-2}$)	Comparative conductivity (%)	Free proline content ($\mu g \cdot g^{-1}$)	POX activity ($\triangle A465 \cdot g^{-1} \cdot min^{-1}$ protein)		PAL activity ($\triangle A290 \cdot g^{-1} \cdot min^{-1}$ protein)	
					Root	Leaf	Root	Leaf
Grafted	35.79[a]	11.38[a]	26.20[b]	54.93[b]	55.94[a]	64.63[a]	23.30[a]	9.02[a]
Non-grafted	14.32[b]	6.71[b]	45.50a	98.23a	33.75[b]	62.18[b]	17.43[b]	6.40[b]
Percent increase by grafting (%)	149.93	69.60	-42.42	-44.08	65.75	3.94	33.68	40.94

Means values in the same column followed by the same letter are not significantly different (P<0.05) according to DMTR.

Table 3. Effects of tomato rootstock on some fruit quality parameters of eggplant.

Treatment	VC content ($mg \cdot 100\ g^{-1}$)	Soluble sugars content (%)	Protein content ($mg \cdot g^{-1}$)
Grafted	24.00[a]	3.17[a]	13.85[a]
Non-grafted	21.70[b]	3.17[a]	13.35[a]

Means values in the same column followed by the same letter are not significantly different (P<0.05) according to DMRT.

Table 4. Effects of tomato rootstock on the populations of bacteria (B), actinomycetes (A) and fungi (F) in rhizosphere on eggplant ($\times 10^5 \cdot g^{-1}$).

Date	Treament	Bacteria	Actinomycete (A)	Fungi (F)	Total number of microbe	B/F	A/F
May 6	Grafted	15251[b]	225[a]	2.24[b]	15478.24[b]	6808.48[a]	100.45[a]
	Non-grafted	9724[c]	92[c]	3.05[a]	9819.05[c]	3188.200[b]	30.16[b]
	Tomato rootstock	17834[a]	114[b]	2.32[b]	17950.32[a]	7687.07[a]	49.14[c]
May 28	Grafted	26162[a]	1991[a]	61.28[c]	28214.28[a]	426.93[a]	32.49[a]
	Non-grafted	19783[c]	708[c]	112.56[a]	20603.56[c]	175.76[c]	6.29[c]
	Tomato rootstock	25432[b]	1231[b]	78.78[b]	26741.78[b]	322.82[b]	15.63[b]
June 19	Grafted	32001[a]	2065[a]	86.77[c]	34152.77[a]	368.80[a]	23.80[a]
	Non-grafted	27357[c]	972[c]	189.64[a]	28518.64[c]	144.26[b]	5.13[b]
	Tomato rootstock	31467[b]	1966[b]	90.16[b]	33523.10[b]	349.25[a]	21.82[a]

Means values in the same column followed by the same letter are not significantly different (P<0.05) according to DMRT A: means actinomyces population; F: means fungal population; and B: means bacterial population.

grafted treatment were increased by 10.59%. There was no significant difference in soluble sugar and protein contents between grafted and non-grafted treatments.

Microbial composition in rhizosphere

Grafting can affect the microbial composition in rhizosphere (Table 4). Microbial population increased significantly with the growth of eggplant and reached maximum at fruit set stage. Bacteria were the most abundant microbes in rhizosphere, followed by actinomyces and fungi. Across the

Table 5. Effects of tomato rootstock on soil microbial biomass C, soil microbial biomass N and soil microbial biomass P in rhizosphere of eggplant.

Treatment	Soil microbial biomass C (μg·g^{-1})			Soil microbial biomass N (μg·g^{-1})			Soil microbial biomass P (μg·g^{-1})			Microbial quotient (MQ)		
	05-06	05-28	06-19	05-06	05-28	06-19	05-06	05-28	06-19	05-06	05-28	06-19
Grafted	110.47^{a}	216.57^{a}	220.88^{a}	0.041^{a}	0.065^{a}	0.063^{a}	8.05^{a}	20.13^{a}	20.20^{a}	0.016	0.034^{a}	0.030^{a}
Non-grafted	98.54^{b}	169.98c	169.17c	0.038^{b}	0.052^{b}	0.050^{b}	6.50^{b}	15.80c	14.90c	0.015	0.030^{b}	0.025^{b}
Tomato rootstock	105.00^{a}	200.34^{b}	209.6^{b}	0.04^{a}	0.063^{a}	0.060^{a}	8.00^{a}	18.60^{b}	18.00^{b}	0.015	0.033^{a}	0.030^{a}

Means values in the same column followed by the same letter are not significantly different (P<0.05) according to DMTR.

survey period, the populations of bacteria and actinomyces in grafted eggplant and tomato rootstock treatments were higher than those in non-grafted eggplant treatment, while the fungi population in grafted eggplant rhizosphere was significantly lower than that in non-grafted eggplant and rootstock treatments. The ratios of the fungi population to the total microbial population in non-grafted eggplant rhizosphere were higher than those of grafted eggplant treatment at all the stages. Grafting increased the population of bacteria and actinomyces, the total microbial population, and A/F and B/F, but decreased the fungi population.

Microbial biomass and microbial quotient in rhizosphere

The amounts of microbial biomass C (MBC), microbial biomass N (MBN) and microbial biomass P (MBP) in grafted and rootstock treatments were significantly higher than those in non-grafted treatment at all three sampling times (Table 5). The MBC in grafted eggplant treatment increased from May 6 to June 19, and reached the maximum (220.88 μg·g^{-1}) at fruit set stage. In contrast to MBC, MBN in grafted treatment increased progressively at first-two stages, but fell at the third stage. The MBP followed the same pattern as that of MBC, and reached the peak value on June 19 (20.20 μg·g^{-1}). The values of microbial quotient (MQ) in grafted treatment were increased from May 6 to 28, but none thereafter. It was similar to that of MBN. And the tomato rootstock treatment had the same level as the grafted eggplant treatment.

Soil enzyme activity in rhizosphere

On May 6, difference in urase activity in grafted treatment was not significant (Figure 1). However, from May 28 to June 19, the urase activities in grafted treatment were significantly increased by 21.66 and 27.25% compared with non-grafted treatment. The catalase activities in grafted treatment were markedly increased by 166.67, 162.55 and 166.00% for each survey stage, and reached the top value on May 28 (Figure 2). The activities in rootstock treatment were similar to the grafted one.

DISCUSSION

V. dahliae infects eggplant roots in the early stage of crop growth, eventually triggering a continuous cropping obstacle. At present, grafting is considered as an effective approach to preventing this soil-borne disease. Using tomato as rootstock demonstrated very high disease resistance (Zhou et al., 1997, 2001; Feng et al., 2000; Song, 2006). And holding intrinsic fruit quality is needed for applying grafting technique. Zhang (2004) showed that the levels of dry matter, protein, vitamin C and sugar of grafted eggplant were higher than those of non-grafted eggplant. In this research, the fruit quality parameter of eggplant was not significantly affected by rootstock except for VC content. The result was in agreement with Zhang (2004) and our previous studies on grafting on wild eggplant rootstock (Yang et al., 2005; Wang et al., 2003). Many reports suggested that grafting could change the intrinsic growth, especially in physiology. Kato (1989) has reported that root volume, root vigor increasing and releasing oxygen ability of chloroplast was enhanced by grafting eggplant onto wild eggplant rootstock. In this study, grafted eggplant had higher root active-absorbing area, chlorophyll content, POX and PAL activities than non-grafted eggplant, however, decrease for comparative conductivity and free proline content. After grafting, the root absorb-ability for nutrient and water and the transportation and distribution of assimilation products within plant were enhanced, and the intensities of photosynthesis and metabolism were enhanced.

Also, grafting could effectively prevent the pathogen infecting and spreading in plant by

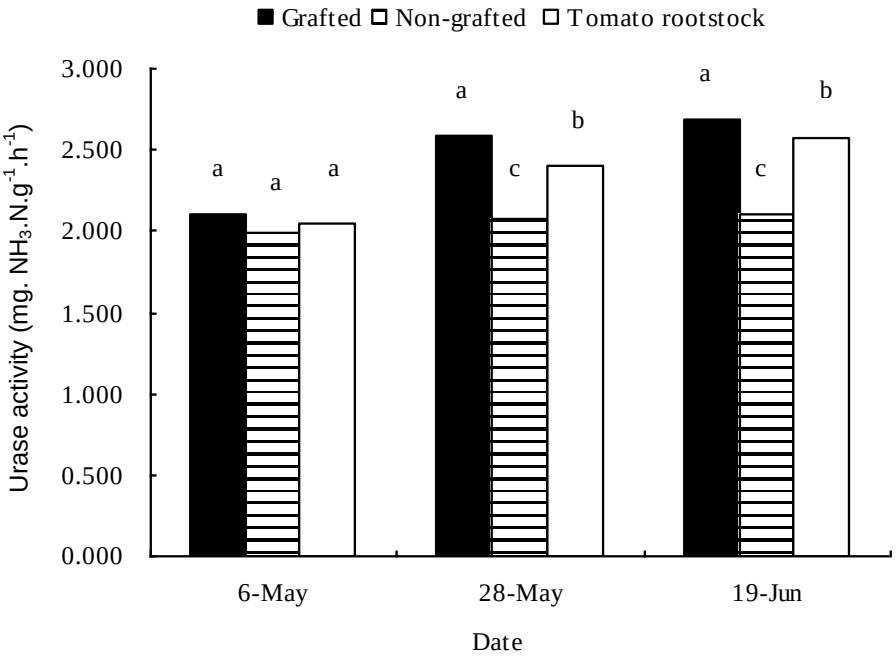

Figure 1. Effect of tomato rootstock on urease activity in soil of eggplant.

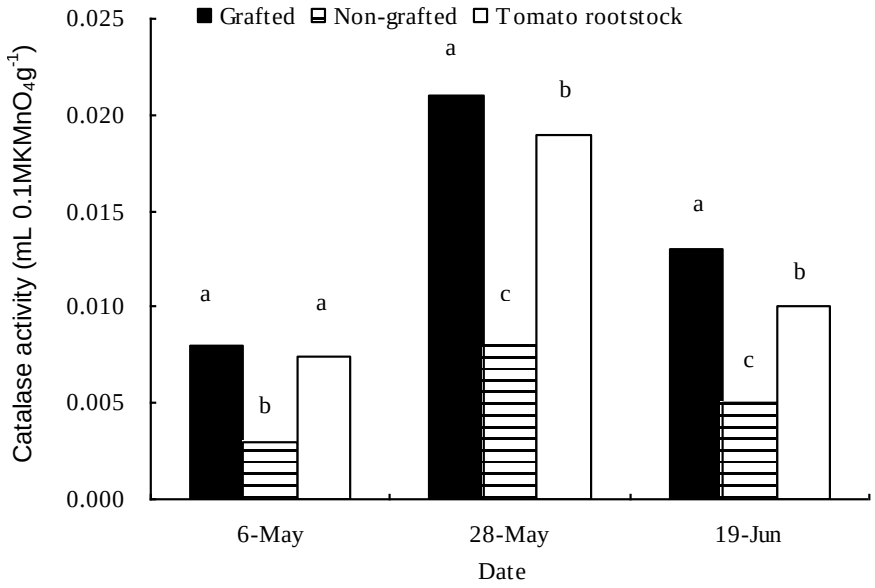

Figure 2. Effect of tomato rootstock on catalase activity in soil of eggplant.

decreasing the comparative conductivity and free proline content. POX and PAL activities, which were related to plant disease resistance, had distinct changes in root, when compared with leaf. It was likely that root could rapidly conduct defense reaction to cope with the stress in soil, aiming to protect the up-ground growth. Grafting modified the physiology of plant and it is the intrinsic reason for rejecting the Verticillium wilt. The regulation to biological characteristics in rhizosphere by grafting is another major reason for the perfect disease resistance. Lockwood (1970) reported that the soil which is abundant in microorganism has less risk for infecting soil-borne diseases than indigent soil. The occurrence of soil-borne diseases is positively associated with the population of fungi, while negative correlation with the population of bacteria and actinomyces (Han et al., 2006). Now, it is recognized that grafting not only modified the root system directly, but also induces metabolic alterations in scion

growth. The components of root exudates could be affected by grafting. And root exudates of grafted eggplant may be different from non-grafted eggplant and tomato rootstock. Root exudates represent one of the largest direct inputs of plant chemicals into the rhizosphere environment (Bertin et al., 2003). Allelochemicals in root exudates can influence plant growth through their impact on the rhizosphere microbial community. In this study, grafted eggplant on tomato rootstock presented better growth than non-grafted eggplant and tomato rootstock. Root exudates released by grafted plant may have some unique substances that were different from non-grafted eggplant and tomato rootstock. So it could be speculated that the components of root exudates from grafted eggplant had altered and induced grafted eggplant change the intrinsic microbial community structure to be beneficial to reduce the risk of soil-borne diseases.

The results corresponded to the studies on soybean and peanut (Li et al., 2006; Sun et al., 2001a). But how and which component of root exudates affect the microbial community structure needs to be determined. Microbial biomass can act as a labile source of nutrients for plants, a pathway for incorporation of organic matter into the soil, and a temporary sink for nutrients. Microbial biomass such as microbial biomass C (MBC), microbial biomass N (MBN) and microbial biomass P (MBP) are small but very dynamic and essential components of nutrient cycling in agro-ecosystems (Zhang et al., 2005). They could directly reflect the soil fertility. Generally, the higher level of soil microbial biomass, the better soil quality (Spedding et al., 2004). Xu et al. (2002) reported that soil MBC and MBN had positive correlation with the contents of total carbon and total nitrogen in soil. Soil MBP was correlated with the contents of MBC, MBN and total phosphorus in soil (He, 1997). In this study, soil microbial biomass in grafted treatment was higher than those in non-grafted treatment, and stayed at high level. It illuminated that the increase of soil microbial biomass after grafting had overcome the decrease of soil fertility and physical and chemical properties, finally, improved the quality and fertility in rhizosphere. Lynch and Panting (1980) reported that soil MBC increased along with root growth and increase of root density of the crop. In this study, a significant increase in MBC during stages of plant growth, no matter in grafted or non-grafted eggplant treatment was observed which was consistent with the study by Lynch and Panting (1980). The increase in root volume, as observed in this study, and possibly a greater amount of root exudates and readily metabolise C are perhaps the most influential factors that contribute to the higher MBC of grafted eggplant than that of non-grafted eggplant.

As fruit setting demands plenty of nitrogen, the soil nitrogen pool will be scarce at fruit set stage. It may be the reason for the decline in MBN on May 28. Shen et al. (1999) and Song et al. (2001) reported similar conclusions to ours in corn studies. The MBP followed the same trend as that of MBC. The reason discussed for the change in the amount of MBC may also be applicable to MBP. Microbial quotient (MQ) which is the ratio of MBC to TOC, provides a measure of organic matter dynamics (Anderson and Domsch, 1989). The increase of MQ in grafted eggplant treatment was an indicator for improving the content of organic matter in rhizosphere. Insam (1991) reported MQ increased along with the increase of yields of soybean, durra, rye and corn. Therefore, higher MQ in soil of grafted eggplant was one of the reasons of the yield of plant biomass increasing after grafting eggplant onto tomato rootstock. Enhancement of soil enzyme activities means the increase of biological activity of soil (Sun et al., 2001b). Soil urase directly participates in the transformation of nitrogenous organic matter. The urase activity can measure the ability of soil nitrogen supply (Hu et al., 2005). The catalase can decompose the peroxide in soil which is harmful to plant (Li et al., 2007). Tarafdar and Claassen (1988) reported that soil enzymes are associated with microorganisms' activity and microbial biomass.

In this research, the soil urase and catalase activities were enhanced by grafting with tomato rootstock. This result corresponded to the aforementioned research on the microbial biomass. From the results mentioned earlier, we can conclude that grafting eggplant on tomato rootstock regulates the state of plant growth and the soil condition to benefit the enhancement of disease resistance of eggplant. Disease resistance is not a simple character, but integrated characters composed of multi-mode and multi-factor. Actually, resistant mechanism is coordinated by multi-mechanism. No matter what resistant mechanism is, the speed and intensity of gene expression of the resistance gene determine the degree of disease resistance. But some inducing factors can accelerate the resistance gene expression. Grafting onto resistant rootstocks can adjust the plant growth and biological characteristics by the interaction of scion-rootstock and the root secondary metabolism to get high disease resistance.

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