Front. Agric. China 2008, 2(4): 441–445
DOI 10.1007/s11703-008-0066-7

RESEARCH ARTICLE

Qiongguang LIU, Landi HE, Jingyi ZHANG, Yutao WANG, Zhenzhong WANG

Reactive oxygen species activity in the interaction of rice with *Erwinia chrysanthemi* pv. *zeae*

Abstract Activities of reactive oxygen species (ROS) were investigated in the interaction between rice and *Erwinia chrysanthemi* pv. *zeae*. Results showed that $O_2 \cdot^-$, H_2O_2 and malondialdehyde (MDA) in resistant variety (128) had higher increases in activity compared to those in the susceptible variety (Texian 13) 24 hours after bacteria inoculation. The activities of superoxide dismutase (SOD) increased in 128 and Texian 13 twenty-four hours after inoculation and then decreased, but the SOD activity in 128 was found to be usually lower than that in Texian 13. The CAT activity in Texian 13 had two peaks at 24 h and 96 h after inoculation, while little change was seen in 128. In conclusion, ROS and its related enzymes could be correlated to rice resistance against *E. chrysanthemi* pv. *zeae*.

Keywords *Erwinia chrysanthemi* pv. *zeae*, active oxygen, resistance, rice

1 Introduction

Rice bacterial foot rot (*Erwinia chrysanthemi* pv. *zeae*) was a newly discovered disease affecting rice in the 1980s. It was widely found in areas of the South Yangtze River, China (Liu et al., 2003; Liu and Zeng, 1999). The symptoms of infected rice were blackening and rotting at the base of stems, curling of leaves, dead hearts, litter booting and white ears. In natural conditions, plants are always under threat from a large number of pathogens due to the lack of natural barriers in the plant structure and toxin production. In addition, plants also have a series of defensive mechanisms. Recent studies have found that reactive oxygen species (ROS) may play a key role in plant defense. ROS and nitric oxide (NO) have aroused great concern because they are widespread in organisms and have a wide variety of physiological functions (Bolwell, 1999). ROS have a direct anti-microbial function (Pang and Kuc, 1992), participating in cell lignification and the cross-linking of the lignification with hydroxyproline-rich acid glycoprotein, which is conducive to plant resistance to pathogen infection (Bradley et al., 1992). In addition, ROS is likely to act as a second messenger to regulate the expression of resistance genes, start the transcription of phytoalexin synthetic genes, and cause hypersensitive cell death (Foyer et al., 1997). Among them, H_2O_2, one of the ROS, has been drawing close attention in recent years.

Both superoxide dismutase (SOD) and catalase (CAT), which can remove ROS, are important defensive enzymes in plants. They participate in the metabolism of secondary materials of plant disease resistance such as lignin, phenolic compounds, phytoalexin and reactive oxidative species, and play an important role in plant defense response. It has been found in recent years that H_2O_2 and salicylic acid, as signal molecules, are involved in sending information about transmission of disease resistance, thereby, stimulating various disease resistance processes as acquired systems in plant cells. The result of formation and content variability of active oxygen as well as metabolic enzymes produced in the interaction between rice and *Magnaporthe grisea*, potato and *Phytophthora infestans*, indicate that H_2O_2 plays an important role in plant disease resistance. After pathogenic infection, the formation and accumulation of ROS may be one of the main reasons for starting membrane lipid peroxidation (Song et al., 1996), while the content variability of MDA may be an important indicator of the degree of membrane lipid peroxidation.

Biological characteristics and infection laws of *Erwinia chrysanthemi* pv. *zeae* have been investigated, but the physiology and biochemistry regarding the resistance

Translated from *Journal of Huazhong Agricultural University*, 2007, 26(4): 451–455 [译自: 华中农业大学学报]

Qiongguang LIU (✉), Jingyi ZHANG, Yutao WANG, Zhenzhong WANG (✉)
College of Natural Resources and Environment, South China Agricultural University, Guangzhou 510642, China
E-mail: qgliu@scau.edu.cn; zzwang@scau.edu.cn

Landi HE
Kunming Institute of Environmental Science, Yunnan Province, Kunming 650032, China

between the pathogen and rice varieties have rarely been reported. In this paper, we studied the activities of ROS and their protective enzymes produced after the interaction between *Erwinia chrysanthemi* pv. *zeae* and rice varieties.

2 Materials and methods

2.1 Rice varieties and pathogen

Both resistant variety (128) and susceptible variety (Texian 13) were provided by the Rice Research Institute of Guangdong Academy of Agricultural Sciences, China. The pathogen *Erwinia chrysanthemi* pv. *zeae* strain Ech7 was preserved in the Laboratory of Bacteria at South China Agricultural University.

2.2 Inoculation and rice sampling

After being cultivated in an ordinary bacterial medium plate at 30°C for 24 hours, Ech7 was diluted into suspensions at a concentration of 9×10^8 cfu·mL^{-1} in sterile water and used to inoculate trefoil stage rice by root dipping. The rice grew under greenhouse conditions. The roots from 8 rice plants were collected 12, 24, 48, 72, 96 and 120 hours after inoculation, rinsed in distilled water, soaked dry with filter paper, and weighed before determination of reactive oxygen species metabolism.

2.3 Extraction and determination of superoxide anion ($O_2\cdot^-$)

Five grams of tested samples were added into the phosphate buffer (PBS) of 50 mL (0.1 mol·L^{-1}, pH 7.0), and were ground into homogenate in ice bath, centrifuged at 12000 × g for 20 min at 4°C. 0.5 mL supernatant was taken, into which 0.5 mL PBS (50 mmol·L^{-1}, pH 7.8) and 1 mmol·L^{-1} hydroxylamine hydrochloride were added, shaken, and reacted for 1 h at 25°C. Then 1 mL sulfanilic acid (17 mmol·L^{-1}) and 1 mL α-naphthylamine (7 mmol·L^{-1}) were added and this was reacted for another 20 minutes at 25°C. Photoabsorption was determined at a wavelength of 530 nm. The standard curve was made according to the NO_2 method: 1 mL sulfanilic acid and 1 mL α-naphthylamine were added into 1 mL $NaNO_2$ at a series concentration of 0, 5, 10, 15, 20, 30, 40, 50 μmol·L^{-1}, and reacted for 20 min at 25°C before they were determined in $OD_{530\ nm}$.

2.4 Extraction and determination of H_2O_2

Five grams of samples were added into 50 mL ice acetone, then ground and centrifuged at 15000 × g for 10 min at 4°C. 1 mL supernatant was taken and 0.1 mL HCl of $TiCl_4$ (20%, V/V) and 0.2 mL strong aqua ammonia were added. After that, the product of the hyperoxide Ti compound was washed 5 times with acetone and dissolved in 3 mL H_2SO_4 (1 mol·L^{-1}). After the acetone was volatilized, extraction was determined in $OD_{410\ nm}$.

2.5 Extraction and determination of MDA

The extraction method was the same as described in Subsection 2.3. One mL supernatant was taken and added into a 4 mL TCA solution (20%, W/V) with 0.5% thiobarbituric acid (W/V), cooled down quickly after being boiled for 30 min, centrifuged at 4000 × g for 3 min, and finally determined in $OD_{532\ nm}$ and $OD_{560\ nm}$ of nonspecific absorption value. The contents of MDA were calculated with the constant 155 mmol·g^{-1}.

$$\mathrm{MDA} = \frac{(\mathrm{OD}_{532\,\mathrm{nm}} - \mathrm{OD}_{560\mathrm{nm}}) \times V_{\mathrm{lq}}}{1.55 \times 10^5 \times m},$$

where V_{lq} indicates the volume of the liquids, and m indicates the fresh weight of the rice root.

2.6 Extraction and determination of SOD

The extraction method was the same as that described in Subsection 2.3. The reaction system of enzyme activity determination involved using the 3 mL mixture mentioned above which was obtained and irradiated in fluorescent for 30 min after adding of 0.05 mL enzymes. At the same time, the mixture was irradiated without the enzyme, and acted as the instrumental zero. $OD_{560\ nm}$ was determined by protein-nucleic acid analyzer and enzyme quantity that could inhibit photochemical reduction at 50% NBT was set as an activity unit (U).

2.7 Extraction and determination of CAT

The extraction method was also the same as in Subsection 2.3 – using H_2O_2 as substrate which was diluted from 0.6 mL (30%) to 100 mL by PBS (0.05 mol·L^{-1}, pH7.0). The reaction system of enzyme activity determination was 1.0 mL substrate, 1.8 mL distilled water, and 0.2 mL enzyme. The substratum without any enzyme acted as a control to determine $OD_{560\ nm}$.

3 Results and analysis

3.1 $O_2\cdot^-$ changes in the interaction between rice and *E. chrysanthemi* pv. *zeae*

In the interaction between rice varieties and *E. chrysanthemi* pv. *zeae*, results showed that $O_2\cdot^-$ increased both in the resistant variety (128) and in the susceptible variety (Texian 13) after 12 h of inoculation. The first peak

appeared 24 h later and the production rate of $O_2 \cdot^-$ had a higher increase in the inoculated rice than in the non-inoculated rice. In addition, the content of $O_2 \cdot^-$ in resistant variety (128) was higher than that of susceptible variety (Texian 13) (Table 1). $O_2 \cdot^-$ production had a second peak 72 hours after inoculation. In this process, the yield rate of $O_2 \cdot^-$ in the resistant variety was always higher than the susceptible variety, which indicated that the overall growth rate of $O_2 \cdot^-$ was higher in the resistant variety than that in the susceptible variety.

3.2 Changes in H_2O_2 during the interaction of rice and *E. chrysanthemi* pv. *zeae*

The results indicated that H_2O_2 in the susceptible variety increased after inoculation with the pathogen. It reached a peak 48 hours after inoculation, and the highest value was measured at 96 hours. During the interaction of rice and *E. chrysanthemi* pv. *zeae*, H_2O_2 in the resistant rice variety was consistently higher than in the susceptible variety rice (Table 2). It showed that resistant rice roots produced H_2O_2 more quickly than susceptible roots. Simultaneously, it appeared that H_2O_2 was closely related to rice resistance in the interaction of rice and *E. chrysanthemi* pv. *zeae*.

3.3 MDA changes during the interaction of rice and *E. chrysanthemi* pv. *zeae*

Results showed that the MDA used to inoculate rice plants at 12 h were all lower than that in their controls. However, MDA was higher in the resistant variety than in the susceptible variety, and it reached its peak at 24 h. Thereafter, the MDA contents of both resistant and susceptible rice were all above the levels of their controls (Table 3). Furthermore, when infected with *E. chrysanthemi* pv. *zeae*, lipid reaction in the resistant variety occurred earlier and was higher than that in the susceptible variety. Therefore, it indicated that MDA content has a positive correlation with rice resistance.

3.4 Changes in SOD during the interaction of rice and *E. chrysanthemi* pv. *zeae*

Results showed that SOD activities had a positive correlation with rice resistance within 24 hours during the interaction of rice and *E. chrysanthemi* pv. *zeae*, however they were negatively correlated after 24 h. Twelve hours after inoculation, SOD activities in both resistant and susceptible varieties were lower than those of their controls and reached a peak at 24 h. Forty-eight hours

Table 1 Changes in $O_2 \cdot^-$ during the interaction of rice and *E. chrysanthemi* pv. *zeae* ($mmol \cdot g^{-1} \cdot min^{-1}$)

items	time after inoculation/h					
	12	24	48	72	96	120
128 control	0.0140 ± 0.0026 a	0.0264 ± 0.0005 c	0.0180 ± 0.0025 bc	0.0300 ± 0.0045 bc	0.0224 ± 0.0015 b	0.0286 ± 0.0007 c
128 treatment	0.0480 ± 0.0025 a	0.0619 ± 0.0016 a	0.0320 ± 0.0057 a	0.0480 ± 0.0024 a	0.0340 ± 0.0025 a	0.0433 ± 0.0028 a
Texian 13 control	0.0118 ± 0.0002 a	0.0196 ± 0.0012 d	0.0131 ± 0.0008 c	0.0266 ± 0.0024 c	0.0260 ± 0.0008 b	0.0386 ± 0.0006 ab
Texian 13 treatment	0.0280 ± 0.0015 a	0.0403 ± 0.0029 b	0.0246 ± 0.0004 ab	0.0380 ± 0.0012 b	0.0328 ± 0.0008 a	0.0370 ± 0.0015 b

Note: Values were the means of three repetitions; the data within a column followed by the same letter are not significantly different at 5% level. The same for Tables 2–5.

Table 2 Changes in H_2O_2 during the interaction of rice and *E. chrysanthemi* pv. *zeae* ($mmol \cdot g^{-1}$)

item	time after inoculation/h					
	24	48	72	96	120	144
128 control	0.0510 ± 0.0015 a	0.0584 ± 0.0040 ab	0.0493 ± 0.0021 ab	0.0680 ± 0.0035 ab	0.0490 ± 0.0019 a	0.0528 ± 0.0026 ab
128 treatment	0.0564 ± 0.0040 a	0.0692 ± 0.0060 a	0.0542 ± 0.0030 a	0.0733 ± 0.0020 a	0.0660 ± 0.0070 a	0.0668 ± 0.0080 a
Texian 13 control	0.0440 ± 0.0062 a	0.0500 ± 0.0058 b	0.0369 ± 0.0016 c	0.0562 ± 0.0049 c	0.0576 ± 0.0020 a	0.0469 ± 0.0011 b
Texian 13 treatment	0.0480 ± 0.0036 a	0.0591 ± 0.0042 ab	0.0418 ± 0.0035 bc	0.0615 ± 0.0013 bc	0.0580 ± 0.0090 a	0.0620 ± 0.0046 ab

Table 3 Changes in MDA during the interaction of rice and *E. chrysanthemi* pv. *zeae* ($mmol \cdot g^{-1}$)

item	time after inoculation/h					
	24	48	72	96	120	144
128 control	0.1126 ± 0.0083 a	0.1185 ± 0.0028 b	0.0606 ± 0.0040 ab	0.0530 ± 0.0021 b	0.0515 ± 0.0012 bc	0.0712 ± 0.0013 a
128 treatment	0.1052 ± 0.0132 ab	0.1352 ± 0.0044 a	0.0660 ± 0.0012 a	0.0860 ± 0.0060 a	0.0700 ± 0.0059 a	0.0613 ± 0.0023 a
Texian 13 control	0.0953 ± 0.0042 ab	0.0993 ± 0.0058 c	0.0477 ± 0.0018 c	0.0434 ± 0.0008 b	0.0454 ± 0.0017 c	0.0540 ± 0.0023 c
Texian 13 treatment	0.0790 ± 0.0049 b	0.0930 ± 0.0040 c	0.0540 ± 0.0021 bc	0.0780 ± 0.0042 a	0.0590 ± 0.0015 b	0.0499 ± 0.0010 c

after rice inoculation, the activity of SOD in the susceptible variety was higher than that in the resistant variety (Table 4).

3.5 CAT changes during the rice and *E. chrysanthemi* pv. *zeae* interaction

It appeared that CAT activity in the susceptible variety was higher than that in its control and reached the highest value after 24 hours of inoculation. However, CAT activity in the resistant variety was lower than that in its control. In contrast, after 48 hours, CAT activity in the resistant variety was higher than that in its control, while that in the susceptible variety was lower. Throughout the interaction between rice and pathogen, it was obvious that CAT activity in the resistant variety was basically unchanged or changed little (Table 5).

4 Discussion

Both $O_2\cdot^-$ and H_2O_2 are types of reactive oxygen species. Foreign scholars utilize oligogalacturonic acid elicitor from fungal cell walls to induce soybean cells to produce H_2O_2 (Aprostoll et al., 1989). In addition, oligogalacturonic acid elicitor from citrus pectin can also induce soybean cells to produce H_2O_2 (Legndre et al., 1993). The rice plant can also be induced to produce H_2O_2 by some inducers such as glucan, galactose and aldehyde from pathogenic fungi (Svalhein and Robertsen, 1993). H_2O_2 can be involved directly in the esterification, cell wall lignification, and structural protein oxidation cross-linking of the host cell because of certain unique characteristics. Furthermore, H_2O_2 can not only induce phytoalexin accumulation to some extent or directly have some toxic effects on pathogens, but it can also transfer resistance information against disease as a signal molecule.

$O_2\cdot^-$ was first reported to participate in the hypersensitive reaction between potato tuber and *Phytophthora infestans* in 1980 (Doke, 1983). From then on, $O_2\cdot^-$ was also reported to participate in the reactions of many plant pathogenic fungi, bacteria, viruses and nematodes. Therefore, it can be considered that $O_2\cdot^-$, as well as its metabolic enzymes (such as SOD, etc.), play an important role in plant disease resistance. The results of our study showed that amounts of $O_2\cdot^-$ and H_2O_2 in the resistant rice variety (128) and the susceptible rice variety (Texian 13) were different. Regardless of being either of resistant or susceptible variety, when inoculated for 12 hours, both $O_2\cdot^-$ and H_2O_2 increased and reached their first peak after 24 hours. However, the growth rates and increments of both $O_2\cdot^-$ and H_2O_2 in the resistant variety were significantly higher than those in the susceptible variety. $O_2\cdot^-$ in the resistant variety increased rapidly before 24 h, while in the susceptible variety it increased after 48 h. The $O_2\cdot^-$ production rate in the resistant variety was higher than that in the susceptible variety at all times within 96 h. After inoculation, the H_2O_2 content in the resistant variety rose and reached the first peak at 48 h, with the second peak at 96 h. The H_2O_2 content in the resistant variety was higher than that in the susceptible variety during the whole process. These results indicate that the resistant variety responded quickly to bacterial infection and the production of $O_2\cdot^-$ and H_2O_2 was fruitful *in vivo*, while the susceptible variety responded slowly to bacterial infection, producing relatively small amounts of $O_2\cdot^-$ and H_2O_2 *in vivo*.

SOD and CAT are both important defensive enzyme systems, which function in getting rid of reactive oxygen in plants. The former can generate H_2O_2 from excessive $O_2\cdot^-$ in plants, while the latter can turn H_2O_2 into H_2O. Our results showed that, in the case of SOD activity, when the rice was inoculated with *Erwinia chrysanthemi* pv. *zeae* at 12 hours, the specific activities of SOD in the resistant and susceptible varieties were both lower than those in

Table 4 Changes in SOD during the interaction of rice and *E. chrysanthemi* pv. *zeae* ($U\cdot g^{-1}\cdot 30\ min^{-1}$)

item	time after inoculation/h					
	24	48	72	96	120	144
128 control	0.3256 ± 0.0108 a	0.2400 ± 0.0058 c	0.2470 ± 0.0067 ab	0.2100 ± 0.0208 c	0.3050 ± 0.0090 b	0.3119 ± 0.0081 c
128 treatment	0.2114 ± 0.0205 b	0.3929 ± 0.0060 a	0.2330 ± 0.0060 b	0.2500 ± 0.0058 b	0.2035 ± 0.0084 d	0.3607 ± 0.0077 b
Texian 13 control	0.2560 ± 0.0038 b	0.1600 ± 0.0100 d	0.2108 ± 0.0051 c	0.1570 ± 0.0046 d	0.2570 ± 0.0133 c	0.2540 ± 0.0066 d
Texian 13 treatment	0.1331 ± 0.0192 c	0.3250 ± 0.0097 b	0.2551 ± 0.0037 a	0.3079 ± 0.0016 a	0.3398 ± 0.0107 a	0.3490 ± 0.0030 a

Table 5 Changes in CAT during the interaction of rice and *E. chrysanthemi* pv. *zeae* ($U\cdot g^{-1}\cdot min^{-1}$)

item	time after inoculation/h					
	24	48	72	96	120	144
128 control	0.0200 ± 0.0012 a	0.0215 ± 0.0009 ab	0.0138 ± 0.0010 bc	0.0192 ± 0.0050 a	0.0147 ± 0.0004 b	0.0170 ± 0.0006 b
128 treatment	0.0203 ± 0.0009 a	0.0212 ± 0.0029 ab	0.0159 ± 0.0006 ab	0.0197 ± 0.0010 a	0.0154 ± 0.0005 b	0.0133 ± 0.0008 b
Texian 13 control	0.0139 ± 0.0005 b	0.0262 ± 0.0006 a	0.0170 ± 0.0006 a	0.0146 ± 0.0012 a	0.0158 ± 0.0005 b	0.0220 ± 0.0021 a
Texian 13 treatment	0.0161 ± 0.0010 b	0.0192 ± 0.0008 b	0.0127 ± 0.0005 c	0.0161 ± 0.0010 a	0.0299 ± 0.0006 a	0.0161 ± 0.0010 b

their controls. But after 24 hours, the SOD activities began to increase, and reached their peaks, while SOD specific activity in the susceptible variety was higher than the resistant variety at 48 hours after inoculation. It indicated that SOD activity was positively correlated with rice resistance within 24 hours when infected with bacteria. In the case of CAT, its activity in the susceptible variety was higher than that in its control during the 12 hours after inoculation and reached the first peak at 24 hours after inoculation, while that in the resistant variety was lower than that in its control. On the contrary, forty-eight hours after inoculation, CAT activity in the resistant variety was higher than that in its control and lower in the susceptible variety. During their study of *Xanthomonas oryzae* pv. *oryzae* and rice interaction, Zeng et al. (1999) discovered that SOD activity in rice leaves decreased while POD activity increased, and the range of enzyme activities in the resistant varieties was higher than that seen in the susceptible varieties. Cao and Xiao (2002) suggested that both SOD and POD activities in rice leaves were enhanced significantly after being infected with *Xanthomonas oryzae* pv. *oryzae*. The reasons could be that, on one hand, different plant disease systems may have different resistant reaction processes. It is impossible that all the disease resistant systems are related to $O_2\cdot^-$ and SOD, etc., and even though they are, there might be differences to some extent. Production mechanisms for $O_2\cdot^-$ may be different in different varieties and production mechanisms for ROS also differs between different plants. Even in the same plant, there might exist several ways to produce ROS, which may eventually lead to different production rates of $O_2\cdot^-$ (Guo and Li, 2000). On the other hand, there is a lack of persuasiveness in speculating on the actions of $O_2\cdot^-$ and H_2O_2 only by analyzing the changes in one to two enzymes in plant resistance reactions. Based on the results from this study, it can be determined that the activities of H_2O_2, CAT and SOD are closely related with the resistance to *Erwinia chrysanthemi* pv. *zeae*.

Pathogen infection can give rise to lipid peroxidation of plants (Keppler and Novacky, 1987). The active oxygen (mainly $O_2\cdot^-$) accumulation in plants can directly attack the unsaturated fatty acids of the membrane system, thereby initiating the peroxidation of lipids (Adam et al., 1989). MDA is the main product of lipid peroxidation and its content generally can be referred to as the degree of lipid peroxidation. In the study of the interaction between rice and *Magnaporthe grisea*, Ge et al. (1998) found that MDA remarkably increased after the rice was infected with *Magnaporthe grisea*, having a positive relation with rice resistance. The results of our study also showed that MDA in the resistant variety was almost higher than that in susceptible variety after rice was inoculated with *Erwinia chrysanthemi* pv. *zeae*. It could be explained that the onset of membrane lipid peroxidation in the resistant variety reaction was earlier than in the susceptible variety reaction, thus leading to higher levels than in the susceptible variety. MDA content was found to have a positive relation to the rice resistance.

Acknowledgements This research was supported by the Key Technologies Research and Development Program of Guangdong Province, China (No. E99032).

References

Adam A, Farkas T, Somiyal G, Hevesi M, Kiraly Z (1989). Consequence of $O_2\cdot^-$ generation during a bacteria induced hypersensitive reaction in tobacco: Deterioration of membrane lipids. Phytopathology, 45: 259–266

Aprostoll A, Heinstein P F, Low P S (1989). Rapid stimulation of an oxidative burst during elicitation of cultured plant cells. Plant Physiol, 90: 109–116

Bolwell G P (1999). Role of active oxygen species and NO in plant defence responses. Cure Opin in Plant Biol, (2): 287–294

Bradley D J, Kjelloom P, Lamb C J (1992). Elicitor induced and wound induced oxidative cross-linking of a praline rich plant-cell wall protein: a novel, rapid defense responses. Cell, 70(1): 21–30

Cao C S, Xiao Y S (2002). Activity changes of some enzymes in hybrid rice combinations with different resistance after inoculation with *Xanthomonas campestris* pv. *oryzae*. Acta Phytopathologica Sinica, 32(2): 181 (in Chinese)

Doke N (1983). Generation of superoxide anion by potato tuber protoplast during the hypersensitive response hyphal wall component of *Phytophthora infestans* and specific inhibition of the reaction by suppressions of hypersensitivity. Plant Pathol, 23: 359–367

Foyer C H, Lopez-delgado H, Dat J F, Scott I M (1997). Hydrogen peroxide and glutathione-associated mechanisms of acclamatory stress tolerance and signaling. Physiol Plant, 100: 241–254

Ge X C, Song F M, Zheng Z (1998). Changes in antioxidant content and activity of ascorbic acid peroxidase in the interaction between rice and *Magnaporthe grisea*. Journal of Zhejiang Agricultural University, 24(4): 387–391 (in Chinese)

Guo Z J, Li D B (2000). Active oxygen species in plant disease resistance. Acta Botanica Sinica, 42(9): 881–891 (in Chinese)

Keppler L D, Novacky A (1987). The initiation of membrane lipid peroxidation during bacteria induced hypersensitive reaction. Plant Patho, 30: 233

Legndre L, Rueter S, Heinstein P F, Low P S (1993). Characterization of the oligogalacturonide included oxidative burst in cultured soybean cells. Plant Physiol, 102: 233–240

Liu Q G, Wang Z H, Chen Y T, Ou W M, Ou Z K (2003). Reinfection and latent infection of *Erwinia chrysanthemi* pv. *zeae* in rice. Journal of Plant Protection, 30(3): 333–334 (in Chinese)

Liu Q G, Zeng X M (1999). Pathogenicity and biological characteristics of *Erwinia chrysanthemi* pv. *zeae* in Guangdong Province. Journal of South China Agricultural University, 20(1): 9–12 (in Chinese)

Pang M, Kuc J (1992). Peroxidase-generated hydrogen peroxide as a source of antifungal activity *in vitro* and on tobacco leaf disks. Phytopathology, 82: 696–699

Song F M, Zheng Z, Ge X C (1996). Role of active oxygen and membrane lipid peroxidation in plant-pathogen interactions. Plant Physiology Communications, 32(5): 377–385 (in Chinese)

Svalhein O, Robertsen B (1993). Elicitation of H_2O_2 production in cucumber hypocotyl segments by oligo 1,4-α-D-galacturonides and an loigo-β-glucan preparation from cell wall of *Phytophthora megasperma* f. sp. *glycinea*. Physiol Plant, 88: 675–683

Zeng F H, Cao C S, Yi K, Wu Y X (1999). Relationship between active oxygen metabolism in the leaves non-induced and systemic acquired resistance. Journal of Xiangtan Normal University, 20(3): 89–93 (in Chinese)