African Journal of Biotechnology Vol. 10(21), pp. 11442-11449, 21 September, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.618
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Enhancement of defense responses by oligandrin against *Botrytis cinerea* in tomatoes

Bing-gan Lou[1], Ai-ying Wang[1], Chai Lin[1], Tong Xu[1], Xiao-dong Zheng[2]*

[1]Institute of Biotechnology, Zhejiang University, Hangzhou 310029, China.
[2]College of Food Science and Biosystem Engineering, Zhejiang University, Hangzhou 310029, China.

Accepted 29 August, 2011

Oligandrin is an elicitin-like protein with a molecular mass of ~10 kDa secreted by *Pythium oligandrum*. Here, the effect of oligandrin on defense response against *Botrytis cinerea* in tomato leaves is reported. Tomato seedlings were pretreated with 5 ml oligandrin (10 µg/ml) by root submerging and then inoculated with *B. cinerea*. Disease severity was subsequently evaluated and compared with the control. Results indicate that oligandrin pretreatment reduced disease index by 78.6% on day 7 after inoculation. On day 3 after inoculation, oligandrin pretreatment caused up-regulation of peroxidases (POD), polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PAL) in leaves by 20.0, 5.56 and 32.88%, compared with inoculation without oligandrin pretreatment, respectively. On day 5 after inoculation, POD, PPO and PAL were up-regulated by 46.24, 32.61 and 57.14%, respectively. 24 h after the treatment with oligandrin, the expression of pathogenesis-related protein (PRs) genes, PR-2a (extracellular β-1,3-glucanase) and PR-3a (extracellular chitinase), were up-regulated by 7.75 fold and 4.56 fold in tomato leaves, compared with the control, respectively. The expression of LeERF2, a member of ethylene-dependent signaling pathway, was also significantly elevated by 7.41 fold. At the same time, the expression of ethylene receptor homologue PR-6 protein was also induced. These results indicate that oligandrin can induce resistance to *B. cinerea* in tomatoes, and the induction of resistance involves the activation of the ethylene-dependent signaling pathway. Oligandrin is potentially useful for gray mould prevention in tomato crop.

Key words: *Botrytis cinerea*, induced resistance, oligandrin, resistance related enzymes.

INTRODUCTION

Oligandrin is an elicitin-like protein with a molecular mass of ~10 kDa secreted by *Pythium oligandrum* (Picard et al., 2000a). It triggers cytological and biochemical modification in tomatoes thereby induces resistance to *Phytophthora parasitica* and *Fusarium oxysporum* f. sp. *radicis-lycopersici* (Picard et al., 2000a; Benhamou et al., 2001). An application of oligandrin on sectioned petioles of tobacco plants induced phloem protein accumulation in the mature sieve tubes in the leaves, and depressed phytoplasma infection (Lherminier et al., 2003). Similarly, application of oligandrin to grapevine roots could protect leaves against *Botrytis cinerea* (Mohamed et al., 2007). Oligandrin-treated plants reacted more rapidly and more efficiently to *F. oxysporum* f. sp. *radicis-lycopersici* invasion, mainly through the massive accumulation of fungitoxic compounds at sites of attempted pathogen penetration (Picard et al., 2000a). However, the mechanism by which oligandrin induces resistance to *B. cinerea* still remains to be elucidated.

Tomatoes (*Solanum lycopersicum*) are cultivated around the world, and gray mould disease caused by *B. cinerea* is one of its most prominent, widespread, and destructive diseases. Control of gray mould in the crop mostly depends on fungicides. However, the use of chemicals has led to customer's concerns about health and environmental risks, as well as the development of resistance to common fungicides (Chen et al, 2006; Yourman et al., 1999). A natural, safe, and effective alternative to the disease control without the use of fungicides may involve the use of elicitors of plant defense.

*Corresponding author. E-mail: xdzheng@zju.edu.cn. bglou@zju.edu.cn.

Previous studies showed that peroxidase (POD), polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PAL) are key enzymes conferring disease resistance in plants (Hammerschmidt et al., 1982; Campos-Vargas and Saltveit, 2002; Picard et al., 2000b). These enzymes are involved in local defense (Ryals et al., 1996). The expression of pathogenesis-related (PR) genes was taken as a marker of the systemic acquired resistance (SAR) (Ward et al., 1991). The expression and activities of PR proteins such as chitinases and β-1,3-glucanase were related to the defense response (Bezier et al., 2002; Robert et al., 2002). Ethylene (ET), the plant growth regulators jasmonic acid (JA) and salicylic acid (SA) play an important role in plant defense responses to pathogens (Glazebrook, 2005).

In this study, activities of POD, PPO and PAL in leaves of tomato plants treated with oligandrin were analyzed. We also analyzed the expression of the pathogenesis-related proteins (PRs) genes, ethylene-dependent signaling pathway genes and JA-responsive gene for the basic PR-6 protein after treatment with oligandrin. The relative effectiveness of oligandrin in reducing *B. cinerea* infection in a greenhouse was evaluated to determine whether an oligandrin dipping is a feasible approach for controlling gray mould.

MATERIALS AND METHODS

Tomato (*S. lycopersicum* HeZuo903) plants were grown to the four leaf stage in plastic pots containing peat and perlite (1:1 mixture v/v) obtained from greenhouse of Zhejiang University. Oligandrin from *P. oligandrum* strain RCU1 was provided by Institute of Biotechnology, Zhejiang University, Hangzhou, China.

Pathogen inoculum

B. cinerea fq01 was isolated from decayed tomato fruit and maintained on potato dextrose agar (PDA). Conidial suspensions of the pathogen were prepared by flooding the 14-day-old culture dishes incubated at 25℃ with sterile distilled water containing 0.05% Tween 80. The spore suspension was adjusted to 1.0×10^5 spores/ml by counting with a haemacytometer. Gray mould disease was evaluated on a scale of 0 to 5 with: 0 = no necrosis, leaf area is completely healthy; 1= less than 5% of the leaf area are with symptoms; 2= less than 15% of the leaf area is with symptoms; 3= less than 25% of the leaf area is with symptoms; 4= less than 50% of leaf area; 5= more than 50% of leaf area is covered with Botrytis symptoms. A disease index was calculated as the sum of the areas of the four leaves using the formula:

$$\text{Disease index} = (\sum^{5}_{i=0} N_i \times i) / (5 \times \sum^{5}_{i=0} N_i)$$

Where, i is the severity (0 to 5, the highest level is 5), and N_i are the number of leaves with a severity of i.

Effect of oligandrin on tomato gray mould

Seedlings were assigned to nine treatment groups and one control group. During early expansion of the fourth leaf seedlings were treated with oligandrin solution at 5 µg/ml (for groups 1 to 3), 10 µg/ml (for groups 4 to 6) and 20 µg/ml (for groups 7 to 9) by root submerging. Leaves were then inoculated by spraying (5 ml/plant) with conidial solution (1.0×10^5 conidia/ml) at 4, 24 and 48 h after oligandrin treatment. The control group was first treated with autoclaved water and then inoculated with conidia. Seedlings were planted in pot and random block design was employed, with each group containing 10 plants, with three replications. After spraying, plants were bagged to maintain moisture for 12 h. Humidity was maintained at >97% and temperature was 20 to 25℃. Disease index was assessed on day 7.

Enzyme activities analysis

Seedlings were assigned to three treatment groups and one control group. The inoculation method was as aforementioned. Based on the results from the effect of oligandrin on tomato gray mould, seedlings were treated with 10 µg/ml oligandrin, and sprayed with gray mould conidia 4 h after oligandrin treatment. Group 1 was treated with oligandrin and then sprayed with gray mould, group 2 was treated with water in place of oligandrin, and then sprayed with gray mould conidia, group 3 was treated with oligandrin without inoculation, while the control group was treated with water without inoculation. Each group contained 20 plants, with three replications. On the first, second, third, fifth, seventh and ninth day after inoculation with *B. cinerea*, tomato leaves were collected, respectively, and stored at -80℃.

All enzyme extraction procedures were conducted at 4℃. For PAL, 1 g of the tissue was ground and mixed with 2 ml extracting buffer [0.2 M boric acid buffer containing 10% (w/v) polyviny poly-pyrolidone (PVPP), 1 mM EDTA, and 50 mM β-mercaptoethanol, pH 8.8]. For PPO and POD, 2 g of the tissue were ground with 10 ml of 100 mM sodium phosphate buffer (pH 6.4) containing 0.2 g of PVPP, homogenized and centrifuged at 12,000 × *g* at 4℃ for 30 min, and the supernatant was collected.

For the PAL assay (Assis et al., 2001), 300 µL of the extract was incubated with 1 ml 0.02 M L-phenylalanine and 2 ml of the PAL extracting buffer at 24℃ for 2 min, and absorbance at 290 nm was measured in an ultraviolet spectrophotometer. The PAL activity was expressed as U290, where 1 U290 = 0.01ΔOD290/mg protein/min. For the PPO assay (Galeazzi et al., 1981), extract (100 µL) was incubated with 2 ml 0.05 M phosphate buffer (pH 7.0) and 0.5 ml of 0.5 M catechol at 24℃ for 2 min, and the absorbance at 398 nm was measured with an ultraviolet spectrophotometer. The PPO activity was expressed as U_{398}, where $U_{398} = 0.01\Delta OD_{398}$/mg protein/min. More also, POD activity was determined using guaiacol as substrate (Ippolito et al., 2000). The reaction mixture consisted of 0.1 ml of crude extract and 2 ml of guaiacol (8 mM, in 100 mM sodium phosphate buffer, pH 6.4), incubated for 30 min at 30℃. The increase in absorbance at 460 nm was measured after 1 ml H_2O_2 (24 mM) was added. The activities of POD were expressed as U_{460}, where $U_{460} = 0.01\Delta OD_{460}$/mg protein/min. Protein content was determined using Nanodrop ND-1000 Spectrophotometer at OD280.

Determination of malondialdehyde content

Experimental design and sample collection time were the same as earlier mentioned. In brief, samples were collected on days 1, 2, 3, 5, 7 and 9 after inoculation with gray mould. The oldest leaves from one of the plants in each replicate were collected. The leaves were sheared, weighed and stored in liquid nitrogen until processing. The malondialdehyde content was determined according to Zhang and Fan (2007). The component was precipitated from the supernatant of the extract with 0.5% thiobarbituric acid and the suspension was boiled for 10 min, then immediately cooled down on ice. After centrifugation at 8,000 ×*g* for 10 min, the absorbance was

measured at 532 and 600 nm.

RNA isolation

Experimental design was the same as described in enzyme activity analysis. Samples were collected at 2, 4, 8, 24 and 48 h after oligandrin treatment. Lower leaves were collected from three plants in each group. Total RNA was extracted using TRIzol Reagent (Invitrogen, USA) according to the manufacturer's instructions. Isolated RNA was dissolved in 20 µl of RNase free H_2O, quantified by spectrophotometry and stored at -80°C.

cDNA preparation

2 µg of total RNA extracted from tomato leaves were reverse transcribed with reverse cDNA kit (Invitrogen) and stored at −80°C.

Real-time PCR

Each reaction (20 µl) contained Bio-Rad Super SYBR 10 µl Green mix, 1.0 µL cDNA (20 ng), 0.2 µl each primer (10 µM) and 8.6 µl ddH_2O. All PCRs were carried out in duplicate. The PCR mixes were dispensed into ABI optical reaction tubes (Applied Biosystems, Foster City, CA, USA). The reaction tubes were centrifuged at 3000 rpm for 10 s to settle the reaction mixtures to the bottom of the wells. The PCR was carried out with an iCycler PCR system (BIO-RAD). Thermal cycles consisted of an initial hold for 3 min followed by 40 cycles of amplification at 95°C for 30 s, 52°C for 30 s, and 72°C for 40 s. All statistical analyses were performed with $2^{-\Delta\Delta Ct}$ methods (Livak and Schmittgen, 2001). The primers were (5'-3'): β-actin: R 5'-ccaccttaatcttcatgctgct-3', F 5'-acattgtgctcagtggtggtact-3'. PR-2a: R 5'- ccagaatgacaaacaaaaggaa -3', F 5'- aaacaagcagccaaaatacaac -3'. PR-3a: R 5'- gctcgttcgtagttagattgg -3', F 5'- gttttggtactgctggtgatg -3'. LeERF2: R 5'- gagaagctcgtaaaatcaggggtaag -3', F 5'-ctccaaagctccatcgagccaccgc -3' PR-6: R 5'-tccttgctcacctacttgttcttgg -3', F 5'-ttccttatgctgtggaaatactttg -3'. Primers used for PR-6 are as published by Gadea (1996). Primers used for β-actin, PR-2a and PR-3a are as published by Wang et al. (2009). Primers used for LeERF2 are as published by Tournier et al. (2003).

Statistical analysis

All statistical analyses were performed with SPSS 10.0. Each experiment had three replicates and all experiments were performed three times and yielded similar results. Measurements from all the replicates were combined and the treatment effects were analyzed.

RESULTS

Effect of oligandrin on tomato gray mould

Results show that the optimal concentration of oligandrin was 10 µg/ml and optimal treatment time was 4 h before inoculation with *B. cinerea* (Table 1). The oligandrin treatment had significant protective and curative effect against gray mould in tomato leaves. An application of oligandrin to tomato roots 4 h before the inoculation decreased disease index to 20.5% (Figure 1), compared with 95.6% in the control. These results indicate that the root treatment with oligandrin (10µg/ml) 4 h before inoculation with *B. cinerea* was the appropriate inducing conditions. Tomato seedlings were treated with distilled water (DW), oligandrin alone, *B. cinerea* alone and oligandrin plus *B. cinerea* (O+B).

Oligandrin treatment decreased the accumulation of malondialdehyde

Malondialdehyde (MDA) is a biomarker of oxidative stress in cells. After mock treatment with water, the control group did not show any significant MDA content change, neither did the group treated with oligandrin alone (Table 2). However, the oligandrin group showed lower MDA content (0.3 to 0.9 nmol/mg lower) compared with the control group. On days 3 and 5, MDA contents in the oligandrin group were 11.3 and 12.5% lower than those in the control group. The group inoculated with *B. cinerea* without oligandrin pretreatment had increased leaf MDA; from 5.3 to 12.2 nmol/mg. The group inoculated after oligandrin treatment also showed an increase in leaf MDA content, but to a lesser extent (0.6 to 3.0 nmol/mg lower) compared with that inoculated with *B. cinerea* alone. On the 3rd and 5th day, the oligandrin treatment 24 h before inoculation reduced leaf MDA contents by 26.1 and 25.0%, respectively, compared with the group inoculated alone without root treatment.

Oligandrin treatment increased activities of antioxidant enzymes in leaves

After application of oligandrin, the activities of leaf POD, PPO and PAL increased gradually, and peaked on day 5. The peak values were 50.3, 45.6 and 69.2% higher than those in the control group, respectively (Figure 2). Also, after inoculation with *B. cinerea* following oligandrin application, activities of leaf POD, PPO and PAL also increased. The activity of POD reached its peak on day 3, and activities of PPO and PAL reached their peaks on day 5. The peak values of POD, PPO and PAL in the group treated with oligandrin and inoculated with *B. cinerea* were 20.0, 32.61 and 57.14% higher than those in the group treated with oligandrin alone (Figure 2). These results show that root treatment with oligandrin stimulated the antioxidant enzyme activities in leaves.

Oligandrin promoted the expression of PR genes

The root treatment with oligandrin (10 µg/ml) enhanced the expression of PR-2a and PR-3a genes in leaves (Figure 3). Transcripts of the PR-2a gene reached its maximal level at 24 h in oligandrin treated tomato plants, about 7.75-fold higher than that in control plants at the same time point (Figure 3A). The control tomato plants showed low mRNA levels of PR-2a throughout the experi-

Table 1. Protective effect of oligandrin against tomato gray mold in greenhouse.

Oligandrin (μg/ml)	Relative protection effect (%)		
	4 h before inoculation	24 h before inoculation	48 h before inoculation
5	61.2[c]	40.5[b]	55.3[b]
10	78.6[a]	50.2[a]	63.5[a]
20	70.5[b]	56.3[a]	61.8[a]

The data are the average of three replicates. The different letters in the same column indicate a significant difference at 5% level.

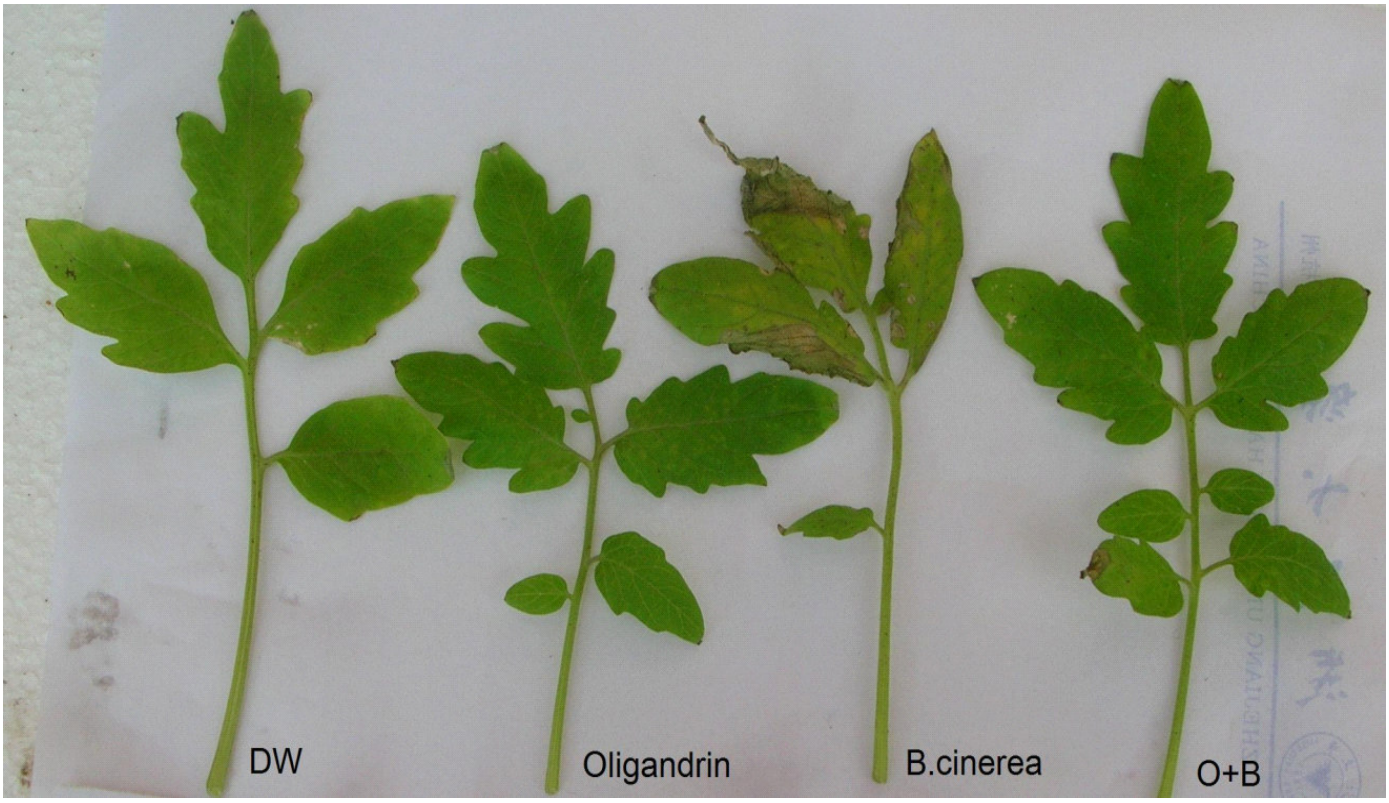

Figure 1. Protective effect of oligandrin against tomato gray mold.

Table 2. Effect of oligandrin treatment on tomato leaf MDA content.

Treatment	MDA content (nmol/mg protein)					
	1* day	2 days	3 days	5 days	7 days	9 days
CK	4.2	4.5	5.3	4.8	5.2	4.6
Oligandrin (10 ug/ml)	3.9	4.1	4.7	4.2	4.3	3.8
B. cinerea	5.3A	6.5A	8.8A	9.6A	10.5A	12.2A
O+B	4.7A	5.4A	6.5B	7.2B	8.3B	9.2B

CK, water control; Oligandrin, oligandrin treatment; *B. cinerea*, inoculation with *B. cinerea*, O+B, oligandrin treatment followed by *B. cinerea* inoculation after 24 h. *Day after inoculation. The numerical values in the table are the average of the three replicates; the letters in the same line show the significant difference at level 0.05.

ment. More also, the pattern of PR-3a mRNA levels was similar to that of PR-2a in the oligandrin treated tomato plants. PR-3a mRNA levels in the oligandrin treated tomato plants were 4.56-fold above that in the

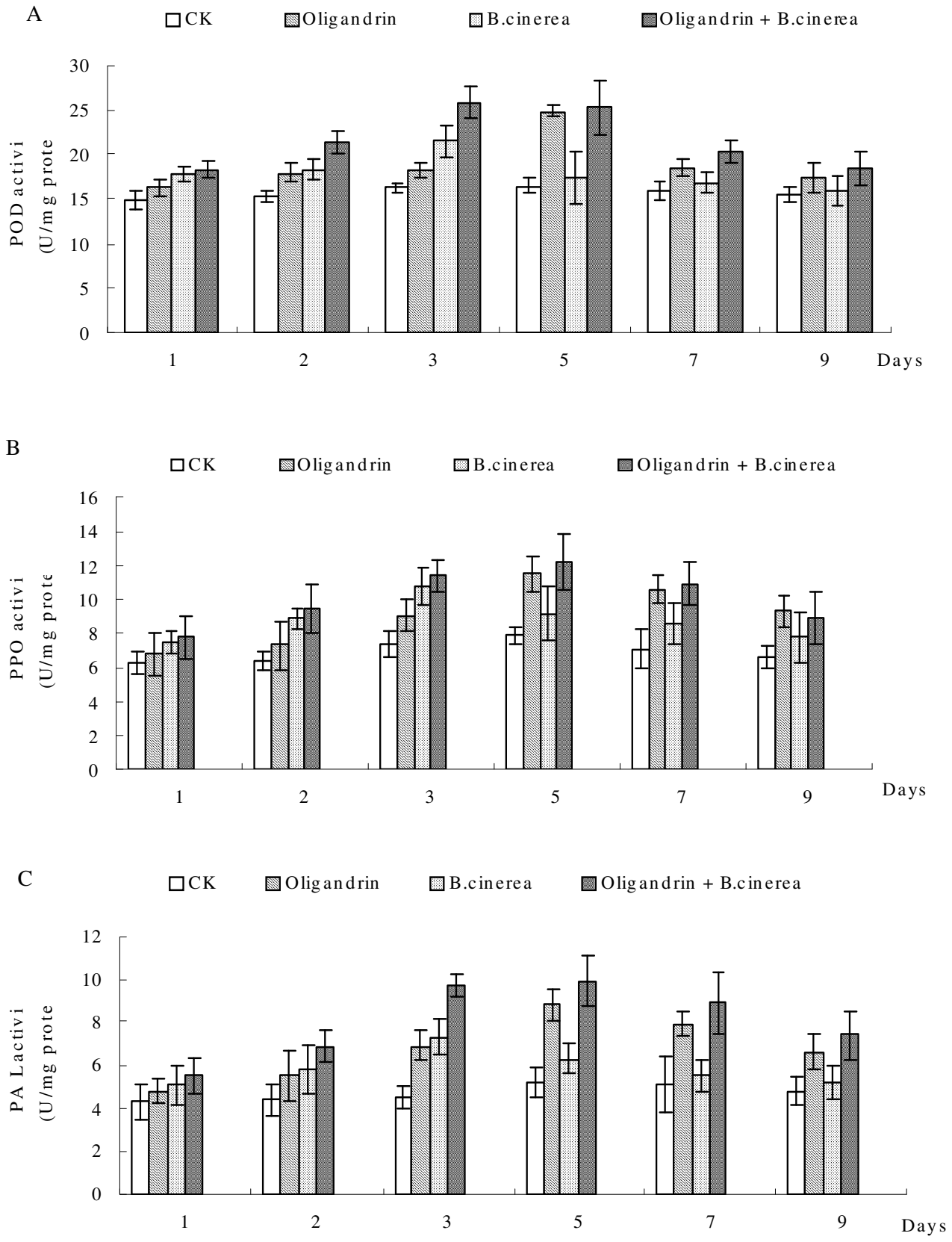

Figure 2. Activity of resistance related enzymes in leaves of tomato treated with oligandrin. CK, distilled water; Oligandrin, oligandrin alone; *B. cinerea*, inoculation using conidia of *B. cinerea* alone; Oligandrin *+ B. cinerea,* oligandrin application 1 day before inoculation with *B. cinerea*.

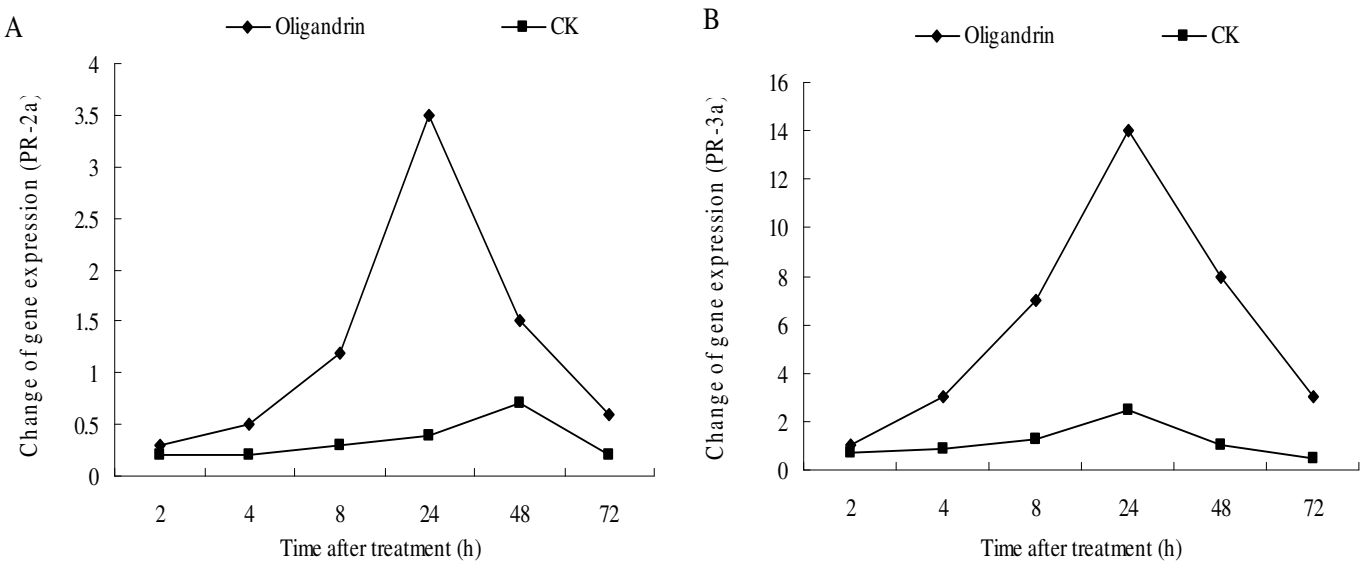

Figure 3. Expression of extracellular β-1, 3 glucanase (A) and extracellular chitinase (B) in leaves of tomato treated by root application of oligandrin at 2, 4, 8, 24, 48 and 72 h.

control plant at its peak stage (Figure 3B).

Oligandrin promoted the expression of LeERF2 and PR-6 genes

Transcripts of ethylene receptor homologue PR-6 and ethylene-inducible transcription factor LeERF2 in the leaves were analyzed. The expression of both PR-6 and LeERF2 genes was transiently induced at 8 and 24 h in oligandrin-treated plants (Figure 4). The expression of PR-6 and LeERF2 genes were up-regulated 3.60 fold and 7.41 fold in tomato leaves, respectively. These results suggest that the transient increase in ethylene production in oligandrin-treated tomato plants is likely associated with the activation of the ethylene mediated signaling pathway and induction of basic PR gene expression, resulting in the reduction of gray mold disease severity.

DISCUSSION

In this study, we examined the ability of oligandrin isolated from a biocontrol agent *P. oligandrum* to induce tomato defense reactions and resistance against gray mould when applied to roots. Results indicate that oligandrin pretreatment can protect tomato from *B. cinerea* invasion. The protective effect was comparatively better if inoculation of *B. cinerea* occurred at 4 h after treatment with oligandrin, compared with inoculation at 24 and 48 h after the treatment. In the experiment, mRNA levels of leaf PR-2a, PR-3a and LeRF2 genes reached their peaks 24 h after the treatment with oligandrin.

These levels were 7.75-, 4.56- and 7.41-fold higher than their counterpart without oligandrin treatment. The main components of fungal cell wall are β-1, 3-glucan and chitin. Oligandrin treatment significantly increased the expression of PR-2a and PR-3a (genes related to pathogenesis). This may be one of the mechanisms by which oligandrin elicits disease resistance. The other mechanism may involve the elevation of defense enzymes POD, PPO and PAL. At peak levels after oligandrin treatment, the activities of POD, PPO and PAL in tomato leaves were 50.3, 45.6 and 69.2% higher than those in the control group. In the group treated with oligandrin followed by B. cinerea inoculation, the peak levels of POD, PPO and PAL were 20.0, 32.61 and 57.14%, higher than those in the control group (Figure 2). The protective effect of oligandrin treatment appeared better when inoculation occurred at 4 h, compared with at 24 and 48 h. But the protective effect appeared better when inoculation occurred at 48 h, compared with 24 h. The higher protection against inoculation at 48 h may be due to elevated levels of the defense enzymes POD, PPO and PAL during this period.

POD is generally considered important in host resistance mechanisms as it catalyzes the last step of lignin biosynthesis (Hammerschmidt et al., 1982), and can oxidize phenolic compounds to quinones (Campos-Vargas and Saltveit, 2002). PPO acts as a defensive enzyme and plays a key role in the defense system in fruit (Picard et al., 2000b). PAL is a key enzyme of the phenylpropanoid pathway and takes part in the synthesis of phenolic compounds, phytoalexin and lignin (Pellegrini et al., 1994; Dixon and Paiva, 1995). These substances are associated with the process of local disease defense (Ryals et al., 1996).

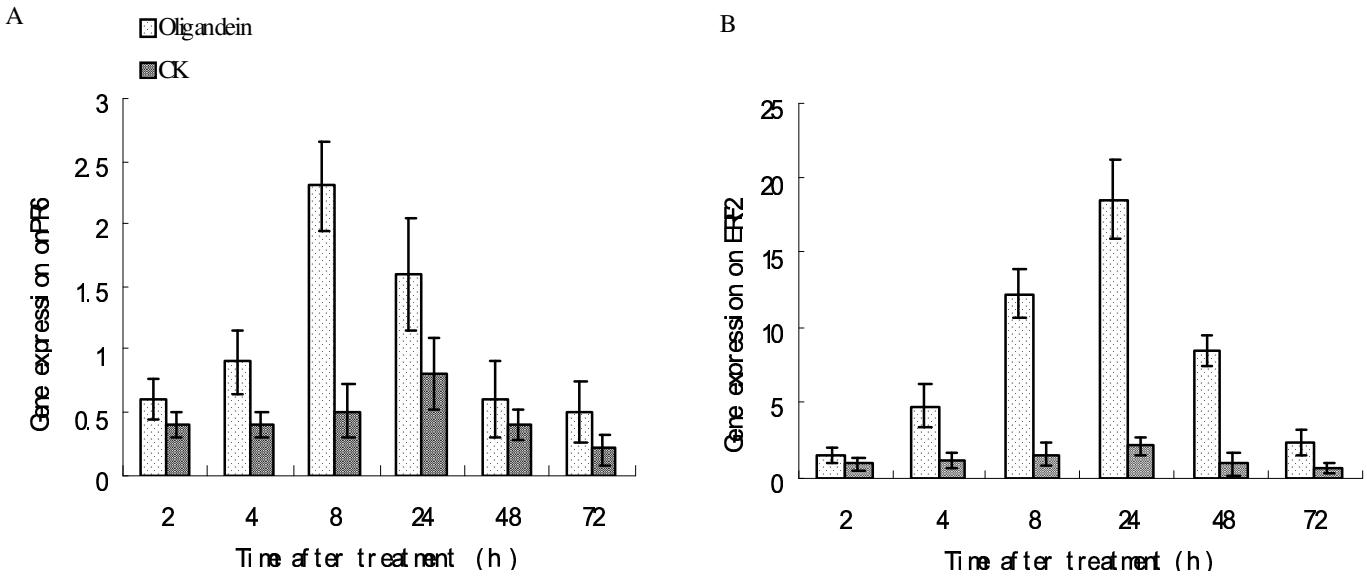

Figure 4. Expression of defense genes PR-6 (A) and LeERF2 (B) in leaves of tomato seedlings treated with oligandrin

PRs can be induced by different stress stimuli and play an important role in plant defense against pathogenic constraints, and in general adaptation to stressful environments (Edreva, 2005). The family of PR-2 catalyze endo-type hydrolytic cleavage of the 1,3-β-*D*- glucosidic linkages in β-1, 3-glucans. PR-3 is an endochitinase that catalyzes the hydrolysis of β-1,4-N-acetylglucosamine linkage, so it can cleaves fungal cell walls *in situ* and plays a major role in disease resistance. A treatment with oligandrin caused a rapid induction of the pathogenesis-related proteins (PRs) genes PR-2a and PR-3a. Transgenic tobacco over-expressing PR-2 and PR-3 has been shown to have improved resistance to *Cercospora nicotianae* (Zhu et al., 1994). Combining chitinases with β-1, 3-glucanases can significantly enhance biocontrol efficacy against many pathogenic fungi (Sela-Buurlage et al., 1993).

The oligandrin treatment of tomato root induced the expression of ET-related transcription factor (ERF2) genes and the JA-responsive gene for the basic PR-6 protein, up-regulated these genes by 7.41 fold and 3.6 fold in tomato leaves, respectively. These results indicate that the JA/ET-signaling pathway is required for oligandrin-induced resistance against *B. cinerea* in the tomato. Furthermore, the levels of the pathogenesis-related proteins (PRs), β-1,3-glucanase and chitinase, were up-regulated in the leaves of treated tomato plants by 7.75 and 4.56 fold, respectively. Oligandrin-induced defense responses in tomato leaves against *B. cinerea* have the features of both ISR and SAR. Oligandrin perhaps simultaneously induced SA and JA/ET signal pathway.

MDA is mainly formed by the ROS (reactive oxygen species)-induced degradation of polyunsaturated lipids (Pryor and Stanley, 1975; Del Rio et al., 2005). ROS are the primary mediators of oxidative damage in plants (Halliwell and Gutteridge, 1989). Excessive generation of ROS, such as superoxide anion and hydrogen peroxide, is presumed to be a significant factor in tissue injury. ROS accumulation in the cell may damage membrane structure and function, trigger the membrane lipid peroxidation and induce the membrane breakdown (Thompson, 1997). These ROS can rapidly attack all types of bio-molecules, causing membrane deterioration, lipid peroxidation, and DNA mutation, leading to irreparable metabolic and structural dysfunctions and cell death (Halliwell and Gutteridge, 1989). MDA is a final product of lipid peroxidation and its amount can be used as a measure of lipid peroxidation (Mei and Song, 2010). In addition, MDA is poisonous to plant cells (Mei and Song, 2010). The overall level of MDA is lower in oligandrin-treated tomato plants indicating that the membrane damage of non-treated seedlings was higher than that of oligandrin-treated seedlings.

In conclusion, the mode of action of oligandrin in the control of diseases in tomato plant is to evoke antioxidant enzyme activities and up-regulate pathogenesis-related proteins (PRs) genes. Oligandrin perhaps induced both SA and JA/ET signal pathways. However, the mechanism by which plants recognize the signal molecule of oligandrin requires further investigation.

ACKNOWLEDGEMENTS

This work was supported by a grant (No. 30471127) from the National Natural Science Foundation of China and a grant (Y3100107) from the Zhejiang Provincial Natural

Foundation.

REFERENCES

Assis JS, Maldonado R, Munoz T, Escribano MI, Merodio C (2001). Effect of high carbon dioxide concentration on PAL activity and phenolic contents in ripening cherimoya fruit. Postharvest Biol. Technol. 23: 33-39.

Benhamou N, Bélanger RR, Rey P, Tirilly Y (2001). Oligandrin, the elicitin like protein produced by the mycoparasite *Pythium oligandrum*, induces systemic resistance to Fusarium crown and root rot in tomato plants. Plant Physiol. Biochem. 39: 681–698.

Bezier A, Lambert B, Baillieual F (2002). Study of defense related gene expression in grapevine leaves and berries infected with *Botrytis cinerea*. Eur. J. Plant Pathol. 108: 111-120.

Campos-Vargas R, Saltveit ME (2002). Involvement of putative chemical wound signals in the induction of phenolic metabolism in wounded lettuce. Physiol. Plant 114: 73–84.

Chen AL, He J, Lian YJ (2006). The fungicide activity of analogs of pentamidine against *Botrytis cinerea*. Acta. Phytophy. Sin. 33(1): 68-72.

Del Rio D, Steward AJ, Pellegrini N (2005). A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. Nutr. Metabol. Cardiovasc Dis. 15: 316-28.

Dixon RA, Paiva NL (1995). Stress induced phenylpropanoidmetabolism. Plant Cell. 7: 1085-1097.

Edreva A (2005). Pathogenesis related proteins: research progress in the last 15 years. Gen. Appl. Plant Physiol. 31: 105–124.

Gadea J, Mayda ME, Conejero V, Vera P (1996). Characterization of defense related genes ectopically expressed in viroid infected tomato plants. Mol. Plant Microbe. 9: 409–15.

Galeazzi MAM, Sgarbieri N, Costantinides SM (1981). Isolation, purification and physiochemical characterization of polyphenol oxidase from dwarf variety of banana (*Musa Cavendishii*). J. Food Sci. 46: 150-155.

Glazebrook J (2005). Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. Ann. Rev.Phytopathol. 43: 205–27.

Halliwell B, Gutteridge JMC (1989). Free Radicals in Biology and Medicine. 2nd ed. Oxford University Press, Oxford, USA.

Hammerschmidt R, Nuckles EM, Kuc J (1982). Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum lagenarium*. Physiol. Plant Pathol. 20: 73–82.

Ippolito A, Elghaouth A, Wilson CL, Wisniewski M (2000). Control of postharvest decay of apple fruit by *Aureobasidium pullulans* and induction of defense responses. Postharvest Biol. Technol. 19: 265–272.

Lherminier J, Benhamou N, Larrue J, Milat ML, Boudon-Padieu E, Nicole M, Blein JP (2003). Cytological characterization of elicitin induced protection in tobacco plants infected by *Phytophthora parasitica* or phytoplasma. Phytopahology, 93: 1308-1319.

Livak KJ, Schmittgen TD (2001). Analysis of relative gene expression data using real time quantitative PCR and the $2^{-\Delta\Delta Ct}$ Method. 25: 402-408.

Mei YQ, Song QS (2010). Response to temperature stress of reactive oxygen species scavenging enzymes in the cross tolerance of barley seed germination. J. Zhejiang Univ. Sci. B. 11(12): 965-972.

Mohamed N, Lherminier J, Farmer MJ, Fromentin J, Beno N, Houot V, Milat ML, Blein JP (2007). Defense responses in grapevine leaves against *Botrytis cinerea* induced by application of a *Pythium oligandrum* strain or its elicitin, oligandrin, to roots. Phytopathology, 97: 611–620.

Pellegrini L, Rohfritsch O, Fritig B, Legrand M (1994). Phenylalanine ammonialyase in tobacco. Molecular cloning and gene expression during the hypersensitive reaction to tobacco mosaic virus and the response to a fungal elicitor. Plant Physiol. 106: 877–886.

Picard K, Ponchet M, Blein J, Rey P, Tirilly Y, Benhamou N (2000a). Oligandrin, a proteinaceous molecule produced by the mycoparasite Pythium oligandrum induces resistance to *Phytophthora parasitica* infection in tomato plants. Plant Physiol. 124: 379-395.

Picard K, Tirilly Y, Benhamou N (2000b). Cytological effects of cellulases in the parasitism of *Pythium oligandrum*. Appl. Environ. Microb. 66: 4305-4314.

Pryor WA, Stanley JP (1975). Letter: A suggested mechanism for the production of malonaldehyde during the autoxidation of polyunsaturated fatty acids. Nonenzymatic production of prostaglandin endoperoxides during autoxidation. J. Org. Chem. 40: 3615-3617.

Robert N, Roche K, Lebeau Y, Breda C, Boulay M, Esnault R, Buffard D (2002). Expression of grapevine chitinase genes in berries and leaves infected by fungal or bacterial pathogens. Plant Sci. 162: 389-400.

Ryals JA, Neuenschwander UH, Willits MG, Molina A, Steiner HY, Hunt MD (1996). Systemic acquired resistance. Plant Cell. 8: 1809–1819.

Sela-Buurlage MB, Ponstein AS, Bre-Vloemans SA, Melchers LS, Van den Elzen PJM, Cornelissen BJC (1993). Only specific tobacco (*Nicotiana tabacum*) chitinases and β-1,3-glucanases exhibit antifungal activity. Plant Physiol. 101: 857–863.

Thompson JE (1997). Membrane deterioration during senescence. Canada J. Botany 5: 867-879.

Tournier B, Sanchez-Ballesta MT, Jones B, Pesquet E, Regad F, Latche A, Pech JC, Bouzayen M (2003). New members of the tomato ERF family show specific expression pattern and diverse DNA-binding capacity to the GCC box element. FEBS Letters, 550: 149–54.

Wang FD, Feng GH, Chen KS (2009). Defense responses of harvested tomato fruit to burdock fructooligosacc haride, a novel potential elicitor. Postharvest Biol. Technol. 52: 110–116.

Ward ER, Uknes SJ, Williams SC, Dincher SS, Wiederhold DL, Alexander DC, Ahl-Goy P, Metraux JP, Ryals JA (1991). Coordinate gene activity in response to agents those induce systemic acquired resistance. Plant Cell 3: 1085-1094.

Yourman LF, Jeffers SN (1999). Resistance to benzimidazole and dicarboximide fungicides in greenhouse isolates of *Botrytis cinerea*. Plant Dis. 83: 569–575.

Zhang L, Fan J (2007). The experiment course of plant physiology. Beijing: China Agric. Uni. Press.

Zhu Q, Maher EA, Masoud S, Dixon RA, Lamb CJ (1994). Enhanced protection against fungal attack by constitutive co-expression of chitinase and glucanase genes in transgenic tobacco. Nat. Biotechnol. 12: 807–812.