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RESEARCH ARTICLE

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Effects of salicylic acid on the behavior of Yali pear infected by *Alternaria kikuchiana* Tanaka

Abstract To investigate the inductive effect of salicylic acid (SA) on the resistance of *Pyrus bretschneider* cv Yali to black spot disease (*Alternaria kikuchiana* Tanaka), the physiological and biochemical characteristics of detached pear leaves at the age of 5 to 10 days were measured after application of SA. The results showed that exogenous SA significantly improved the resistance of Yali pear (*Pyrus bretschneider* cv Yali) leaves to black spot disease. For the SA treatment at 0.02 $mmol \cdot L^{-1}$ SA concentration, the disease index was the lowest, and the induced resistance reached up to 63.9%. Furthermore, SA induced local and systemic resistance of Yali pear against the black spot disease. Expression of systemic resistance in leaves was detectable 3 d after SA treatments and lasted for 10 d. POD, PPO, and PAL activities of Yali pear leaves increased by SA treatment. It is suggested that exogenous SA solution as a chemical activator could induce the resistance of Yali pear to black spot disease.

Keywords Yali pear, *Alternaria kikuchiana* Tanaka, salicylic acid, induced resistance

1 Introduction

Yali pear is one of the best pear cultivars cultivated extensively in Northern China. However, it often suffered serious losses from black spot disease caused by *Alternaria kikuchiana* Tanaka. To reduce black spot disease occurrence, lots of chemical pesticides are used. Although the common chemical pesticides have positive effects in the disease control, it would cause environmental pollution and pesticide residues for a long time. Therefore, seeking for new methods to control pear black spot disease is an important topic for researchers and pear growers.

Salicylic acid (SA) is a kind of phenolic compound, existing in plants widely. SA can control many important metabolic processes in plants (Raskin, 1992). At present, the study of SA is mainly focused on the induction of plant defense responses (Loake and Grant, 2007). It has been reported that SA induces a variety of crops to produce a wide range of disease resistance, such as cucumber (Shi et al., 2004), tobacco (Park et al., 2007), turf-type fescue (Gu and Wang, 2003), and so on. However, whether or not SA has the ability to induce the resistance of Yali pear leaves to black spot disease had not been reported. Our experiment was to evaluate the ability of SA, whether it could induce resistance to black spot disease in the leaves of Yali pear.

2 Materials and methods

2.1 Biological materials and fungal strains

Yali pear plants (*Pyrus bretschneider* cv Yali) were selected from the orchard of the Agricultural University of Hebei, and healthy young leaves of 5 to 10 days were collected as trial materials.

The pathogenic fungus *A. kikuchiana* Tanaka was obtained from the College of Plant Protection in the university. Fungal was grown on solid potato dextrose agar (PDA) for seven days at 25°C. Then, conidia were washed with sterile water by centrifugation and suspended in 0.1% Tween solution. The conidial concentration was adjusted to about 2×10^5 $conidia \cdot mL^{-1}$.

2.2 Methods

2.2.1 Determination of colony growth

After filtration through a nylon filter with 0.25 μm pore diameter, the SA solution was mixed with PDA and adjusted to different concentrations (0, 0.002, 0.02, 0.2, and 2 $mmol \cdot L^{-1}$). Drops (about 20 μL) of the conidial

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suspension were placed at the center of the PDA medium. The experiments were conducted with three replicates. After inoculation, the PDA media were placed in a phytotron at 25°C to observe the morphology of mycelia, measure the diameter of colony, and calculate inhibition rate 5 days later.

2.2.2 Foliage protection experiments with SA treatment

SA was adjusted to different concentrations (0.002, 0.02, 0.2, and 2 mmol·L^{-1}) and sprayed onto young leaves of Yali pear with distilled water treated as a control. Three days later, the branches were cut off and inserted into conical flask for hydroponic culture. Then, conidial suspension was brushed onto the leaves with a writing brush. The inoculated branches were incubated in a moist chamber at 20°C in the dark for 48 h and transferred to a phytotron, maintaining at 25°C. Experiments were repeated for three times. In each experiment, 20 branches per treatment were used. Seven days later, the disease index was calculated. The classification standard of black spot disease was referenced to the research method of plant disease by Fang (1998) (Table 1).

2.2.3 Determination of pharmacodynamics during SA treatments

SA (0.02 mmol·L^{-1}) or distilled water (control) was sprayed onto young leaves of Yali trees. The treated leaves were detached from trees 1, 2, 3, 4, 5, 6, 7, 10, 15, and 20 day(s) after treatment, respectively. Then, the detached leaves were inoculated with *A. kikuchiana* Tanaka and cultured in phytotron at a steady temperature of 25°C. Seven days later, the disease index was calculated. Twenty shoots were inoculated per treatment, and the experiment was repeated three times.

2.2.4 Experiments of local and systemic resistance

Young leaves of the test shoots were divided into left and right parts, with about three leaves each. Leaves from the left part were carefully brushed with SA at 0.02 mmol·L^{-1} (SL) or distilled water as a control (CL), while those from right part were smeared nothing, namely, SR and CR were brushed with neither SA nor water. Then, conidial suspension was sprayed to SL, CL, SR, and CR. Seven days after inoculation, the disease index of the two parts was calculated separately. Twenty branches were used per treatment with three replicates.

2.2.5 Assays of enzymatic activities

In the initial experiment, young leaves of Yali trees were sprayed with different concentrations of SA (0, 0.002, 0.02, 0.2, 2 mmol·L^{-1}), and were sampled three days later. Enzyme activities of the samples were analyzed or stored at −70°C for later use. Thereafter, SA (0.02 mmol·L^{-1}) or distilled water (control) was sprayed onto the young leaves of Yali trees, and the enzyme activity assays of the treated leaves were made at 1, 2, 3, 4, 5, 6, 7, 10, 15, and 20 day(s), respectively, followed by determinations of POD (Moerschbacher et al., 1998), PPO (Zhang, 1990), and PAL (Mozzetti et al., 1995) activities.

3 Results

3.1 Growth of *Alternaria kikuchiana* Tanaka

Alternaria pathogen was cultivated on the PDA medium, which contained 0, 0.002, 0.02, 0.2, and 2 mmol·L^{-1} salicylic acid, respectively. Five days after inoculation, colony morphology was recorded, and bacteriostasis rate was calculated. Results showed that the colony diameter had no significant difference between the PDA medium treated with SA (0.002–0.2 mmol·L^{-1}) and control (Table 2). The colony shape and color after treatment with SA were similar to those of the control, and bacteriostasis rate was less than 2%. However, when SA concentration of the PDA medium reached to 2 mmol·L^{-1}, the mycelium was inhibited significantly.

3.2 Effect of induced resistance treated by SA at different concentrations

Young leaves of Yali pear were sprayed with different SA concentrations three days before the conidial suspension of *A. kikuchiana* Tanaka was applied. Seven days after inoculation, the disease index of the leaves treated with SA (0.02 mmol·L^{-1}) was the lowest, and the inductive effect

Table 1 The classification standard of black spot disease

level	disease severity	representing number
1	no symptom	0
2	area with symptom 0–25%	1
3	area with symptom 26%–50%	2
4	area with symptom 51%–75%	3
5	area with symptom more than 75% or withered leaf	4

Table 2 Effect of SA on colony growth of *Alternaria kikuchiana* Tanaka

concentration of SA /(mmol·L^{-1})	colony diameter/mm	inhibition rate/%	colony shape	colony color	density and growth potential of mycelia
0 (control)	59.4	0	round-shaped floc	black green	thick and flourish
0.002	58.2	1.94	round-shaped floc	black green	thick and flourish
0.02	58.3	1.77	round-shaped floc	black green	thick and flourish
0.2	61.6	−3.79	round-shaped floc	dark gray	thick and flourish
2	53.7*	9.52	round-shaped floc	dark green	very thick center with thin margin , flourish

Note: * denotes significant at 0.05 level; ** denotes significant at 0.01 level.

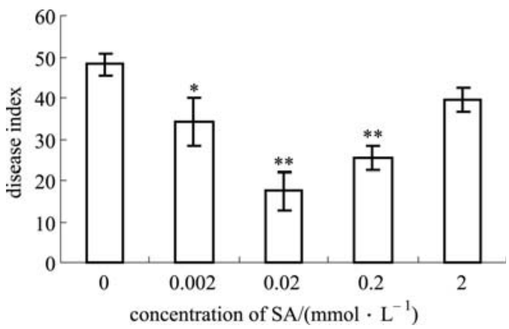

Fig. 1 Effect of SA treatment before inoculation on pear black spot disease index in Yali pear
Note: * denotes significant at 0.05 level; ** denotes significant at 0.01 level.

reached 63.9% (Fig. 1).

3.3 Effect of SA treatment on pharmacodynamics duration after *A. kikuchiana* Tanaka infection

Young leaves of Yali pear were sprayed with SA (0.02 mmol·L^{-1}) 1–20 days before *A. kikuchiana* Tanaka inoculation to the leaves (Fig. 2). The results obtained after SA treatment showed the disease index of the leaves was always lower than that of the control. A peak of significant protection in respect to control appeared in the trees treated on the 3rd day, with −49.3% severity. Thereafter, the action of SA lost its effectiveness gradually. The treatments at both 15th and 20th day showed no significant protection, and the inductive effect was lower than 15%. It suggested that the inductive effect could last for more than 10 days.

3.4 Local and systemic resistance

The SA treatment decreased the disease index of all the treatments by 40.3% and 32.6% compared to the control, respectively (Fig. 3). However, when the leaves were brushed with SA on the 2nd or the 4th day after infection with *A. kikuchiana* Tanaka, the incidence was not significantly reduced, indicating that SA could not cure the leaves infected by the pathogen of black spot disease.

3.5 Effect of SA treatment on antioxidant enzyme activities

3.5.1 Changes of enzymatic activities with SA treatment at different concentrations

The activities of POD, PPO and PAL were measured using Yali leaves sprayed with different concentrations of SA or treated with water (control). With the increase of SA concentration the activities of three enzymes increased at first and then dropped slowly (Fig. 4). SA at 0.02 mmol·L^{-1} could significantly increase the activities

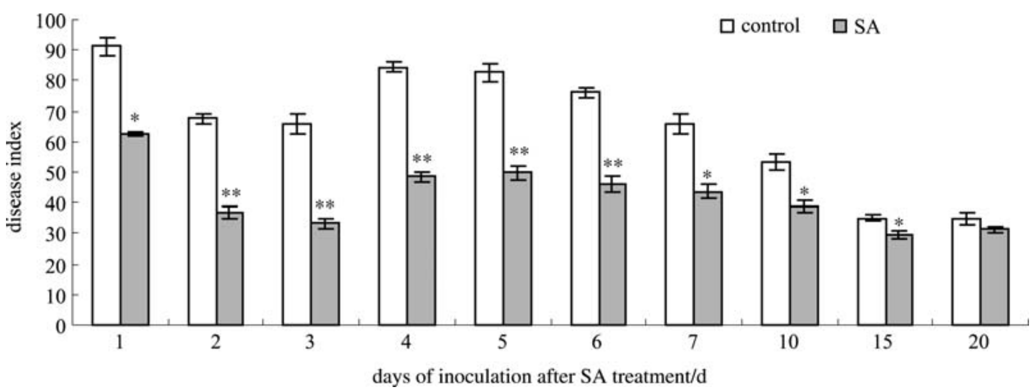

Fig. 2 Disease index on leaves of Yali pear after different time intervals between SA treatment and inoculation
Note: * denotes significant at 0.05 level; ** denotes significant at 0.01 level.

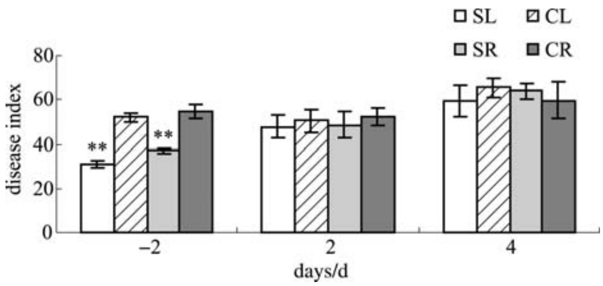

Fig. 3 Effect of SA treatment before or after inoculation on the black spot disease index in Yali pear

Note: SL, CL, SR and CR represent the left leaves after treatment with 0.02 mmol·L^{-1} SA, the left leaves after treatment with water, the right leaves after treatment with 0.02 mmol·L^{-1} SA and the right leaves after treatment with water, respectively. * denotes significant at 0.05 level; ** denotes significant at 0.01 level.

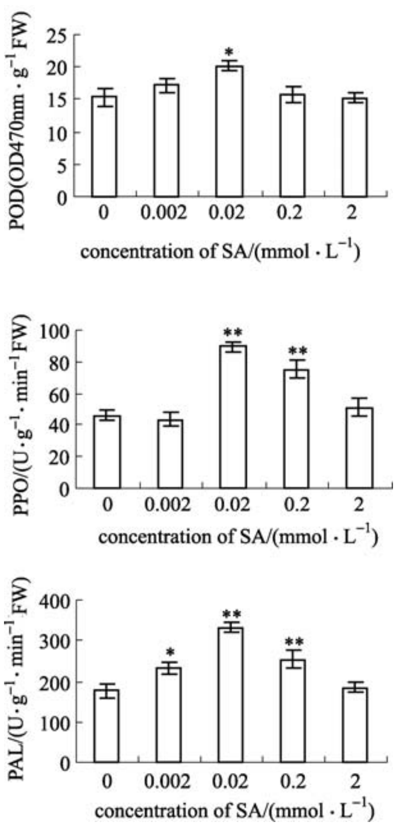

Fig. 4 Effects of different concentrations of SA on activities of POD, PPO and PAL in Yali pear leaves

Note: * denotes significant at 0.05 level; ** denotes significant at 0.01 level.

of these three enzymes by 31.4%, 93.8% and 89.3%, respectively, compared to the control.

3.5.2 Assays for enzymatic activities after SA treatment

The change of POD activity shown in Fig. 5 indicates that the Yali leaves treated with 0.02 mmol·L^{-1} SA from 4 to 20

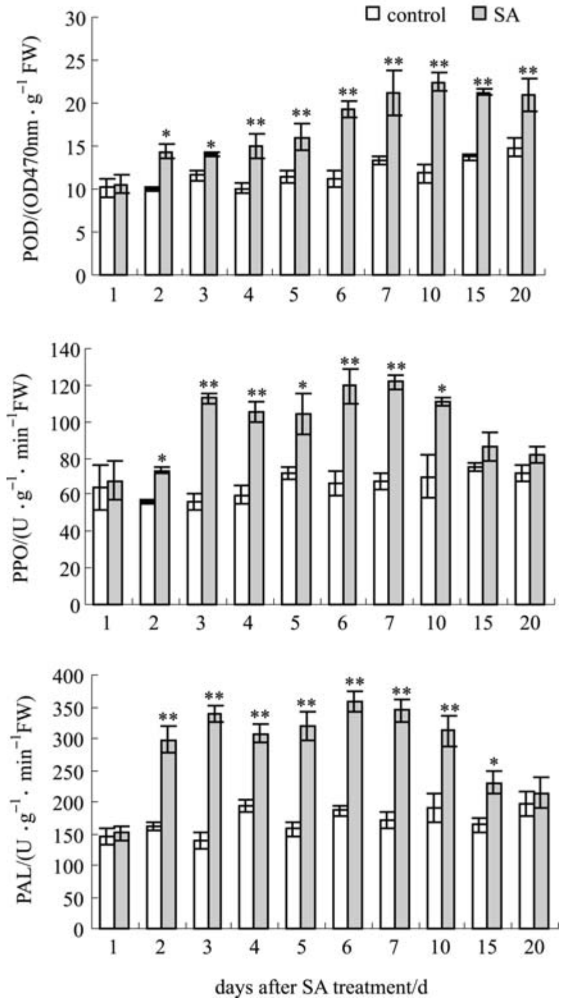

Fig. 5 Effects of exogenous SA on activities of POD, PPO, and PAL in Yali pear leaves

Note: * denotes significant at 0.05 level; ** denotes significant at 0.01 level.

days could be significantly increased more in the POD activity when compared to their respective controls, reaching up to the maximum (90.0% in respect to control) on the 10th day.

The changes of PPO and PAL activity were similar. They all had a rapid rise and then decreased slowly (Fig. 5). The activities of PPO and PAL reached their peaks at the third day, when the activities of the two enzymes were increased by 101.6% and 145.0%, respectively, in respect to their controls. The PPO and PAL activities were significantly lower at 10th day after SA treatment. Furthermore, at the 20th day, the activities of the two enzymes were not significantly different from those of their controls.

4 Discussion

Induced resistance responses contribute to the natural protection of plants against pathogens. An emerging strategy in plant protection is the chemical induction of systemic acquired resistance (SAR) (Lucas, 1999). Considered as a SAR inducer, a chemical should fulfill at least the following criteria (Sticher et al., 1997) including (a) showing indirect antimicrobial activity, (b) protecting against a range of pathogens without specificity, and (c) activating host defense mechanisms. Salicylic acid and its derivatives showed a typical SAR response in cucumber and tobacco and effectiveness against different pathogens (Métraux et al., 1990; Seskar et al., 1998).

Although few studies on induced resistance responses have been performed on trees, emerging data suggest that the SAR paradigm may work in these plants too. In particular, the benzothiadiazole derivative acibenzolar-S-methyl (BTH) as a SAR inducer have been reported to be effective in the protection of apples from fire blight disease caused by *E. amylovora* (Brisset et al., 2000; Maxson-Stein et al., 2002). Notably, in the signal transduction pathway of SAR, BTH acts as downstream of SA (Malamy et al., 1990). Therefore, the young leaves of Yali pear were selected as the experimental materials, and the expression of resistance was studied after the SA treatments. We suggest that SA, when sprayed onto Yali leaves 2 days before *A. kikuchiana* Tanaka inoculation, can make a significant protection, inducing not only the local defense response but also the systemic resistance (Fig. 3).

The resistance of Yali pear leaves would not continue to increase with the increased SA concentration. The best induced concentration was $0.02\ \mathrm{mmol \cdot L^{-1}}$ (Fig. 1). Mao et al. (2004) found that different plants might need different concentrations of SA to induce the expression of their resistance, which was related to the level of endogenous SA in plants. The concentration of SA inducing the resistance of Yali pear leaves was lower than that of many other plants (0.07–$0.7\ \mathrm{mmol \cdot L^{-1}}$) (Raskin, 1992). It might be speculated that the leaves of Yali pear contained a higher concentration of salicylic acid.

Studies have shown that many antioxidant enzymes are related to plant defense responses (Wang et al., 2005; Liang et al., 2006). Moreover, the activities of these enzymes are regarded as an important indicator of plant defense responses. Our results showed a significant local accumulation of these enzymes and a persistent tendency of their systemic accumulation. Therefore, SA was not only directly involved in the resistance against *Alternaria kikuchiana* Tanaka but also inducted the activity of defense-related enzymes to prevent pathogen attack.

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