African Journal of Biotechnology Vol. 11(72), pp. 13670-13677, 6 September, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.3573
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

Induction of defensive enzymes (isozymes) during defense against two different fungal pathogens in pear calli

Juan Zhao[1,2], Yuguo Wang[2], Jishu Zhang[1]*, Yuanhuai Han[2], Zhifen Yang[3], and Wenxin Feng[2]

[1]College of Life Science, Northwest A & F University, Yang ling 712100, Shan xi Province, People's Republic of China.
[2]Agricultural College, Shanxi Agricultural University, Tai gu 030801, Shan xi Province, People's Republic of China.
[3]College of Arts and Sciences, Shanxi Agricultural University, Tai gu 030801, Shan xi Province, People's Republic of China.

Accepted 23 February, 2012

Activities of defensive enzymes peroxidase (POD), superoxide dismutase (SOD), catalase (CAT), polyphenol oxidase (PPO) and esterase (EST) and their isozymes in pear calli were studied to reveal their role in the defensive response to different fungal infections and to find some clues to enhance their antimicrobial properties. The results confirm the fact that the activities and isozymes of these five enzymes showed differences in response to different fungal infections. After the inoculation of two different fungi for the same calli, its defensive enzymes' activities changed relatively when compared with those of the control and in *Botryosphaeria berengriana* f.sp. *piricola* (BBP)-infected calli, the enzymes' activities changed more significantly than those of *Monilinia fructigena Honcy* (MFH). Meanwhile, more new isozymes were induced by BBP infection. These are in agreement with the fact that the BBP-infected calli decay was slower than that of the MFH. These results suggest that enhancing defensive enzymes' activities and inducing new isozymes may be related to mitigating pathogen-induced oxidative damage which result in the decrease of calli decay, and this implies that antioxidant defense response may be involved in the mechanisms of plant against fungal pathogen.

Key words: Pear callus, fungi infection, defense enzyme, isozyme, biochemical defense mechanism.

INTRODUCTION

Pear ring rot (caused by *Botryosphaeria berengeriana* f. sp. *Piricola*) and brown rot (caused by *Monilinia fructigena Honcy* (MFH)) are two of the most serious fungal diseases occurring in pear plants and fruits. Periodic application of fungicides to control them has been of great concern to the general public because of their potential harmful effects on human health. So it is important to determine the defense mechanisms to control fungal diseases in pear plants. At present, few reports are available for systematic research on the pathogenesis of these two pathogenic fungi and little is known about the defense mechanism of their host.

Plants have evolved elaborate defense mechanisms to protect themselves against attack by pathogens such as fungi, bacteria and viruses. The new defensive enzymes isozyme can be quickly induced after the pathogen infection of plants which is an important host defensive mechanism against the infection (Nam, 2008). Since the 1960s, many scholars have studied the relationship between POD isozymes and diseases including Wheat Scab, Barley yellow leaf disease and Verticillium wilt of cotton blight. Accumulating evidence indicated that plants' antifungal abilities are correlated with the expression of POD, as a regulator of the release of reactive oxygen species (ROS) (Low and Merida, 1996; Tzeng and

*Corresponding author. Email: zjs2918@163.com.

Abbreviations: POD, Peroxidase; **SOD,** superoxide dismutase; **CAT,** catalase; **PPO,** polyphenol oxidase; **EST,** esterase; **BBP,** *Botryosphaeria berengriana* f.sp. *piricola*; **MFH,** *Monilinia fructigena Honcy*; **PAGE,** polyacrylamide gel electrophoresis.

Devay, 1993). Recently, numerous reports have demonstrated the relationship between the expression pattern of POD isozymes and their different antifungal abilities (Sandra et al., 2006; Wang et al., 2008). In addition to POD, several other enzymes have been identified to play a role in defending fungi infection, including polyphenoloxidase (PPO) and esterase (EST) (Xu et al., 2008). However, for another two enzymes, superoxide dismutase (SOD) and catalase (CAT), which are functionally related with POD and involved in regulating the release of ROS, little is known about their functions in plant immunity.

Plant cell cultures can often serve as model systems to study the biochemical changes in relation to plant defense responses against pathogens (Kneer et al., 1999). Cell culture systems not only have a higher rate of metabolism than differentiated plants, but are also relatively easy to manipulate by empirical means, allowing for a better control of external factors that can interfere with the metabolic activities, and thus having advantages over studies on *in vivo* plant–pathogen interactions (Strat-mann et al., 2000). In recent past, many elicitor-induced cell cultures of different plant species were used as model systems to understand plant–pathogen interactions both at biochemical and molecular levels (Shein et al., 2003). Plant cell and organ cultures grown *in vitro* usually exhibit changes in physiological and biochemical responses upon exposure to biotic and abiotic elicitors (Sircar and Mitra, 2008).

In this research, in order to investigate whether or not the defensive enzymes' activities and isozymes show significant changes and, if they do, what may be the relationship between these changes and the fungal infection at the same genetic background, the pear calli which has different responses to infection of the two pathogenic fungi was selected. Isozyme electrophoretic analysis of POD, SOD, CAT, PPO and EST was conducted on the pear calli that was infected by these two pathogenic fungi in order to deeply explore their defense ability against pathogenic bacteria and to generally understand the biochemical defense mechanisms of pear calli against pathogenic fungus.

MATERIALS AND METHODS

Plant material and fungi

Jinmi pear (*Pyrus bretschneideri* Rehd.) was taken from the Institute of Pomology, Shanxi Academy of Agriculture. Callus was induced from ripe fruit, grown and screened for uniform texture as experimental material. Callus was inoculated with *B otryosphaeria berengriana* f.sp. *Piricola* (BBP) and *Monilinia fructigena Honcy* (MFH) and sampled every 12 h with completely randomized method until 120 h. The samples were stored at -70°C and frozen in liquid nitrogen for later analysis.

Enzyme extraction

All operations were performed at 4°C. For total enzyme extracts, 0.2 g of callus were ground to fine powder with liquid nitrogen and homogenized with a mortar and pestle in 2 ml of ice cold 50 mM Tris-HCl buffer (pH 6.8) containing 0.1 mM Ethylene-diaminetetraacetic acid (EDTA), 5 mM cysteine and 1% PVP. The homogenate was centrifuged at 8000 g for 15 min and the supernatant fraction was filtered through a column containing 1 ml of Sephadex G-50 equilibrated with the same buffer used for the homogenization. The filtered fraction was used for enzyme activity and isozyme electrophoresis.

Enzyme assays

CAT activity was assayed following the method of Wang et al. (2004). POD activity was determined following the method described by Yao and Tian (2005). SOD activity was determined according to the method of Wang et al. (2004). PPO activity was assayed following the method of Murr and Morris (1974). Protein content was measured according to the method of Bradford (1976), using BSA as the standard protein.

Analysis of isozymes

Polyacrylamide gel electrophresis (PAGE) was carried out as described by Wang et al. (2004) with some modifications. The gel was prepared with 4% stacking gel and 7% separating gel and each sample (20 µL) was run at 4°C, with bromophenol blue as a front indicator. After electrophoresis, gels were stained for POD with modified benzidine, SOD with riboflavin, CAT with starch and KI, PPO with OPD and Hydroquinone Quinol, and EST with α-naphthyl acetate, β-naphthyl ester and fastness blue R salt. Gels were photographed and analyzed with a gel imaging system (Beijing Liuyi Instrument, WD-9413A-type). All experiments were repeated three times.

Data analysis

All data were analysed by one-way analysis of variance (ANOVA). Mean separations were performed by Duncan's multiple range test and the differences at $p \leq 0.05$ were considered as significant.

RESULTS

The morphological change of calli infected by two kinds of fungi

The morphological change of calli was obviously different after inoculation with two kinds of fungi. The MFH-infected calli decayed and its colour turned brown in an earlier phase, and the degree of rot was significantly higher than that of BBP-infected calli (Figures 1a to e). Twenty-four hours after inoculation with MFH, the calli was slightly brown, with the colour of its tissue gradually changing to brown and rotting followed by rapid growth of fungi. At the 96^{th} hour of infection, the tissue became rotten and shrunk significantly having an intense brown color. There was no obvious change in BBP-infected calli after BBP inoculation for 24 h. Its colour began to change to brown at the 48^{th} h, but the degree of rot was slower than that of MFH-infected calli. After 96 h of infection, there were still parts of the tissue that did not show any

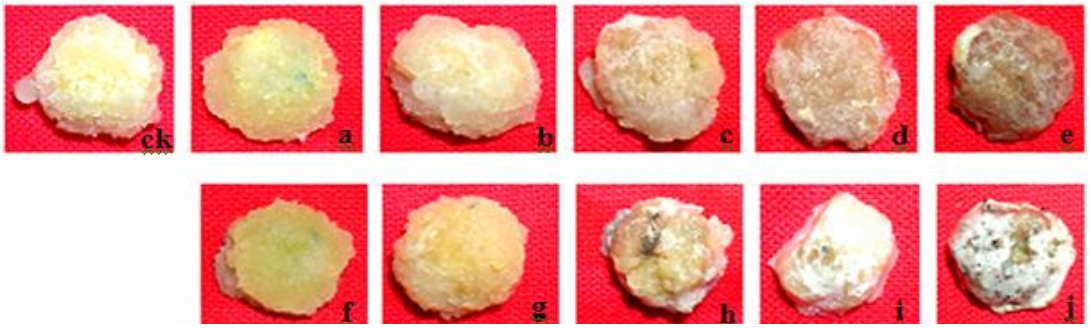
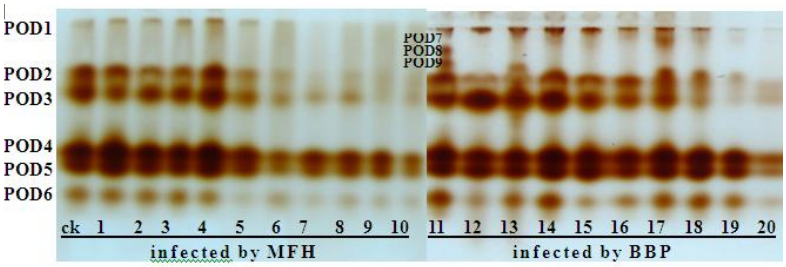


Figure 1. Effect of fungi on pear calli at different time after inoculation. a -e: calli infected by MFH (inoculation for 24, 48, 72, 96 and 120 h). f-j: calli infected by BBP (inoculation for 24, 48, 72, 96 and 120 h).



CK: control; 1 ~ 10: 12, 24, 36, 48, 60, 72, 84, 96, 108 and 120 h.

After infected by MFH; 11 ~ 20: 12, 24, 36, 48, 60, 72, 84, and 96 h.

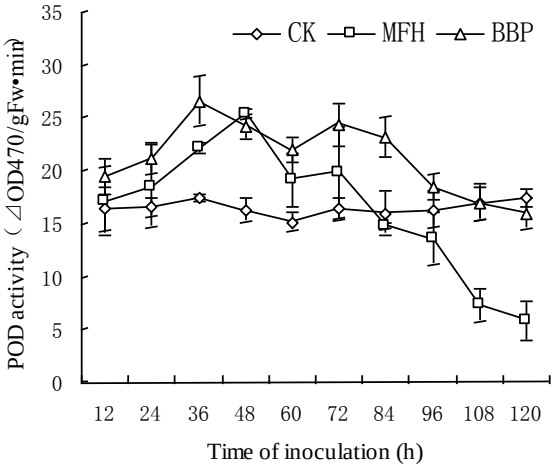


Figure 2. Changes in POD activity and isozyme in pear calli infected with MFH and BBP. Lanes 1-10: change in the expression level of POD isozyme in MFH-infected; Lanes 11-20: for BBP-infected calli, isozyme bands POD3-5 gradually increased, and the enzyme expression was significantly higher than that which was previously observed.

brown taint. More serious rotting and browning of the tissue appeared at the 120th h, while the extent was still lighter than that of the MFH infection (Figure 1, f-j).

Gel and specific activities of POD

When the POD activity in MFH-infected calli was compared with the control, it showed a continuous increase before 48 h inoculation and then declined to a relatively low level (Figure 2). There was also an obvious change in the expression level of POD isozyme in MFH-infected calli (lanes 1 to 10), but no new isozyme was identified. After 60 and 72 h infection, POD1 (Rf 0.09), POD2 (Rf 0.21) and POD3 (Rf 0.3) were lost respectively. BBP infection enhanced POD activity and the performance was better than that of MFH. For BBP- infected calli (lanes 11 to 20), the isozyme bands of POD3-5

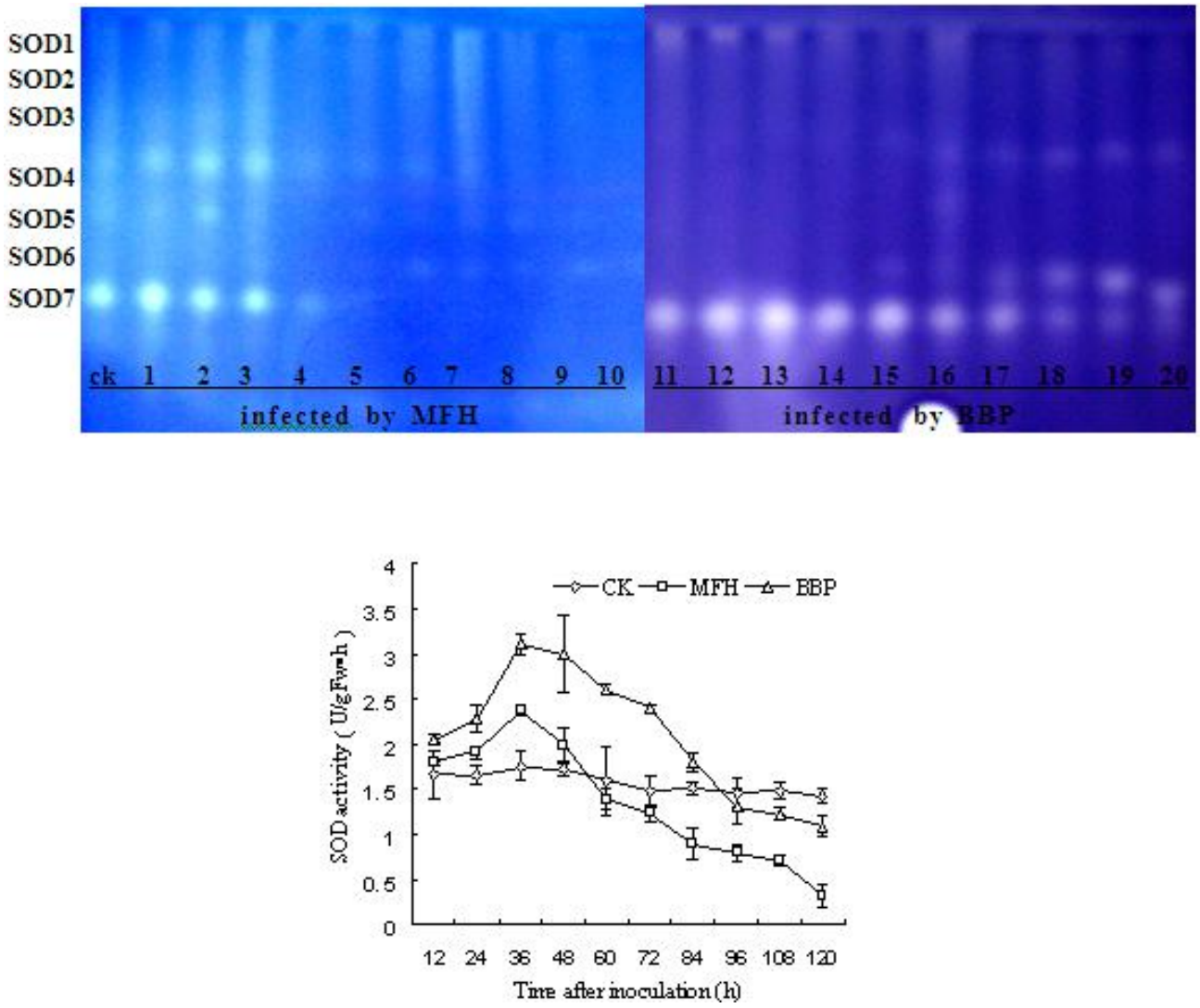


Figure 3. Changes in SOD activity and isozyme in pear calli infected with MFH and BBP. Lanes 1-10: After infection by MFH, the main isozyme band SOD7 (Rf 0.76) gradually increased until the 48th h, and later it gradually reduced; Lanes 11-20: after infection by BBP, the main isozyme band, SOD7, gradually increased and the enzyme expression in anaphase of infection was significantly higher than that which was previously observed.

gradually increased, and the enzyme expression was significantly higher than that which was previously observed. Three new isozyme bands (POD7: Rf 0.11, POD8: Rf 0.15, and POD9: Rf 0.18) appeared 12, 36 and 84 h after infection, respectively. It shows that the new POD isozymes can be induced by BBP-infection which plays a key role in defense against pathogen infection.

Gel and specific activities of SOD

As revealed in Figure 3, SOD activity significantly increased in both treatments compared with the control ($p < 0.05$). SOD activities in BBP-infected calli were induced and they showed approximately 74.5% increase within 36 h ($p < 0.05$) and decreased thereafter. Meanwhile, the main isozyme band, SOD7, gradually increased and the expression amount of SOD4 (Rf 0.38) seemed to have improved, though a new isozyme band, SOD6, appeared after 60 h of infection. Similar trends occurred in MFH-infected calli, but the SOD activity in BBP-infected calli was always higher than that in MFH-infected calli. Isozyme band SOD7 (Rf 0.76) gradually increased for 36 h, and later it gradually reduced. A new isozyme band, SOD6, appeared with a small amount of expression after 36 h of infection, though it was observed that most isozyme bands were lost at the anaphase of infection. When compared with the control (lane 0), there were significant changes in SOD isozymes. After infection by MFH (lanes 1 to 10), the main isozyme band, SOD7 (Rf 0.76), gradually increased for 48 h, and later it gradually reduced. A new isozyme band appeared with a small

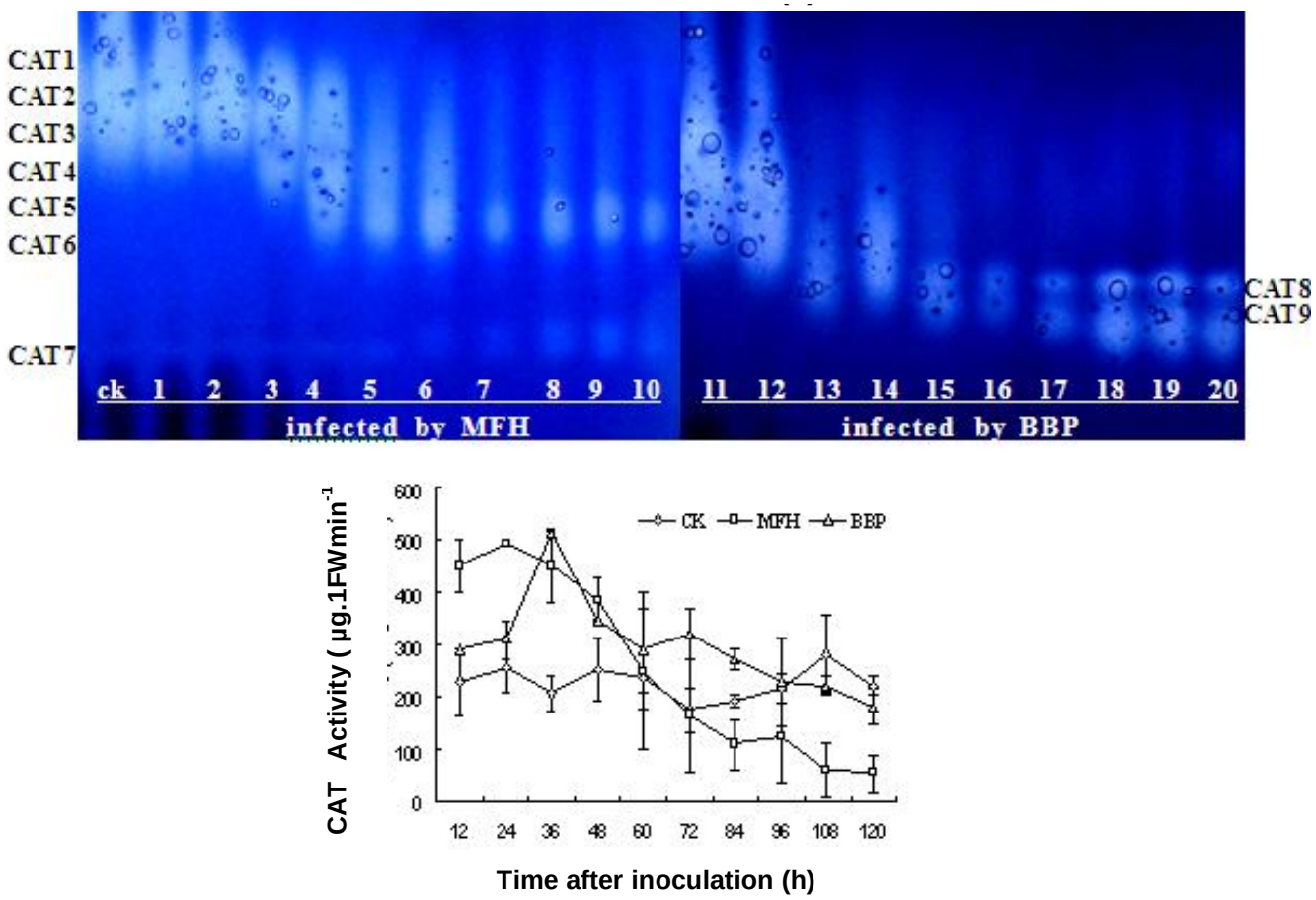


Figure 4. Changes in CAT activity and isozyme in peal calli infected with MFH and BBP. Lanes 1-10: For MFH-infected calli, band CAT1 (Rf 0.22) reduced gradually and disappeared 48 h after inoculation; Lanes 11-20: in the case of CAT isozyme, CAT1～4 enzyme bands were weaker than those with MFH and they disappeared 24 h after infection.

amount of expression after 36 h of infection. Most isozyme bands were lost at the anaphase of infection. After infection by BBP (lanes 11 to 20), the main isozyme band, SOD7, gradually increased and the enzyme expression in the anaphase of infection was significantly higher than that which was previously observed. The expression amount of SOD4 (Rf 0.38) also seems to be improving, and it was observed than a new isozyme band, SOD6, appeared after 60 h of infection. The result shows that both fungi can induce the production of a new SOD isozyme in pear calli after infection. When BBP was compared with MFH, BBP induced the new isozyme band much than MFH did, but its expression amount in the anaphase was greater than that of MFH.

Gel and specific activities of CAT

CAT activity (Figure 4) got to a peak in MFH-infected calli and was about 247% higher than that of the control at the 36th h ($p < 0.05$), and then it rapidly declined. For MFH-infected calli (lanes 1 to 10), band CAT1 (Rf 0.22) reduced gradually and disappeared at the 48th h after inoculation. Moreover, bands CAT2～4 disappeared at the 84th h after inoculation, while band CAT7 increased gradually. New isozyme bands, CAT5 (Rf 0.43) and CAT6 (Rf 0.51), appeared 36 and 48 h after infection, respectively, while the CAT activity in BBP-infected calli changed gently after the peak of the activity emerged at 24 h. In the case of CAT isozyme (Lanes 11 to 20), CAT1～4 enzyme bands were weaker than those with MFH and they disappeared 24 h after infection. Band CAT7 increased gradually and its expression was significantly higher than that with MFH at infection anaphase. New isozyme bands, CAT8 (Rf 0.55) and CAT9 (Rf 0.63), appeared at the 36th h and their intensity increased to the last time point.

Gel and specific activities of PPO

As shown in Figure 4, PPO activity in both treatments rapidly increased and was significantly higher than the control at 12 h after inoculation, after which it then

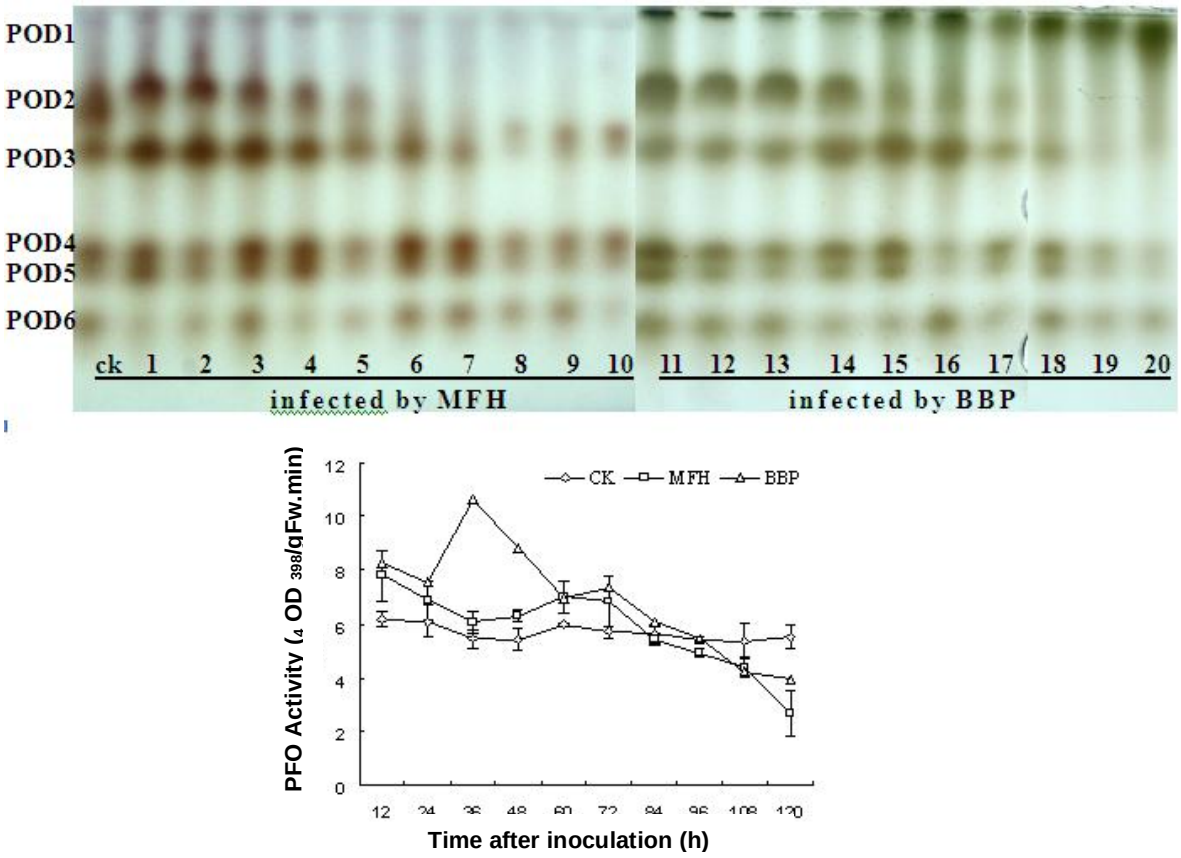


Figure 5. Changes in PPO activity and isozyme in peal calli infected with MFH and BBP. Lanes 1-10: PPO1 decreased with MFH; Lanes 11-20: PPO1 increased with BBP over time.

decreased. PPO activity increased rapidly following a slight decrease, and reached the highest level at the 36th h in BBP-infected calli, which was significantly higher than the control ($p < 0.05$), and then it slowly decreased. On the contrary, there was no significant PPO activity peak in MFH-infected calli but a slight increase was shown after 48 to 72 h inoculation, followed by a rapid decline.

There were several changes in PPO isozymes in response to the two fungal infections (Figure 5). While no new isozyme band was induced during the infection, PPO2 (Rf 0.27) band disappeared and the intensities of most bands declined at later stages. There are two major differences between MFH and BBP. First, PPO1 decreased with MFH but increased with BBP over time. Second, PPO3 showed increased intensity with both MFH and BBP, but the intensity peaked early with MFH than with BBP.

The EST isozyme analysis

The EST isozmyes responded to the fungal infection with changed expression levels and induction of new isozymes (Figure 6). When compared with the control (lane 0), there were significant differences in specific isozyme bands of EST infected by the two fungi. For MFH-infected calli (lanes 1 to 10), the EST specific isozyme bands did not significantly change, but a new isozyme band EST5 (Rf O.81) appeared 72 h after infection. FOR BBP-infected calli (lanes 11 to 20), other EST specific isozyme bands did not significantly change, but new isozyme bands EST8 (Rf 0.32) and EST9 (Rf O.45) appeared with a high expression amount 24 h after infection. It was observed that both fungi can induce pear calli to produce new isozymes. When compared with MFH, BBP induced pear calli to produce more EST isozyme bands with a significantly higher expression amount.

DISCUSSION

The processes plants adopt in order to defend themselves against pathogens are indeed complex. Some defense enzymes, including POD, SOD, CAT, PPO and EST play a very important role in these processes. The correlations between aggravation of diseases and enzymes activity were significant (Silva et al., 2004; Jetiyanon, 2007). In this experiment, some significant

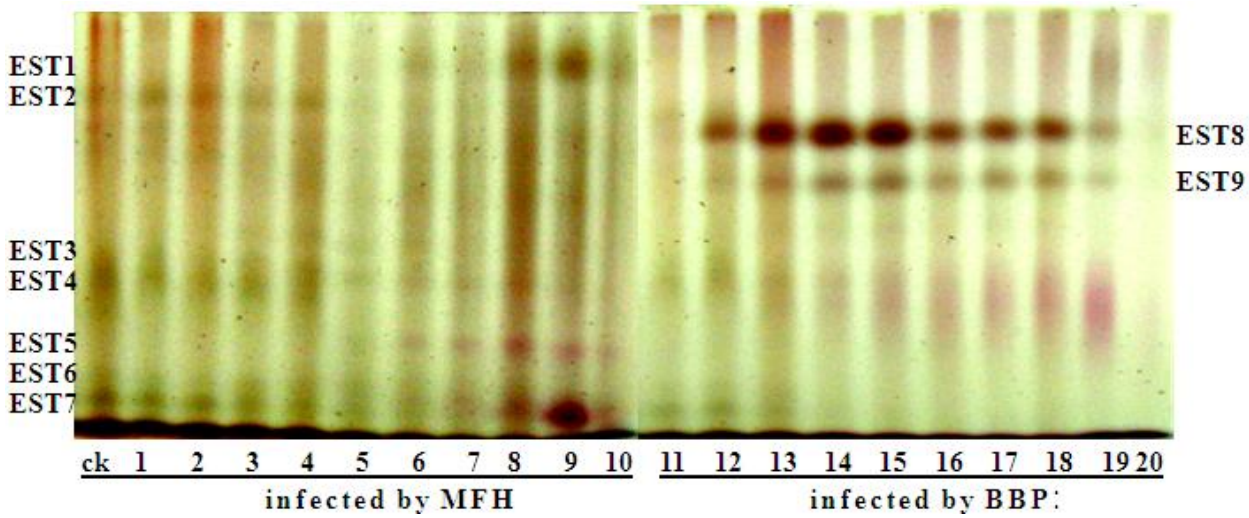


Figure 6. Changes in gel of EST isozyme in peal calli infected with MFH and BBP. Lanes 1-10: For MFH-infected calli, the EST specific isozyme bands did not change significantly, though a new isozyme band EST5 (Rf O.81) appeared 72 h after infection; Lanes 11-20: For BBP-infected calli, other EST specific isozyme bands did not significantly changed, though new isozyme bands EST8 (Rf 0.32) and EST9 (Rf O.45) appeared with a high expression amount 24 h after infection.

changes in the activities and isozymes of defense enzymes in pear calli infected by MFH and BBP were observed (Figures 2 to 6).

ROS are often detected in plant-pathogen interactions and are associated with symptom development. In this experiment, we found that the four defensive enzymes' activities were effective in controlling brown rot caused by BBP and MFH in pear calli. In order to further verify whether or not the defensive enzymes' isozymes influenced pathogen-induced oxidative damage, the isozymes were analyzed. The results show that more new isozymes were induced during BBP infection than MFH infection, implying that the isozymes had potent efficacy in alleviating pathogen-induced oxidative damage in pear calli.

The mechanism by which defensive enzymes mitigated pathogen-induced oxidative stress is complicated. Generally, an appropriate intracellular balance between ROS generation and scavenging exists in all cells. This redox homeostasis requires the efficient coordination of reactions in different cell compartments and is governed by complex signal transduction pathways. Plants possess an array of antioxidants that can protect cells from oxidative damage by scavenging ROS. The scavengers include ascorbate, glutathione and hydrophobic molecules (tocopherols, carotenoids and xanthophylls) and detoxifying enzymes that operate in the different cellular organelles (Noctor and Foyer, 1998). These enzymes include SOD, CAT and POD, which work together with other enzymes of the ascorbate-glutathione cycle to promote the scavenging of ROS (Hernandez et al., 2001). SOD catalyzes the dismutation of O_2 to H_2O_2 and O_2. CAT is present in the peroxisomes of nearly all aerobic cells (Dionisio-Sese and Tobita, 1998). It can protect the cell from H_2O_2 by catalyzing its decomposition into O_2 and H_2O (Foyer and Noctor, 2000). POD is widely distributed in all higher plants and protects cells against the destructive influence of H_2O_2 by catalyzing its decomposition through the oxidation of phenolic and enodiolic co-substrates (Asada, 1992; Borsani et al., 2001). In this study, BBP and MFH significantly stimulated the activities of antioxidant enzymes POD, SOD and CAT (Figures 2 to 5), and the expressions of their isozymes were also increased compared to the control. The antioxidant enzymes' activities in BBP-infected calli were always higher than those in MFH-infected calli, due to the fact that many new isozyme bands were induced by BBP and not MFH. These results indicate that the high levels of POD, SOD and CAT played important roles in reducing damage caused by pathogen, which partially account for the observed delay in symptom development in pear calli. Thus, we considered that the mechanisms by which BBP-infected calli inhibit decay development may be related to alleviation of pathogen-induced ROS.

Thipyapong and Steffen (1997) found that PPO has a wide range of responses to multiple induction signals such as infection of bacteria or fungi, physiological stress, mechanical damage and signal molecules (methyl jasmonate, salicylic acid, ethylene and cAMP). These experimental treatments can induce PPO gene expression and strengthen the stability of the corresponding mRNA (Bashan et al., 1985; Sommer, 1994). PPO is also involved in the oxidation of polyphenols into quinones using molecular oxygen as an electron acceptor and lignification of plant cells during microbial infection (Chittoor et al., 1999). Alterations in oxidative processes and phenolic metabolism are still debatable because it is not certain whether they can play roles in hypersensitive

plant cells or not. A number of studies suggested that PPO may participate in defense reactions and confer hypersensitivity to plants resistant to diseases, for example, potato, cotton and vicia. The new generation of PPO isozymes and the performance of disease resistance for organization are positively correlated. Our results show that both the infection of MFH and BBP did not induce new generation of PPO isozymes. It showed that PPO isozyme changes are related to the interaction of the host and pathogen which is different from existing reports. Different pathogens may induce a similar resistant response and activate different signal transduction pathways with different defense responses when pathogens infected the same plant.

At present, few reports are available about the change of EST activities and isozyme after fungi infection. This study's results demonstrated that the new EST isozyme bands can be induced by BBP and MFH, but the color, width and changes in specific enzyme bands induced by MFH are very different from those induced by BBP. The isozymes induced by BBP have a large amount of isozyme bands and high gene expression. However, the role played by EST isozymes in plant defense response should be further studied.

Conclusion

The metabolism of plant defense response including its physiological and biochemical reactions catalyzed by enzymes is very complex. In this experiment, on the one hand, the defensive enzymes' activities and isozymes of BBP-infected calli changed significantly. The tissue induced many new bands which are particularly expressed more after infection. This is in agreement with the tissue being inoculated with BBP which causes it to decay slowly. On the other hand, after inoculation with two different fungi for the same kind of tissue, its defensive enzymes' activities and isozymes changed vigorously following further changes that were observed relatively. This indicates that the defensive enzymes' activities and isozymes are closely related to disease resistance of fruit, and they play an important role in the process of tissue biochemical defense. As such, it is a kind of performance for plant system to acquire resistance ability.

REFERENCES

Asada K (1992). Ascorbate peroxidase: a hydrogen peroxide-scavenging enzyme in plants. Plant Physiol. 85:235-241.

Bashan Y, Okon Y, Henis YP (1985). Polyphenoloxidase and phenols in relation to resistance against Pseudomonas sy-ringae pv. tomato in tomato plants. Can. J. Bot. 65:366-372.

Borsani O, Valpuesta V, Botella MA (2001). Evidence for a role of salicylic acid in the oxidative damage generated by NaCl and osmotic stress in Arabidopsis seedlings. Plant Physiol. 126:1024-1030.

Bradford MM (1976). A Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72:248-254.

Chittoor, JM., Leach JE, White FF (1999). Induction of peroxidase during defense against pathogens. in: PathogenesisRelated Proteins in Plants. S. K. Datta and S. Muthukrishnan, eds. CRC Press. Boca Raton, FL, U.S.A. pp.171-193

Dionisio-Sese ML, Tobita S (1998). Antioxidant responses of rice seedlings to salinity stress. Plant Sci. 135:1-9.

Foyer CH, Noctor G (2000). Oxygen processing in photosynthesis: regulation and signalling. New Phytol. 146: 359-388.

Hernandez JA, Ferrer MA, Jimenez A, Barcelo AR, Sevilla F (2001). Antioxidant systems and O^{2} − /H_2O_2 production in the apoplast of pea leaves. Its relation with saltinduced necrotic lesions in minor veins. Plant Physiol. 127:827-831.

Jetiyanon K (2007). Defensive-related enzyme response in plants treated with a mixture of Bacillus strains (IN937a and IN937b) against different pathogens. Biol. Control 42:178-185.

Kneer R, Poulev AA, Olesinski A, Raskin I (1999). Characterization of the elicitor-induced biosynthesis and secretion of genistein from roots of *Lupinus luteus* L. J. Exp. Bot. 50:1553-1559.

Low PS, Merida JR (1996). The oxidative burst in plant defense: function and signal transduction. Physiol. Plant 96:533-542.

Murr DP, Morris LL (1974). Influence of O_2 and CO_2 on o-diphenol oxidase activity in mushrooms. J. Am. Soc. Hortic. Sci. 99:155-158.

Nam JK (2008). Inhibition of powdery mildew development and activation of antioxidant enzymes by induction of oxidative stress with foliar application of a mixture of riboflavin and methionine in cucumber. Sci. Hortic-Amsterdam 118: 181-188.

Noctor G, Foyer CH (1998). Ascorbate and glutathione: keeping active oxygen under control. Ann. Rev. Plant Biol. 49:249-279.

Sandra R, Marijana RS, Branka PK (2006). Influence of NaCl and mannitol on peroxidaseactivity and lipid peroxidation in *Centaurea ragusina* L.roots and shoots. J. Plant Physiol. 163:1284-1292.

Shein V, Andreeva ON, Polyakova GG, Zrazhevskaya GK(2003). Effect of pine callus elicitation by the Fusarium strains of various pathogenicityon the content of phenolic compounds. Russ. J. Plant Physiol. 50:634-639.

Silva HSA, Romeiro RS, Macagnan D, Halfeld-Vieira BA, Pereira MCB, Mounteer A (2004). Rhizobacterial induction of systemic resistance in tomato plants non-specific protection and increase in enzyme activities. Biol. Control 29:288-295.

Sircar D, Mitra A (2008). Evidence for p-hydroxybenzoate formation involving enzymatic phenylpropanoid side-chain cleavage in hairy roots of *Daucus carota*. J. Plant Physiol. 165:407-414.

Sommer A, Neeman E, Steffens JC, Mayer AM, Harel E (1994). Import targeting and processing of a plant polyphenol oxidase. Plant Physiol. 105:1301-1311.

Stratmann J, Scheer J, Ryan CA (2000). Suramin inhibits initiation of defense signaling by systemin, chitosan, and a b-glucan elicitor in suspension-cultured Lycopersicon peruvianum cells. Proc. Natl. Acad. Sci. 97:8862-8867.

Thipyapong P, Steffens JC (1997). Tomato polyphenol oxidase (differential response of the polyphenol oxidase F promoter to injuries and wound signals). Plant Physiol. 115: 409-418.

Tzeng DDS, Devay JE (1993). Role of oxygen radicals in plant disease development. Plant Pathol. 10:1-34.

Wang Y, Li X, Bi Y, Ge YH, Li YC, Xie F (2008), Postharvest ASM or harpin treatment Induce resistance of muskmelons against *Trichothecium roseum*. Agric. Sci. China 7(2):217-223.

Wang YS, Tian SP, Xu Y, Qin GZ, Yao HJ (2004). Changes in the activities of pro- and anti-oxidant enzymes in peach fruit inoculated with Cryptococcus laurentii or *Penicillium expansum* at 0 or 20°C. Postharvest Biol. Tech. 34:21-28.

Xu XB, Qin GZ, Tian SP (2008). Effect of microbial bio control agents on alleviating oxidative damage of peach fruit subjected to fungal pathogen. Int. J. Food Microbiol. 126:153-158.

Yao HJ, Tian SP (2005). Effect of pre- and post-harvest application of salicylic acid or methyl jasmonate on inducing disease resistance of sweet cherry fruit in storage. Postharvest Biol. Tech. 35:253-262.