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RESEARCH ARTICLE

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Role of cell wall degrading enzymes in the interaction of poplar and *Melampsora larici-populina* Kleb.

Abstract The activity of cell wall-degrading enzymes, produced in poplar cultivars infected *Melampsora larici-populina* Kleb., was studied. The results show that PMG, PMTE, C_X and β-glucosidase played roles during the infection. After inoculation, the activity of PMG in both susceptible and resistant cultivars had two peak values in 2 dpi and 5 dpi. The activities of PMTE and β-glucosidase had a peak value in 3 dpi, and C_X in 2 dpi. Among these cell wall-degrading enzymes, the activities of PMG and PMTE were higher and the activities of C_X and β-glucosidase were relatively lower. The activities of these cell wall-degrading enzymes were significantly higher in susceptible cultivars than those in resistant cultivars. All these demonstrated that these cell wall-degrading enzymes played certain roles in the infection of *M. larici-populina*.

Keywords *Melampsora larici-populina*, cell wall-degrading enzymes, poplar

1 Introduction

Melampsora larici-populina Kleb., a widespread and frequent disease of poplar seedlings and saplings, has the characteristics of frequent pathogenic variations and various host species, and will lead to serious economic losses and constraints of commercial and ecological poplar forest development (Tian et al., 1999). In recent years, many studies focused mainly on pathogenic differentiation, host resistance and the like, but there is a lack of research on its pathogenesis mechanism (Pei and McCracken, 2005) which is the theoretical foundation of resistance breeding.

The plant cell wall is the first barrier for the invasion of fungal pathogens. Therefore, during the invasion, the cell wall degrading enzymes (CWDEs), which is secreted by pathogens, is a crucial factor for the successful development of the disease in host. These related enzymes have a close relationship with pathogenesis (Zhao and Zhang, 2002). Many researches have indicated that CWDEs play an important role during the invasion of necrotrophic pathogens. But studies related to the role of CWDEs in biotrophic pathogens are rarely reported (Li et al., 2000; Lu, 2005). Research on wheat rust, bean rust and other rust pathogens revealed that CWDEs exist during the invasion stage in biotrophic pathogens (Xu and Mendgen, 1997; Lu, 2005). After studying cell wall degrading enzymes in the interaction of wheat and rust, Lu (2005) found that pectin methylgalacturonase (PMG), pectin methyl trans-eliminase (PMTE), carboxymethyl cellulose (C_X) and β-glucosidase (β-G), which were secreted much less during the interaction than in necrotrophic pathogens, also played important roles in wheat stripe rust invasion. Although research on dynamic changes of host enzymes during the interaction stage of *Melampsora* spp. and its host were reported in recent years (Wei, 1984; Fan et al., 1989), there is still a lack of research on whether the CWDEs were secreted by *Melampsora* during the interaction. Therefore, our study focused on this issue and demonstrated dynamic changes of CWDEs secreted by *Melampsora* in different compatible interactions with poplar. The function of CWDEs during the interaction of poplar and *Melampsora* will be revealed and these researches also provide a theoretical basis on better understanding the pathogenic mechanism of *M. larici-populina*.

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2 Materials and methods

2.1 Plant materials and fungal strains

The study was conducted on the poplar cultivars *Populus szechuanica* Schneid. and *P. purdomii* Rehd. Cuttings were

collected from one-year-old poplar plants and were grown in a greenhouse.

M. larici-populina strain Bq was collected from Yanqing County in Beijing and strain Sb from Baoxing, Sichuan Province. These two strains belong to different physiological races according to previous studies. All these studies indicated that the reaction type of strain Bq on *P. szechuanica* was [0], while that on *P. purdomii* was [4]. As to strain Sb, the reaction type on *P. szechuanica* was [1] and on *P. purdomii* was [4] (Cao et al., 1998). The methods on strain propagation, storage and reaction type criteria referenced Cao et al. (1998).

2.2 Inoculation and sampling method

Inoculation was carried out on mature leaves of greenhouse-grown plants with the leaf plastochron index (LPI) of 5 to 8. The LPI was basipetally counted from the reference leaf (lamina approximately 20 mm long) at the shoot apex (Larson and Isebrands, 1971). Urediniospores, which were diluted into suspension with a concentration of 2×10^4 spore/mL by distilled water, were applied to the lower (abaxial) surfaces of the poplar leaves with a glass stick with a density of 100 mL/m^2. Control poplar plants were inoculated only with distilled water. All inoculated plants were placed in the greenhouse after keeping them humid for 24 h.

The activities of CWDEs from both inoculated and control plants were measured before inoculation treatment. Samples were collected in 1, 2, 3, 5 and 7 days post-inoculation (dpi) and the dynamic activities of CWDEs were measured.

2.3 Measurement of CWDEs activity

The measurements of CWDE activity were carried out by a spectrophotometer (Li et al., 2000; Lu, 2005). The activities of pectin methylgalacturonase (PMG), pectin methyl trans-eliminase (PMTE), carboxymethyl cellulose (C_X) and β-glucosidase (β-G) were measured and the samples were collected strictly at about 1 g. All the inoculation, sample collection and CWDE activity measurement were repeated three times.

3 Results

3.1 Dynamic changes of pectin methylgalacturonase (PMG)

The activities of PMG in *P. szechuanica* and *P. purdomii* increased in 1 dpi with different pathogenic strains. The change of PMG in inoculated poplars was more significant than in control cultivars, but the difference value of PMG activity (ΔPMG) in inoculated and control plants varied in the two hosts. In the *Melampsora larici-populina–P. szechuanica*, the ΔPMG changed significantly after inoculation with Bq strain in 2 dpi and the peak value appeared in 2 dpi and 5 dpi. The peak value, which was 0.4403 U/mg in 5 dpi, was much higher than that of control treatment at any given time during the research stage. When inoculated by the Sb strain, the ΔPMG rose rapidly and reached its peak value in 2 dpi at the amount of 0.1768 U/mg (Fig. 1). As to the interaction of *Melampsora larici-populina* and *P. purdomii*, the difference in value grew rapidly after it was inoculated by the Bq strain. The peak value appeared in 5 dpi when the activity of inoculated leaves was 1.5220 U/mg, higher than that of control leaves. On the contrary, the peak value appeared in 3 dpi after it was inoculated by the Sb strain and the difference value was 0.4027 U/mg (Fig. 2). Figures 1 and 2 indicated that the ΔPMG in susceptible cultivars was 2.3–3.5 folds higher than that in resistant cultivars, and the ΔPMG in the two cultivars was 1.2–4.4 folds higher after inoculation by the Bq strain than those after inoculation by the Sb strain.

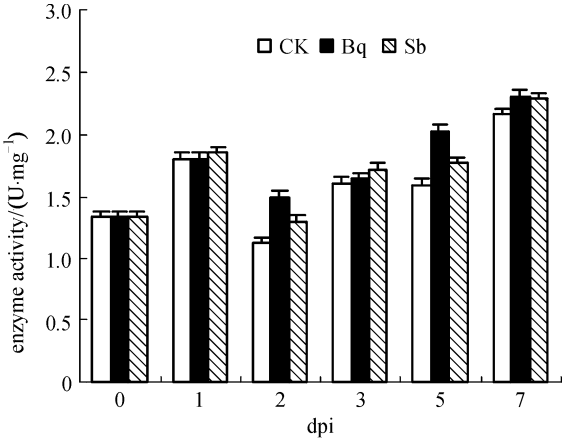

Fig. 1 Changes of PMG activity in *Populus szechuanica* infected by *Melampsora larici-populina*

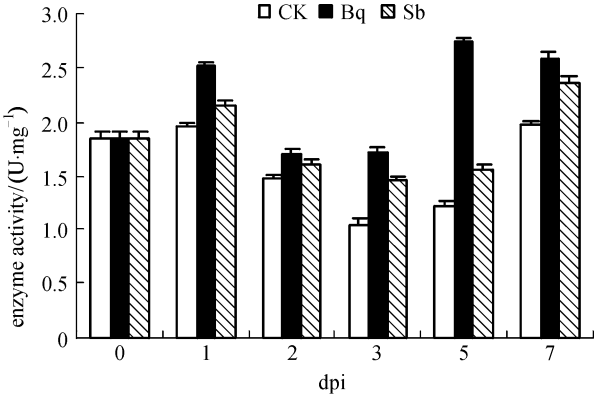

Fig. 2 Changes of PMG activity in *Populus purdomii* infected by *Melampsora larici-populina*

3.2 Dynamic changes of pectin methyl trans-eliminase (PMTE)

The changes of PMTE activity varied in different resistant cultivars, and the ΔPMTE, which was equal to the difference value of inoculated leaf and control leaf, also varied in different pathogen-host interactions during the whole experiment process. In the *Melampsora larici-populina–P. szechuanica* interaction, the ΔPTME activity increased after the inoculation with Sb strain, and reached a peak value at the amount of 0.2809 U/mg in 3 dpi. From then on, the ΔPTME gradually decreased with the development of pathogen invasion. There were two peak values in the *P. szechuanica* when infected by the Bq strain, one appearing in 1 dpi with an amount of 0.1819 U/mg and the other in 3 dpi. Thereafter, the ΔPTME decreased after reaching 0.1410 U/mg in 3 dpi (Fig.3). In the susceptible cultivars, the ΔPMTE rose until 7 dpi after *P. purdomii* was infected by the Sb strain. No peak value appeared during the research. However, after inoculation by the Bq strain, the ΔPMTE increased gradually to a peak value in 3 dpi and the amount reached 0.7473 U/mg. During the whole interaction of the Bq strain and *P. purdomii*, we found that there existed only one peak value in 3 dpi and the ΔPTME activity decreased after the peak value (Fig. 4). Through the observation on the dynamic changes of PTME activity, we found that the PMTE activity in susceptible cultivars was about 2.7–3.2 folds higher than that in resistant cultivars.

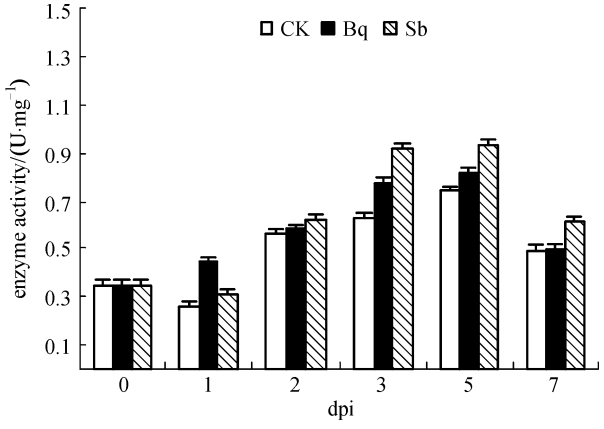

Fig. 3 Changes of PMTE activity in *Populus szechuanica* infected by *Melampsora larici-populina*

3.3 Dynamic changes of carboxymethyl cellulose (C_X)

The C_X activity fluctuated and the difference value of C_X activity in infected and control leaves varied especially in different poplar cultivars after inoculation with different pathogen strains. In *P. szechuanica*, the trends of C_X activity were similar after inoculation by Bq and Sb strains. The peak value appeared in 2 dpi and thereafter, the activity gradually decreased. However, the peak value of the difference value varied in the two interactions with the

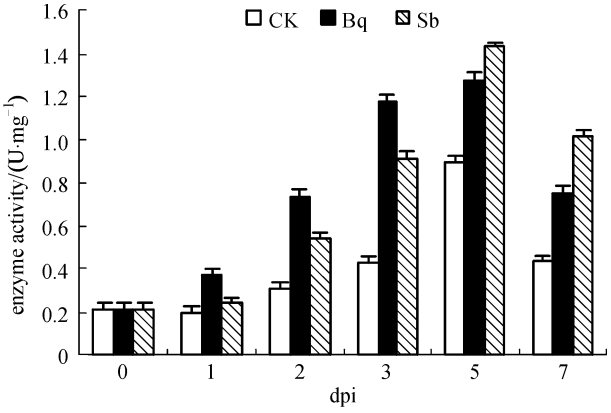

Fig. 4 Changes of PMTE activity in *Populus purdomii* infected by *Melampsora larici-populina*

amount of 0.0895 U/mg in the Sb–*P. szechuanica* interaction and with the amount of 0.0564 U/mg in the Bq–*P. szechuanica* interaction, respectively (Fig. 5). In *P. purdomii*, the changes on the difference value of C_X activity were similar after infected by the two different strains. The difference value increased to a certain amount and then slightly decreased until the end of observation. By contrast, the amount and occurring time of peak value were different. The peak value existed in 3 dpi at the amount of 0.28629 U/mg after inoculation by the Sb strain and the peak value appeared in 2 dpi at the amount of 0.2785 U/mg in the leaves infected by the Bq strain (Fig. 6). Generally, the activity of C_X was much lower in resistant cultivars than in susceptible cultivars during the whole research.

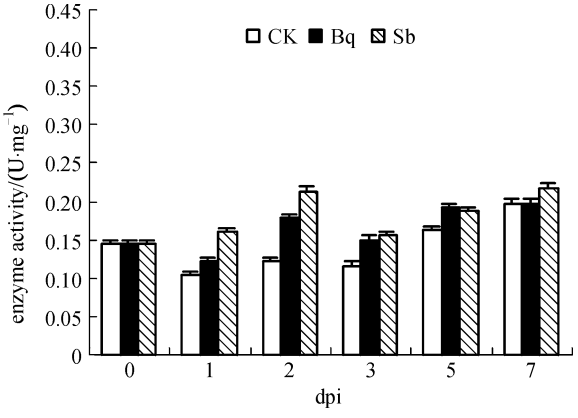

Fig. 5 Changes of C_X activity in *Populus szechuanica* infected by *Melampsora larici-populina*

3.4 Dynamic changes of *β*-glucosidase (*β*-G)

The activity of *β*-G in *P. szechuanica* changed distinctly after infection by the Sb strain, and the difference value (Δ*β*-G) reached 0.0451 U/mg in 1 dpi. Thereafter, the Δ*β*-G decreased after reaching the peak value in 2 dpi with the amount of 0.0623 U/mg. As to the *P. szechuanica* infected by Bq strain, the peak value of Δ*β*-G was 0.0333 U/mg in 3

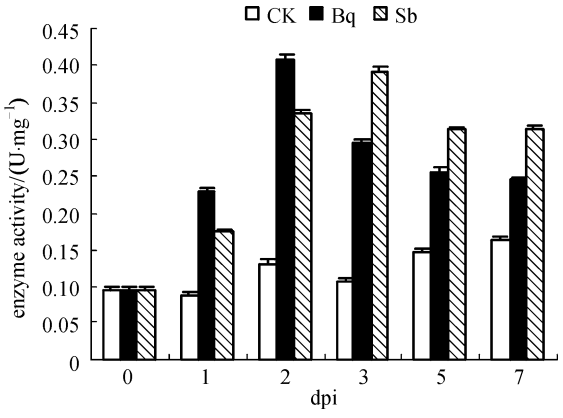

Fig. 6 Changes of C_X activity in *Populus purdomii* infected by *Melampsora larici-populina*

dpi (Fig. 7). In *P. purdomii* infected by Sb and Bq strains, the $\Delta\beta$-G changed similarly and the peak value appeared in 3 dpi. Nevertheless, the peak values of different interactions also varied. In the Sb–*P. purdomii* interaction, the peak value was 0.1791 U/mg. In the leaves infected by the Bq strain, the peak value was 0.2105 U/mg in 3 dpi (Fig. 8).

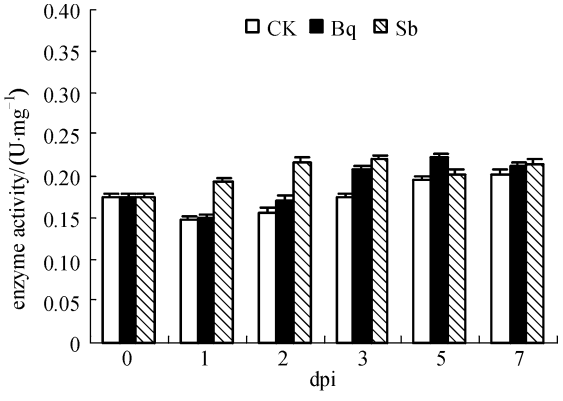

Fig. 7 Changes of β-G activity in *Populus szechuanica* infected by *Melampsora larici-populina*

4 Discussion

Based on the dynamic changes of cell wall-degrading enzymes (CWDEs) during the interaction of poplar cultivars and *Melampsora larici-populina*, the pectin methylgalacturonase (PMG), pectin methyl trans-eliminase (PMTE), carboxymethyl cellulose (C_X) and β-glucosidase (β-G) could be detected. However, the activities of these CWDEs were lower during the interaction with their host than those secreted by necrotrophic pathogens such as *Cladosporium cucumerinum* Ellis & Arthur, *Botrytis cinerea* Pers. ex Fr., *Curvularia lunata* (Wakker) Boed and so on (Li et al., 2000, 2003; Feng et al., 2002; Chen et al., 2006). Meanwhile, the CWDEs activities in susceptible cultivars were about 2–5 folds higher than those in resistant

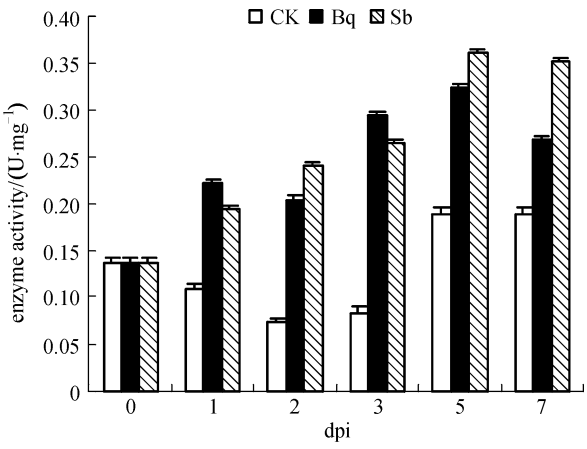

Fig. 8 Changes of β-G activity in *Populus purdomii* infected *Melampsora larici-populina*

cultivars. All these indicated that *M. larici-populina* could secrete these kinds of CWDEs just as other biotrophic pathogens during the invasion stage, and the results also show that the CWDEs play a vital role in pathogenic differentiation.

The plant cell wall is the first barrier that must be breached by fungal pathogens. Therefore, pathogens must secrete a series of CWDEs in order to penetrate into plant tissues and eventually absorb the nutrients from host species. Among these CWDEs, PMG and PMTE can degrade the pectin while the C_X and β-G decompose the cellulose which eventually dehydrates into glucose through metabolism. These kinds of CWDEs promote the nutrition absorption of pathogen by comprehensive action (Zhao and Zhang, 2002). The biotrophic pathogens such as rust and powdery mildew have different ways of nutrition absorption in comparison with necrotrophic pathogens and semi-biotrophic pathogens which destroy the host tissue in order to uptake useful nutrition. The biotrophic pathogens degrade cells in the infection instead of destroying the tissue thoroughly. So it can be supposed that the way of fungal degradation of host walls or wall appositions seems to follow complicated schemes in order to achieve successful invasion and colonization rather than undermining the host cell, thereby maintaining the integrity of the host tissue (Christop et al., 1999). After ultrastructural study of the *Melampsora* during the penetration process, Tian et al. (2002) found that the hypha of *Melampsora* elongated through cell wall space and the pathogen did not destroy the host cell during the invasion. In the present study, we also found that the activities of PMG and PMTE were higher than the activities of C_X and β-G both in the resistant and susceptible cultivars. Through these findings, we speculate that in order to elongate quickly through the cell wall space without destroying the tissue, PMG and PMTE degrade the pectin substances which exist in the thin layer of cell wall space and primary wall. As to C_X and β-G, they play important roles in hypha invasion and coloniza-

tion by decomposing the cellulose in the secondary cell wall. Besides, the conclusion that the activities of PMG and PMTE were much higher than the activities of C_X and β-G indicated that pectinase plays more important roles than cellulose enzyme during the invasion stage of *M. larici-populina*.

Many researches have revealed that the dikaryotic urediospores of U. *viciae-fabae* secrete a number of cell wall degrading enzymes in a highly regulated manner during the pathogen development in the host tissue. Cellulase production occurs already during appressorium formation. Pectin methylesterases are secreted during hypha development, and polygalacturonate lyase is produced during differentiation of haustorial mother cells (Heiler et al., 1993; Deising et al., 1995). In our study, we also found that in the *M. larici-populina* and poplar cultivars interaction, the peak value of ΔPMG appeared in 1 dpi and 5 dpi both in susceptible and resistant cultivars. Other CWDEs activities also had similar trends as the difference value of PMG activity. All these can reveal that the secretion of CWDEs in *Melampsora larici-populina* has strict schemes during the hyphal invasion. The pathogens of *M. larici-populina* control the secretion process and the process cannot be influenced by the host defense system. So the secretion stage of these CWDEs are similar both in compatible and incompatible combination.

The CWDEs is not only the main pathogenic factor of pathogen, but also the inducible factor of host resistant reaction (Zhao and Zhang, 2002). In an incompatible combination of *Cladosporium cucumerinum* and its host, the activities of CWDEs increased to a large amount at the beginning of the interaction. These phenomena indicate that these CWDEs play important roles in host defense reaction induction just as elicitor (Li et al., 2000). We also found that the activities of PMTE, PMG, and C_X appeared at similar peak values in 1 or 2 dpi during the interaction of poplar and leaf rust. It can be speculated that these CWDEs in biotrophic pathogens play the same roles as necrotrophic pathogens. In order to maintain the integrity of the host tissue, these CWDEs can induce the host defense reaction which can produce a series of host protection enzymes to prohibit the development of hypha at the threshold of host and pathogen interaction.

CWDEs have a close relationship with infection and colonization of *M. larici-populina* in the poplar cultivars. During the research for many years, we found that the activities of CWDEs might be affected by environmental temperature and humidity (Li et al., 2003). The activities of CWDEs could decrease while the temperature increased. By contrast, they rose in accordance with high humidity. We used the difference value of CWDEs activities to accurately reveal the function of these enzymes in infected and control leaves during the process of invasion. But, the CWDEs activities could not be analyzed in a given time because of environmental factors. Therefore, we should focus on the determination of suitable temperature and relative humidity in order to reveal the temporal and spatial expression of CWDEs in the interaction of poplar and leaf rust. Meanwhile, further ultrastructural studies on the effects of CWDEs on the host and on interaction mechanism of pectinase and cellulose enzyme during the pathogen development should be carried out in order to reveal the function of CWDEs in the interaction of biotrophic pathogen and host.

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References

Cao Z M, Li Z Q, Hu J J (1998). Physiological specialization in *Melampsora larici-populina*Kleb. in the Qinling Mountains. J Northwest For Coll,13(1): 53–57 (in Chinese)

Chen X J, Zhang H, Xu J Y, Tong Y H, Ji Z L (2006). Cell wall degrading enzymes produced by *Rhizoctonia solani*and their pathogenicity to rice plants. Jiangsu J Agr Sci, 22(1): 24–28 (in Chinese)

Deising H, Frittrang A K, Kunz S, Mendgen, K (1995). Regulation of pectin methylesterase and polygalacturonate lyase activity during differentiation of infection structures in *Uromyces viciae-fabae*. Microbiology, 141:561–571

Fan Z H, Shen R X, Zhou Z M. (1989). The relationships between the activities of peroxidase and phynelalanine ammonialyase and the resistance of poplar to leaf rust. Acta Phytopathol Sin, 19(2): 95–100 (in Chinese)

Feng J, Gao Z G, Xue C S, Zhuang J H, Chen J, Bai S Y (2002). The pathogenesis of the cell-degrading enzymes produced by *Curvularia lunata*. Rain Fed Crop, 22(3): 164–166 (in Chinese)

Heiler S, Mendgen K, Deising H (1993). Cellulotic enzymes of the obligately biotrophic rust fungus *Uromyces viciae-fabae* are regulated differentiation-specifically. Mycol Res, 97: 77–85

Larson P R, Isebrands J G. (1971). The plastochron index as applied to developmental studies of cottonwood. Can J For Res, 1: 1–11

Li B J, Chen L Q, Meng W J, Wang F J (2003). Effect of temperature and humidity on cell wall degrading enzymes produced by *Botrytis cinerea* during infection of tomato plant. Acta Phytopathol Sin, 33(3): 209–212 (in Chinese)

Li B J, Zhou C L, Zhao K H, Li F Y (2000). Pathogenic mechanism of scab of cucumber caused by *Cladosporium cucumerinum* II: The cell wall degrading enzymes and its pathogenic action. Acta Phytopathol Sin, 30(1): 13–18 (in Chinese)

Lu X F (2005). Study on Proteins in Interaction System between Wheat and Stripe Rust Fungi. Yangling: Northwest A & F University (in Chinese)

Pei M H, McCracken A R (2005). Rust Diseases of Willow and Poplar. CABI Publishing, 1–264

Tian C M, Li Z Q, Kang Z S (1999). Advances in researches of cathay poplar leaf rust (*Melampsora larici populina* Kleb.). J Northwest For Coll, 14(2): 81–88 (in Chinese)

Tian C M, Liang Y M, Kang Z S, Li Z Q, Zhao Y X (2002).

Ultrastructure of poplar leaf Infected by rust fungus (*Melampsora larici-populina* Kleb.). Acta Phytopathol Sin, 32(1): 71–78 (in Chinese)

Wei Y N (1984). A study on activities of polyphenol oxidase and peroxidase, and tendency in changes of isozyme spectra in Chinese white poplar (*Populus tomentosa* Carr.) leaves after infected with *Melompsora magnusiana*Wagner. J Beijing For Univ, 17(3): 73–92 (in Chinese)

Xu H, Mendgen K (1997). Targeted cell wall degradation at the penetration site of cowpea rust basidiosporelings. Am Phytopathol Soc, 10(1): 87–94

Zhao L, Zhang T Y (2002). The role of degrading enzymes in plant-pathogen interaction. Microbiology, 29(1): 89–93 (in Chinese)