Expression and functional analysis of a PR-1 Gene, \textit{MuPR1}, involved in disease resistance response in mulberry (\textit{Morus multicaulis})

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\textbf{ABSTRACT}

In the present study, the cDNA (designated \textit{MuPR1}) of PR1 gene was obtained from mulberry. Our results showed that \textit{MuPR1} shares some conserved characteristics with its homologues from different plant families. \textit{MuPR1} was localized exclusively to the extracellular spaces, and the \textit{MuPR1} gene was expressed constitutively in all selected mulberry tissues, although its expression levels varied substantially. It was also showed that \textit{MuPR1} expression was induced by pathogen, MeJA, SA and GA\textsubscript{3}, and transgenic Arabidopsis overexpressing \textit{MuPR1} showed enhanced resistance to \textit{Botrytis cinerea} and \textit{Pst. DC3000}. Moreover, the peptide derived from \textit{MuPR1} protein C-terminus with a PxGNNxxxxx\textit{PY} motif was suggested to play a role in activating plant resistance. Furthermore, the \textit{MuPR1} gene may have roles in mediating the rates of oxygen radical formation and detoxification. Therefore, the \textit{MuPR1} gene may be a candidate gene for breeding resistant mulberry varieties in the future.

\textbf{Abbreviations:} CAPE1-like: CAP-derived peptide 1-like; DAB: 3,3′-Diaminobenzidine; GA\textsubscript{3}: gibberelic acid; GFP: green fluorescent protein; MeJA: methyl jasmonate; NBT: nitroblue tetrazolium; PR: pathogen-related protein; \textit{Pst}. DC3000: Pseudomonas syringae pv tomato DC3000; SA: salicylic acid.

\section{1. Introduction}

Mulberry (\textit{Morus}) is a perennial woody tree species and is widely cultivated all over the world, and its leaves have been used as the natural food for silkworms for thousands of years (He et al. 2013). It is susceptible to various diseases throughout its life, and these diseases will seriously affect the yield and quality of mulberry leaves, which will have a serious impact on sericulture production (Kumar and Gupta 2004). Because silkworm is very sensitive to pesticides, the improper use of pesticides will have adverse effects on silkworm growth and development, and even cause the death of silkworm (Ji et al. 2008). In addition, the application of pesticides will also generate adverse environmental consequences (Zhang et al. 2018). Therefore, it is not feasible to use agrochemicals in mulberry orchards, and many researchers are committed to breed resistant varieties to defend mulberry from diseases (Khurana and Checker 2011). However, the conventional mulberry breeding is restricted by the complex disease resistance, limited resistant germplasm resources and poor heredity of mulberry trees. Transgenic approaches can overcome the limitations of traditional breeding and is a good alternative way to breed disease-resistant varieties (Hou et al. 2014; Ali et al. 2018a). However, the available resistance genes of mulberry are very limited, and the molecular mechanism of interaction between mulberry and pathogens is still poorly understood. Therefore, it is very important and essential to understand the molecular mechanism of plant–pathogen interactions and explore the disease resistance genes for mulberry improvement.

Plants, being sessile, are often exposed to various unfavorable environmental conditions that impact growth and development of plants (Claey and Inzé 2013). In the long term evolution, plants have possessed complex and sophisticated mechanisms to resist pathogen infection and inhibited disease development (Velásquez et al. 2018). The invading pathogens or their elicitors can trigger the defense responses in plant and result in extensive transcriptional reprogramming. Among these responses, many of pathogen-related protein (PR) genes are induced, and the proteins were significantly accumulated in plants (Peng and Kaloshian 2014; Wen et al. 2017). Since the PR proteins were first described in tobacco leaves (\textit{Nicotiana tabacum L.}) they have been identified from many other plant species belonging to several families. Presently, PR-proteins are grouped into 17 families with diverse properties and functions (Christensen et al. 2002), and PR1s are regard as marker proteins of induced plant immune and have important and constructive functions in systemic acquired resistance (SAR) establishment (Glazebrook 2005; Ali et al. 2018a). PR1 proteins among plants are highly conserved in structure and have highly similarity in some domains, indicating that they have general roles in the response of plant to biotic stress (Lincoln et al. 2018). Previous studies showed that the transcription and translation level of \textit{PR1} was significantly increased in the plants under biotic stresses (Ali et al. 2018b; Shi et al. 2019; Tunsagool et al. 2019; Yang et al. 2019). When the \textit{PR1} genes were over-expressed in different plants, the transgenic plants generally exhibit enhanced increased disease resistance toward many plant disease-causing bacteria, fungi, and even viruses, and conversely, the \textit{PR1} gene silenced plants showed more susceptibility than wild-type to pathogen infection (Cutt et al. 2019).
data, and the RT and Arabidopsis plants were transformed with the Agrobacterium tumefaciens introduced into

The gene was ligated into the expression vector pBI121 and

MODEL online tools (http://swiss-model.expasy.org/).

derived molecular pattern (Chen et al. 2014; Chien et al. 2015; Santén et al. 2017). Moreover, the PR1 gene

c-terminus. The CAP-derived peptide 1-like (CAPE1-like) of the C-termi

protocol (Clough and Bent 1998). After transformation, the sterilized T1 seeds were plated on kanamycin (50 μg mL⁻¹) selection medium to select transformed plants, and the T3 generations were used for further functional studies.

2.4. Determination of MuPR1 subcellular localization

The MuPR1 was fused with green fluorescent protein (GFP) gene, and the fusion gene was ligated into the expression vector pBI121 under the control of 35S to produce 35S::MuPR1-GFP and 35S::GFP, respectively, and then the vectors constructed were introduced into GV3101 strain which was used to transformat Arabidopsis plants. The GFP expression was examined using a fluorescence microscope (Olympus BX51; Tokyo, Japan).

2.5. Mulberry seedling treatments

For Pseudomonas syringae pv. mori inoculation, the abaxial surface of young mulberry leaves were brushed with the bacterial suspension (10⁶ CFU mL⁻¹). As for Colletotrichum dematium inoculation, sterilized filter paper discs (8-mm-diameter) were soaked with the conidial suspension (2.5 × 10³ conidia mL⁻¹) and then were applied onto the adaxial surface of young mulberry leaves. Then inoculated leaves were covered with polyethylene bags for 48 h. The seedlings inoculated with 10 mmol L⁻¹ MgCl₂ were used as controls.

2.6. qRT-PCR analysis

RNAs were extracted and cDNAs were synthesized as describe above, and qRT-PCR analysis was performed with the SYBR Premix Ex Taq™ kit (Takara, Dalian, China). The qRT-PCR primers were designed based on our available mulberry transcriptome data. The comparative cycle threshold (Ct) method (Livak and Schmittgen, 2001) was used to evaluate the relative mRNA amount with the actin gene as an internal reference mRNA. All samples were assayed in triplicate.

2.7. Promoter analysis

The promoter region was isolated with the Tail-PCR method. The promoter obtained was subcloned into the vector pBI121 to replace the 35S promoter and fused with GUS. The vector constructed was introduced into GV3101 strains, and the transformed strains were used to transform Arabidopsis plants for stable expression. Moreover, tobacco leaves were infiltrated with the transformed strains for transient expression analysis using the method described before (Arpat et al. 2012). GUS activity was detected by histochemical staining method as described by Jefferson et al. (1987).

2.8. Arabidopsis plant treatments

The rosette leaves of four-week-old Arabidopsis were inoculated with P. syringae pv tomato DC3000 (Pst. DC3000) by injecting 50 μL of the bacterial suspensions (10⁶ CFU mL⁻¹) with a syringe. The rosette leaves inoculated with 10 mmol L⁻¹ MgCl₂ were used as mocks. The rosette leaves were inoculated with Botrytis cinerea by placing 5-μL droplets of spore suspension (2 × 10⁶ conidia mL⁻¹). Inoculated plants were placed in a plastic tray and covered with a transparent
2.9. Determination of resistance against pathogens

The 4-week-old wild type and MuPR1 transgenic Arabidopsis seedlings were used for the determination of resistance. The seedlings were challenged with *Pst.* DC3000 or *B. cinerea* as described above. The inoculated seedlings were examined daily for disease incidence and severity. Meantime, the inoculated leaves were ground and then were serially diluted and plated on King’s B medium. After incubation, the colonies on the medium were counted to determine the number of bacteria in the leaves. All the experiments were conducted independently three times.

2.10. Bioactivity analysis of CAPE1

The MuCAPE1 peptide was synthesized by Beijing Protein Innovation Co., Ltd. Arabidopsis and mulberry seedlings were sprayed with 100 nmol L\(^{-1}\) CAPE1, respectively, for 2 h prior to challenge with pathogen. Plants were sprayed with sterile water served as controls. Then the Arabidopsis seedlings were inoculated with *Pst.* DC3000 or *P. syringae pv. mori* as described above. The infection symptoms in Arabidopsis and mulberry were observed at 3 and 5 d after inoculation, respectively. At the same time, the bacterial numbers in the leaves of Arabidopsis and mulberry were calculated.

2.11. Hydrogen peroxide and superoxide assays

Superoxide (O\(_2\)\(^{-}\)) production in the leaves was determined by nitroblue tetrazolium (NBT) staining. Arabidopsis leaf was firstly infiltrated with NBT solution (6 mmol L\(^{-1}\)), after that they were infiltrated with lacto-glycerol-ethanol solution (1:1:4, v/v/v) and subjected to 5 min boiling in water to

Figure 1. Amino acid sequences and structural analysis of MuPR1 protein. (A) Nucleotide and amino acid sequences of MuPR1 with putative phosphorylation sites which are marked with asterisks. (B) The MuPR1 protein was predicted to contain a conserved motif at residues 29–164 aa that belonged to the SCP-PR1 like super family. (C) The proposed 3-D structures of MuPR1 protein established by homology-based modeling.

Figure 2. Multiple sequence alignment of the MuPR1 protein sequence with other plant PR1 proteins. Amino acid residues conserved in all proteins were black shaded and similar amino acids were gray shaded. Signal peptide regions are lined above the sequence in blue and the cleavage site predicted was indicated by the arrowhead. The conserved CNYx and PxGNxxxxxPY motifs were lined above the sequence in red. The conserved cysteine residues are marked with black dot, and the positions of the three disulphide bonds (C-C), the four \(\alpha\) helices and the four \(\beta\) parallel strands are marked.
remove the green coloring (chlorophyll). The purple formazan deposit reflects O$_2^-$ production within leaf tissues. The accumulation of hydrogen peroxide (H$_2$O$_2$) in leaf tissues was detected with 3,3′-Diaminobenzidine (DAB) staining. The leaf samples were submerged in 1 mg mL$^{-1}$ DAB solution (pH 3.8) and incubated at 25°C for 12 h in the dark. After staining, the leaves were soaked in 95% ethanol to clear the tissues of chlorophyll. The H$_2$O$_2$ was visualized as brown precipitation with leaf tissue.

3. Results

3.1. Isolation of PR1 gene cDNAs from mulberry and structural analysis of its deduced proteins

The MuPR1 cDNA (GenBank accession No. AHA43767.1) was isolated from Nongsang 14 by PCR, and the gene codes a protein of 171 amino acids with a mass of 18.59 kDa with an isoelectric point of 8.41. There were 9 serines, 5 threonine and 6 tyrosine which were predicted as potential phosphorylation sites in the deduced MuPR1 protein with the software NetPhos (http://www.cbs.dtu.dk/services/NetPhos) (Figure 1(a)). Moreover, a conserved motif belonged to the SCP-like super family was found at the residues 29–164 aa of the deduced MuPR1 protein (Figure 1(b)). Molecular modeling results indicated that the predicted 3D structure of MuPR1 protein contained four α-helices and four β-strands (I-IV) (Figure 1(c)). From the alignment of PR-1 proteins, it was showed that there were a hydrophobic signal sequence and a conserved CNYx and P$_x$GNxxxxxxxPY motif at the N-terminal and C-terminal end of the primary translation products, respectively. There were three disulphide bridges formed with six conserved cysteine residues and four highly conserved α-helices and β-strands in the mature PR-1 proteins, indicating that all the PR-1 proteins have the same α-β-α sandwich structural fold (Figure 2). It was showed that all the PR-1 type proteins from different plant families, including both mono- and dicotyledons shared 64% sequence identity suggesting there was a high level of sequence conservation among PR-1 type proteins. The phylogenetic relationships of PR1 with its homologs showed that all the proteins resulted different clusters. The MuPR1 and all the PR1s of Morus members are classed into the same cluster, and they were nearest to the PR1 of Ficus pumila followed by Trema orientalis. However, MuPR1 was diverged from the PR1s of monoyledons which are distinctly grouped in another cluster (Figure 3).

3.2. Expression patterns of MuPR1 gene and subcellular localization of MuPR1 protein

To understand MuPR1 functions, its tissue expression profile in mulberry was examined using qRT-PCR. It was showed that MuPR1 gene was constitutively expressed in the tissues examined, although the levels of expression varied substantially. MuPR1 was detected in the primary roots and flowers with a relatively high level, but it was expressed at very low levels in the secondary roots, stem, leaves and fruits (Figure 4(a)).
To confirm these results, the promoter of *MuPR1* (*pMuPR1*) was cloned and fused with β-glucuronidase (GUS) gene and then the fused gene was expressed in Arabidopsis plants which were subjected to histological GUS assay. The results showed that high GUS activities were found in the primary roots, flowers and immature siliques, but no or weak GUS signal was found in the secondary roots, leaf, stems, and mature siliques (Figure 4(b)). Therefore, the *MuPR1* gene is not only induced after pathogen infection but is also regulated by developmental signals.

To analyze the subcellular localization of *MuPR1* protein, the *MuPR1* gene was fused with the green fluorescent protein (GFP) gene and introduced into Arabidopsis plants. As shown in Figure 4(c), the *MuPR1*-GFP fluorescence distributed exclusively to the extracellular spaces, whereas the GFP fluorescence distributed in the whole cell (Figure 4(c)), suggesting that the *MuPR1* protein is localized to the extracellular spaces.

**3.3. MuPR1 expression is induced by pathogens and some plant hormones**

To explore the potential plant defense functions of *MuPR1*, its expression profile following fungal or bacterial pathogen infection was analyzed by qRT-PCR. The results showed that the expression of *MuPR1* is very low without pathogen infection, but it was induced evidently either by *P. syringae pv. mori* or *C. dematium* infection (Figure 5(a)). At the same time, the *pMuPR1* fused with GUS was used to further analyze the expression profile of *MuPR1* upon pathogen infection. Transient expression of the GUS gene driven by 35S or *pMuPR1* in tobacco leaves was performed, and histochemical staining results showed that the *pMuPR1*-GUS fusion gene was induced by *B. cinerea* or *Pst.* DC3000, but there was no detectable GUS expression in the leaves inoculated with MgCl2. However, the 35S-GUS gene was clearly induced either by *B. cinerea*, *Pst.* DC3000 or MgCl2, and no GUS activity was detected in the leaves inoculated with the vector (GV3101) whether induced by *Pst.* DC3000, *B. cinerea* or MgCl2 (Figure 5(b)). When the transgenic Arabidopsis plants containing *pMuPR1*-GUS fusions were subjected to histochemical staining, similar results were obtained for GUS activity which was visible in the leaves infected with *B. cinerea* or *Pst.* DC3000 and was not detected in the transgenic Arabidopsis leaves inoculated with MgCl2 (Figure 5(c)). Therefore, the expression of *MuPR1* can be induced by pathogens. Moreover, high levels of GUS expression were also detected in the leaves treated by SA and MeJA, but low GUS expression was found in the leaves treated by GA3 or H2O. Therefore, the expression of *MuPR1* was also strongly induced by SA and MeJA but not obviously induced by GA3 (Figure 5(d)).

**3.4. MuPR1 shows enhanced protection against fungal and bacterial pathogens**

To analyze the role of *MuPR1* in the plant defense against pathogens, the leaves from the transgenic *MuPR1* Arabidopsis plants (OE) and wild-type plants were detached and inoculated with *B. cinerea*. After four days of inoculation, many hyphae of *B. cinerea* strains were observed on the leaf surface of wild-type plants inoculated, and dark necrotic lesions were found to develop around the inoculation sites, and the leaves began to appear chlorosis. In contrast, these disease signs were not observed on the leaves of *MuPR1*-overexpressing plants at that time (Figure 6(a)). These results suggested that the overexpressing of *MuPR1* in the Arabidopsis plants enhanced plant resistance to *B. cinerea*. Moreover, the transgenic *MuPR1* Arabidopsis plants following were inoculated with *Pst.* DC3000 to explore the role of *MuPR1* in plant response to bacterial pathogens. Although the chlorotic lesions were observed on both the wild-type and transgenic plants, the chlorotic lesions around the inoculation sites in the leaves of wild-type plants was much larger than those in the leaves of transgenic plants (Figure 6(b)),
suggesting that the MuPR1-overexpressing plants conferred resistance to Pst. DC3000. Furthermore, the bacterial growth of Pst. DC3000 in the inoculated leaves was monitored to evaluate whether the apparent lack of symptom development reflected the restriction of pathogen multiplication in the MuPR1-overexpressing plants. The monitoring results showed that the strain Pst. DC3000 multiplied approximately 2 orders of magnitude from 12 to 60 h post inoculation in the leaves of wild-type Arabidopsis, whereas the growth of Pst. DC3000 in the leaves of MuPR1-overexpressing plants was extremely limited (Figure 6(c)). Therefore, the results presented above indicated that MuPR1-overexpression in Arabidopsis resulted in enhanced resistance to Pst. DC3000 and B. cinerea.

3.5. MuPR1 may have roles in mediating the rates of oxygen radical formation and detoxification

To unravel the molecular regulatory mechanisms of MuPR1 in mediating plant responses to pathogen infection, two types of reactive oxygen species such as H2O2 and O2− accumulation were examined in plants inoculated with B. cinerea or Pst. DC3000. The results indicated that there was no significant difference in the production of H2O2 between the MuPR1 transgenic Arabidopsis and wild-type plants without Pst. DC3000 or B. cinerea infection. DAB staining indicated that there was more H2O2 accumulation in the leaves of transgenic MuPR1 plants than in wild type plants inoculated with B. cinerea or Pst. DC3000 (Figure 7(a)). However, NBT staining showed that overexpression of MuPR1 repressed O2− radical formation in the leaves inoculated with Pst. DC3000 or B. cinerea (Figure 7(b)). Therefore, MuPR1 may have roles in mediating the rates of oxygen radical formation and detoxification.

3.6. Peptide derived from the C-terminus of MuPR1 play roles in enhancing plant resistance

It was reported that the peptide designated CAPE1 derived from the C-terminus of the precursor protein of tomato PR-1b, which contains conserved CNYx and PxGNxxxxxPY motifs, is highly conserved across different plants (Chen et al. 2014; Chien et al. 2015). It is interesting that MuPR1 also contains the conserved CNYx and PxGNxxxxxPY motifs which were designed as MuCAPE1 (PPGNYGERPY) (Figure 8). To examine whether the peptide MuCAPE1 derived from MuPR1 induce significant antipathogen responses, the synthetic peptide was presprayed onto the leaves of Arabidopsis and mulberry plants, respectively, and sterilized water was used as control. After the treatment for 2 h, the leaves of Arabidopsis and mulberry were challenged with Pst. DC3000 and P. syringae pv. mori, respectively. The Arabidopsis and mulberry plants pretreated with sterile water showed severe disease symptoms, but the Arabidopsis and mulberry seedlings pretreated with MuCAPE1 displayed no significant disease symptoms after the pathogen challenges (Figure 9(a,b)). Moreover, bacterial growth was also extremely limited in the leaves of plants pretreated with MuCAPE1 (Figure 9(c,d)). Therefore, the MuCAPE1 peptide might be act as a DAMP elicitor to activate defense responses and enhance plant resistance against pathogens.

4. Discussion

PR1 proteins are induced faster by pathogenic infections and used as markers for SAR (El-Komy 2014). Our results also showed that MuPR1 gene expression in mulberry leaves was induced in response to C. dematium or P. syringae pv. mori infection (Figure 5(a)) and the activity of pMuPR1 were induced by Pst. DC3000 or B. cinerea (Figure 5(b,c)). In addition, it was reported that PR1 genes are also induced by plant growth hormones, but the PR1 genes in different plants may have different expression patterns when they were induced by the same hormone (Vos et al. 2015). Our data also revealed that the pMuPR1 gene contains some cis-acting elements involved in MeJA and GA3 responsiveness (Table 1), and the analysis of the pMuPR1 activity showed that it was induced by MeJA and GA3 (Figure 5(d)). Therefore, MuPR1 may be regulated through different signaling pathways and regulators. In addition, it was reported that the expressions of PR genes were also induced in response to some abiotic environmental stresses, such as salt, low temperature, and

Figure 6. Relative susceptibility analysis of the transgenic Arabidopsis plants overexpressing MuPR1 to B. cinerea and Pst. DC3000. A. Relative susceptibility analysis of the transgenic Arabidopsis plants to B. cinerea; B. Relative susceptibility analysis of the transgenic Arabidopsis plants to Pst. DC3000; C. Growth of Pst. DC3000 strains within infected Arabidopsis leaves. The bacterial numbers were calculated at 12-h intervals after inoculation and represented as colony-forming units (CFU) per gram of leaf tissue. The data represent the means and SD of three biological samples. Double asterisks indicate significant differences versus WT (P < 0.01). WT. Wild-type Arabidopsis; OE1, OE2 and OE3: Transgenic Arabidopsis lines overexpressing MuPR1. OE: Transgenic Arabidopsis plants overexpressing MuPR1.
certain light conditions (Edreva 2005; Gao et al. 2015). Cis-acting elements involved in light, low-temperature, circadian control, and anaerobic induction responsiveness were also found in the pMuPR1 (Table 1). This indicates that the MuPR1 gene may also be regulated by diverse abiotic stresses and play important roles in plant resistance to abiotic stresses.

In addition, PR1 genes have been shown to be expressed constitutively in different plant organs and tissues (Bonasera et al. 2006; Wanderley-Nogueira et al. 2012). Several studies showed that the acidic isoforms of tobacco PR proteins, such as PR-la, PR-lb and PR-lc, present in the extracellular spaces (Dixon et al. 1991; Elvira et al. 2008; Rivière et al. 2008). Our data showed that the MuPR1 is an acidic protein with an isoelectric point of 5.9. Prediction analysis of the MuPR1 protein sequence by SignalP 4.1 server (http://www.cbs.dtu.dk/services/SignalP/) showed that there was a cleavable signal sequence at the N-terminus and the protein was localized outside of the cell (score = 0.980). Our data also showed that MuPR1-GFP fusion was localized exclusively to the extracellular spaces (Figure 4(c)). This is consistent in that some PR1s were detected in the phloem saps of several plants, including mulberry, cucurbits, rice, and Arabidopsis (Walz et al. 2004; Deeken et al. 2008; Gutierrez-Carbonell et al. 2015; Gai et al. 2018), and some PR1s exhibit vascular-specific expression patterns (Carr et al. 1987; Tornero et al. 1997). Therefore, MuPR1 proteins could be secreted into the phloem and may play an important role in SAR.

Moreover, it has been shown that H₂O₂ was induced in the tomato leaves treated by CAPE1 (Chen et al. 2104). Our

Figure 7. Accumulation of hydrogen peroxide (H₂O₂) (A) and superoxide (O₂⁻) (B) in Arabidopsis leaves infected with Pst. DC3000 or B. cinerea. The brown staining indicates the formation of a brown polymerization product when H₂O₂ reacts with DAB, and the purple coloration indicates the formation of insoluble formazan deposits that are produced when NBT reacts with superoxide. WT. Wild-type Arabidopsis; OE. Transgenic Arabidopsis plants.

Figure 8. Identification of conserved CAPE1 sequences of MuPR1 based on tomato CAPE1.
results also showed that there was more H2O2 accumulation in the leaves of MuPR1 transgenic plants than that in the wild type plant leaves when they were challenged with Pst. DC3000 or B. cinerea (Figure 7(a,b)). Therefore, CAPE1 derived from MuPR1 may also be an elicitor and trigger the generation of H2O2, which has direct antimicrobial activity and may be a signal of systemic acquired resistance. However, the NBT staining showed that overexpression of MuPR1 repressed O·− radical formation in the leaves inoculated with Pst. DC3000 or B. cinerea (Figure 7(c,d)). Therefore, MuPR1 may have roles in mediating the rates of oxygen radical formation and detoxification.

5. Conclusions

In conclusion, we have isolated and functionally characterized the PR1 from mulberry. The expression of MuPR1 gene was induced by pathogens and phytohormones and that the heterogeneous expression of MuPR1 in Arabidopsis enhances transgenic plant resistance to Pst. DC3000. Further analysis showed that the peptide with a PsGNxxxxxPY motif derived from C-terminal of MuPR1 may play a role in activating mulberry resistance against pathogens. Moreover, MuPR1 may have roles in mediating the rates of oxygen radical formation and detoxification. Our data has provided some new information on understanding the functionary of PR1 gene and perhaps facilitates mulberry genetic improvement in the future.

Disclosure statement

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