Preparation and Characterization of Antiserum against Halostachys caspica Pathogenesis-Related Protein HcPR10 (Postprint)

Authors: Zhang Hua, Yang Tao, Heng Youqiang, Wang Yan

Date: 2020-08-02T00:00:00+00:00

Abstract

Pathogenesis-related proteins (PRs) play important roles in plant disease resistance and stress tolerance. The Halostachys capsica pathogenesis-related protein gene HcPR10 (GenBank: KF673356) was obtained from a salt-suppressed subtractive library of Halostachys capsica under 600 mmol · L^{-1} NaCl stress. To investigate the mechanism underlying the biological function of Halostachys capsica pathogenesis-related protein HcPR10, the HcPR10 recombinant protein was expressed and purified in vitro, and specific HcPR10 polyclonal antibodies were prepared. In this study, a prokaryotic recombinant expression vector pET28a-HcPR10 was constructed via double enzyme digestion and transformed into Escherichia coli BL21 for induced expression. The conditions for soluble induction expression of the recombinant protein were optimized through orthogonal analysis, the fusion protein was purified using Ni-NTA affinity chromatography column, and BALB/c mice were immunized to prepare polyclonal antibodies. Based on the purified His-HcPR10 recombinant protein and total protein from HcPR10-transgenic Arabidopsis thaliana, the antiserum titer and specificity were detected by ELISA and Western blotting, respectively. The results showed that the recombinant expression vector pET28a-HcPR10 was successfully constructed. Orthogonal analysis revealed that large amounts of soluble target protein could be induced under the conditions of induction temperature 27 °C, induction speed 200 r · min^{-1}, IPTG concentration 0.7 mmol · L^{-1}, and induction time 6 h. ELISA detection showed that the anti-HcPR10 serum titer reached 1:243,000. Western blotting results demonstrated that the prepared antiserum could specifically bind to the recombinant protein and heterologously expressed HcPR10 protein in transgenic Arabidopsis thaliana. High-titer, high-specificity antiserum against Halostachys capsica pathogenesis-related protein HcPR10 was obtained, laying a foundation for further studies on the subcellular localization and biological function of HcPR10.

Full Text

Preparation and Identification of Antiserum Against HcPR10 from *Halostachys capsica*

Hua ZHANG, Tao YANG, Youqiang HENG, Yan WANG*

(Xinjiang Key Laboratory of Biological Resources Genetic Engineering, College of Life Science and Technology, Xinjiang University, Urumqi 830046, China)

Abstract

Pathogenesis-related proteins (PRs) play crucial roles in plant defense against pathogens and various environmental stresses. The *Halostachys capsica* pathogenesis-related protein gene *HcPR10* (GenBank: KF673356) was isolated from a salt-suppression subtractive cDNA library of *H. capsica* under 600 mmol · L^{-1} NaCl stress. To investigate the biological function mechanism of *H. capsica* pathogenesis-related protein HcPR10, we prepared specific polyclonal antibodies against HcPR10 through in vitro expression and purification of the recombinant protein. In this study, the prokaryotic recombinant expression vector pET28a-HcPR10 was constructed via double digestion and transformed into *Escherichia coli* BL21 for induced expression. The conditions for soluble induction of the recombinant protein were optimized through orthogonal analysis. The fusion protein was purified using Ni-NTA affinity chromatography and used to immunize BALB/c mice for polyclonal antibody preparation. The antiserum titer and specificity were detected by ELISA and Western blotting based on purified His-HcPR10 recombinant protein and total protein from *HcPR10*-transgenic *Arabidopsis*. The results demonstrated successful construction of the recombinant expression vector pET28a-HcPR10. Orthogonal analysis revealed that maximum soluble target protein expression was achieved under the following conditions: induction temperature of 27 °C, rotation speed of 200 r · min^{-1}, IPTG concentration of 0.7 mmol · L^{-1}, and induction time of 6 h. ELISA detection showed that the anti-HcPR10 serum titer reached 1:243,000. Western blotting results indicated that the prepared antiserum could specifically bind to both the recombinant protein and heterologously expressed HcPR10 protein in transgenic *Arabidopsis thaliana*. We successfully obtained high-titer, high-specificity antiserum against *H. capsica* pathogenesis-related protein HcPR10, laying a foundation for further studies on the subcellular localization and biological function of HcPR10.

Keywords: pathogenesis-related protein 10 from *Halostachys capsica*, prokaryotic expression, protein purification, antibody preparation and identification

Introduction

Pathogenesis-related proteins (PRs) are a class of specific proteins induced in plants when subjected to biotic or abiotic stresses, playing important roles in

plant disease defense and stress responses (Wen et al., 2008). PR proteins are widely distributed in both monocotyledonous and dicotyledonous plants. Based on their electrophoretic mobility, plant origin, serological relationships, and amino acid sequence homology, PR proteins are classified into 17 families, with PR10 attracting particular attention due to its inclusion of over 100 members (Van et al., 2006; Sels et al., 2008).

PR10 was first discovered in cultured parsley (*Petroselinum crispum*) cells (Somssich et al., 1988). Subsequently, PR10 proteins have been identified in rice (*Oryza sativa*), sugarcane (*Saccharum officinarum*), *Panax notoginseng*, and chickpea (*Cicer arietinum*) (Wu et al., 2016; Peng et al., 2017; Tang et al., 2019; Chatterjee et al., 2019). These proteins have molecular weights of 15–19 kDa, acidic isoelectric points, no signal peptide sequences, and are intracellular proteins (Radauer et al., 2008). They represent a class of structurally conserved proteins with diverse biological functions (Yang and Wang, 2017). Overexpression of PR10 genes from various sources can significantly enhance transgenic plant resistance to multiple pathogens (Zandvakili et al., 2017; Tang et al., 2019). Currently, RNase activity is widely considered the key to PR10-mediated biotic stress resistance (Peng et al., 2017; Finkina et al., 2017). In vitro studies have shown that tobacco (*Nicotiana tabacum*) NtPR10 with RNase activity exhibits antifungal activity against *Alternaria alternata* (Zhang et al., 2018). Overexpression of jasmonic acid-induced pathogenesis-related protein 10 (JIOsPR10) enhanced rice tolerance to rice blast fungus (*Magnaporthe oryzae*) (Wu et al., 2016). Maize (*Zea mays*) ZmPR10 also demonstrated broad-spectrum antifungal activity (Zandvakili et al., 2017).

In recent years, some studies have found that PR10 genes are also induced by abiotic factors such as salt, drought, and cold, playing important roles in plant abiotic stress defense. Overexpression of *Salix matsudana* SmPR10 increased salt stress resistance in *Arabidopsis*, while overexpression of rice RSOsPR10 enhanced drought tolerance in rice and drought and salt tolerance in creeping bentgrass (*Agrostis stolonifera*) (Takeuchi et al., 2016; Han et al., 2017; Wang et al., 2018). In addition to disease resistance and stress tolerance functions, recent studies have revealed that overexpression of LlPR10 from Easter lily (*Lilium longiflorum*), rice JIOsPR10, and rice OsPR10A regulates plant growth and development (Hsu et al., 2014; Wu et al., 2016; Zhang et al., 2019). Although the plant PR10 family has been extensively studied, the mechanisms underlying its functions remain unclear.

Protein function is closely related to its subcellular location (Scott et al., 2005; He et al., 2013), making subcellular localization a key characteristic in protein function studies. Currently, most subcellular localization studies of PR10 proteins employ fusion reporter genes for microscopic observation. For example, construction of a GFP fusion expression vector revealed that cytoplasmic localization of the pepper (*Capsicum annuum*) PR10-LRR1 complex is a prerequisite for triggering cell death in pepper leaf cells (Choi et al., 2012). Chickpea CaABR18 protein is a nuclear-localized protein with dual action modes

that exerts antifungal activity by increasing fungal membrane permeability and nuclear disintegration (Chatterjee et al., 2019). Additionally, immunogold labeling showed that *Vitis pseudoreticulata* VpPR10.2 protein is distributed in chloroplasts and cell walls, where it exerts enzymatic activity and resists early pathogen invasion (He et al., 2013). These results demonstrate that different PR10 proteins localize to different organelles and exert biological functions through different mechanisms.

Halostachys capsica is the most widely distributed constructive and dominant species in saline-alkaline environments of Xinjiang, representing a pioneer halophyte with extremely strong vitality (Xi et al., 2006; Tao, 2007; Zeng et al., 2015). *HcPR10* (GenBank: KF673356) is a salt-responsive gene in *H. capsica*. Previous studies have found that this gene is induced by various abiotic stresses and can significantly improve salt and drought tolerance in transgenic plants (unpublished data), but its mechanism of biological function remains unclear. PSORT (http://psort.hgc.jp/form.html) online prediction suggests that *H. capsica* pathogenesis-related protein HcPR10 may be localized to mitochondria matrix, cytoplasm, and peroxisomes at the subcellular level. Therefore, this study constructed the prokaryotic expression vector pET28a-HcPR10, expressed and purified the fusion protein His-HcPR10 using the *E. coli* system, immunized mice, and prepared high-titer, high-specificity polyclonal antibodies against *H. capsica* pathogenesis-related protein HcPR10, laying a foundation for studies on HcPR10 tissue and cellular localization and biological function.

Materials and Methods

1.1.1 Experimental Materials

Arabidopsis thaliana of the Columbia-0 (Col-0) ecotype was used. Two homozygous transgenic *Arabidopsis* lines overexpressing *HcPR10*, designated OE1 and OE9, were previously obtained in our laboratory via *Agrobacterium*-mediated floral dip transformation. *Escherichia coli* DH5α and BL21 competent cells were purchased from Beijing TransGen Biotech Co., Ltd. DH5α-pET-28a(+) and DH5α-pMD18-T-HcPR10 strains were preserved in our laboratory.

1.1.2 Reagents

Restriction endonucleases Hind III, EcoR I, Taq polymerase, and T4 DNA ligase were purchased from TaKaRa. Protein and DNA markers were obtained from Sangon Biotech (Shanghai) Co., Ltd. BCA and Bradford protein concentration assay kits were purchased from Beijing Solarbio Science & Technology Co., Ltd. All reagents for SDS-PAGE electrophoresis were products of Beijing Saichi Biotechnology Co., Ltd. Horseradish peroxidase-labeled goat anti-mouse IgG antibody was purchased from Beijing CoWin Biosciences Co., Ltd. DAB color development kit was obtained from Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd. Other common reagents were domestic analytical grade.

1.2.1 Construction of Prokaryotic Expression Vector pET28a-HcPR10

Based on the previously cloned *H. capsica* pathogenesis-related protein *HcPR10* gene sequence (GenBank accession: KF673356), primers were designed with EcoR I and Hind III restriction sites at the upstream and downstream ends, respectively. The upstream primer HcPR10-EP1: 5 -cggGAATTCATGGGTGTATTTACAT-3 and downstream primer HcPR10-HP2: 5 -tgtAAGCTTTCAAGCATAAAGCTGAGG-3 were synthesized (underlined sequences indicate restriction sites, lowercase letters indicate protective bases). Using pMD18-T-HcPR10 plasmid as template, PCR amplification was performed and detected by 1% agarose gel electrophoresis. The HcPR10 PCR product and pET28a expression vector were double-digested, recovered, and ligated with T4 ligase at 16 °C overnight. The ligation product was transformed into DH5α strain. The recombinant plasmid pET28a-HcPR10 was identified by double digestion and sequenced by Sangon Biotech (Shanghai).

1.2.2 Prokaryotic Expression, Optimization, and Purification of Recombinant Protein

The correctly sequenced recombinant plasmid pET28a-HcPR10 was transformed into *E. coli* BL21. Target protein expression was induced following the method of Zhang et al. (2018). Pre-induction, post-induction, post-induction supernatant, and post-induction pellet samples were centrifuged at 4 °C, 12,000 r $\cdot$ min^{-1} for 2 min, and 20 L of each was loaded for detection by 15% SDS-PAGE. Based on the percentage of soluble fusion protein relative to total protein content, a four-factor three-level orthogonal experiment was designed to optimize induction conditions including IPTG concentration (0.4, 0.7, 1.0 mmol $\cdot$ L^{-1}), temperature (27, 31, 37 °C), rotation speed (180, 200, 220 r $\cdot$ min^{-1}), and time (4, 5, 6 h) . An orthogonal analysis table was designed to analyze the effects of these four factors on HcPR10 recombinant protein solubility and optimize the expression system.

Proteins induced under nine different conditions from the orthogonal experiment were subjected to SDS-PAGE electrophoresis. Gel bands were scanned by ImageJ for grayscale analysis to determine the optimal prokaryotic induction conditions for HcPR10. Under optimal conditions, recombinant protein His-HcPR10 was expressed, bacterial cells were collected and sonicated, then centrifuged at 8,000 r $\cdot$ min^{-1} for 10 min to collect the supernatant. The supernatant was loaded onto a nickel column for protein purification, then eluted with 200 mmol $\cdot$ L^{-1} imidazole. Eluted protein was detected by SDS-PAGE, quantified using the BCA protein assay kit, aliquoted, and stored at -80 °C.

1.2.3 Antiserum Preparation

Purified His-HcPR10 fusion protein was used as antigen, with pre-immune serum from BALB/c mice serving as negative control. For primary immunization, 50 g

antigen was emulsified with an equal volume of Freund' s complete adjuvant and injected intraperitoneally. Booster immunizations with 50 g antigen emulsified in Freund' s incomplete adjuvant were administered on days 10, 29, and 33. Blood was collected by orbital bleeding 4 days after the fourth immunization. Serum was separated by incubating at 37 °C for 2 h, then at 4 °C overnight, followed by centrifugation at 5,000 $r \cdot min^{-1}$ for 15 min, and stored at -80 °C.

1.2.4 Polyclonal Antibody Titer Detection

Purified His-HcPR10 fusion protein (1 g per well) was used to coat ELISA plates at 4 °C overnight. Plates were washed three times with TTBS for 10 min each, then blocked with 200 L of 1% BSA blocking solution at 37 °C for 1 h. Pre-immune and immune sera diluted in 1% BSA blocking solution (100 L per well) were added and incubated at 37 °C for 1 h. After washing three times with TTBS for 3 min each, HRP-labeled goat anti-mouse IgG secondary antibody (1:1,000) was added and incubated at 37 °C for 1 h. Plates were washed four times with TTBS for 3 min each, then developed with TMB substrate. After gradient blue color development, the reaction was stopped by adding 50 L of 2 $mol \cdot L^{-1}$ H_2SO_4 per well, and absorbance at 450 nm was measured. Using pre-immune serum as control, A_{450} values of pre-immune (N) and immune (P) sera were measured, with $P/N > 2.1$ as the criterion for effective serum titer.

1.2.5 Western Blotting Detection

Four-week-old *Arabidopsis* plants grown in nutrient soil were used for total protein extraction following the method of Koteyeva et al. (2011). Protein concentration was determined using the Bradford assay. Ten micrograms of His-HcPR10 recombinant protein and 10 g of *Arabidopsis* total protein were separated by 15% SDS-PAGE and transferred to NC membrane. Membranes were blocked with PBST containing 5% skim milk at 4 °C overnight, then incubated with primary antibody (1:1,500 v/v) at 37 °C for 2 h. After washing three times with PBST for 10 min each, membranes were incubated with HRP-labeled goat anti-mouse IgG (1:2,000 v/v) at 37 °C for 2 h. Following three washes with PBST for 10 min each, bands were visualized by DAB color development.

Results

2.1 Construction and Identification of Prokaryotic Expression Vector pET28a-HcPR10

Recombinant plasmid pET28a-HcPR10 was extracted and identified by double digestion with EcoR I and Hind III. Agarose gel electrophoresis (1%) revealed target gene fragments and vector fragments of the expected 486 bp size [Figure 1: see original paper]. Correct sequencing results confirmed successful construction of the prokaryotic expression vector pET28a-HcPR10.

2.2.1 Prokaryotic Expression of Recombinant Protein HcPR10

The recombinant plasmid pET28a-HcPR10 was transformed into *E. coli* BL21. Four BL21-pET28a-HcPR10 monoclonal colonies were randomly selected for preliminary induction expression under 0.7 mmol · L^{-1} IPTG, 37 °C, and 220 r · min^{-1} for 4 h. SDS-PAGE results showed that compared with pre-induction, all four monoclonal colonies exhibited enhanced protein bands at approximately 25 kDa after IPTG induction [Figure 2A: see original paper], with no expression level differences among colonies, indicating successful protein expression in BL21. Induced proteins were sonicated and analyzed by 15% SDS-PAGE for pre-induction, post-induction, post-induction supernatant, and post-induction pellet samples. Results showed that recombinant protein HcPR10 existed in both soluble and inclusion body forms [Figure 2B: see original paper].

2.2.2 Optimization and Purification of Recombinant Protein HcPR10

To obtain large quantities of soluble HcPR10 recombinant protein, proteins expressed under nine different conditions from the orthogonal experiment were sonicated and analyzed by SDS-PAGE. Band grayscale scanning using ImageJ was performed for quantitative calculation and statistical analysis [Figure 3: see original paper]. Data showed that among the nine orthogonal groups, group 8 had the lowest soluble protein content, while group 4 had the highest, approximately 3.5-fold greater than group 8. Therefore, the maximum soluble HcPR10 recombinant protein expression was achieved under the following conditions: IPTG concentration 0.7 mmol · L^{-1}, temperature 27 °C, rotation speed 200 r · min^{-1}, and induction time 6 h. Large-scale expression was performed under optimal conditions using bacteria containing pET28a-HcPR10 recombinant plasmid. *E. coli* cells were collected, sonicated, and centrifuged at 8,000 r · min^{-1} for 10 min to collect the supernatant. The supernatant was purified by histidine-tag affinity chromatography. SDS-PAGE analysis of pre-induction, post-induction, post-induction supernatant, post-induction pellet, and purified His-HcPR10 protein showed that under optimal conditions, most induced target protein existed in soluble form, and purified recombinant protein HcPR10 of approximately 25 kDa was obtained [Figure 4: see original paper]. The purified protein concentration was determined to be 500 g · mL^{-1} using the BCA protein assay kit.

2.3 Analysis of Polyclonal Antibody Titer and Specificity

Indirect ELISA was used to detect antiserum titer. Antiserum was diluted with 1% BSA blocking solution at ratios of 1:1,000, 1:3,000, 1:9,000, 1:27,000, 1:81,000, and 1:243,000 for detection. As shown in [Figure 5A: see original paper], when antiserum was diluted 243,000-fold, the absorbance value of mouse antiserum was greater than 2.1 compared with the negative control, indicating an antiserum titer of 1:243,000. Western blotting identification was performed using purified His-HcPR10 recombinant protein, wild-type *Arabidopsis*, and two transgenic *HcPR10 Arabidopsis* lines. Results showed a hybridization band at

approximately 25 kDa for purified recombinant protein [Figure 5B: see original paper]. Both transgenic *Arabidopsis* lines exhibited specific hybridization bands at approximately 18 kDa [Figure 5C: see original paper], consistent with the expected molecular weight, while no hybridization band appeared in wild-type *Arabidopsis* total protein. This indicates that the prepared HcPR10 antiserum can bind not only to recombinant His-HcPR10 protein but also specifically recognize heterologously expressed HcPR10 protein in *Arabidopsis*. High-titer, high-specificity antiserum against His-HcPR10 fusion protein was successfully prepared.

Discussion and Conclusion

An efficient protein expression system is fundamental for studying protein structure, localization, and function. The core objective of this study was to purify target protein and prepare high-titer, high-specificity antiserum against *H. capsica* pathogenesis-related protein HcPR10. *Escherichia coli* is the most commonly used prokaryotic expression system, offering advantages including clear genetic background, genetic stability, high expression levels, low cost, easy purification of expressed products, and wide applicability (Nuc & Nuc, 2006). Additionally, pET28a was selected as the expression vector not only because it contains the bacteriophage T7 promoter for high-level target protein expression, but also because it encodes six continuous histidine residues at both N- and C-termini. After transforming pET28a-HcPR10 into *E. coli* BL21, induced protein was expressed in both soluble and inclusion body forms, affecting large-scale protein acquisition and subsequent purification. Induction temperature, IPTG concentration, induction time, and rotation speed are major factors affecting prokaryotic expression and protein solubility. Orthogonal experimental design reduces trial numbers, shortens experimental cycles, and can determine whether factor effects on results are significant and identify interactions among factors, making it commonly used for exploring optimal combinations of multiple factors and levels (Wen et al., 2014). This study employed orthogonal design to optimize prokaryotic induction conditions, ultimately determining that maximum soluble fusion protein HcPR10 content in *E. coli* was achieved at 27 °C, 0.7 $mmol \cdot L^{-1}$ IPTG, 6 h induction time, and 200 $r \cdot min^{-1}$ rotation speed, thereby increasing HcPR10 recombinant protein yield and purification efficiency and facilitating protein purification and polyclonal antibody preparation. Zhao et al. (2015) selected pET32a as expression vector and used the control variable method to study effects of induction temperature, induction time, IPTG concentration, and initial host cell density on protein expression, finding that 30 °C, 0.4 $mmol \cdot L^{-1}$ IPTG, initial host cell density A_{600} of 0.8, and 8 h induction time were optimal for soluble protein expression. Zhang et al. (2018) selected pCold II low-temperature expression vector and showed that compared with 37 °C, fusion protein expression was greater and more soluble at 15 °C. Therefore, optimal protein induction conditions appear to be closely related to the selected expression vector and the intrinsic properties of the target protein itself.

Recombinant protein was induced under optimal conditions and purified by Ni^{2+} affinity chromatography to obtain target protein for mouse immunization. ELISA detection showed that the prepared HcPR10 antiserum titer reached 1:243,000. Antibody specificity for antigen protein is critical for antibody applications. This study extracted total protein from wild-type and *HcPR10*-transgenic *Arabidopsis* to identify HcPR10 antiserum specificity by Western blotting. Results showed specific bands near 18 kDa in both transgenic lines OE1 and OE9, while no hybridization band appeared in wild-type *Arabidopsis* total protein, demonstrating high specificity of the prepared HcPR10 antiserum. These results establish a foundation for using this antiserum to further investigate HcPR10 protein tissue and cellular localization and biological function.

In summary, this study successfully constructed the prokaryotic expression vector pET28a-HcPR10 for *H. capsica* pathogenesis-related protein HcPR10. Through a four-factor three-level orthogonal experiment, optimal induction conditions for soluble His-HcPR10 recombinant protein expression were obtained: temperature 27 °C, IPTG 0.7 $mmol \cdot L^{-1}$, time 6 h, and rotation speed 200 $r \cdot min^{-1}$. Purified recombinant protein at 500 $g \cdot mL^{-1}$ was obtained. The prepared polyclonal antibody reached a titer of 1:243,000, and Western blotting demonstrated that this serum could specifically bind to heterologously expressed HcPR10 in transgenic plants, making it suitable for HcPR10 subcellular localization studies.

References

CHATTERJEE M, CHAKRABORTY J, DAS S, 2019. Abscisic acid–responsive 18 (CaABR18) protein from chickpea inhibits the growth of the wilt-causing *Fusarium oxysporum* f. sp. *ciceri* race1[J]. *Plant Molecular Biology Reporter*, 37(3): 170-185.

CHOI DS, HWANG IS, HWANG BK, 2012. Requirement of the cytosolic interaction between pathogenesis-related protein10 and leucine-rich repeat protein1 for cell death and defense signaling in pepper[J]. *The Plant Cell*, 24(4): 1675-1690.

FINKINA EI, MELNIKOVA DN, BOGDANOV IV, et al., 2017. Plant pathogenesis-related proteins PR-10 and PR-14 as components of innate immunity system and ubiquitous allergens[J]. *Current Medicinal Chemistry*, 24(17): 1772-1787.

HAN XJ, HE XL, QIU WM, et al., 2017. Pathogenesis-related protein PR10 from *Salix matsudana* Koidz exhibits resistance to salt stress in transgenic *Arabidopsis thaliana*[J]. *Environmental and Experimental Botany*, 141: 74-82.

HE MY, XU Y, CAO JL, et al., 2013. Subcellular localization and functional analyses of a PR10 protein gene from *Vitis pseudoreticulata* in response to *Plasmopara viticola* infection[J]. *Protoplasma*, 250(1): 129-140.

HSU SW, LIU MC, ZEN KC, et al., 2014. Identification of the tapetum/microspore-specific promoter of the pathogenesis-related 10 gene and its regulation in the anther of *Lilium longiflorum*[J]. *Plant Science*, 215-216: 124-133.

KOTEYEVA NK, VOZNESENSKAYA E, ROALSON EH, et al., 2011. Diversity in forms of C4 in the genus *Cleome* (Cleomaceae)[J]. *Annals of Botany*, 107(2): 269-283.

NUC P, NUC K, 2006. Recombinant protein production in *Escherichia coli*[J]. *Postepy Biochemii*, 52(4): 448-456.

PENG Q, SU YC, LING H, et al., 2017. A sugarcane pathogenesis-related protein, ScPR10, plays a positive role in defense responses under *Sporisorium scitamineum*, SrMV, SA, and MeJA stresses[J]. *Plant Cell Reports*, 36(9): 1427-1440.

RADAUER C, LACKNER P, BREITENEDER H, 2008. The Bet v1 fold: an ancient, versatile scaffold for binding of large, hydrophobic ligands[J]. *BMC Evolutionary Biology*, 8(1): 286-295.

SCOTT MS, GOSLINE SJC, THOMAS DY, et al., 2005. Refining protein subcellular localization[J]. *PLoS Computational Biology*, 1(6): e66.

SELS J, MATHYS J, CONINCK BMAD, et al., 2008. Plant pathogenesis-related (PR) proteins: A focus on PR peptides[J]. *Plant Physiology and Biochemistry*, 46(11): 941-950.

SOMSSICH IE, SCHMELZER E, KAWALLECK P, et al., 1988. Gene structure and in situ transcript localization of pathogenesis-related protein 1 in parsley[J]. *Molecular and General Genetics*, 213(1): 93-98.

TAKEUCHI K, HASEGAWA H, GYOHDA A, et al., 2016. Overexpression of RSOsPR10, a root-specific rice PR10 gene, confers tolerance against drought stress in rice and drought and salt stresses in bentgrass[J]. *Plant Cell, Tissue and Organ Culture*, 127(1): 35-46.

TANG BF, LI X, PU LM, et al., 2019. A pathogenesis-related protein 10 gene *PnPR10-3* was involved in molecular interaction between *Panax notoginseng* and *Fusarium solani*[J]. *Australasian Plant Pathology*, 48(5): 447–456.

TAO DY, 2007. Research progress of *Halostachys caspica*[J]. *Journal of Anhui Agricultural Sciences*, 35(20): 6083-6084.

VAN LC, REP M, PIETERSE CMJ, 2006. Significance of inducible defense-related proteins in infected plants[J]. *Annual Review of Phytopathology*, 44(1): 135-162.

WANG ZX, LIU N, NI ZY, et al., 2018. Cloning and expression analysis of *GhPR-10* gene related to drought stress in *Gossypium hirsutum*[J]. *Genomics and Applied Biology*, 37(5): 1993-2002.

WEN YA, ZHU Z, SHI Y, et al., 2014. Research on optimization expression conditions of plectasin prokaryotic expressions by the orthogonal experiment[J]. *Pharmaceutical Biotechnology*, 21(5): 424-428.

WEN YJ, HE HW, HUANG QS, et al., 2008. Roles of pathogenesis-relative protein 10 in plant defense response[J]. *Plant Physiology Journal*, 44(3): 585-592.

WU JN, KIM SG, KANG KY, et al., 2016. Overexpression of a pathogenesis-related protein 10 enhances biotic and abiotic stress tolerance in rice[J]. *Plant Pathology Journal*, 32(6): 552-562.

YANG T, WANG Y, 2017. Research progress on plant pathogenesis related protein PR-10[J]. *Plant Physiology Journal*, 53(12): 2057-2068.

ZANDVAKILI N, ZAMANI M, MOTALLEBI M, et al., 2017. Cloning, overexpression and in vitro antifungal activity of *Zea Mays* PR10 protein[J]. *Iranian Journal of Biotechnology*, 15(1): 42-49.

ZENG YL, LI L, YANG RR, et al., 2015. Contribution and distribution of inorganic ions and organic compounds to the osmotic adjustment in *Halostachys caspica* response to salt stress[J]. *Scientific Reports*, 5(1): 13639.

ZHAI JB, TIAN CY, YAN P, et al., 2006. Primary research on the halophyte flora in Xinjiang[J]. *Chinese Journal of Eco-Agriculture*, 14(1): 7-10.

ZHANG J, ZHANG LL, ZHANG BB, et al., 2018. Prokaryotic expression of *Halostachys caspica* HcDMC1 and preparation of its antiserum[J]. *Bulletin of Botanical Research*, 38(3): 399-405.

ZHANG T, GUO YL, CHEN Y, et al., 2019. Expression characterization of rice OsPR10A and its function in response to drought stress[J]. *Chinese Bulletin of Botany*, 54(6): 711-722.

ZHANG Y, WANG J, ZHOU SQ, et al., 2018. Biological activity of tobacco PR10 protein and expression analysis induced by *Alternaria alternata*[J]. *Journal of Plant Protection*, 45(3): 455-462.

ZHAO L, MA LG, WANG ZS, et al., 2015. Cloning and expression analysis of pathogenesis-related protein 10 gene of *Salvia miltiorrhiza*[J]. *Acta Botanica Boreali-Occidentalia Sinica*, 35(6): 1078-1084.

Note: Figure translations are in progress. See original paper for figures.