



# **Cloning and Expression of a Pathogenesis- Related Protein Gene *NtPR10* in Tobacco (*Nicotiana tabacum*)**

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# Outline

- Background
- Methods and Results
- Conclusions



# ● Background

## 1. The research progress of pathogenesis-related (PR) proteins.

- PR proteins are defined as plant proteins that are induced in pathogenical or related situations.
- Based on their structural and functional properties, PR proteins are currently classified into 17 families.
- They play an important role in responding to biotic and abiotic stresses, also regulating plant growth and development.



Table 1. Recognized and proposed families of PR proteins

Family	Type member	Properties
PR-1	Tobacco PR-1a	unknown
PR-2	Tobacco PR-2	$\beta$ -1,3-glucanase
PR-3	Tobacco P, Q	chitinase type I, II, IV, V, VI, VII
PR-4	Tobacco “R”	chitinase type I, II
PR-5	Tobacco S	thaumatin-like
PR-6	Tomato Inhibitor I	proteinase-inhibitor
PR-7	Tomato P	endoproteinase
PR-8	Cucumber chitinase	chitinase type III
PR-9	Tobacco “lignin-forming peroxidase”	peroxidase
PR-10	Parsley “PR1”	“ribonuclease-like”
PR-11	Tobacco class V chitinase	chitinase type I
PR-12	Radish Rs-AFP3	defensin
PR-13	ArabidopsisTHI2.1	thionin
PR-14	Barley LTP4	lipid-transfer protein
PR-15	Unknown	germin
PR-16	Pepper GLPs	germin-like protein



## 2. The research progress of PR10 proteins.

- The PR10 protein was first reported in parsley, Now more than 100 PR10 or PR10-related sequences have been identified from various plants, including hot pepper, peanut, wheat, rice, grape, maize *et al.*.
- Scientists have found PR10 proteins are involved in plant defense and other biological processes.

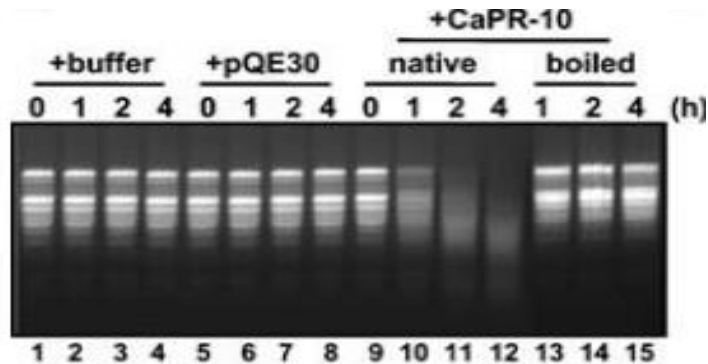


(The pictures come from the Internet)

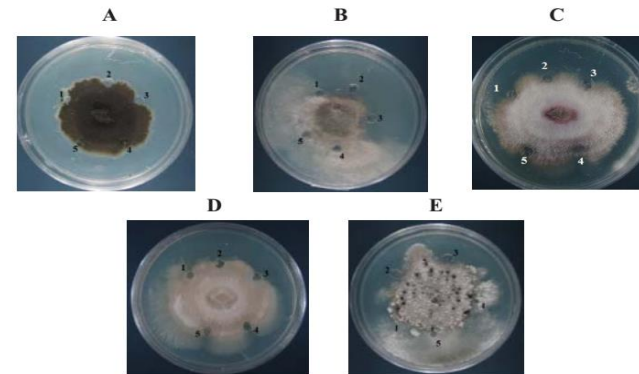


- Many PR10 proteins including Bet\_v\_1 and P-loop domains.
  - Bet\_v\_I is a conserved domain of the PR protein family, has a defensive function against pathogen infection.
  - P-loop motif (GxGGxGxxK), is presumed to function as a binding site for nucleotides involved in RNase activity for some PR10 proteins.

- Most of PR10 proteins have been reported to exhibit ribonuclease activity and antifungal activity.

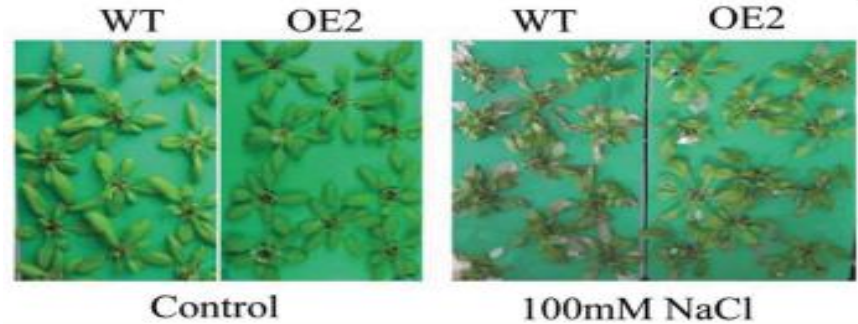
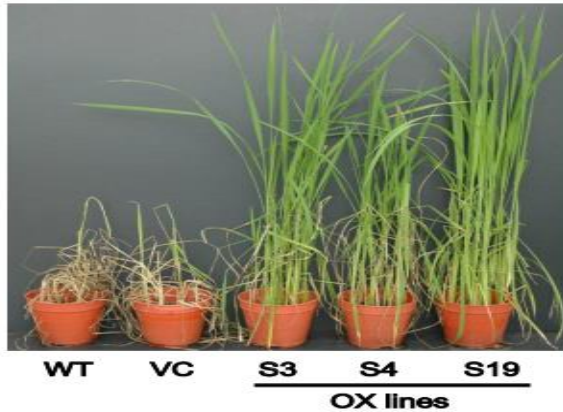


- Hot pepper PR10 (CaPR10) showed ribonuclease activity. (Park et al., 2004)



- Maize PR10 exhibited broad spectrum antifungal activity. (Zandvakili et al., 2017)

- Many PR10 proteins also respond to various abiotic stresses.



- Overexpression of a rice *PR10* gene, confers tolerance against drought and salt stresses in Rice and bentgrass. (Takeuchi *et al.*, 2016)
- PR10 from Koidz exhibits resistance to salt stress in transgenic *Arabidopsis thaliana*. (Han *et al.*, 2017)





### 3. Scientific questions?

- Why are there no research reports on tobacco PR10 proteins?
- What functions do tobacco PR10 proteins have?
- Are tobacco PR10 proteins involved in disease resistance and stress tolerance?

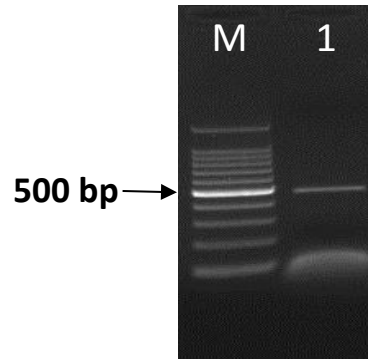




## ● Methods/Results

### • 1. Cloning of Tobacco PR10 gene *NtPR10*

- In silico cloning and RT-PCR techniques were performed to isolate an *NtPR10* gene in tobacco induced by tobacco mosaic virus (TMV).



**The PCR amplification of *NtPR10* gene.**  
M: DNA Marker; 1: The amplified fragment.



## 2. Bioinformatics analysis of *NtPR10*

➤ The ORF of *NtPR10* was 483bp in length, encoding 160 amino acids containing Bet\_v\_I domain, without trans-membrane domain and signal peptide, was predicted to locate in cytosol. In addition, *NtPR10* protein has the “P-loop” domain, relating to nuclease activity.

Solanum tuberosu	MGVTSYTHEETTTPIAFTIRLRFKALVVDSDNLIFFKLMFQ.VK	39
Capsicum annuum.	MGVYTFTEDESTASVVPSSRLFKALVIDFNILVSKLIPD.VE	39
Nicotiana tabacu	MGVTTYTHEASTTVAPTRLRFKALVLDADNLIFFKLMFQVVK	40
Solanum lycopers	MGVTTYTHEDETSTVSENRLLFKALVIDGDNLIFFKLMFN.VK	39
Consensus	mgv t e p rlfkalv d nl kl p v	
Solanum tuberosu	NIEAE.GDG...SIKKMNFVEGSPISKYLKHKIHVVDDKNL	75
Capsicum annuum.	SIENVEGDGGPFTIKKITFVEGGPMPKYLKHKIHVIDDKNL	79
Nicotiana tabacu	NIETVIEGDGGVCSIKKMNFVEGGPIKYLKHKLHVIDDKNL	80
Solanum lycopers	NVETE.GDG...SIKKINFVEGGPIKYLKHKIHAIDDKNL	75
Consensus	e gdg ikk fveg p kylkhh h ddknl	
Solanum tuberosu	VTKYSMIEGDVLGDKLESISYDLKFEAHGNGGCVCKSITE	115
Capsicum annuum.	VTKYSLIEGDALADKAESVDYDAKLEGSANGGCVATTVTV	119
Nicotiana tabacu	VTKYSLIEGDVLGDKLESITYDKFEETSAGGCICKTSTTE	120
Solanum lycopers	VTKYSLIEGDVLGDKLESITHDVKFEFAGNPAGNGGCVCKTKTE	115
Consensus	vtkys iegd l dk es d k e ggc t	
Solanum tuberosu	YHTKGDYVVLKDEEHNNEGQKQGMELFKIVEAYLLANPSVYA	155
Capsicum annuum.	YHTKGDYVVTVEEHNVHKGRANDIVKAI EAYLLANPSAYA	159
Nicotiana tabacu	YHTKGDYVFKEEHNNEGKEKAMELEKVVEDYLLANSTVYA	160
Solanum lycopers	YHTKGDYVVLKDEEHNNEGKKHAMELEKAVEDYLLANPSIYA	155
Consensus	yhtkgdyv eehn k e yllan ya	

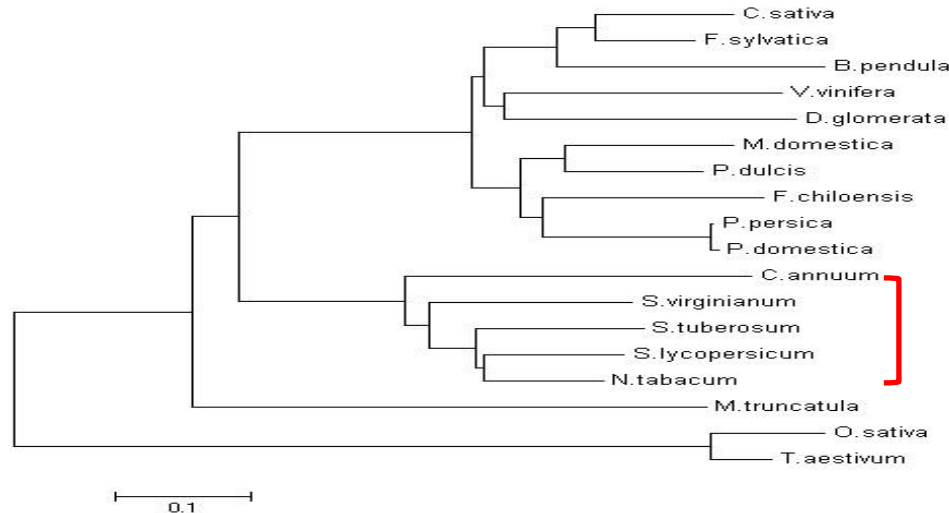
The homologous analysis of PR10 proteins.

The red box represents the "P-Loop" domain



### • 3. Phylogenetic analysis

- **Construct:** A rootless phylogenetic tree was constructed by using the neighbor-joining method with other 18 plant PR10 proteins.
- **Result:** 5 PR10 proteins of Solanaceae form an evolutionary clade with close relatives.

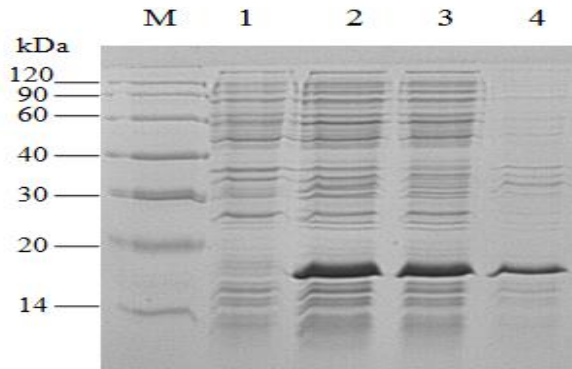


The phylogenetic tree of PR10 proteins



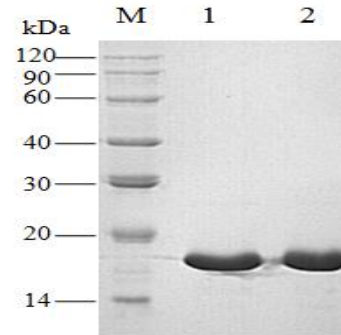
## 4. Prokaryotic expression and purification of NtPR10 protein

- We constructed the prokaryotic expression system of NtPR10, and obtained soluble protein inducing under the 15°C, 0.1 mmol/L IPTG, overnight.
- The high-quality NtPR10 fusion protein was purified.



**The solubility analysis of NtPR10 fusion protein.**

M: Molecular weight marker; 1: Non-induced; 2: Induced crude clone; 3: Supernatant of cell lysate; 4: Precipitation of cell lysate.

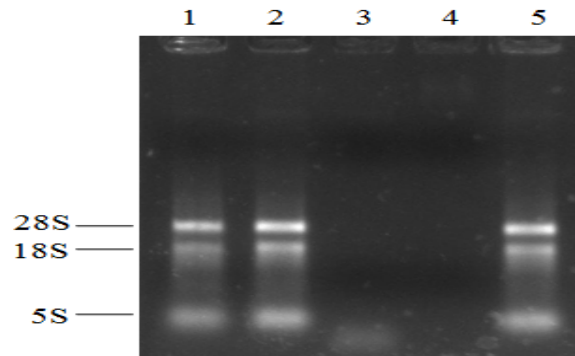


**The purification of NtPR10 fusion protein.**

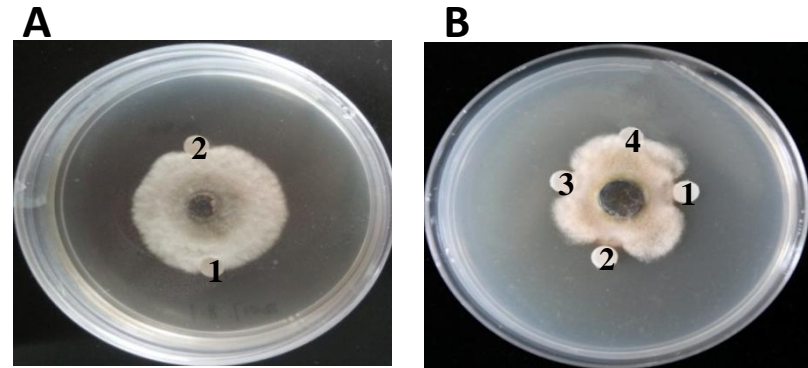
M: Molecular weight marker; 1-2: The purified NtPR10 protein.

## • 5. Analysis of the activity of NtPR10 protein

- The activity analysis exhibited that NtPR10 can degrade Tobacco total RNA and inhibit *Alternaria alternata* growth, indicating that NtPR10 has ribonuclease activity and antifungal activity.



**The ribonucleolytic activity of NtPR10**  
1: DEPC water treating; 2: The protein solution buffer; 3: RNase A; 4: The purified NtPR10; 5: Ribonuclease Inhibitor.



**A: The antifungal activity of fungicide.**

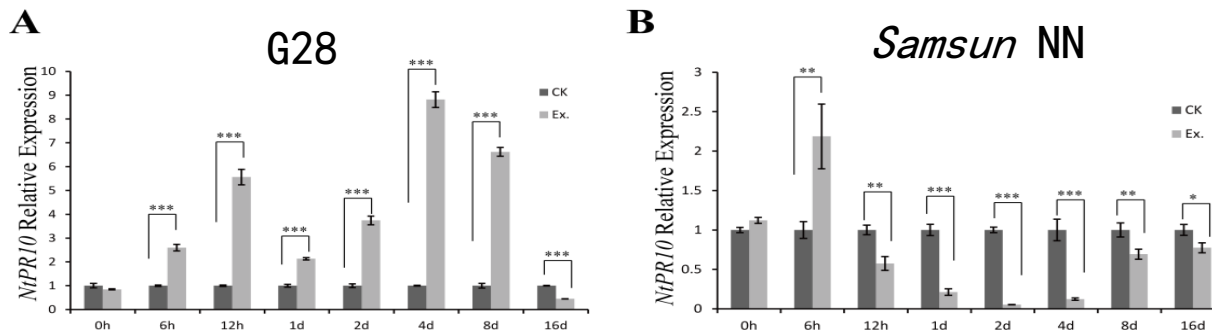
1: ddH<sub>2</sub>O; 2: fungicide (20 µg/µL).

**B: The antifungal activity of different concentrations of NtPR10 solution.**

1: NtPR10 (1 µg/µL); 2: NtPR10 (0.5 µg/µL); 3: NtPR10 (0.25 µg/µL); 4: The solution buffer.



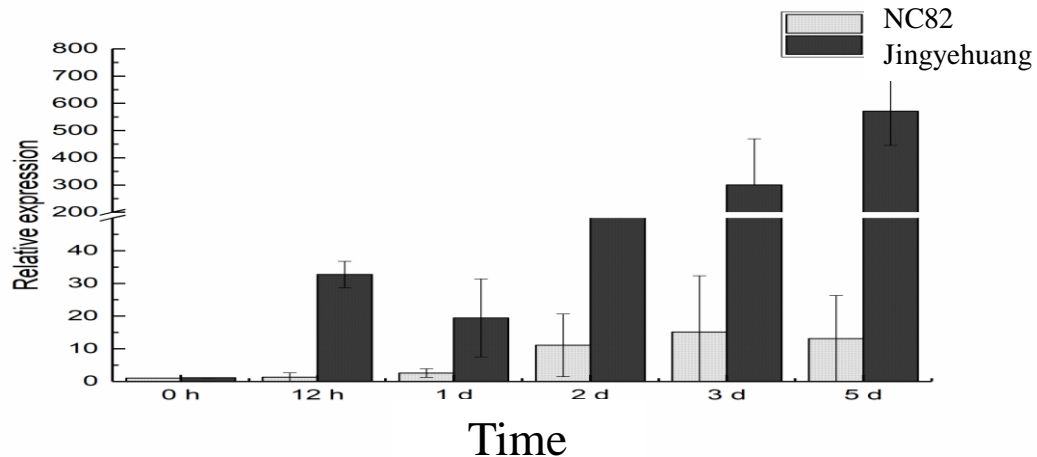
- **6. The expression pattern of the *NtPR10* induced by TMV and *Alternaria alternata* in the resistant and susceptible cultivars**
- Under the induction of TMV, the expression of *NtPR10* was significantly up-regulated in the susceptible variety. While in resistant variety, *NtPR10* was significantly up-regulated 6 hours postinoculation, then down regulated until 16th day, and gradually increased to the level before the infection.



The expression pattern of *NtPR10* in different period after TMV induction



- Under the induction of *Alternaria alternata*, The *NtPR10* gene was significantly up-regulated in both resistant and susceptible cultivars , However, in resistant cultivar, the response rate and expression level were significantly higher than susceptible cultivar.



The expression pattern of *NtPR10* in different period after *Alternaria alternata* induction





● **7. The reason of the expression difference induced by TMV and Alternaria alternata.**

- Mainly because of the mechanism difference in resistant to TMV and Alternaria alternata.
- *Samsun* NN has the *N* gene mediated resistance to TMV, *N* gene is a single dominant gene.
- The resistance to Alternaria alternata of tobacco is a quantitative trait, controlled by a number of genes.



## ● Conclusions

- We first cloned the *NtPR10* gene, and NtPR10 protein contain Bet\_v\_1 and P-loop domains
- NtPR10 has ribonuclease activity and antifungal activity.
- *NtPR10* responded to the infection of TMV and *Alternaria. alternata* infection, suggesting that the *NtPR10* might have an important function in the process of defense pathogen.

A scenic landscape featuring a bright blue sky with wispy white clouds. Below the sky is a body of dark blue water with gentle ripples. In the middle ground, there are several lush green islands or peninsulas covered in dense vegetation. The overall scene is bright and clear, suggesting a sunny day.

*Thank You*