

## Studies on the Interactions between Pto and Pti1s Proteins from Maize by a Yeast Two-Hybrid System

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**Abstract:** To check whether the protein ZmPto can interact with the protein ZmPti1s, in this study, the gene sequences of ZmPto and ZmPti1a, ZmPti1b were inserted into yeast expression vectors pEG202 and pJG4-5, respectively. Yeast two-hybrid was then conducted to verify their interactions. Results showed that yeast strains neither carrying empty plasmid nor carrying only one single fusion sequence of ZmPti1 or ZmPto can activate reporter genes. Only the yeast strains carrying both fusion sequence of ZmPti1 and ZmPto can activate reporter genes indicating that ZmPto can interact directly with ZmPti1 in the yeast two-hybrid system.

**Keywords:** Maize, Yeast two-hybrid system, ZmPto, ZmPti1

### INTRODUCTION

During growth and development, plants often encounter various environmental stresses, including nutrient deficiency, pathogens, high or low temperature, salinity, drought and waterlogging. To survive and complete the life circle under those stresses, plants have developed many complex mechanisms perceiving external signals and manifesting adaptive responses (Bohnert *et al.*, 2006; Fujita *et al.*, 2006; Zhu, 2002). In these events, reversible protein phosphorylation catalyzed by protein kinases and protein phosphatases plays a central role (Yang *et al.*, 1997). An important form of disease resistance known as gene-for-gene resistance depends on the pathogen delivering a specific "avirulence signal," generated by the expression of an Avirulence (Avr) gene and on the plant perceiving and responding to that signal, an ability conferred by a corresponding Resistance (R) gene (Dangl, 1994; Keen, 1990). The direct or indirect interactions between the plant R proteins and the pathogen Avr proteins trigger a cascade of defense responses including Hypersensitive Response (HR) and localized cell death at the site of pathogen ingress halting pathogen spread (Gabrie and Rolfe, 1990).

Pto cloned from tomato is a member of R gene family and can enhance resistance to the common bacterial pathogen *Pseudomonas syringae* pv. tomato expressing *avrPto* (Martin *et al.*, 1993). In its resistance pathway, *avr* gene products are delivered into the plant cell by a type 3 secretion system, as an intracellular receptor for the AvrPto signal molecule, Pto can thus interact with AvrPto (Ackerveken and Bonas, 1997). This interaction of Pto-AvrPto is strictly correlated with

the onset of disease resistance in plants (Tang *et al.*, 1996; Scofield *et al.*, 1996). Pto-interacting (Pti) proteins are proved to be downstream components of Pto by using the yeast two-hybrid system. Pti1, a serine/threonine protein kinases specifically phosphorylated in vitro by Pto, but cannot phosphorylate Pto, is also involved in the hypersensitive response (Zhou *et al.*, 1995). Over expression of *Pti1* in transgenic tobacco results in an accelerated HR when the transgenic plants are challenged with the tobacco pathogen *P. syringae* tabaci expressing *avrPto* (Zhou *et al.*, 1995). Recent study shows that *GmPti1*, a *Pti1* homologue cloned from soybean, can be induced by Salicylic Acid (SA) and wounding (Tian *et al.*, 2004). All these above researches suggest that Pti1 may participate in the Pto-mediated disease resistance pathway.

From 2005 on, our group has engaged in the study on the biological functions of Pto/Pti1 pathway. A number of *Pto* and *Pti1*-like genes have been cloned from maize and named as *ZmPto*, *ZmPti1a* and *ZmPti1b*, respectively. Further studies show that the expressions of *ZmPto* and *ZmPti1s* are all not only induced by SA, but also induced by ABA, salt, low temperature and osmotic, indicating that Pto/Pti1 pathway in maize may not only play roles in biotic stresses, but also in abiotic stresses signal transduction (Zou *et al.*, 2006; Zou *et al.*, 2010; Zou *et al.*, 2011; Li *et al.*, 2011). In this study, yeast two-hybrid was used to analyze the interactions of ZmPto with ZmPti1s cloned by our lab. This can help us to further study the mechanisms of the interaction of Pto-like protein and Pti1 proteins and make it possible to clear their biological functions in stresses resistance pathway.

## MATERIALS AND METHODS

**Strains, plasmids and reagent:** *E. coli* strain DH $\alpha$ , plasmids inserted with *ZmPto*, *ZmPti1a* and *ZmPti1b*, respectively were stored in our lab. Plasmids (pEG202 and pJG4-5) and yeast strain EGY48 (*his3*, *trp1*, *ura3*, *LexAop-LEU2*, *p8op-lacZ*), Y864 (*MATa ade2-101 trp1-901 leu2-3*, *112 ura3-52 his3-D200 gal4D gal80D plexAlacZ: URA3*) were kindly provided by Yang (1997) (The Chinese Academy of Agricultural Sciences, China). Enzymes and DNA marker were bought from Takara (Dalian China). Other reagent was all bought from Sigma.

**Construction of yeast expression vector:** The sequence of *ZmPto* gene was amplified by PCR from plasmid pGreen0029 containing *ZmPto* cDNA with adding *Mfe* I sites into 5' and 3' primers, respectively. The PCR product was then digested with *Mfe* I. The plasmid pEG202 was digested with *EcoR* I. The digested products including the sequence of *ZmPto* gene and the pEG202 vector fragment were ligated with T4 DNA ligase to produce the recombinant plasmid pEG202-*ZmPto*.

The sequence of *ZmPti1a* gene was amplified by PCR from plasmid pGreen0029 containing *ZmPti1a* cDNA with adding *EcoR* I and *Xho* I sites into 5' and 3' primers, respectively. The PCR product was then digested with *EcoR* I and *Xho* I. The plasmid JG4-5 was digested with *EcoR* I and *Xho* I. The digested products including the sequence of *ZmPti1a* gene and the JG4-5 vector fragment were ligated with T4 DNA ligase to produce the recombinant plasmid pJG4-5-*ZmPti1a*.

The sequence of *ZmPti1b* gene was amplified by PCR from plasmid pGreen0029 containing *ZmPti1b* cDNA with adding *EcoR* I into 5' and 3' primers, respectively. The PCR product was then digested with *EcoR* I. The plasmid JG4-5 was digested with *EcoR* I. The digested products including the sequence of *ZmPti1b* gene and the JG4-5 vector fragment were ligated with T4 DNA ligase to produce the recombinant plasmid pJG4-5-*ZmPti1b*.

**The yeast two-hybrid system:** The basic procedures for the yeast two-hybrid system were conducted as described previously (Yang *et al.*, 2005).

This study was conducted in Yangtze University in 2010.

## RESULTS AND DISCUSSION

**Construction and identification of yeast expression vector:** After a series of restriction endonucleases digest, ligation and transformation work as described in materials and methods, the cDNA sequences of *ZmPto*, *ZmPti1a* and *ZmPti1b* were eventually ligated into the

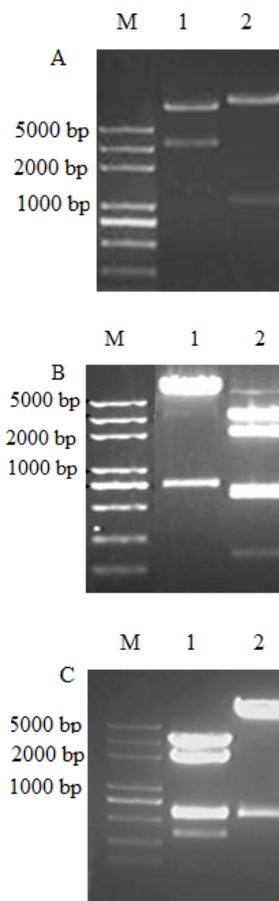


Fig. 1: Identification of yeast expression vector constructions by digestions with various restriction enzymes (A) results of recombinant plasmid pEG202-*ZmPto* digested with restriction enzymes: (M) DNA marker, (1) *Sac* I/*Bam*H I, (2) *Eco*R I/*Bam*H I, (B) results of recombinant plasmid pJG4-5-*ZmPti1a* digested with restriction enzymes: (M) DNA marker, (1) *Nco* I/*Eco*R I, (2) *Hind* III, (C) results of recombinant plasmid pJG4-5-*ZmPti1b* digested with restriction enzymes: (M) DNA marker, (1) *Hind* III/*Stu* I, (2) *Xho*I

vectors of pEG202 and pJG4, respectively. The recombinant plasmids were named as pEG202-*ZmPto*, pJG4-5-*ZmPti1a* and pJG4-5-*ZmPti1b*, respectively. After ligation, identifications were conducted by digestions with relative endonucleases. As shown in Fig. 1, the digestion products were in consistency with the expected. Then, the sequencing results further proved the right recombinant plasmids (data not shown). These showed that the yeast expression vectors were successfully constructed and could be used for the next experiments.

**Identification of interactions of *ZmPto* with *ZmPti1s*:** *Pto* has a higher sequence homology with *ZmPto*, *Pti* has a higher sequence homology with

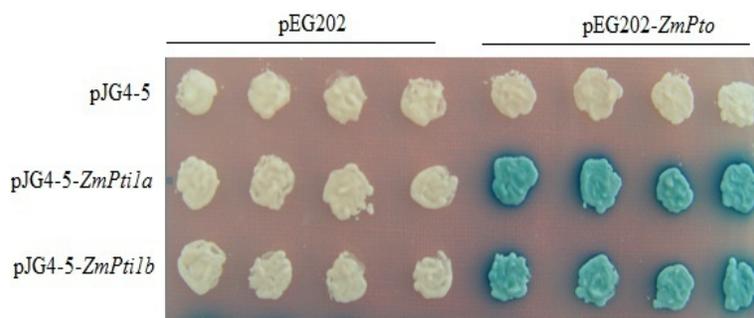


Fig. 2: Interaction of *ZmPto* with *ZmPti1a* and *ZmPti1b*. The cDNA of *ZmPto* was fused into the DNA-binding domain of plasmid pEG202 and transformed into yeast strain EGY48. Otherwise, the cDNA of *ZmPti1a* and *ZmPti1b* were fused into the DNA-activation domain of plasmid pJG4-5 and transformed into yeast strain Y864. The transformed Y864 and EGY48 strains were mated with liquid media lacking histidine, uracil and tryptophan, after which they were grown on SD+Galactose+BU Salts+X-gal medium. The plates were incubated at 30°C for 1 day and photographed. Three independent, representative colonies are shown for each bait-prey combination

*ZmPti1a* and *ZmPti1b* too. All these above indicate that *ZmPto* and *ZmPti1s* may have the similar biological functions with Pto and Pti1. Based on this knowledge, it is very important to identify whether the Pto-like protein from maize interact with Pti1-like proteins from maize just like that in tobacco. Therefore a yeast two-hybrid assay was used to determine the interaction of *ZmPto* with *ZmPti1*. As shown in Fig. 2, yeast strains neither carrying empty plasmid nor carrying only one single fusion sequence of *ZmPti1s* or *ZmPto* can activate reporter genes. Only the yeast strains carrying both fusion sequence of *ZmPti1a* or *ZmPti1b* and *ZmPto* can activate reporter genes indicating that *ZmPto* can interact directly with *ZmPti1a* and *ZmPti1b* in the yeast two-hybrid system.

### CONCLUSION

The interactions between proteins are basic for cell signal transduction, metabolism and many other biological processes. Studies on the interactions between proteins can help us discover many principal questions in life. Yeast two-hybrid assay thus provides an important technology to study the protein interactions. Yeast two-hybrid assay is conducted in eukaryotic yeast and analyzed in live cells, so this assay can represent the live metabolism in a large degree. In this study, the gene of *ZmPto* and *ZmPti1a/b* were inserted into yeast expression vectors, respectively. Only the yeast co-expressing of *ZmPto* and *ZmPti1a* or *ZmPti1b* proteins can keep alive on auxotrophic medium and thus activate the direct interactions of *ZmPto* and *ZmPti1s* proteins from maize. The result would lay a good foundation for further study on the Pto/Pti1 signal pathway and its biological functions.

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### REFERENCES

- Ackerveken, G.V. and U. Bonas, 1997. Bacterial avirulence proteins as triggers of plant disease resistance. *Trends Microbiol.*, 5: 394-398.
- Bohnert, H.J., Q. Gong, P. Li and S. Ma, 2006. Unraveling abiotic stress tolerance mechanisms--getting genomics going. *Curr. Opin. Plant Biol.*, 9: 180-188.
- Dangl, J.L., 1994. The enigmatic avirulence genes of phytopathogenic bacteria. *Curr. Microbiol. Immunol.*, 192: 99-118.
- Fujita, M., Y. Fujita, Y. Noutoshi, F. Takahashi, Y. Narusaka, K. Yamaguchi-Shinozaki and K. Shinozaki, 2006. Crosstalk between abiotic and biotic stress responses: A current view from the points of convergence in the stress signaling networks. *Curr. Opin. Plant Biol.*, 9: 436-442.
- Gabrie, D.W. and B.G. Rolfe, 1990. Working models of specific recognition in plant-microbe interactions. *Annu. Rev. Phytopathol.*, 28: 365-391.
- Keen, N.T., 1990. Gene-for-gene complementarities in plant-pathogen interactions. *Annu. Rev. Genet.*, 24: 447-463.
- Li, Z.L., M.D. Bian, Z.Y. Wu, X.H. Zhang, Q. Yang and C.L. Huang, 2011. Isolation and drought-tolerant function analysis of *ZmPti1-1*, a homologue to *Pti1*, from maize (*Zea mays* L.). *Afr. J. Bio.*, 10: 5327-5336.
- Martin, G.B., S. Brommonschenkel, L. Chunwongse, A. Frary, M.W. Ganai, R. Spivey, T. Wu, E.D. Earle and S.D. Tanksley, 1993. Map-based cloning of a protein kinase gene conferring disease resistance in tomato. *Science*, 262: 1432-1436.

- Scofield, S.R., C.M. Tobias, J.P. Rathjen, J.H. Chang, D.T. Lavelle, R.W. Michelmore and B.J. Staskawicz, 1996. Molecular basis of gene-for-gene specificity in bacterial speck disease of tomato. *Science*, 274: 2063-2065.
- Tang, X., R.D. Frederick, J. Zhou, D.A. Halterman, Y. Jia and G.B. Martin, 1996. Initiation of plant disease resistance by physical interaction of AvrPto and Pto kinase. *Science*, 274: 2060-2063.
- Tian, A.G., G.Z. Luo, Y.J. Wang, J.S. Zhang, J.Y. Gai and S.Y. Chen, 2004. Isolation and characterization of a *pti1* homologue from soybean. *J. Exp. Bot.*, 396: 535-537.
- Yang, Y., J. Shah and D.F. Klessig, 1997. Signal perception and transduction in plant defense responses. *Genes. Dev.*, 11: 1621-1639.
- Yang, J., R. Lin, J. Sullivan, U. Hoecker, B. Liu, L. Xu, X.W. Deng and H. Wang, 2005. Light regulates COP1-mediated degradation of HFR1: A transcription factor essential for light signaling in *Arabidopsis*. *Plant Cell*, 17: 804-821.
- Zhou, J., Y.T. Loh, R.A. Bressan and G.B. Martin, 1995. The tomato gene *Pti1* encodes a serine/threonine kinase that is phosphorylated by Pto and is involved in the hypersensitive response. *Cell*, 83: 925-935.
- Zhu, J.K., 2002. Salt and drought stress signal transduction in plants. *Annu. Rev. Plant Biol.*, 53: 247-273.
- Zou, H.W., Z.Y. Wu, Q. Yang, X.H. Zhang, M.Q. Cao, W.S. Jia, C.L. Huang and X. Xiao, 2006. Gene expression analyses of *ZmPti1*, encoding a maize Pti-like kinase, suggest a role in stress signaling. *Plant Sci.*, 171: 99-105.
- Zou, H.W., Z.Y. Wu, X.H. Zhang, Y.Q. Wang and C.L. Huang, 2010. Over-expression of *ZmPti1*: A homologue to *Pti1*, increases salt tolerance of *Arabidopsis thaliana*. *Afr. J. Biotech.*, 9: 656-662.
- Zou, H.W., Z.J. Song, Z.Y. Wu, X.H. Zhang, H.F. Liu, G.H. Ma and C.L. Huang, 2011. Isolation and analysis of *ZmPto* from maize, a homologue to Pto. *Plant Omics J.*, 4: 53-59.