INTERACTOME ANALYSIS OF APOPTOTIC ATPASE PROTEIN REVEALS CROSSTALK OF RICE RESPONSES TO BACTERIAL INFECTION BY *XANTHOMONAS ORYZAE* PV. *ORYZAE* AND NITROGEN DEFICIENCY

Amy Thein, Chao Yu, Yutong Chen, Fang Tian, Huamin Chen, Chenyang He

State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, China

Abstract

Apoptosis is mediated by many kinds of enzymes including caspases, Dnases, protein kinases and ATPase in plants. However, the role of apoptotic ATPase in cell death platform and in response to biotic and abiotic stress condition is not yet clearly understood. Our previous experiments showed that Os08g42700 (apoptotic ATPase) played an important role in rice in overlapping responses to infection by the bacterial blight pathogen *Xanthomonas oryzae* pv. *oryzae* (Xoo) and nitrogen deficiency stresses. According to GO analysis, it was found that, NB-ARC domain of Os08g42700 is 32.1% homology with that of RPM1 from Arabidopsis. In order to detect the underlying mechanism of crosstalk between Xoo infection and nitrogen deficiency, we carried out yeast two-hybrid (Y2H) screening using Os08g42700 as the bait protein and a cDNA library for rice treated with both stresses. A total of 127 clones were selected from Y2H screening on their colony appearance, and their sequences were analyzed by blast at rice genome annotation project (RGAP). In these candidate interactors, we found five of them particularly interesting, which are Os11g47970, Os09g39400, Os08g44680, Os06g46770, and Os09g12750. Therefore, it is very likely that these proteins function as a complex in the apoptosis pathway like their homologous proteins in Arabidopsis. We are currently constructing the full length CDS of each protein into protein expression vector for GST pulldown approach. Further *in vitro* and *in planta* experiments will confirm the specificity of interaction and subcellular localization of interacting proteins. The present study will provide new insights towards the molecular mechanisms of disease resistance response and nitrogen metabolism.

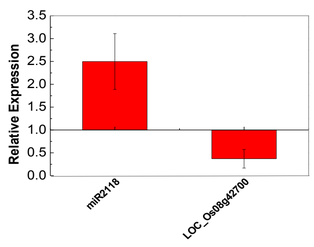


Fig.1 - Relative expression of Os08g42700 in response to Xoo challenged inoculation under low nitrogen condition.

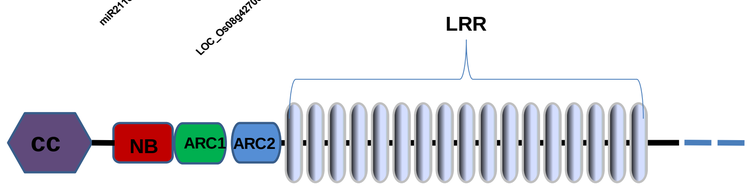
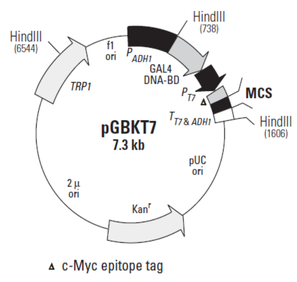


Fig.2 - Schematic representation of domains found in Os08g42700. (Domains are not drawn to scale).

Fig. 3 - Construction of pGBKT-7 with Os08g42700 and confirmed by digestion of pGBKT-7:Os08g42700 with NdeI and SalI



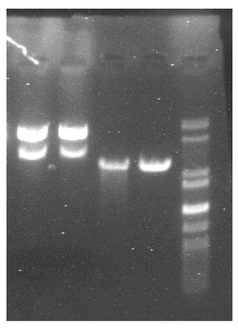
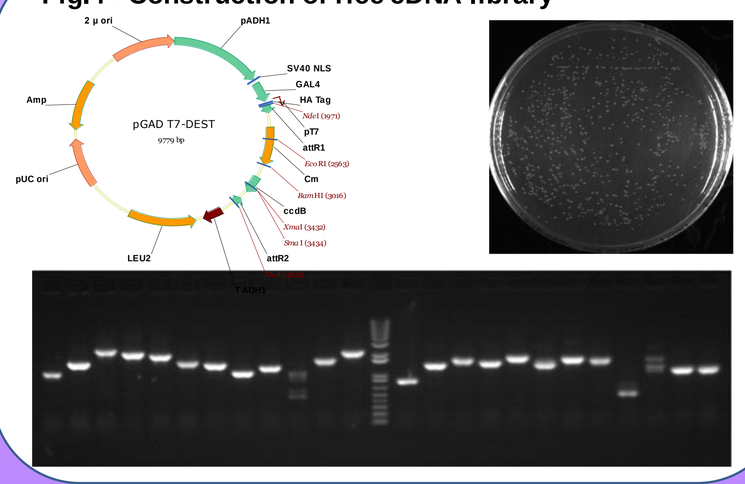


Fig.4 - Construction of rice cDNA library



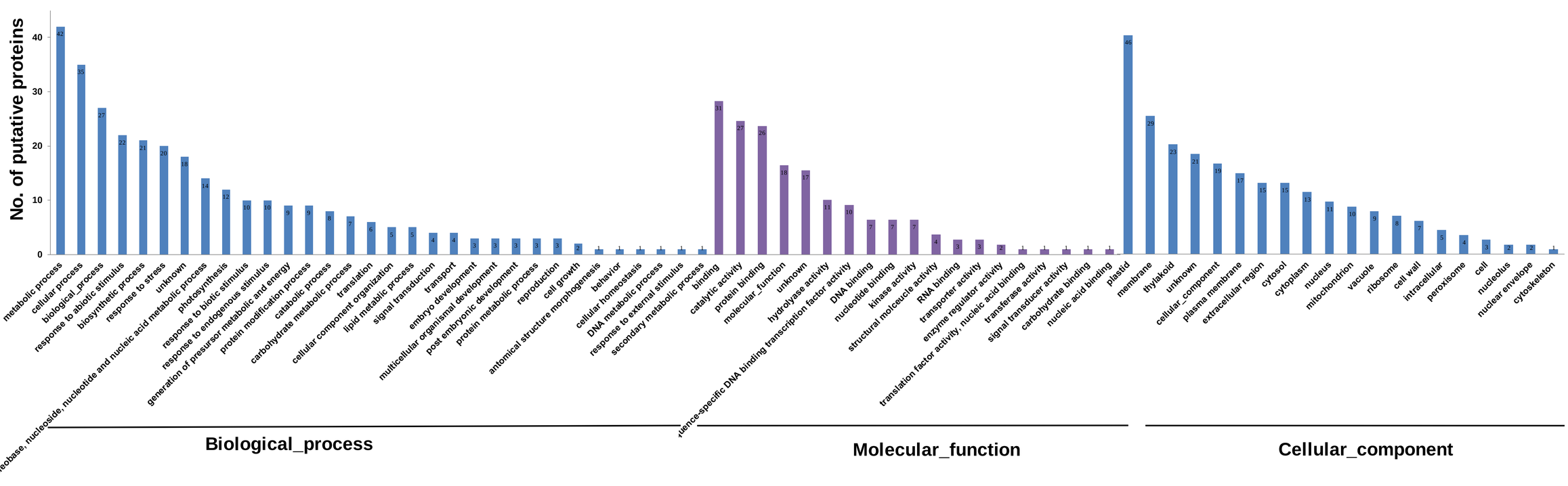
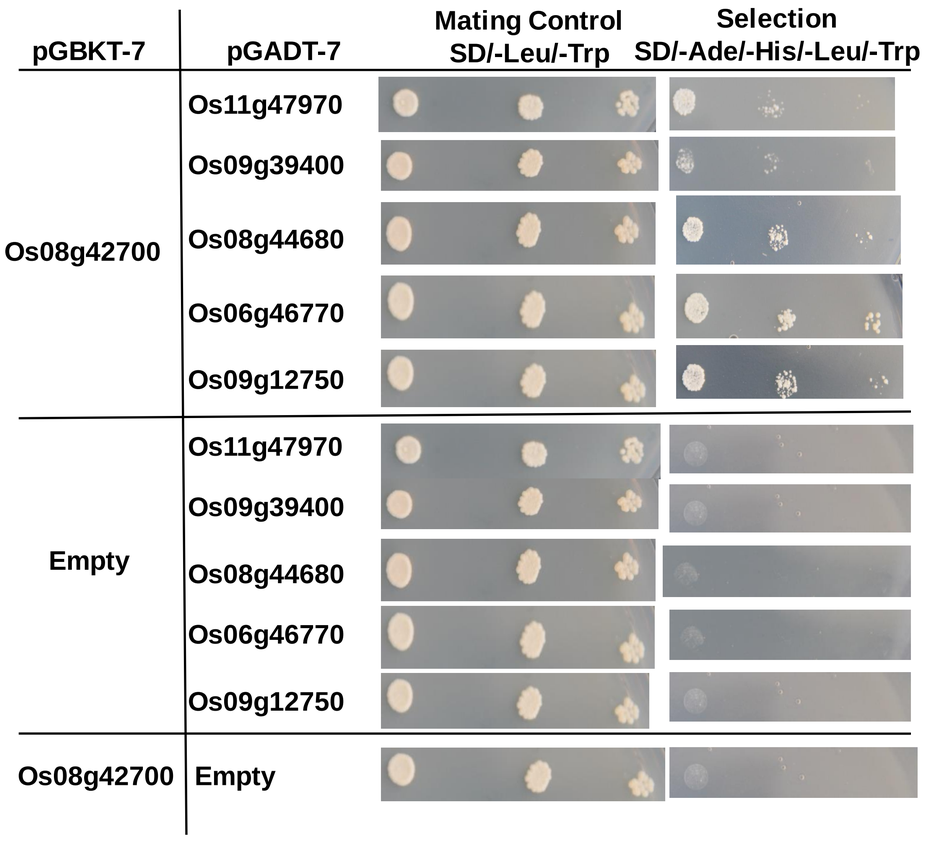


Fig. 5 - GO classification histogram of 127 proteins from rice identified in Y2H assay as putative interactors with Os08g42700.

Fig. 6 - Potential interactors of Os08g42700 in Y2H assays.



Conclusion

- **By Y2H screening using Os08g42700 as a bait, 127 putative proteins were sequence analysed and GO analysis by using Rice genome annotation project.**
- **According to colony morphology and other references, some positive interactors were chosen and confirmed by Y2H assays.**
- **Os11g47970(AAA-type ATPase family protein, Os09g39400(histidine-containing phosphotransfer protein), Os08g44680(photosystem I reaction center subunit II), Os06g46770 (ubiquitin family protein), Os09g12750 (Myb-like DNA binding domain containing protein) were found to be positive interactors in Y2H assays.**
- **Further confirmation for specific binding will be done by using GST pulldown approach.**
- **Transient protein expression analysis in *N. benthamiana* and confocal microscopy analysis will help to understand the functional role of proteins involved in this pathway.**