Dissecting Rice Blast Resistance Gene *Pi-ta/AVR-Pita* Signaling Pathway Using the Yeast Two-hybrid System

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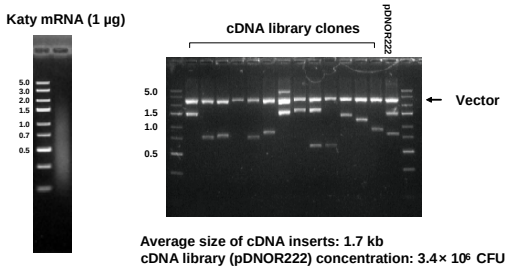
Research Objective

During infection the blast pathogen *M. oryzae* produces the AVR-Pita metalloprotease. AVR-Pita is predicted to directly interact with the Pi-ta protein in the rice plants triggering resistance responses. The objective of the present study is to identify and characterize interacting proteins with AVR-Pita and Pi-ta using the yeast two-hybrid system (Y2H).

Construction of a rice cDNA library

A cDNA library was constructed using a pool of leaf mRNA of the cultivar Katy (*Pi-ta/Pi-ta*) harvested 6, 12, and 24 hrs after inoculation with an incompatible race IB-49 (ZN61) or a compatible race IE-1k (TM2). Expected disease reactions below were documented 7 days after inoculation.

mRNA qualification **cDNA library qualification**

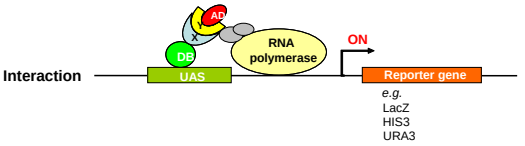


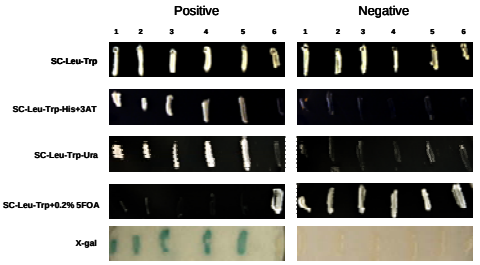
Average size of cDNA inserts: 1.7 kb
cDNA library (pDNOR222) concentration: 3.4× 10^6 CFU

Principles of the yeast two-hybrid system (Y2H)

Yeast cell expressing both the bait (GAL4 DB) and prey (GAL4 AD) fusion protein.

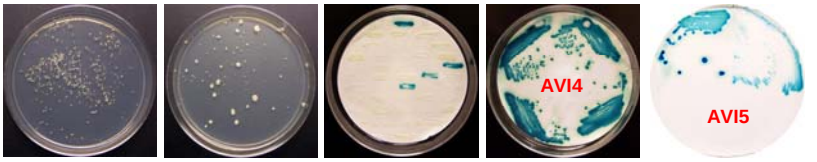
Two proteins interact and the GAL4 AD fusion protein is localized to the promoter and transcription is activated.



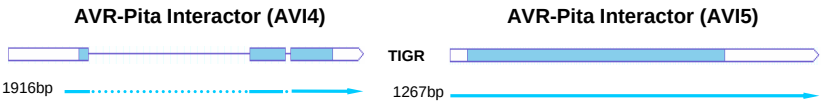


Blue color indicates positive interaction, white color indicates negative interaction.

Y2H screening for AVR-Pita interactors

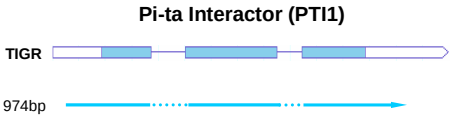


SC-Leu-Trp SC-Leu-Trp-Ura- His+3AT X-Gal (SC-Leu-Trp-Ura-His+3AT, 50mM)

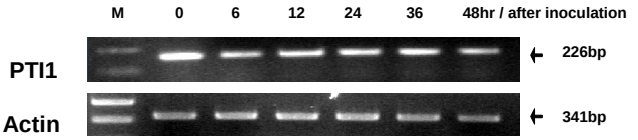


Y2H screening for Pi-ta interactors

X-Gal (SC-Leu-Trp-Ura-His+3AT, 50mM)

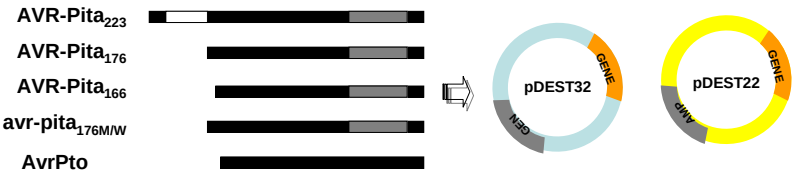


Semi-quantitative RT-PCR



Construction of AVR-Pita bait or prey plasmids

Four AVR-Pita Y2H fusion proteins were constructed to determine whether interactions of AVI4 and AVI5 are specific to avirulent AVR-Pita. The bait for AvrPto was also used to examine the interaction specificity with AVI4 and AVI5.



Summary and Future Focus

- The average size of inserts in this cDNA library is 1.7 kb and the library covers 9 × of the genes in the rice genome indicating this library can be used to identify any rice gene using Y2H.
- Two AVR-Pita interacting proteins AVI4 and AVR5 and one Pi-ta interacting protein PTI1 will be further analyzed for their interaction specificities with AVR-Pita or Pi-ta and roles in disease resistance.

Acknowledgements

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Website: http://www.ars.usda.gov/news/events