Development of Assay for flg22-Induced ROS

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Abstract

A gene from Magnaporthe oryzae, called 17227, was previously identified as a putative effector because it was found to suppress BAX-mediated programmed cell death (PCD) in Nicotiana benthamiana (Fig. 1). A known effector, AvrPiz-t also possesses this activity (Fig. 1.0). AvrPiz-t is also able to suppress PAMP (pathogen-associated molecular pattern) induced ROS (reactive oxygen species)[4], the PAMP-triggered immunity (PTI). Interestingly, induction of PTI by flg22 can also inhibit PCD induced by avirulent pathogens (Fig. 2.0, [3]). However, if BAX-mediated PCD is also suppressed by PTI, effector assays cannot distinguish between induction of PTI (which then suppresses PCD) or direct suppression of PCD. Here, I developed the assay for flg22 for Dr. Ebbole's lab and tested if flg22 can suppress BAX-induced PCD.

Research Objectives and Outcomes

Research Objectives:

- Determine if flg22 suppresses BAX-mediated plant cell death; of interest because many research groups use suppression of BAX as evidence of effector activity, whereas an alternate interpretation of such results is that heterologous expression of proteins in plants may induce PTI.
- Demonstrate that flg22 in the laboratory was active as a PTI inducer by developing leaf disk ROS assay [7]; relevant for our lab since a related research project is to assess the ability of 17227 to suppress ROS induction by flg22.

Outcomes:

- My results (Fig. 3.0) indicated that the developed system can work for the detection of flg22 induction of ROS, and that flg22 induction of PTI inhibits PCD induction by BAX (Fig. 4.0)

Summary: Flg22 is able to suppress PCD induced by BAX. Based on this, I conclude that to claim an effector suppresses host defenses one should first show that the effector is not inducing PTI as the mechanism for BAX suppression. Given that 17227 suppresses BAX-induced PCD, it will be important to next show if 17227 does or does not induce PTI. Here, I developed the leaf disk assay for the lab and demonstrated that the reagents and system are functioning properly. A comparison of BIO-RAD reagents with stock chemicals showed that the stock chemicals are sufficient for this application, and the cost of the reagents for the assay are negligible. The leaf disk assay will be used to test if 17227 can suppress flg22-induced ROS. The first experiment for this objective will be to use AvrPiz-t or another known effector to demonstrate suppression. With the appropriate positive and negative controls we should then be able to demonstrate if 17227 is able to suppress induction of PTI.

Connections to Academic Knowledge

My introductory biology and ecology coursework introduced me to the kingdom fungi, detailing the five major lineages: the chytrids, zygomycetes, glomeromycetes, ascomycetes, and basidiomycetes [5]. While my research was based on developing an assay to be used to study the Had1 gene from M. oryzae, an ascomycete fungus, the scope of the research conducted was from simple biology towards the more complex fields of molecular biology, genetics, and plant pathology. As of yet, I have not taken courses related to those three subjects, but the knowledge I have gained throughout this experience has established a basis of knowledge that will be beneficial to me come time to take the aforementioned coursework.

Connections to Discipline

Rice is a staple food product for approximately half the world’s population, and is essential to the economic integrity of many nations. Approximately 30% of rice crop is lost worldwide due to M. oryzae, which is the equivalent of depriving nearly 60 million people of food. Mitigating its spread via fungicide can cost nearly $70 per hectare; a costly venture that poses not only economic, but environmental concerns [2]. M. oryzae’s impact on the welfare of humans, the economy, and the environment make the research conducted within this lab all the more relevant. By using molecular biological methods and understanding the underlying principles of genetics, a more comprehensive understanding of what genes are responsible for M. oryzae’s ability to suppress PAMP-induced ROS is gained. With the experimental data gained from the leaf disc assay developed within this lab combined with the research of other scientists, resistant rice strains could be developed as a result, potentially alleviating the economic, human welfare, and environmental concerns associated with M. oryzae.

Relationship to Career Goals

I intend on pursuing a career as an environmental attorney, and hope to have a place within the legislative or bureaucratic framework of either the local, state, or federal government, so as to direct our policies toward ensuring environmental sustainability. Although these future goals do not directly pertain to molecular biological research, I believe to be both competent in the studies of science and the language of jurisprudence is a great asset to have as an environmental lawyer and as a legislator. This experience has:

- impressed upon me the importance of scientific research, for the implication of this research alone could benefit the economic integrity of many nations and the lives of countless people;
- allowed me to gain technical experience as it relates to the genetic modification of species, thereby granting me a more informed opinion of GMOs.

Ultimately, this experience has bestowed upon me a deeper understanding of the scientific process, and will allow me to be a more environmentally literate lawyer.

Acknowledgements

I would like to thank Hong Juan Nian and Meilin Chen for mentoring me during the extent of my research. Their aide was vital to both my conceptual and practical understanding of the topics investigated. I would also like to thank the BURS program and Agrilife Research for their generous sponsorship. Sponsors for high impact experiences for BESC and the BESC poster symposium include the Department of Plant Pathology and Microbiology, the College of Agriculture and Life Sciences, the Office of the Provost and Executive Vice President for Academic Affairs.