Plant/pathogen interactions: new method to monitor H_2O_2 production in living cells

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Introduction

Every plant cell has the possibility to detect the presence of pathogens and to emit signals which control the spatial and temporal development of the resistance mechanisms. Recent data showed that specific ROS molecule, H_2O_2 in particular, is directly implicated in the physiological regulation of different signal transduction pathways related to the host defense. However the large number of ROS production sites in the cell and the difficulty associated with their detection make it hard to study the spatio-temporal resolution of ROS generation. In this poster we illustrate an experimental set up which makes use of a recently synthesizes H_2O_2 specific fluorescent probe to monitor, by microscopy, the H_2O_2 production during early stages of the *Phytophthora infestans* infection.

A method to check the H_2O_2 production

1. In our approach we have chosen *Arabidopsis thaliana* and *P. infestans* as reference organisms. A bright field micrograph with sporangia on the epithelial cell of the plant (figure 1) is illustrated.

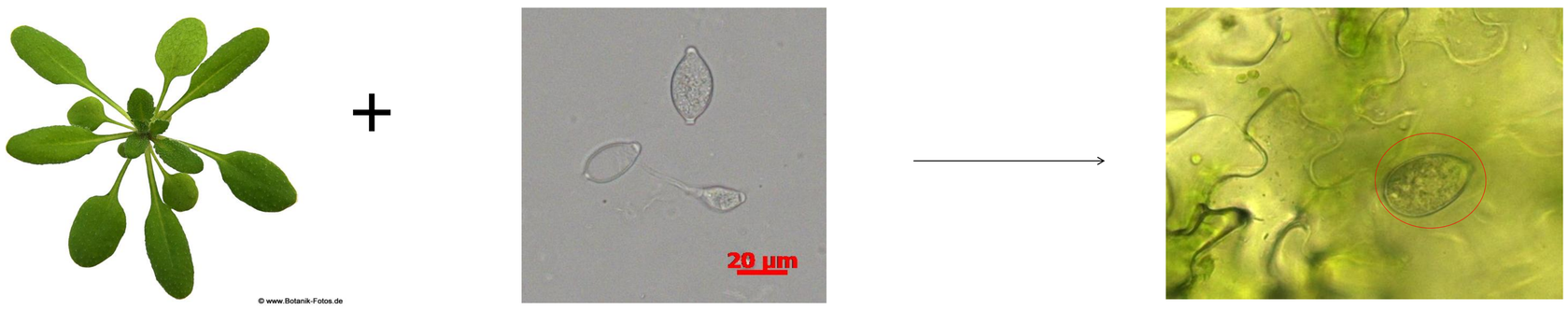


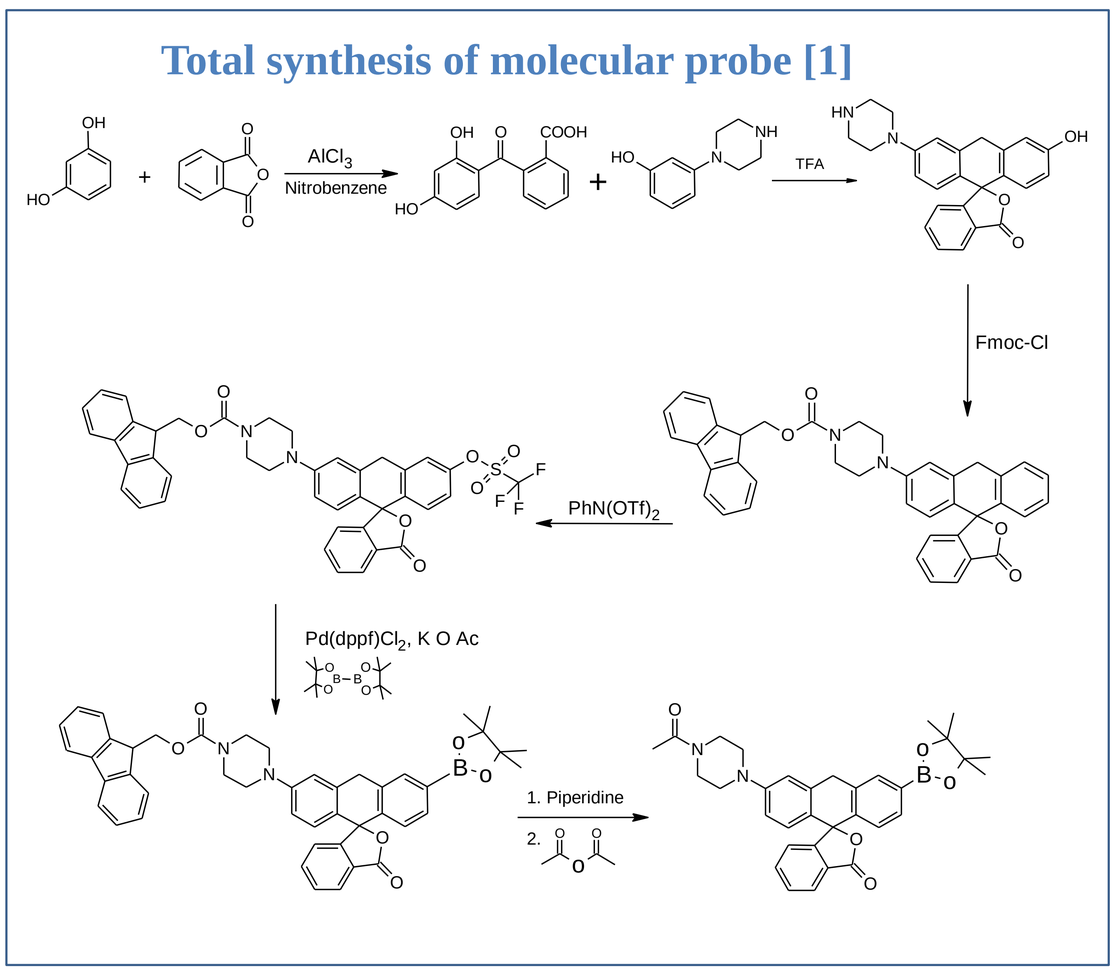
Figure 1: Arabidopsis thaliana and micrographs of sporangia.

2. We use a microscope Axion Imager.A1m with a multichannel acquisition. Figure 2 shows the fusion between a bright field micrograph and a micrograph with fluorescence.

Figure 2: Micrographs, GX1000, multidimensional acquisition., filter Set 46, blue aniline. Appressorium of P. infestans and callose production by A. thaliana..

3. We have constructed a molecular probe which reacts specifically with H_2O_2 to produce a fluorescence (figure 3) [1]. Figure 4 shows the difference of probe emission with H_2O_2 and without. Contrary to other techniques of detection, this probe is specific and non-dependent on the activity of a peroxidase and its substrate.

Total synthesis of molecular probe [1]



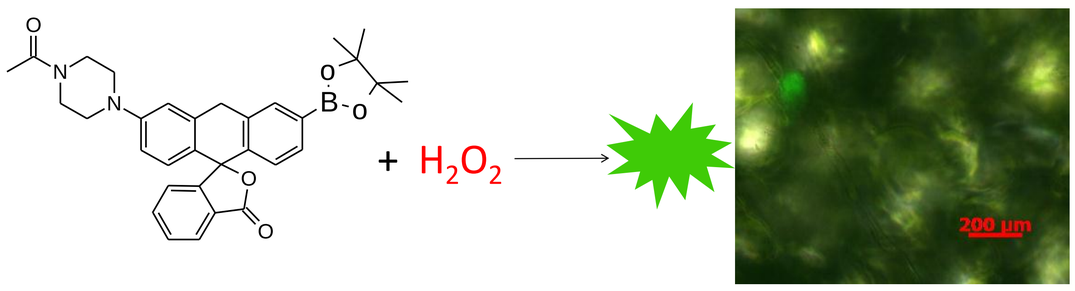


Figure 3: Molecular probe and micrographs of probe fluorescence during the interaction plant-pathogen

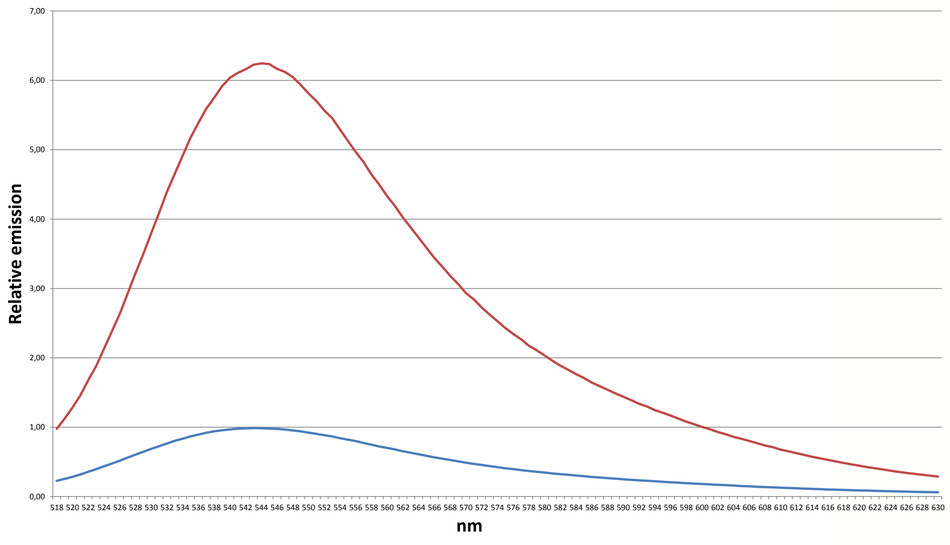


Figure 4: Relative emission spectrum of probe alone (blue line) and probe with H_2O_2 (red line).

Prospects

These tools will be used to observe the H_2O_2 dynamic into the living cell during the interactions of *A. thaliana* with *P. infestans*. A better understanding of these processes will contribute to support the PLANTINTERACT project.

[1]Dickinson, Bryan C. et Chang, Christopher J. 2008. A targetable fluorescent probe for imaging hydrogen peroxide in the mitochondria of living cells. *Journal of the American Chemical Society.* 2008, Vol. 130, pp. 9638-9639

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