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

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Introduction

Every plant cells have 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 into 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 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 on *Arabidopsis thaliana*.

A method to check the H_2O_2 production

A molecular probe which reacts specifically with H_2O_2 to produce a fluorescence was synthetized (figure 1) [1]. Figure 2 and 3 show the response of the probe with different hydrogen peroxide concentrations and the specificity of this probe. Contrary to other techniques of detection, this probe is specific and non-dependent on the activity of a peroxidase and its substrate.

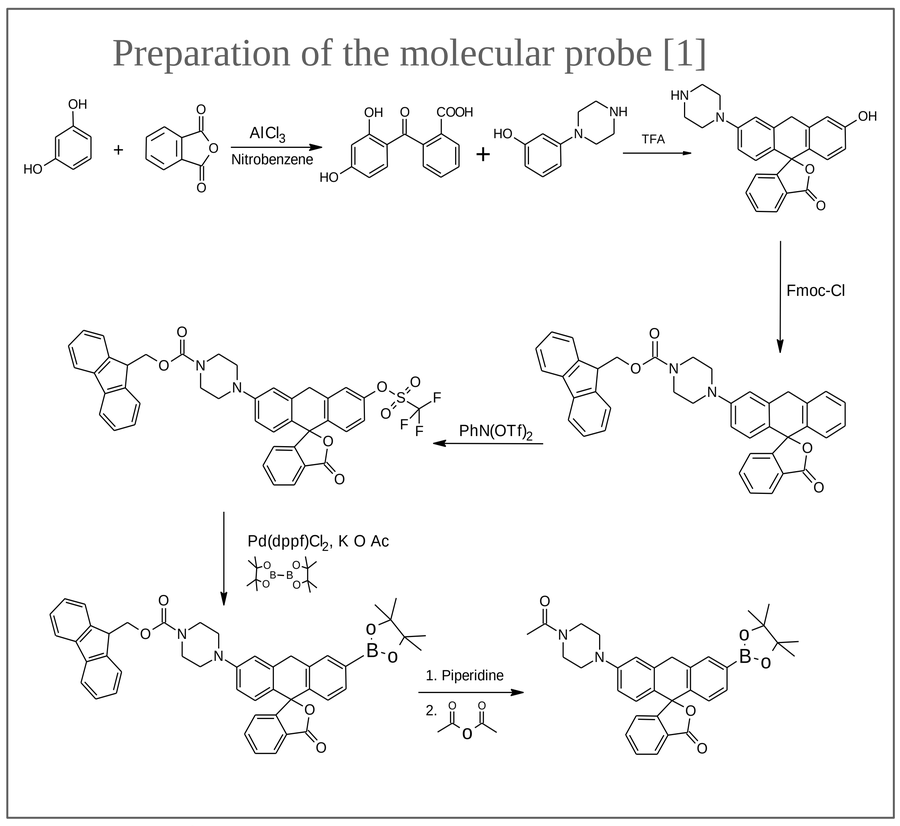

Figure 1: Total synthesis of the molecular probe [1].

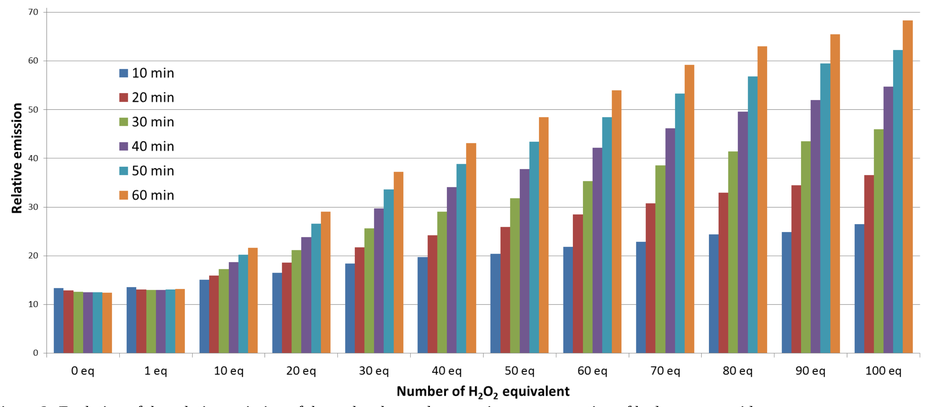

Figure 2: Evolution of the relative emission of the molecular probe to various concentration of hydrogen peroxid.

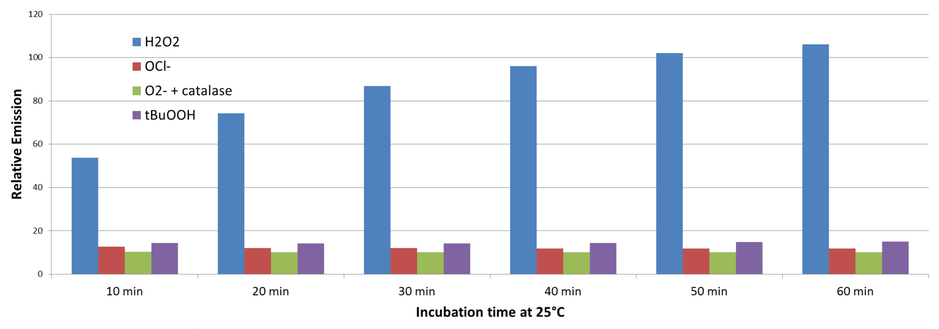

Figure 3: Relative emission of the molecular probe with various oxidative species.

Protocol

Leaf discs were inoculated with 20 µl droplets of sporangia of *P. infestans* suspension containing the molecular probe. A cover slide was placed over the leaf discs to obtain by capillarity the suspension in the center of leaf discs and around the well (figure 4). The slides with leaf discs were incubated on humidified absorbing paper in large petri dishes. Two hours later, the cover slide was removed to enable gas exchange. After incubation, the leaf discs were mounted in glycerol, the reaction was observed by fluorescence microscopy with a microscope Axion Imager.A1m.

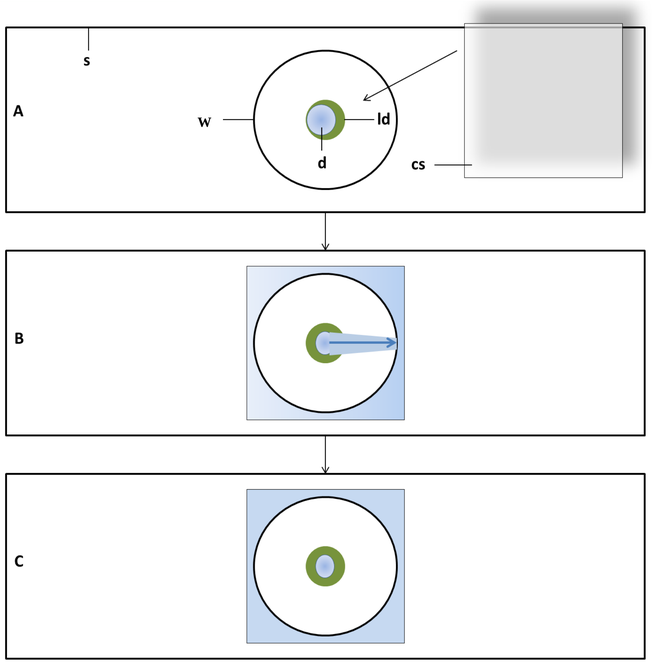

Figure 4: s, slide; w, well; d, droplet; ld, leaf disc; cs, cover slide. A: Leaf discs were inoculated with 20 µl droplets of sporangia suspension containing ContPY. B: A cover slide was deposed on leaf discs, the suspension moves (blue arrow) by capillarity. C: A moiety of suspension stay in center of leaf disc and the other moiety go around the well.

First Results

At 48h post infection, we can observe green fluorescent spots corresponding to the hydrogen peroxide production in the cells of the plant and the pathogen during infection (figure 5 and 6). The figures illustrate the fusion between a micrograph with fluorescence and a bright field micrograph. This hydrogen peroxide production would be locate at the infection site. But the protocol must still be optimized and repeated to understand the process. However, these pictures show our capacity to study this process.

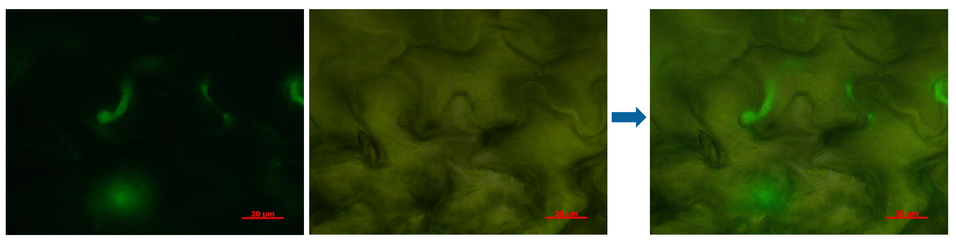

Figure 5: Micrograph of interaction site between P. infestans and A. thaliana. A micrograph in fluorescence and an other in bright field were combined to locate the peroxide hydrogen production in the plant cell. (GX100)

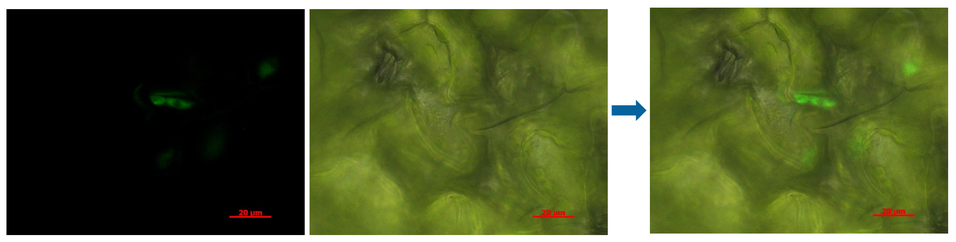

Figure 6: Micrograph of interaction site between P. infestans and A. thaliana. A micrograph in fluorescence and an other in bright field were combined to locate the peroxide hydrogen production in the plant cell. (GX100)

Prospects

These tools will be used to observe the H_2O_2 dynamic into the living cells 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**