THE HYPERSENSITIVE RESPONSE OF OLEA EUROPAEA L. ELICITATED BY PSEUDOMONAS SAVASTANOI

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In plants, the Hypersensitive Response (HR) is an early defense mechanism that is elicited by the recognition of an incompatible pathogen, with the objective of restricting its spread and therefore preventing a generalized infection throughout the organism. One of the earliest events in the HR is the rapid and significant increase in Reactive Oxygen Species (ROS) levels in the cells. This Oxidative Burst (OB) is part of an intracellular signal transduction pathway that triggers a variety of events, in particular the induction of the synthesis of secondary metabolites like phythoalexins (compounds with anti-microbial activity) and lignin (to reinforce the cell wall), and ultimately leading to a programmed cell death of the infected cells.

In this work, we used a previously established in vitro elicitation system to study the interaction between Olea europaea L. and the bacteria Pseudomonas savastanoi, a pathogen responsible for the olive-knot, a disease that drastically affects olive oil production in Portugal. This system is composed suspension cell cultures of the resistant O. europaea variety Galega Vulgar and an avirulent strain of the pathogen P. savastanoi.

Fig. 1 – Olea europaea, one of Portugal's top agricultural species. Pseudomonas savastanoi, the causal agent of olive knot, is responsible for the formation of galls in leaves and stems (insert).

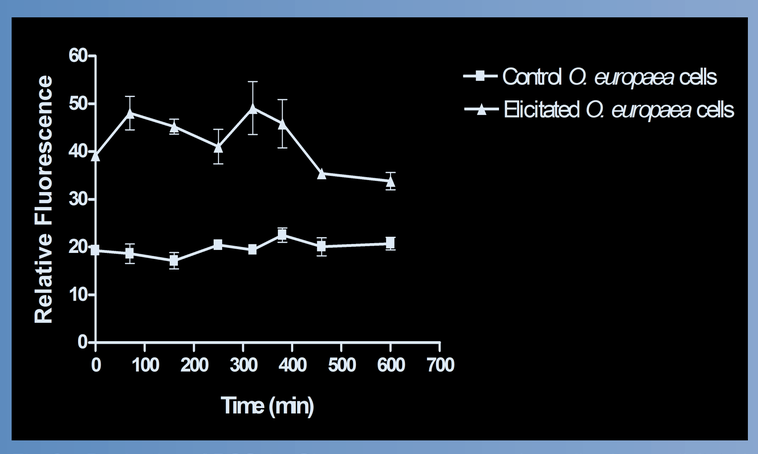

Fig. 2 – Production of ROS by Olea europaea suspension cell cultures elicitated with an avirulent strain of the bacteria Pseudomonas savastanoi.

§ We used the fluorescent probe 2',7'-dichlorodihydrofluorecein diacetate (H2DCFDA) to quantify the generation of ROS by spectrofluorimetric analysis of the supernatant (Parsons et al, 1999).

§ The results show that the challenged cells present a pattern of ROS production typical of the OB found in incompatible interactions, with two bursts at around 100 and 300 minutes after elicitation.

§ This fact points out that the Olea europaea variety Galega Vulgar is in fact resistant to the strain of Pseudomonas savastanoi used in the in vitro elicitation system.

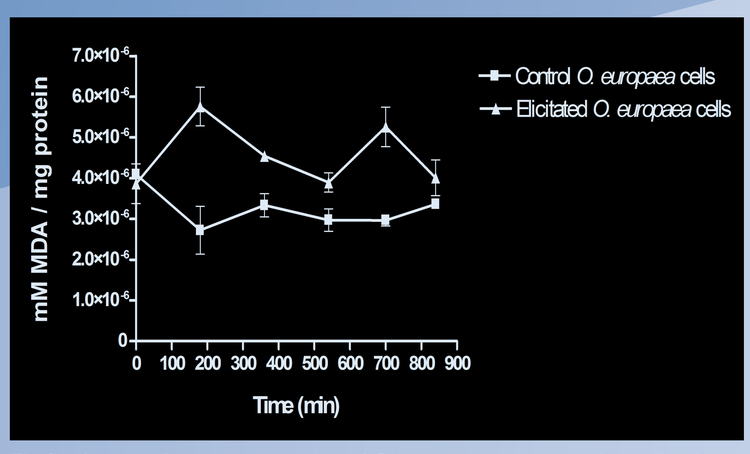

Fig.3 – Levels of lipid peroxidation of Olea europaea suspension cell cultures eliciated with an avirulent strain of Pseudomonas savastanoi.

§ The levels of lipid peroxidation were determined by the TBA test, which quantifies MDA as an end product of lipid peroxidation (Loreto et al, 2001). This test allows us to assess the extent of oxidative injury to the cells.

§ Figure 3 shows two peaks of lipid peroxidation during the time course of elicitation (200 and 600 minutes approximately).

§ Lipid peroxidation bursts immmediatly followed ROS production during the OB, indicating them as the origin of the increased levels of oxidative damage suffered by the cells.

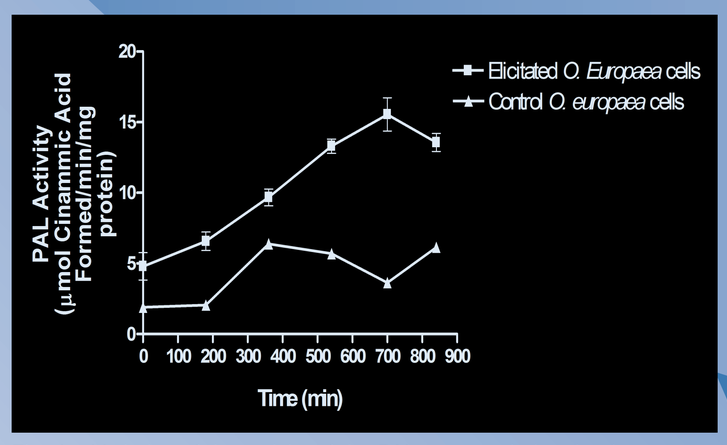

Fig. 4 – Variarion of the activity of the PAL enzyme during the time course of elicitation of Olea europaea suspension cell cultures with an avirulent strain of Pseudomonas savastanoi.

§ Phenylalanine ammonia-lyase (PAL) is the first enzyme of the phenylpropanoid pathway. The activity was evaluated by the quantification of trans-cinnamic acid determined spectrophotometrically at 290 nm.

§ The results point toward changes in the production of secondary metabolites, since the levels of PAL activity dramatically increase in challenged cells, as shown in figure 3.

§ This prominent difference between control and challenged cells indicate that the latter indeed activate certain defense responses, demonstrated by the increased activity of the first enzyme of a metabolic pathway that leads to the production of compounds with an important role in the resistance mechanisms.

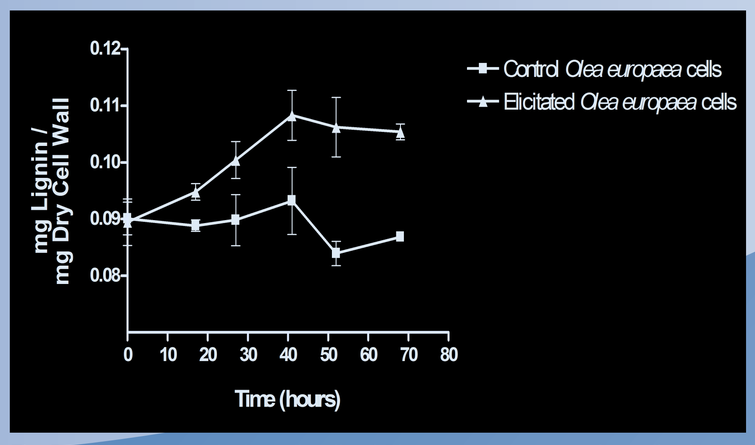

Fig. 5 – Lignin content in the cell walls of Olea europaea suspension cell cultures challenged with an avirulent strain of Pseudomonas savastanoi.

§ Lignin is one of the end-products of the phenylpropanoid pathway and a main component of the cell wall of plants.

§ The elicitated cells have higher lignin levels in their cell walls, comparing to non-elicited cells. The increase in lignin content is in accordance with the increase of PAL activity in challenged O. europaea cells.

§ The reinforcement of the cell wall is a part of the resistence mechanism of Olea europaea var. Galega Vulgar to Pseudomonas savastanoi.

§ When elicitated with an avirulent strain of the pathogen, the Olea europaea variety used in this system triggers certain defense mechanisms.

§ This set of responses start with the activation of specific signal transduction pathways, and includes:
  - § Elevated levels of ROS production, that fuction as an intracellular signal and act directly on the pathogen.
  - § Production of secondary metabolites essential in the resistance phenomena.
  - § Reinforcement of the cell boundaries, by the increased levels of lignin present in the cell wall.

§ Realçar resistencia ou deixar assim?

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Acknowledgments:

This work was supported by F
(POCTI/AGR/36239)

André Cruz is supported by FCT G
(SFRH/BD/6654/2001)