DEVELOPMENT OF TOOLS FOR STUDYING Olea europaea - Pseudomonas savastanoi INTERACTION

A. Braga da Cruz and R.M. Tavares

Centro de Ciências do Ambiente, Biology Department,
Minho University, 4710-057Braga, Portugal

A. Braga da Cruz >>> Tel. + 351 253 604047 >>> Fax +351 253 678980 >>> E-mail: andrex@bragatel.pt

Introduction

The production of olive oil is a major factor in portuguese agriculture. Therefore, pathogens that affect olive trees have a negative impact in our economy. The bacteria Pseudomonas savastanoi is the origin of the olive knot, one of the most serious diseases infecting portuguese olive orchards', causing a severe reduction in both the production and the quality of the fruits, due to the apperance of tumours in the stems and leaves. Consequently, the responses of the olive varieties that are known to be resistant to the infection – like the Galega Vulgar – must be studied, with the aim of better understanding the underlying defense mechanisms. In view of that, we developed a set of tools that will contribute to achieve this goal in the future, helping to find clones more adapted to challenge stress conditions.

Fig. 1 – Olea europaea, one of Portugal's top agricultural species. In the insert it is possible to see the galls which are characteristic of the olive knot, a disease originated by the infection with the bacteria Pseudomonas savastanoi.

Development of an in vitro Elicitation System

It is of crucial importance to develop a model that allowed the study of the interaction of O. uropaea cells and the bacteria P. savastanoi, especially in short temporal frames. Hence, we stablished an in vitro system of elicitation using suspension cell cultures of O. europaea. cells mid exponential phase growth. The cells were harvested, washed, ressuspended in phosphate uffer with 1% sucrose, and after a period of adaptation at the same conditions of growth, a uspension of the bacteria was added to the medium. We then added the tetrazolium dye XTT sodium,3'-(1-[phenylamino-carbonyl]-3,4-tetrazolium)-bis(4-methoxy-6-nitro) benzene-sulfonic cid) to the suspension, at a final concentration of 0,5 mM. This dye permits the quantification of e perhydroxyl / superoxide ($H_2O^{\cdot}/O_2^{-}$) radical acid-base pair, one of the Reactive Oxygen Species ROS) associated with the oxidative burst, allowing the evaluation of the Hypersensitive Response HR). The results demonstra the existence of two bursts of superoxide production during the time ourse of eliciation, at 100 and 300 minutes respectively (fig. 3). The first one is supposed to act ke a sort of transduction signal in the cell, and occurs in both compatible and incompatible teractions, while the second is characteristic of the HR that occurs in the incompatible interactions, nd will eventually lead the infected cells to a programmed cell death.

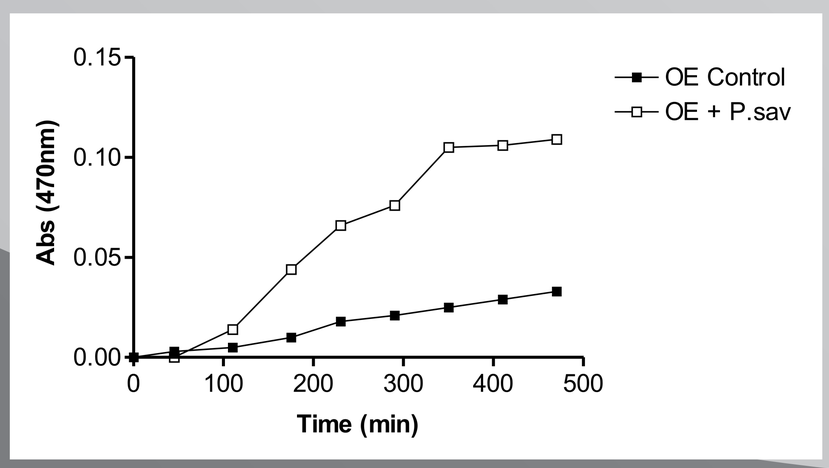

Fig. 3 – Cumulative reduction of XTT by Olea europaea suspension cell cultures eliciated with an avirulent strain of Pseudomonas savastanoi.

Establishment and Characterization of Suspension Cell Cultures

Suspension cultures of Olea europaea cells were initiated from calli of the variety Galega Vulgar. Both types of cultures were maintained in modified MS medium and grown at 26ºC in the dark, with the suspension cell cultures incubated in an orbital shaker at 100 r.p.m..

To better characterize the suspension cell cultures, their growth was accompanied by the determination of the dry weight of samples harvested at regular intervals. With the obtained results it was possible to determine a growth rate of 0,1 Day^{-1}. The inital carbon source of the medium is sucrose at a concentration of 3%, which is swiftly hidrolised to frutose and glucose, possibly due to the action of an extracelular invertase. The growth of the suspension cultures stops arround the 25^{th} day, as seen in fig. 2, upon total depletion of the carbon sources of the culture medium.

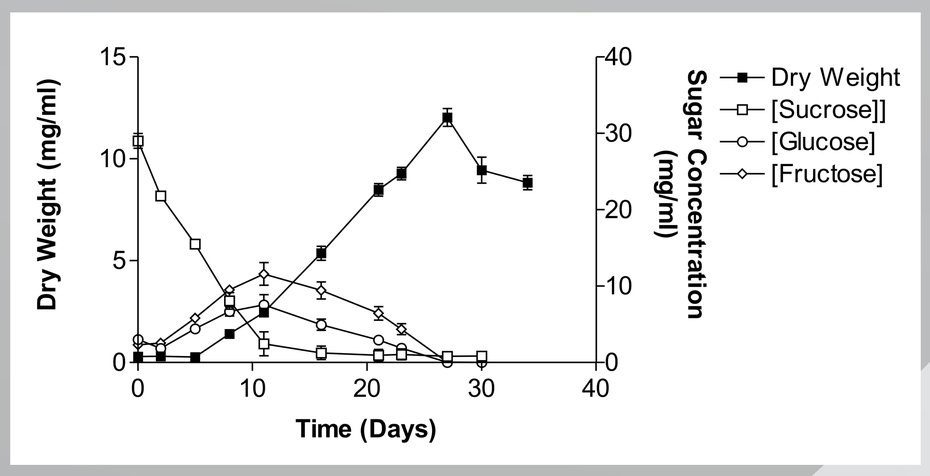

Fig. 3 – Growth of Olea europaea suspension cell cultures, at 26ºC and pH 5,8, in modified MS medium with 3% saccharose as sole carbon source.

Construction of a cDNA Library

A cDNA library is a central tool for molecular studies, since it enables us to find the complete coding regions of genes of interest for our work, thus allowing for more complete studies of their patterns of expression.

The first step was optimising a method for the extraction of the total RNA from suspension cell cultures. This was accomplished using a variation of the method described by Ainsworth et al (1992), and allowed us to obtain RNA samples of high purity and integrity (fig. 4), a crucial factor in the construction of a cDNA library. The RNA used for this process was derived from elicitated O. europaea suspension cells, harvested 300 and 450 minutes after elicitation, due to the higher level of expression of defense mechanisms related genes. mRNA was purified using the Dynabeads mRNA Direct Kit (Dynal) system, and the library was constructed using the ZAP Express vector (Stratagene). The titer of the primary libraryt was 1,6 x 10^5 pfu / ml , and of the amplified library is 7,45 x 10^8 pfu / ml, allowing us to state that the library is representative.

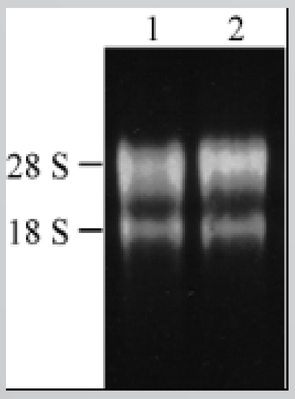

Fig. 4 – Total RNA samples analysed in denaturating formaldehyde gel (1,2% agarose).

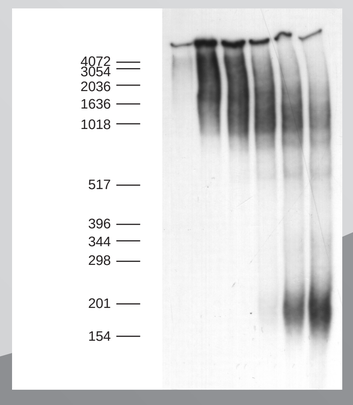

Fig. 5 - Analysis of the cDNA fractions obtained by gel filtration cromatography on a 5% nondenaturating acrylamide gel.

Acknowledgments >>> A. Braga da Cruz is supported by the Foundation for Science and Technology, grant ref. SFRH/BD/6654/2001.

This work was supported by FCT (POCTI / AGR / 36239 /99-00)