DEVELOPMENT OF TOOLS FOR STUDYING *OLEA EUROPAEA* - *PSEUDOMONAS SAVASTANOI* INTERACTION

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Introduction

Since Portugal is one of the world's top olive oil producers, the culture of olive trees (*Olea europaea* L.) assumes a considerable importance in our country's economy. In order to find clones more adapted to challenge stress condition, the responses of our olive varieties to environmental stresses and pests must be studied. One of the diseases that most seriously affects Portuguese olive orchards' is the olive knot, caused by the bacteria *Pseudomonas savastano*i (*Pseudomonas syringae* pv. *savastanoi*). The production and quality of fruits is strongly reduced, due to the appearance of tumours in the stems and leaves (1) (Fig.1). Some Portuguese *O. europaea varieties* (eg. Galega Vulgar) are known to be resistant to the infection, while others are extremely susceptible. With the objective of better understanding *O. europaea* defence mechanisms against *P. savastanoi*, we developed a set of tools that will contribute to this goal in a near future.

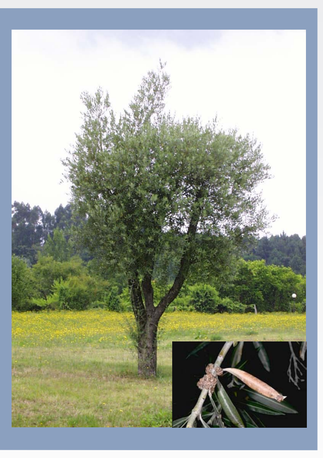

Fig. 1 – *Olea europaea*, one of Portugal's top agricultural species. In the insert it is possible to see the galls which are characteristic of the olive knot, a disease originated by the infection with the bacteria *Pseudomonas savastanoi*.

Establishment of Suspension Ciultures

Suspension cultures of Olea europaea cells were initiated from calli of the variety Galega Vulgar (Fig. 2). Both types of cultures were maintained in modified MS medium and grown at 26ºC in the dark. Suspension cell cultures were incubated in an orbital shaker at 100 rpm.
Suspension cell culture growth was accompanied by dry weight determination of samples, harvested at regular intervals. It was possible to evaluate an exponential growth of the cell culture, with a specific growth rate of 0,11 Day-1. The growth stopped at around 25 days of culture, upon total depletion of the medium's carbon source (data now shown).

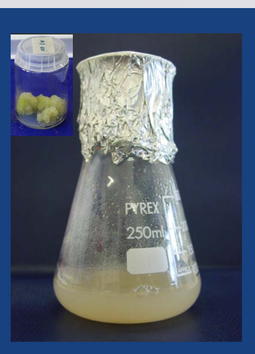

Fig. 2 – *Olea europaea* var Galega Vulgar suspension cell cultures. In the insert are represented the *calli* from which they were established.

Development of an *In Vitro* Eliciation System

An in vitro system of eliciation was developed using *O. europaea* suspension cell cultures. Cells in mid exponential phase growth were washed, ressuspended in phosphate buffer and incubated with a suspension of an avirulent strain of *P. savastanoi*. This model was used to study the production of Reactive Oxygen Species (ROS) which is associated with the oxidative burst, occurring during the Hypersensitive Response (HR). For this purpose, the tetrazolium dye XTT ([sodium,3'-(1-[phenylamino-carbonyl]-3,4-tetrazolium)-bis(4-methoxy-6-nitro) benzene-sulfonic acid]), which allows the quantification of the perhydroxyl / superoxide (H2O./O2-) radical acid-base pair, was added to the suspension to a final concentration of 0.5 mM.Our results showed the existence of two bursts during the time course of eliciation, at 100 and 300 minutes respectively, as seen in Fig. 3. The first one is supposed to act like a sort of transduction signal in the cell, and occurs in both compatible and incompatible interactions (2,3), while the second is characteristic of the HR that occurs in the incompatible interactions, and will eventually lead to a programmed cell death of infected cells

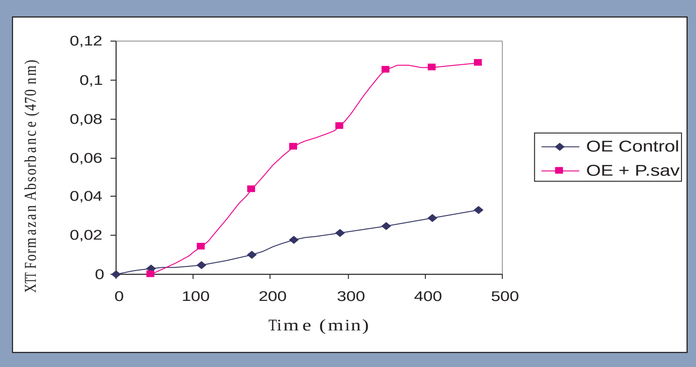

Fig. 3 – Cumulative reduction of XTT by Olea europaea suspension cell cultures eliciated with an avirulent strain of Pseudomonas savastanoi.

Construction of a cDNA Library

A cDNA library is a valuable tool for the research of complete coding regions of interesting genes for our work, allowing more complete studies of their expression patterns.
A method for the extraction of the total RNA from suspension cell cultures was optimised. This was accomplished using a variation of Ainsworth et al (4) protocol, and allowed us to obtain RNA samples of high purity and integrity (Fig. 4). The RNA used for this process was derived from eliciated *O. europaea* suspension cells, harvested 300 and 450 minutes after eliciation. mRNA was purified using the Dynabeads mRNA Direct Kit (Dynal) and used as a template for the synthesis of the 1st strand cDNA. After the 2nd strand synthesis, cDNA was cloned in the ZAP Express vector (Stratage) and packaged using lamba packaging extracts (Stratagene).

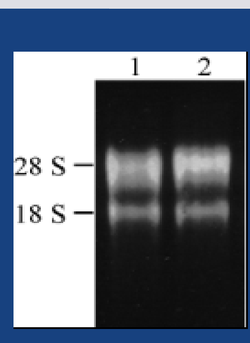

Fig. 4 – Total RNA samples analysed in denaturating formaldehyde gel (1,2% agarose).

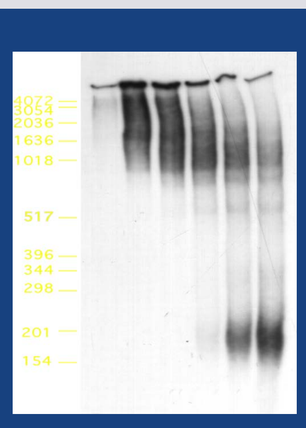

Fig. 5 - Analysis of the cDNA fractions obtained by gel filtration cromatography on a 5% nondenaturating acrylamide gel.

References

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Acknowledgments

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