Eliciting System of an In Vitro Development of 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 climate changes, the responses of olive trees 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 savastanoi. The production and quality of fruits is strongly reduced, due to the appearance of tumours in the stems and leaves (1). With the objective of better understanding P. savastanoi, we developed a set of tools that will contribute to this goal in the near future.

Establishment of Suspension Cultures

An in vitro system of elicitation was developed using O. europaea suspension cell cultures. Cells in mid-exponential phase growth were washed, resuspended 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'-phenylamino-carbonyl]-3,4-tetrazolium)-bis(4-methoxy-6-nitro) benzene-sulfonic acid), which allows the quantification of the perhydroxyl / superoxide (H2O2/O2-) generation, was used. The growth stopped at around 25 days of culture, upon total depletion of the medium’s carbon source.

Development of an In Vitro Elicitation System

Supernatant cultures of Olea europaea cells were incubated with 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'-phenylamino-carbonyl]-3,4-tetrazolium)-bis(4-methoxy-6-nitro) benzene-sulfonic acid), which allows the quantification of the perhydroxyl / superoxide (H2O2/O2-) generation, was used. The growth stopped at around 25 days of culture, upon total depletion of the medium’s carbon source.

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 Arnaud et al. (2) protocol, and allowed us to obtain RNA samples of high purity and integrity (Fig. 4). The RNA used for this process was derived from elicited O. europaea suspension cells, harvested at 300 and 450 minutes after elicitation. mRNA was purified using the DynaBeads mRNA Direct Kit (Dynal) and used as a template for the synthesis of the 1st strand cDNA. In the insert it is possible to see the gels which are characteristic of the HR that occurs in the incompatible interactions, and will eventually lead to a programmed cell death of infected cells.

References