

DEVELOPMENT OF AN IN VITRO CALLUS CULTURE SYSTEM TO STUDY THE INDUCTION OF SECONDARY METABOLITES IN OLIVE (OLEA EUROPAEA L.)

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There is a great interest about the knowledge of plant defence mechanisms and the expression of resistance genes induced by pathogens in plant crops of economic interest. Our research aims to study the secondary metabolic pathways possibly involved in olive defence mechanisms. We produced olive callus cultures to analyse their metabolites and genes expression after treatment with elicitors. To establish callus cultures from in vitro and in vivo leaf explants, we selected the olive cultivars Ogliarola Salentina and Leccino, and we evaluated different media with different hormones combinations. Specifically, we defined the optimal media for induction of callus of Ogliarola Salentina: Murashige e Skoog '72 basal medium (MS) with addition of Zeatin (1 mg/ml) and IBA (0.1 mg/ml); and the other one for Leccino: Nitsch & Nitsch' 69 (NN) medium with BAP (1 mg/L) and 2.4D (2 mg/L). Callus production was higher in Ogliarola Salentina compared to Leccino.

Calluses of cv. Ogliarola were analyzed by mass spectrometry and showed a

high content of polyphenolic and at less extent of terpene compounds, which are known to be involved in plant defence. The most abundant metabolites were verbascoside, caffeic acid, and hydroxytyrosol, which is a precursor of oleuropein.

We then performed a second experiment using the elicitor methyl-jasmonate (MeJa) at different exposure timing (5 h, 24 h, 72 h) and doses (50 μ M, 100 μ M, 500 μ M) to study its effect on the expression of selected genes and on the content of metabolites. The next step will be the testing of callus response after treatment with bacterial lysate of *Xylella fastidiosa* subsp. *pauca*.

First results provided cultivar's specific protocols for olive callogenesis and a preliminary overview of polyphenolic and terpenic compounds produced by olive callus cultures. Our system represents a promising tool to assess the synthesis and control of secondary metabolites involved in response of olive to biotic stresses.