

D. Inácio<sup>1</sup>, T. Monteiro<sup>2</sup>, M. R. Félix<sup>3</sup>, C. Campos<sup>2</sup>, M. Patanita<sup>2</sup>, J. A. Ribeiro<sup>2</sup>, A. Albuquerque<sup>2</sup>, F. Santos<sup>3</sup>, A. da Rosa<sup>1</sup>,  
A. Peixe<sup>3</sup>, M.D. Campos<sup>2</sup>

<sup>1</sup>Master student in Agronomic Engineering, Universidade de Évora, Largo dos Colegiais 2, 7004-516 Évora, Portugal.

<sup>2</sup>MED - Mediterranean Institute for Agriculture, Environment and Development & CHANGE - Global Change and Sustainability Institute, Institute for Advanced Studies and Research, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal. \*Email: mdcc@uevora.pt

<sup>3</sup>MED - Mediterranean Institute for Agriculture, Environment and Development & CHANGE - Global Change and Sustainability Institute, Departamento de Fitotecnia, Escola de Ciências e Tecnologia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.



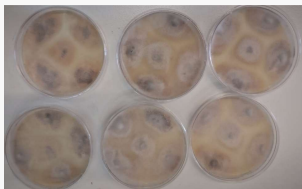
## INTRODUCTION AND OBJECTIVES

One of the most economically important fruit trees in Mediterranean countries is the olive tree (*Olea europaea* L.). Foliar and fruit fungal pathogens are responsible for economically important diseases in most olive-growing areas. Within these pathogens, we highlight *Colletotrichum* spp., the causal agent of the serious anthracnose disease. Under favourable conditions, the disease can devastate entire production fields. Symptoms typically occur on fruits at maturation under wet autumn conditions causing severe quantitative and qualitative yield losses of olive oil and fruits. The best control strategy currently relies on the use of synthetic fungicides, but there is regulatory pressure in agriculture worldwide to limit their use.

In search of a sustainable approach to disease management, the present study reports the transcriptional changes of genes that encode enzymes actively involved in the olive tree's defence response to *C. nymphaeae* infection. 'Galega vulgar', an olive cultivar known to be highly susceptible to the disease, was selected for the studies.

## MATERIALS AND METHODS

### 1) Growth of the fungus *C. nymphaeae*



*C. nymphaeae* belonging to the collection of the Mycology Lab, MED, UÉvora.

### 2) Transplantation of olive plants 'Galega vulgar' to pots



*In vitro* plants obtained from the Plant Breeding and Biotechnology Lab, MED, UÉvora, to ensure their healthy status.

### 3) Olive inoculation with *C. nymphaeae*

Leaves sampling:

- Before inoculation (T0)
- 10 days after inoculation (T1)
- 35 days after inoculation (T2)



### 4) Confirmation of plant infection by *C. nymphaeae* and plant target gene expression



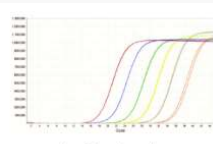
Olive leaves maceration



- gDNA extraction
- RNA extraction and cDNA synthesis



qPCR Machine



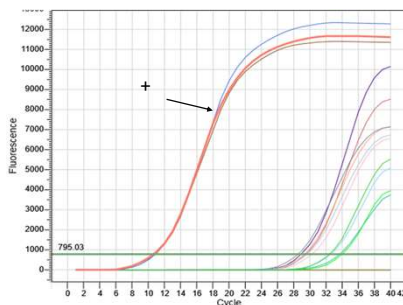
Amplification plot

qPCR analysis

### SELECTED TARGET GENES POTENTIALLY INVOLVED IN DEFENSE RESPONSE:

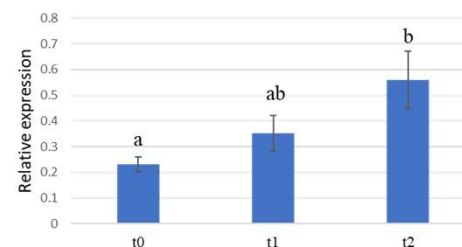
- Superoxide dismutase (*SOD*)
- Endochitinase EP3-like (*CHI*)
- Glutathione S-transferase L3-like (*TransFL3*)
- Glutathione peroxidase 2 (*PEROX2*)
- Glutathione S-transferase THETA 1 (*THETA*)
- Glutathione S-transferase DHAR2-like (*TransDHAR*)
- Glutathione peroxidase 5 (*PEROX5*)
- Glutathione peroxidase 8 (*PEROX8*).

## RESULTS AND FINAL REMARKS



Amplification of gDNA of *C. nymphaeae* by qPCR. +: positive control

✓ The presence of *C. nymphaeae* was confirmed in inoculated olive plants.



Transcript accumulation of Endochitinase EP3-like (*CHI*) in non-inoculated olive plants (T0) and at 10 days (T1) and 35 days (T2) after inoculation with *C. nymphaeae*. Significant differences are identified with different lowercase letters.

✓ Transcriptomic profiling of the selected target genes revealed a general up-regulation in inoculated olive plants (not shown).

✓ *CHI1* revealed a significantly up-regulation ( $p < 0.005$ ) between T0 and T2 in response to *C. nymphaeae* infection.

✓ *CHI* is a promising candidate to be later used in functional analysis.

➤ This work, still in course, will give an important contribution for the identification of key functional genes in olive susceptible responses to infection by *C. nymphaeae* and in the understanding of the molecular basis of compatible interactions.

➤ We emphasize the importance of this study for the identification of candidate genes to incorporate new sources of resistance of olive trees to anthracnose with the promotion of the development of sustainable management strategies.