Determination of the subcellular localisation of GIP and NPP1 proteins in *Phytophtora cinnamomi* tagged with green fluorescent protein

Abdessalem CHAHED[1,2], Noureddine CHATTI[2], Rodrigo COSTA[1,3], Maria JOÃO DE SOUSA [1] and Altino CHOUPINA[1]*

[1]Mountain Research Centre (CIMO), ESA, Polytechnic Institute of Bragança, Campus de Santa Apolónia, 1172, 5300-253 Bragança, Portugal
[2]Laboratory for Research on Genetics Biodiversity and Bioresources Valuation of (LR11ES41), ISBM, University of Monastir, Monastir 5000, Tunisia
[3]Department of Microbiology and Genetics, CIELUS-IBSAL, University of Salamanca / CSIC, Plaza de Drs. Queen s/n, 37007 Salamanca, Spain
*albracho@ipb.pt

Introduction :

P. cinnamomi is considered one of the world's worst invasive pathogens with a serious threat to a wide range of plant species throughout the world. These species cause several billions of dollars of damage on crop, ornamental, and native plants and so far no efficient solutions have been found to control the disease.

A notable characteristic of the interaction between plants and their microbial pathogens is the secretion by both partners of proteins that are associated with attack, defense, and counterdefense thus it's fundamental to improve our knowledge about these proteins with the intention of finding a solution to the disease affecting the Chestnut.

Objective :

Cloning targets genes into pTOR-EGFP vector containing a *ham34* promoter and a *GFP* gene which allows to discriminate the place and determination of the subcellular localization of GIP end NPP1 proteins.

Methods :

1.PCR amplification of the ORFs GIP and NPP1

Primers with adapters having specific restriction sites were designed to allow the insertion of the PCR product into pTOR-EGFP vector. The design of primers was done using bioinformatic tools that take into consideration the restriction sites in pTOR-EGFP vector.

2.Transformation of P.Cinnamomi with the recombinant vector pTOR - EGFP

The recombinant plasmid was used to transform *Phytophthora cinnamomi* by electroporation, then the transformants were cultivated on medium containing ampicillin (30 ug / ml) and incubated for ten days at 25 °C in the dark.

3. Prediction of the subcellular localization of the GIP and NPP1 proteins

. The softwares publicly available CELLO, LOCtree, EuK-mPLoc2.0, Esl pred and SignalP 3.0 have been used for the protein localization prediction.

Results :

1.*Phytophtora cinnamomi* with the recombinant vector pTOR-eGFP

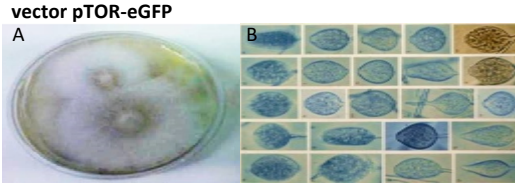

Figure 1: A. *P.cinnamomi* culture in V8 meduim.
B. *Phytophthora* sporangia microscope observation

2.Fusion of the ORFs gip and npp1 genes into pTOR - EGFP

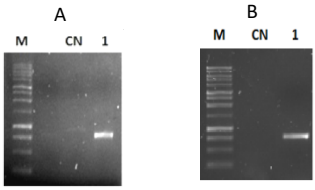

Figure 2: **A.** Amplification of the gene GIP (810 pb); **B.** Amplification of the gene NPP1 (771 pb).

2.Visualization of the recombinant pTOR - EGFP

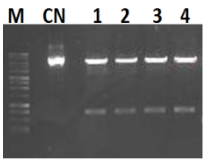

Figure 3: Visualization of the recombinant pTOR-eGFP vector digestion.

3. Prediction of the subcellular localization of GIP and NPP1 proteins

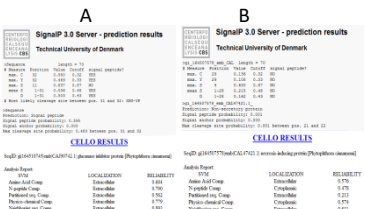

Figure 4:
A.Subcellular localization prediction of GIP protein;
B.Subcellular localization prediction of GIP protein

Conclusion :

➢The cloning of the *gip* and *npp1* ORF in the pTOR-eGFP vector having the gene encoding for the GFP was successfully achieved for future subcellular localization.

➢The transformation of *P. cinnamomi* zoospores by electroporation was successfully.

➢Subcellular localization of the proteins shows that the protein GIP is secreted through the classical ER-Golgi pathway while for NPP1 protein the secretion is assured by unconventional protein secretion processes