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Silencing the *gip* gene of *Phytophthora cinnamomi* by iRNA and subcellular localization of GIP and NPP1 proteins

Poster N°:XXX

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Introduction :

Ink Disease is considered one of the most important causes of the decline of chestnut orchards. The break in yield of *Castanea sativa* Mill is mainly caused by *Phytophthora cinnamomi* one of the most aggressive and widespread plant pathogen causing enormous economic losses and up to now no efficient treatments are available to fight these pathogens.

Because of the importance of chestnut at economical and ecological levels especially in Portugal, it becomes essential to explore the molecular mechanisms that determine the interaction between *Phytophthora* species and host plants through the study of proteins GIP and NPP1 produced by *P.cinnamomi* during the infection.

Objective:

The main goal of this work is to contribute to a better understanding of the function of the proteins GIP and NPP1 involving in the mechanism of infection of chestnuts by *P.cinnamomi,* an essential step for the implementation of control strategies.

Methods :

1.Design of silencing cassette (shRNA) for gene gip

Specific primers were designed following Tiscornia *et al* protocol (2003) to amplify the silencing cassette selected within the ORF of gip gene.

The forward and reverse primers contain the Apa I restriction site to allow the insertion of the silencing cassette (526pb) after PCR, into the Pth210 vector driven by the hsp70 promoter and the ham34 terminator (Bremia lactucae).

2.Transformation of E. coli with the recombinant vector

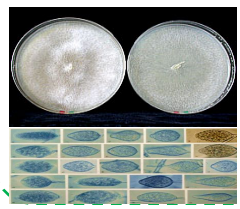
In order to propagate the silencing cassette, 50 µl of competent cells of the strain NZY5α were mixed with the PTH210 recombinant vector (having the cassette sequence).

After transformation , the extraction of recombinant vector was done followed by enzymatic digestion with the enzyme Apa I in order to confirm the clonage of the sliencing cassette into the PTH210 vector.

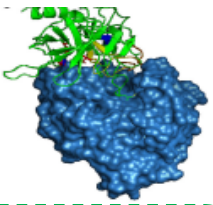
3.PCR amplification of gene gip ORF

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.

Phytophthora cinnamomi.



3D structure of GIP protein



Results :

1.Silencing cassette construction

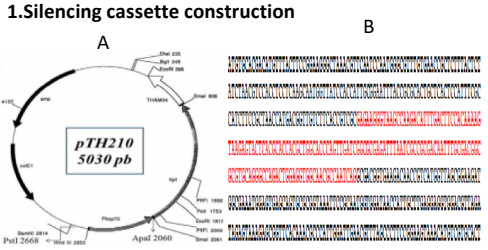
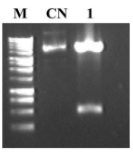


Figure 1 : A : The map of pTH210 vector . B : Sequence of gene gip silencing cassette (black for sense and antisense sequences, red for the loop sequence).

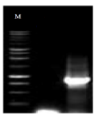
2.Visualization of enzymatic digestion products



The products resulting from the enzymatic digestion were analyzed in agarose gel . We observed two bands, one of pTH210 vector with 5030 pb and another of 526 pb corresponding to the cassette.

Figure 2 : Enzymatic digestion of recombinant pTH210 with Apa I (M : DNA ladder; CN : Negative control for the non recombinant pTH210 supercoiled plasmid ; I :pTH210 recombinant digested with Apa I).

3.Visualization of PCR product



After irradiation of gel it was possible to view the size and intensity of the desired band (811pb) by comparison with the DNA ladder 1kb.

Figure 3 :PCR product of gip gene (M : DNA ladder).

Conclusion :

The final results will allow us to determine the secretory destination of both GIP and NPP1 proteins (after insertion of recombinant pTOR-EGFP vector in *P.cinnamomi* and observation with confocal microscopy).

Also the results will confirm RNAi as a potential alternative biological tool in the control and management of *P.cinnamomi* (after transformation of *P.cinnamomi* with recombinant pTH210 vector and infecting *C.sativa* with transformants).