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Knockdown of *Phytophthora cinnamomi gip* gene by iRNA

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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 GIP protein produced by *P.cinnamomi* during the infection.

Objective :

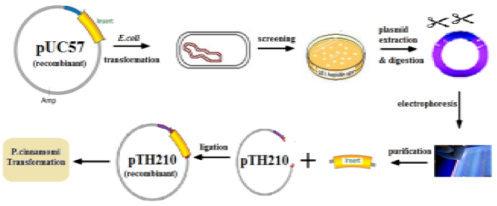
The main goal of this work is to contribute to a better understanding of the function of GIP protein involved in the mechanism of infection of chestnut by *P.cinnamomi,* an essential step for the implementation of control strategies.

Methods :

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

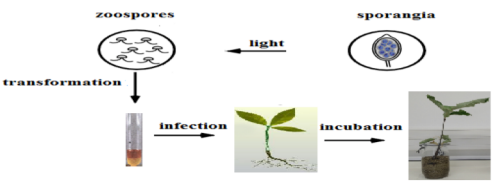
Specific primers were designed to amplify the silencing cassette selected within the ORF of *gip* gene.
The cassette was made by joining a 5'sequence fragment to another from the 3' end of the same sequence in an inverted orientation separated by a loop DNA

2.Transformation of *Phytophthora cinnamomi* zoospores with the recombinant pTH210 vector



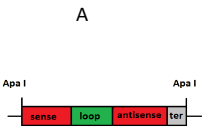
To confirm the transformation of *P. cinnamomi, the genomic DNA was extracted to amplify a region of 551 bp corresponding to the cassette sequence and other region of 1090 bp within the hygromycin gene (hpt).*

3.*Castanea sativa* infection with *P. cinnamomi* strains



Results :

1.Silencing cassette construction



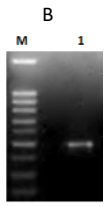
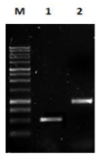


Figure 1: A : Structure of the cassette. B : Visualization of the synthesized cassette on agarose gel M) DNA ladder of 100 bp; 1) the gip silencing cassette (551 pb)

2.Confirmation of *P. cinnamomi* transformation



The PCR screening proves the integration of the recombinant plasmid pTH210 in the genome of *P. cinnamomi*

Figure 2: Visualization of the silencing cassette and the hygromycin fragment PCR products M) DNA ladder of 1 kb; 1) PCR product of the silencing cassette 551 bp; 2) PCR product of the hygromycin fragment 1090 bp

3.Morphological analysis of the chestnut plants

Figure 3: Aspect of chestnuts after infection with non-transformed and transformed *P. cinnamomi*

Conclusion :

➢ A silencing cassette for *P. cinnamomi gip* gene can be produced by PCR-based cloning method

➢ The plants infected by the transformed *P. cinnamomi (transformed with the silencing cassette) revealed a smaller percentage of wilting leaves and root necrosis*

➢The integration of the plasmid pTH210 in the genome of *P. cinnamomi* was confirmed by PCR screening

➢The GIP protein is considered responsible for the suppression of plant defense responses enhancing susceptibility of disease symptoms development