Molecular aproach on the study of infection of *Pinus pinaster* Ait. by the fungus *Lophodermium seditiosum*

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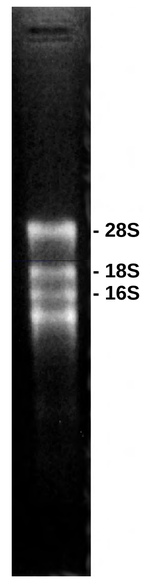
Introduction

Maritime pine (*Pinus pinaster* Ait.) is amongst the most relevant forest species in SW Europe. It occupies 29% of the total forest area in Portugal, but its distribution has diminuished by 26% in the past 25 years. The genus *Lophodermium* comprises several species of fungi known to intervene in the natural decomposition process of pine needles. Amongst these species, only *L. seditiosum* is known to be pathogenic, as it is able to infect healthy needles in young pine populations, spreading from stomata and leading to the death of the needle (needle cast disease). The disease, by attacking young plants, ultimately leads to two main events: on the one hand, it prevents the lack of self regeneration in natural populations, also acting as an agent for the complete destruction of pine nurseries. The purpose of our study is to understand the nature of the infection of *P. pinaster* by *L. seditiosum* at both a physiologycal and molecular level.

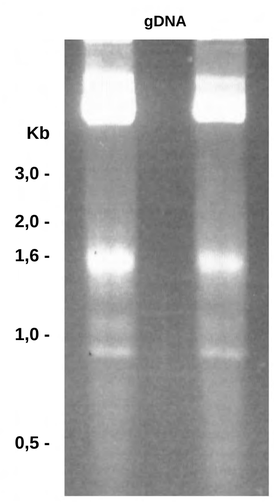
RNA

A protocol was developed for the isolation of RNA from *P. pinaster*, resulting in a high integrity product, as shown by electrophoretical analysis.



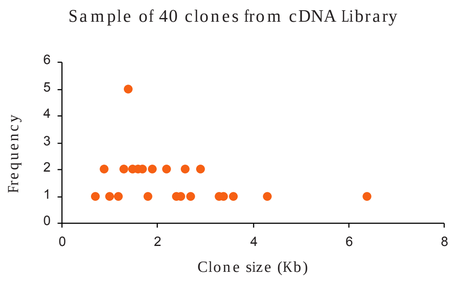
gDNA

Being *P. pinaster* a recalcitrant species, genomic DNA with high integrity and purity was obtained after developing a protocol adapted from the CTAB method which excludes CTAB precipitation.



Construction of a cDNA Library

The *P. pinaster* library was constructed using the *ZAP Express* vector system and amounted to 10^9 cDNA clones. High gene integrity was obtained, as shown by the size of 40 randomly selected clones sampled from the library.

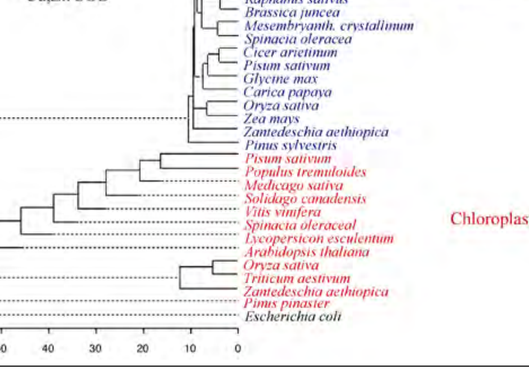


Screening the cDNA Library

Screening for pathogenesis related genes using heterologous probes is underway, having already resulted in the isolation of a gene encoding for the enzyme Superoxide Dismutase (SOD).

Cu,Zn SOD

Phylogenetic analysis of a cDNA clone encoding the enzyme Cu,Zn SOD from *P. pinaster*.



Work in progress

Use of degenerate primers for conserved regions of pathogenesis related genes and further sequencing.
Development of *P. pinaster* suspension cell cultures as a system for elicitation studies.
Elicitation of 1 month old *P. pinaster* seedlings.

Future prospects

The establishing of a model for the studyof the elicitation process, together with the development of molecular tools, will hopefully increase our insight on the severeal aspects which are envolved on the response of *P. pinaster* to infection: signalling and Systemic Aquired Resiatance, the Hypersensytive Response and the production of secundary metabolites.

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