



Phytophthora cinnamomi POPULATIONS ON Quercus FORESTS FROM SPAIN AND PORTUGAL



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INTRODUCTION

Phytophthora cinnamomi is the causal agent of the root infection that produces decline and mortality of cork (*Quercus suber*) and holm oak (*Q. rotundifolia*) in southern Spain and Portugal.

Objectives:

- To study the fingerprint from *P. cinnamomi* isolates of *Quercus* spp.
- To explore the intraspecific variation
- To investigate the genetic diversity and population structure in *P. cinnamomi* species.



Sudden oak death caused by *P. cinnamomi*



Oak die-back caused by *P. cinnamomi*

MATERIALS AND METHODS

Fungal isolates

Isolates from different geographical areas in Portugal and Spain severely affected by Mediterranean oak decline were collected.

DNA extraction from mycelium growing on clarified V8 juice agar was performed with DNeasy Plant Mini kit.

Molecular analysis

Digestion oomycete genomic DNA (300ng) of 56 isolates was digested for 2 h at 37 °C with *EcoRI* (2.5 U) and *MseI* (2.5 U) restriction enzymes.

Adaptor Two oligonucleotide adaptors, complementary to the restricted sites of the DNA fragments were ligated.

Pre-amplification Primers with an additional selective 3' nucleotide (A or T) to *EcoRI* and *MseI*.

Selective amplification Five primer combinations (*EcoRI*-ACC,ATG, AAG and *MseI*-ATT, ACC) were used.

Visualization Amplification products radioactively labelled were separated on 6% polyacrylamide gels and visualised after autoradiography (Figure 1).

Data analyses

Binary matrix of 56X149 bands (1;0)

Similarity matrix based upon Jaccard coefficient; SIMQUAL program NTSYS-pc software package.

Cluster analysis performed SAHN program in NTSYS-pc 2.0

Dendrogram was obtained with unweighted pair-group mean analysis (UPGMA) POPGENE 32 software (Figure 2).

Genetic diversity and genetic structure were performed with AFLP-SURV 1.0.

Analysis of molecular variance based on Euclidian distances between AFLP multilocus phenotypes, was conducted with ARLEQUIN 3.01.

Figure 1. Example of AFLP profiles of *P. cinnamomi* isolates, using the primer combination Eco-ACC/Mse-ATT and Eco-ACC/Mse-ACC.

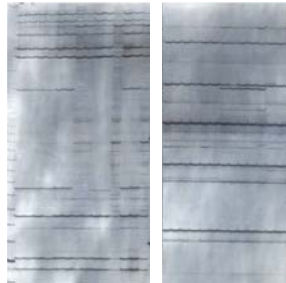
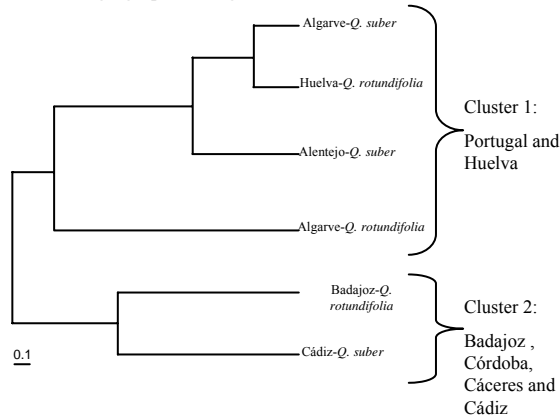


Figure 2. Unweighted pair group mean analysis dendrogram illustrating Nei's genetic distance based on amplified fragment length polymorphism of *Phytophthora cinnamomi* from different geographical origin and hosts



RESULTS

Table 1. Indicators of genotypic diversity for six *P. cinnamomi* populations based on amplified fragment length polymorphic (AFLP) fingerprinting ^a

Population size	% of polymorphic loci	Ht ^a	Hw ^b	Hb ^c	Fst ^d
56	67,8%	0.2267	0.2070	0.00198	0.086

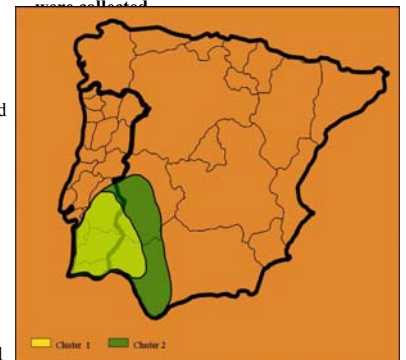
^a Ht Total diversity ^b Hw, Average diversity within populations ^c Hb, average diversity among populations ^d Fst, differentiation between populations. A total of 149 marker loci were obtained.

Table 2. Analysis of molecular variance (AMOVA) for 56 *P. cinnamomi* isolates grouped into six populations.

Variance component	d.f.	variance	% of variation	Φst ^a
Among subpopulations	5	0.75184	11.41	0.114
Among individuals/within subpopulations	50	5.83992	88.59	

^a Φst : genetic differentiation among populations.

Figure 3. Map of Iberian Peninsula showing the locations where the samples were collected.



DISCUSSION

Two different populations have been detected among *P. cinnamomi* isolates infecting Mediterranean *Quercus* species in southern Spain and Portugal by using molecular tools as AFLP fingerprintings. In previous studies dealing with the morphological characterisation of *P. cinnamomi* isolates coming from *Quercus* roots located in this geographical area (southwest of the Iberian Peninsula), two different hyphal patterns were detected (Figure 4). As the first group of isolates came from *Q. suber* samples and the second one came from *Q. rotundifolia*, it was hypothesized that these differences could be related to the host origin. According to our results it seems that these morphological differences formerly detected were better associated with the geographical origin of the isolates. The differences among *P. cinnamomi* isolates coming from the southwest of the Iberian Peninsula were also noticed when their cardinal temperatures of growth were studied. Isolates coming from the eastern part of southern Iberia showed a lower optimal growth temperature (26.9°C) and higher growth rates on Carrot Agar medium than the isolates coming from the west (optimum temperature 30.1°C). These results, together with the molecular data presented here, suggest that there are two different populations of *P. cinnamomi* causing root rot on *Quercus* species in southern Iberia, one coming from Portugal and now also colonizing the southwestern part of Spain, and a second Spanish population of *P. cinnamomi* infecting oak forests in the eastern part of the geographical area affected by oak decline. Nevertheless, there does not appear to be differences in pathogenicity between both populations when artificial inoculations were performed.

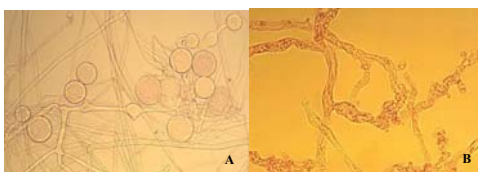


Figure 4. Hyphal patterns detected. A. Straight hyphae, with abundant spherical swellings, coming from western Spain and Portugal. B. Botryose-coralloid mycelium coming from isolates collected in the eastern part of southern Spain affected by oak decline.