

**A Tobacco necrosis virus D isolate from *Olea europaea* L.:
viral characterization and coat protein sequence analysis**

J. M. S. Cardoso¹, M. R. Félix^{1,2}, S. Oliveira^{1,3}, and M. I. E. Clara^{1,2}

¹I.C.A.M. Instituto de Ciências Agrárias Mediterrânicas, Évora, Portugal

²Departamento de Sanidade Animal e Vegetal, Universidade de Évora, Évora, Portugal

³Departamento de Biologia, Universidade de Évora, Évora, Portugal

Received May 12, 2003; accepted December 1, 2003

Published online February 16, 2004 © Springer-Verlag 2004

Summary. A virus isolated from *Olea europaea* L. grown in Portugal, was identified as a member of the species *Tobacco necrosis virus D* (TNV-D, genus *Necrovirus*, family *Tombusviridae*), based on the molecular and serological properties of the purified virus particles. The genomic region encoding the coat protein (CP) of this isolate (named GP isolate) was amplified by RT-PCR and the cDNA was cloned and sequenced. The CP gene encodes a predicted protein of 269 amino acids showing high identity (86.2%) to TNV-D coat protein sequence. Phylogenetic analysis based on necroviruses CP sequences, confirmed GP as a TNV-D isolate. The alignment with homologous TNV-D CP sequences revealed four conserved amino acids involved in Ca²⁺ binding as well as the plant virus icosahedral capsid protein “S” signature. Based on the determined nucleotide sequence, specific primers were designed and successfully used in RT-PCR for virus diagnosis in naturally infected olive trees.

Introduction

Tobacco necrosis virus D (TNV-D) is a small icosahedral virus ca. 28 nm in diameter with a single stranded positive-sense RNA genome approximately 3.8 kb long [6, 34]. It belongs to the genus *Necrovirus* [27] and is naturally transmitted by the soil inhabiting fungus *Olpidium brassicae* (Wor.) Dang [32].

Strains of TNV have been grouped into two distinct species, TNV-A and TNV-D [2, 34]. The complete nucleotide sequences of TNV-A and TNV-D were reported by Meulewaeter et al. (1990) and Coutts et al. (1991) respectively. A similar genome organization of these two species has been deduced from the determined

Note: Nucleotide sequence data reported is available in the GenBank database under the accession number AY263376.