

Genotyping and Phylogeny of Phaeomoniella Genus Associated with Grapevine Trunk Diseases in Algeria

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Abstract : Phaeomoniella is a fungus genus in the mitosporic ascomycota which includes Phaeomoniella chlamydospora specie associated with two declining diseases on grapevine (Vitis vinifera) namely Petri disease and esca. Recent studies have shown that several Phaeomoniella species also cause disease on many other woody crops, such as forest trees and woody ornamentals. Two new species, Phaeomoniella zymoides and Phaeomoniella pinifoliorum H.B. Lee, J.Y. Park, R.C. Summerbell et H.S. Jung, were isolated from the needle surface of Pinus densiflora Sieb. et Zucc. in Korea. The identification of species in Phaeomoniella genus can be a difficult task if based solely on morphological and cultural characters. In this respect, the application of molecular methods, particularly PCR-based techniques, may provide an important contribution. MSP-PCR (microsatellite primed-PCR) fingerprinting has proven useful in the molecular typing of fungal strains. The high discriminatory potential of this method is particularly useful when dealing with closely related or cryptic species. In the present study, the application of PCR fingerprinting was performed using the micro satellite primer M13 for the purpose of species identification and strain typing of 84 Phaeomoniella -like isolates collected from grapevines with typical symptoms of dieback. The bands produced by MSP-PCR profiles divided the strains into 3 clusters and 5 singletons with a reproducibility level of 80%. Representative isolates from each group and, when possible, isolates from Eutypa dieback and esca symptoms were selected for sequencing of the ITS region. The ITS sequences for the 16 isolates selected from the MSP-PCR profiles were combined and aligned with sequences of 18 isolates retrieved from GenBank, representing a selection of all known Phaeomoniella species. DNA sequences were compared with those available in GenBank using Neighbor-joining (NJ) and Maximum-parsimony (MP) analyses. The phylogenetic trees of the ITS region revealed that the Phaeomoniella isolates clustered with Phaeomoniella chlamydospora reference sequences with a bootstrap support of 100 %. The complexity of the pathosystems vine-trunk diseases shows clearly the need to identify unambiguously the fungal component in order to allow a better understanding of the etiology of these diseases and justify the establishment of control strategies against these fungal agents.

Keywords : Genotyping, MSP-PCR, ITS, phylogeny, trunk diseases

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