PCR-based techniques amended for detection of grapevine trunk pathogens from southern Brazil

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The emergence of PCR techniques has brought sensibility and reliability for plant disease diagnostics. Moreover, the molecular techniques sometimes appear as the only suitable alternative in cases of poor or puzzling symptoms, lack of pathogen structures and for surveys involving soil microorganisms or even samples with cross-contaminations. Indeed, pathogen identification in grapevine trunk diseases (GTD) can be considered a hard task as these disorders are frequently caused by complex fungal associations, encompassing *Phaeoacremonium* spp (Pan), *Phaeomoniella chlamydospora* (Pch), *Cylindrocarpon* spp or Botryosphaeriaceae species. In order to detect Brazilian GTD isolates, specific primers for Pan (Pch1/Pch2) and Pch (Pmo1f/Pmo2r) were employed. Also, universal primers for ITS region were used associated with Restriction Fragment Length Polymorphism (RFLP). For RFLP, the endonucleases *Cfo*I, *Hae*III and *Hinf*I were used. The preliminary tests involved DNA samples extracted from mycelial growth cultures and, subsequently tests were conducted with DNA samples extracted from symptomatic grapevine stems. The fungal isolates were confirmed by ITS amplifications which showed different restriction band patterns for each enzyme. Regarding PCR with specific primers, the isolates could be ensured through Tegli et al. (2000) method, with some modifications. But, parallel to specific detections, there were occurrences of unexpected false positives for some isolates yet. Optimizations of the actual methods are still required for warranting the more accurate detection band pattern possible for southern Brazil GTD isolates.

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