Title Molecular insights into fungicide resistance in sensitive and resistant *Penicillium digitatum* strains infecting citrus

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Abstract

The continuous use of chemical fungicides on citrus postharvest has led to the development of resistant strains against the fungicides in use, representing a considerable threat because the control systems are no longer effective. Evaluation of the sensitivity of 75 *Penicillium digitatum* strains to seven different fungicides revealed the presence of a significant number of TBZ- (84%) and IMZ-resistant (77%) strains, i.e., those fungicides most used in citrus postharvest. Molecular characterization of different *P. digitatum* genes involved in fungicide resistance was carried out. All *P. digitatum* genes were selected based on particular mechanisms of resistance due to fungicide target or mode of action. TBZ-resistance was characterized by a unique point mutation in the β-tubulin gene sequence corresponding to amino acid 200, confirming previous work on this subject. Moderate to low resistance to strobilurins did not reveal any mutation in the cytochrome *b* gene. DMI-resistance was evaluated by examining the *CYP51* gene and four different ABC transporters *PMR1*, *PMR3*, *PMR4* and *PMR5*. The *CYP51* gene did not exhibit any mutation relating to DMI-resistance, but a five tandem repeat sequence previously described was found in the *CYP51* promoter in 3 of the 75 isolates examined, whereas DMI-sensitive isolates and the other DMI-resistant isolates of *P. digitatum* had only one tandem repeat. Of all the ABC transporters studied, only *PMR1* and *PMR5* appear to be involved in fungicide resistance and several mutations were found in the promoter and the coding region for *PMR5* in resistant strains compared to sensitive ones. In all cases, the resistance mechanism was consistent in both orchard or packing-house isolates and no differences conferred by either origin or fungicide pressure were observed. Consequently, since different processes have been described that confer fungicide resistance to the same compounds, such as DMIs, the hypothesis that multiple mechanisms could be acting simultaneously gains strength.