Title Molecular mechanism and detection of azoxystrobin-resistant *Penicillium digitatum*

Author Zhang ZF, Chen GJ, Wang JY and Li HY

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Abstract

Green mold, caused by *Penicillium digitatum* (Pers.:Fr) Sacc, is the most destructive postharvest disease of citrus all over the citrus-producing regions. An important control method for this disease is fungicide treatment with imazalil (IMZ), thiabendazole (TBZ). However, the emergence of fungicide-resistant strains with the resulting decrease of failure of disease controls follows the successive and intensive use of these fungicides. Azoxystrobin, belonging to QoIs, is active against a wide range of important plant pathogens including *P. digitatum*. It has been registered for use in postharvest citrus recently in USA. However, the risk of fungicide resistance, the molecular mechanism of resistance, and the molecular detection of *P. digitatum* resistance against azoxystrobin remains unknown. To evaluate the risk of resistance of *P. digitatum* against azoxystrobin, 5 spontaneous mutants and 23 UV-induced mutants were screened in the azoxystrobin-amended PDA. All 28 mutants were stable for azoxystrobin-resestance indicated by the growth in azoxystrobin-amended PDA after 6-successive transfers 7d for each in azoxystrobin-free PDA. The minimum inhibitory concentrations (MICs) of these mutants were above 1000 μg/ml, 1000 times higher of that of parental strain. *In vitro* test of adaptability indicated by mycelial growth and conidial production demonstrated that 2 out of 5 spontaneous mutants and 3 out of 23 UV-induced mutants had and equal adaptability to that of parental strain, suggesting that there was a high risk that *P. digitatum* could produce azoxystrobin-resustant populations. To elucidate the molecular mechanism conferring *P. digitatum* resistance against azoxystrobin, partial sequences of the cytochrome b (*Pdcytb*) gene were amplified and sequenced from all azoxystrobin-resistant mutants as well as the parental strain. Additionally, their putative amino acid sequences were deduced. Alignment of the amino acid sequences indicated that the residual at 143 for parental strain is Glycine, while for all 28 mutants it was Alanine. This suggests that mutation of amino acid at 143 of *Pdcytb* from Gly to Ala was a response to the rise of resistance of *P. digitatum* against azoxystrobin. Based on the resistant molecular mechanism of *P. digitatum* against to azoxystrobin, 1 forward primer was screened and an allele specific-PCR (AS-PCR) system was optimized to detect azoxystrobin-resistant *P. digitatum*. By combining this AS-PCR method with the method of rapid extraction of fungal genomic DNA, we could determine the resistant scenario of a *P. digitatum* isolate within a half day.