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Review

Advances in understanding molecular mechanisms of fungicide resistance and molecular detection of resistant genotypes in phytopathogenic fungi

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Abstract

Although fungicide treatments are a key component in the integrated management of many plant diseases, the appearance of resistance has become an important factor in limiting the efficacy and useful lifetime of fungicides developed at increasingly higher costs. Extensive molecular studies have led to advances in our understanding of mechanisms of fungicide resistance and in developing effective, rapid methods for detection of resistant genotypes of pathogens. This paper reviews recent advances in our understanding of resistance mechanisms of phytopathogenic fungi to some major classes of fungicides (benzimidazoles, demethylation inhibitors [DMIs], Qo respiration inhibitors [QoIs], and dicarboximides [DCFs]) at a molecular level and developments in molecular detection of fungicide-resistant fungi.

Keywords: Benzimidazoles; Demethylation inhibitors (DMIs); Dicarboximides; Fungicide resistance; Qo respiration inhibitors (QoIs); Strobilurins

Contents

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1. Introduction

Fungicides are essential for maintaining healthy, reliable, and high-quality agricultural products. Prior to 1970, nearly all fungicides used for the control of plant pathogens were multi-site inhibitors acting as disease protectants. Despite their widespread use, resistance to these compounds was a rare event. However, since the introduction of the site-specific fungicides in the late 1960s, fungicide resistance in phytopathogenic fungi has become a major problem in crop protection (Brent, 1995).

Fungicide resistance is a stable, inheritable adjustment by a fungus to a fungicide, resulting in reduced sensitivity of the fungus to the fungicide. Resistance may result from single or multiple gene mutations. Resistant isolates typically arise from a very low natural rate of genetic mutation, and these isolates are less affected or not inhibited at all by a labeled application rate of a fungicide. Since the fungicide can effectively control sensitive isolates, resistant isolates may become dominant in pathogen populations under selection pressure of fungicide use over time, therefore disease control failures may eventually occur. The ecological fitness of fungicide-resistant fungal isolates will determine the persistence of resistant genotypes once they are selected. In many instances, since resistant isolates may have lower fitness than sensitive isolates, they cannot survive well in the absence of fungicide selection pressure. In this case, the frequencies of resistant isolates in pathogen populations will decrease once the fungicide applications cease. Alternatively, resistant isolates can be as fit as sensitive isolates and persist for a long time even without any use of the fungicides. This has exemplified by the resistance of several phytopathogenic fungi to benzimidazole or strobilurin fungicides (Koenraadt et al., 1992; Baraldi et al., 2003).

Fungicide resistance can be conferred by various mechanisms (Gisi et al., 2000; Gullino et al., 2000; Fluit et al., 2001; MgGrath, 2001), including: (I) an altered target site, which reduces the binding of the fungicide; (II) the synthesis of an alternative enzyme capable of substituting the target enzyme; (III) the overproduction of the fungicide target; (IV) an active efflux or reduced uptake of the fungicide; and (V) a metabolic breakdown of the fungicide. In addition, some unrecognized mechanisms could also be responsible for fungicide resistance. Here, we review (i) recent developments in elucidating resistance mechanisms of phytopathogenic fungi to benzimidazoles, demethylation inhibitors (DMIs) in sterol biosynthesis, Qo respiration inhibitors (QoIs), and dicarboximide fungicides (DCFs) and (ii) developments in molecular detection of fungicide resistance in plant fungal pathogens by providing examples from a few pathosystems from our research programs.

2. Molecular mechanisms of fungicide resistance

2.1. Benzimidazoles

Resistance to benzimidazole fungicides has been detected in many fungal species. In most cases, resistance was correlated with point mutations in the β-tubulin gene, which result in altered amino acid sequences at the benzimidazole-binding site. Results from numerous studies have shown changes at codon 6, 50, 167, 198, 200, and 240 in the β-tubulin gene could cause benzimidazole resistance in field isolates of pathogenic fungi (Table 1). The direct involvement of the mutations in conferring resistance to benzimidazoles has been confirmed by site-directed mutagenesis followed by gene replacement (Li et al., 1996). Biochemical confirmation was presented by Hollomon et al. (1998) who expressed β-tubulin as a fusion with a maltose binding protein (this fusion protein is soluble) and observed that benzimidazoles bound to the recombinant maltose binding protein fusion β-tubulin, and that this binding was reduced by the mutation at codon 198 from glutamic acid to glycine.

Mutations at different codons in the β-tubulin gene may result in different resistance levels to benzimidazoles. In *Monilinia fructicola*, the mutation at codon 6 and 198 led to a low and a high resistance level, respectively (Ma et al., 2003b). In *Venturia inaequalis*, the mutations at the codon 198 and 200 caused a medium and a high resistance level, respectively (Koenraadt et al., 1992). Additionally, different substitutions at the same codon may also cause different resistance levels. Mutants of *Tapesia yallundae* with changes at the codon 198 from Glu to Ala, Gly, Lys, and Gln had 50% effective concentration (EC_{50}) values to carbendazim ranging from 0.5 to more than 25 μg/ml (Albertini et al., 1999).

Benzimidazole-resistant isolates of fungi with mutations at the β-tubulin gene may have pleiotropic inhibitory effects on mycelial growth at high or low temperatures. A laboratory-induced benomyl-resistant mutant of *Fusarium moniliforme* with a mutation at codon 50 in the β-tubulin gene showed cold-temperature sensitivity (Yan and Dickman, 1996). Field benzimidazole low-resistant isolates of *M. fructicola* with a mutation at codon 6 also showed low temperature sensitivity (Ma et al., 2003b), however, field highly resistant isolates of *M. fructicola* with a mutation at codon 198 and low resistant isolates of *M. laxa* with a mutation at codon 240 were sensitive to high temperatures (Ma et al., 2003b; Ma et al., 2005). Such pleiotropic effects may interfere with the fitness of the resistant isolates and impose a selective disadvantage on these isolates under field conditions. Additionally, information on the temperature sensitivity of resistant isolates could directly impact the fungicide application