

Sequence variation in the *CYP51* gene of *Blumeria graminis* associated with resistance to sterol demethylase inhibiting fungicides

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Abstract

Resistance to sterol 14 α -demethylase inhibiting fungicides (DMIs) has been correlated with mutations in the *CYP51* gene, which encodes the target enzyme eburicol 14 α -demethylase. To test the hypothesis that variation in the *CYP51* gene explains variation for DMI sensitivity in barley and wheat powdery mildew species, this gene was sequenced from isolates of *Blumeria graminis* f.sp. *hordei* (*Bgh*) and f.sp. *tritici* (*Bgt*), respectively, which differed in their responses to DMIs in agricultural populations in the UK. Two single-nucleotide mutations in the *CYP51* gene, which resulted in the amino acid substitutions Y136F and K147Q, were detected. K147Q is a novel mutation present only in *Bgh* isolates expressing very high levels of resistance. Sequence analysis of the *CYP51* gene from the progeny of a cross between DMI-sensitive and resistant *Bgh* isolates showed that both mutations segregate with resistance, which is consistent with *CYP51* controlling a major portion of DMI resistance. However, genetic analysis of resistance to the DMI triadimenol indicates that mutation of the *CYP51* gene is not the only mechanism of resistance operating in *B. graminis*.

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1. Introduction

Ergosterol is the primary plasma membrane sterol in fungi, responsible for maintaining membrane fluidity and stability (Parks and Casey, 1995; Rodriguez et al., 1985). Cytochrome P450 14 α -demethylase (P45014DM) is a key enzyme in the ergosterol biosynthesis pathway (reviewed by Yoshida, 1993). In filamentous fungi, P45014DM catalyses the 14 α -demethylation of eburicol (24 methylene 24, 25 dihydrolanosterol), and is encoded by the *CYP51* gene (Aoyama et al., 1996). In powdery mildew fungi, *CYP51* is a single-copy gene consisting of three exons interrupted by two short introns (Délye et al., 1997b, 1998).

P45014DM is the enzymatic target for a major group of antifungal agents, the demethylase inhibitors (DMIs). The site-specific mode of action and intensive use of these fungicides has led to the development of resistance in many organisms of agricultural and medical importance (Brown et al., 1992; Délye et al., 1997a,b; Sanglard et al., 1998). The genetic basis of resistance to DMIs is complex (De Waard, 1996; De Waard et al., 1995; Van Tuyl, 1977) and may be determined by three patterns of inheritance: (1) a single resistance locus with several alleles (monogenic control), e.g., resistance of *Pyrenophora teres* to triadimenol (Peever and Milgroom, 1992); (2) numerous loci, each contributing a small degree of resistance (polygenic control), e.g., in *Nectria haematococca* (Kalamarakis et al., 1991); or (3) a few resistance genes, each of which controls a distinct level of resistance (oligogenic control), e.g., in *Blumeria graminis* (syn. *Erysiphe graminis*) f.sp. *hordei* (*Bgh*) (Blatter et al., 1998; Brown et al., 1992).

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