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

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Received 3 December 2004; accepted 12 April 2005

Available online 23 May 2005

Abstract

Resistance to sterol 14α-demethylase inhibiting fungicides (DMIs) has been correlated with mutations in the CYP51 gene, which encodes the target enzyme eburicol 14α-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 triadime-nol indicates that mutation of the CYP51 gene is not the only mechanism of resistance operating in B. graminis.

Keywords: Blumeria graminis; Erysiphe graminis; Demethylase inhibiting fungicides; Fungicide resistance; CYP51 gene; Cytochrome P450 14α-demethylase; Triadimenol

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 14α-demethylase (P45014DM) is a key enzyme in the ergosterol biosynthesis pathway (reviewed by Yoshida, 1993). In filamentous fungi, P45014DM catalyses the 14α-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 tria-dimenol (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).