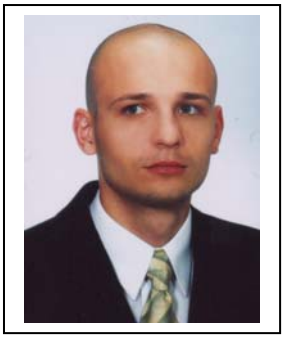

Molecular diagnostics of fungicide resistance to azoles

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Abstract

The increasing resistance of fungal pathogens to fungicides has become an important factor limiting their the effectiveness and usefulness in agricultural applications. Selection pressure resulting from regular, long-term fungicide application, leads to the emergence and spread of new strains with increased resistance to new groups of compounds. At the moment there are several well-known molecular mechanisms directly reducing the efficacy of fungicides. Among the best known is the spread of mutations in the sequences encoding target proteins, overexpression of genes encoding the target proteins and the adaptation of transport proteins to increase efflux of substances with antifungal activity. With the available information on the mechanisms of fungal resistance to fungicide substances, it is now possible to design, test and deploy targeted molecular diagnostics procedures in order to allow rapid analysis of samples sent by farmers and industry. As part of the design process, we compiled an internal database containing available nucleotide and amino acid ABC and MFS transporters likely to participate in fungicide efflux, as well as site-of-action genes (e.g. sterol demethylase). In particular, an ABC transporter gene from *Fusarium graminearum* (FGSG_02865) as well as its homologs from other plant pathogenic fungi appear to provide one of the best candidates for wide specificity MDR resistance gene capable of exporting fungicides. FGSG_02865 is closely related to established resistance factors (FLR1) and its orthologs are present in a number of cereal pathogens. As part of the *in vitro* testing, we conducted bioassays on fungicide sensitive (e.g. *Fusarium verticilioides*) and resistant (*Alternaria alternata*) strains in order to measure 50% mycelia growth inhibition (EC_{50}).On the basis of compiled sequences sets of markers detecting polymorphisms of these genes were designed, and a representative sample sequenced in order to detect variable regions associated with the function.