MOLECULAR ASPECTS OF FUNGICIDE RESISTANCE´

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Molecular detection of fungicide resistance is a rising field in agrochemical research, as the support of current fungicides becomes more and more important due to increasing reports of resistance or at least insufficient performance of fungicide applications. Thus, the management of fungicide resistance evolution in plant pathogens is an important issue not only to the industry, but also to officials, advisers, and to farmers. The recent progress in molecular technologies in elucidating the genetic backgrounds to phenotypes and the possibilities of detection of genetic changes in single individuals or fungal populations allows today a comparably fast development and application of test systems to quantitative measure fungicide resistance in field populations of plant pathogens.

Resistance is naturally occurring when fungal pathogens are controlled by fungicides, via selection of genotypes already present at very low frequency in field populations. The more a fungicide class is applied (number of application and area treated) the higher the probability of selecting resistance. Resistance is caused by different mechanisms such as target site mutations, overexpression of target enzymes, metabolism, or efflux. All these mechanisms are controlled genetically by genes in the nucleus, mitochondria, or extra chromosomal elements.

Fungicide resistance is usually measured by bioassays, carried out on artificial media (*in vitro*), on detached leaves, leaf discs, or in planta (*in vivo*), and either with single individuals (single spore cultures) or with mixed samples. The test design needs to be adapted to the kind of resistance present in the investigated species to the particular fungicide, such as disruptive or continues resistance, and the results should be compared to reference isolates with known sensitivity. Molecular techniques can complement the bioassay results by quantitative information (frequency of resistance) in bulked samples in a high through-put, short response time, and high specificity. However, in order to apply a molecular technic the mechanism of resistance (mutation) needs to be known in a particular pathogen and sufficiently correlated to the phenotype, preferably with both, lab,

greenhouse, and field observations. Known mechanisms of resistance include, e.g. QoI (*cyt* b), DMI/SBI (*cyp*51), or SDHI (*sdh* b, c, d).

For the detection of alterations in genes leading to fungicide resistance different technologies are available, for example diverse PCR methods (real time, allele specific Q-PCR, Scorpion PCR) and sequencing (Sanger sequencing, pyrosequencing). Especially sequencing technologies have made enormous progress in recent years, allowing deep sequencing of bulked DNA samples to detect all changes in different gene fragments and, consequently, the detection of mutations at a high sensitivity. These technologies are of special interest when field samples should be tested for several genetic changes, leading to fungicide resistance. Most of todays´ technologies used focus on a particular resistance based on one or few genetic changes leading to resistance. However, it becomes more and more obvious that more than one mutation (in one gene or several genes) is responsible for resistant phenotypes, e.g. for DMI or SDHI fungicides.

This study shows examples of molecular resistance research and monitoring with DMIs, QoIs, SDHIs, and important plant pathogens, including major soybean diseases, and discuss consequences of the findings for the use of major fungicide classes in disease management strategies.