Metabolomics in Pesticide R&D: Probing Fungal Resistance Mechanisms to Benzimidazole Fungicides Performing ^{1}H NMR Metabolomics

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• *Introduction*: The agrochemical industry represents the backbone of the agricultural sector world-wide. The improvement of food production, increasing concerns over food and environmental safety, and the emergence of resistant to pesticides pests and pathogens, are among the major challenges that the sector is facing. The latter results in heavy yield losses due to the decreased efficacy of the applied crop protection agents. Within this context, the understanding of changes at the metabolome level and their correlation to the observed resistance is important for combating this issue, and represents a newly emerged and promising field in pesticide R&D. Here, we have developed a robust ^{1}H NMR metabolomics-based protocol for the discovery and study of *Fusarium graminearum* resistance mechanism(s) to benzimidazole fungicides.

• *Methods*: The sensitive strain of *Fusarium graminearum* CBS 110261 (WT) and four carbendazim-resistant isolates; two bearing point mutations in target β_2-tubulin gene (FG-3 and FG-6) and two of unknown resistance (FG-1 and FG-2), which were obtained from the WT by mutagenesis, were used. The stains were grown for 10 days on cellophane on PDA amended or not with 2 μg mL^{-1} carbendazim (MBC) (sub-lethal concentration for WT) at 25^{o}C. Mycelia were harvested, lyophilized, and extracted with D_2O. Extracts were analyzed using a Bruker 500MHz analyzer and data were processed in order to be subjected to multivariate analysis. Orthogonal projections to latent structures-discriminate analysis (OPLS-DA) was applied for the detection of trends and biomarkers and the correlation between metabolome and genome.

• *Preliminary Results*: Results of OPLS-DA revealed that mutation(s) resulted in distinct metabolic profiles of the strains being analyzed, which is indicative of the impact that they have on the fungal metabolism and its regulation. In controls, the metabolic profiles of the strain FG-6 were lying closer to the WT compared to the rest strains indicating that the mutation(s) of this strain had a small impact on its metabolism. Interestingly, the strains FG-1 and FG-3 shared similar NMR profiles, which plausibly indicates the presence of analogous mutation(s) leading to the observed changes in the metabolome level. Treatment with 2 μg mL^{-1} MBC slightly altered the clustering observed in the controls, causing a shift of FG-1 close to FG-6. This observation in combination with the results of the analysis of controls clearly indicates that the mutation(s) for FG-1 and FG-2 have occurred in different biochemical site(s). Amino acids, carboxylic acids, and

carbohydrates involved in various biosynthetic pathways were identified among the biomarkers that drive the observed discriminations. 2D NMR experiments are underway for further validation of the observed biomarkers and the biosynthetic pathways involved. The present work represents a proof of concept of the applicability of NMR metabolomics for the robust and high-throughput screening of mutations in fungi leading to resistance to fungicides, and the study of their biochemical basis. Results can be exploited in genetic engineering and crop protection for combating resistance against fungicides.

- *Novel Aspects*: Original metabolomics approach for the study of fungal resistance to fungicides based on ^{1}H NMR spectroscopy.