A secondary metabolite is involved in recognition of the rice blast fungus

J. Collemare (1), H. U. Böhnert (1), M. Pianfetti, I. Fudal (1), D. Tharreau (2), M.-H. Lebrun (1)

(1) UMR2847 CNRS-Bayer Cropscience, Physiologie des plantes et des champignons lors de l'infection, Bayer Cropscience, 14-20 rue P Baizet, 69263 Lyon Cedex 09
(2) CIRAD - UMR BGPI, Campus International de Baillarguet, TA41 / K, 34398 Montpellier Cedex 05

Recognition of the blast fungus *Magnaporthe grisea* by resistant rice cultivars frequently involves interaction between a fungal avirulence gene and a specific rice resistance gene, as observed for the avirulence gene *ACE1* that controls the production of a signal specifically recognized by rice cultivars carrying the resistance gene *Pi33*. *ACE1* encodes for a polyketide synthase fused to a non-ribosomal peptide synthetase, an enzyme involved in the biosynthesis of a microbial secondary metabolite. *ACE1* is specifically expressed in mature appressoria during penetration of the fungus into host plant leaves. The protein Ace1 is only detected in the cytoplasm of appressoria and does not seems to move into infectious hyphae produced inside host plant tissues. Deletion analysis of *ACE1* promoter has led to the identification of a 58-bp region required for its appressorium-specific transcription. This region contains putative binding sites for a *Ste12* transcription factor and a fungal binuclear zinc finger transcription factors. Site-directed mutagenesis of these putative binding sites will be used to assess their role in the control of *ACE1* expression. *Ace1-ks0*, a non-functional *ACE1* allele obtained by site-directed mutagenesis of an essential amino acid of the polyketide synthase KS domain, is unable to confer avirulence. This result suggests that the avirulence signal recognized by *Pi33* resistant rice is not the Ace1 protein, but is likely the secondary metabolite synthesized by Ace1. In order to characterize this metabolite, we have performed a metabolic profiling of *M. grisea* appressoria by LC-MS-MS, using appressoria from either virulent or avirulent isolates differentiated on onion epidermis. Fungal metabolites were detected but none was specific of avirulent isolate. Close to *ACE1,* we identified 15 genes predicted to encode enzymes involved in secondary metabolism, including two enoyl-reductases and a binuclear zinc-finger transcription factor. All these 15 genes are located within a region of 70-kb and display the same penetration-specific expression pattern as *ACE1*, defining a cluster of co-expressed genes. The inactivation of these genes in an avirulent isolate is underway to evaluate their role in the biosynthesis of the avirulence signal recognized by *Pi33* resistant rice cultivars.