CYTOLOGICAL AND MOLECULAR ANALYSIS OF THE BARLEY-LEAF STRIPE INTERACTION


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Leaf stripe of barley (Hordeum vulgare L.) is caused by the seed borne fungus Pyrenophora graminea. We investigated at the microscopic and molecular levels the reaction of barley embryos to leaf stripe infection. In the resistant genotype NIL3876-Rdg2a, abortion of fungal growth occurred at the level of a embryo position defined by the scutellar node, while in the near isogenic line (NIL) Mirco-rdg2a no fungal growth arrest was observed and the pathogen colonized the embryo structures. A quantitative difference between resistant and susceptible NILs was observed for the level of tissues reacting with autofluorescence under UV light excitation. This response correlated with a positive reaction to toluidine blue staining, indicating accumulation of phenolic compounds in the resistant line. Suppression subtractive hybridisation (SSH) and cDNA-AFLP analyses were used to identify genes differentially expressed in barley embryos in response to leaf stripe infection and low abundant sequences of fungal origin. SSH led to the isolation of genes involved in generation of reactive oxygen species and detoxification mechanisms. Microarrays containing the entire set of cDNA-AFLP fragments and 100 genes selected from publicly available databases were used to study gene expression changes at 7 and 14 days after inoculation (dai) in the resistant and susceptible NILs. Gene clusters grouping 238 significantly modulated genes were identified. Genes that responded to leaf stripe infection included pathogenesis-related (PR) genes, genes involved in oxidative stress generation and protection, jasmonate (JA) synthesis and several other different pathways such as vesicle trafficking, protein ubiquitination and signal transduction. Interestingly, we observed induction of several genes involved in secondary metabolic pathways and cell wall reinforcement, supporting microscopic observations and suggesting that this may represent a major defense response of barley embryos infected with leaf stripe. In addition, during the cDNA-AFLP analysis we identified six differentially expressed fungal genes that are likely involved in pathogenicity.