Transgenic expression of Medicago truncatula PR10 and PR5 promoters in alfalfa shows pathogen-induced up-regulation of transgene expression

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Genetic modification of alfalfa to introduce novel traits requires promoters for controlling gene expression. Promoters that are constitutively activated for expression of genes that enhance disease resistance pose a great energy load on the plant and exert a strong selective pressure on the pathogens. Promoters that are induced upon pathogen invasion are needed for engineering plants with disease resistance. Medicago truncatula promoter regions of pathogenesis-related (PR) genes, PR5 and PR10, were identified as being highly up-regulated during the initial stages of infection by root and foliar pathogens. These promoters were PCR amplified and cloned into plant transformation vectors ahead of the β-glucuronidase (gus) gene. Agrobacterium mediated transformation was used to create transgenic lines of alfalfa (cultivar Regen SY27x). The transgenic plants were stained for GUS activity. In uninoculated plants, GUS activity was primarily seen in the root vascular tissues. No activity was observed in uninoculated leaves. With fungal pathogen infection, staining was greatly enhanced and allowed for stain visualized in the leaves. Quantitative PCR assays were done to quantify pathogen-induced GUS expression, as well as expression of PR5 and PR10 in infected leaves. RNA was extracted from symptomatic infected leaves after inoculation and converted to cDNA. Using specific primers, transcript accumulation was compared between cDNA from mock inoculated and inoculated plant tissue. In plants with the PR10:GUS or PR5:GUS constructs, GUS transcripts accumulated 41- to 378-fold over the mock inoculated plants at 7 days after inoculation with Phoma medicaginis, depending on the plant line. GUS transcripts were also strongly up-regulated in response to Colletotrichum trifolii and Pseudomonas syringae pv. syringae. Consistently, the PR10 promoter had greater fold amplifications and greater activity than the PR5 promoter. In response to P. medicaginis, transcripts of the PR10 gene were up-regulated 31- to 221-fold at 7 days after inoculation and transcripts of the PR5 gene were up-regulated 44- to 60-fold. These experiments show that the M. truncatula PR10 promoter is functional in alfalfa for expression of transgenes and up-regulates genes after infection by a range of alfalfa pathogens.