

Constitutive expression of an inducible β -1,3-glucanase in alfalfa reduces disease severity caused by the oomycete pathogen *Phytophthora megasperma* f. sp. *medicaginis*, but does not reduce disease severity of chitin-containing fungi

Sameer A. Masoud, Qun Zhu, Chris Lamb and Richard A. Dixon

cDNA sequences coding for an acidic glucanase (Aglul) that is expressed in elicited alfalfa cell suspension cultures, and a rice basic chitinase (RCH10) that is induced by elicitor and wounding, were placed into constitutive expression cassettes under control of the cauliflower mosaic virus 35S promoter or 35S enhancer sequences, and introduced in alfalfa plants of the regenerable cultivar Regen SY by *Agrobacterium*-mediated transformation. Southern and northern blot analysis confirmed stable incorporation and transcription, respectively, of the chimaeric genes in the transgenic plants. Active rice chitinase was expressed in alfalfa leaves, and leaves of plants transformed with the Aglul sequence exhibited increased glucanase activity and the appearance of an additional glucanase band on activity gels. A glucanase of similar native electrophoretic mobility was constitutively present in root extracts of non-transformed alfalfa plants, and was induced in pathogen-infected leaves, presumably reflecting the expression pattern of the endogenous Aglul gene. Thus, expression of the chimaeric Aglul transgene increased the amount, and broadened the tissue-type constitutive expression, of the Aglul protein compared to control plants. Transgenic alfalfa plants containing a binary vector with both chimaeric genes in tandem expressed each gene to a much lesser extent than transgenic plants containing a single chimaeric gene. Expression of RCH10 in transgenic alfalfa did not appear to affect negatively the *Rhizobium*/alfalfa interaction. Analysis of primary transformants for response to several fungal pathogens of alfalfa indicated statistically significant symptom reduction only in the case of *Phytophthora megasperma* f. sp. *medicaginis* (Pmm), and only in plants overexpressing Aglul. Resistance against Pmm segregated with glucanase expression in a cross between transgenic Regen SY and the commercial alfalfa cultivar Apollo.