

## A Transgenic Approach to Alfalfa Crown Rot Control

Andrew Sathoff, Siva Velivelli, Dilip Shah, Deborah Samac

Alfalfa crown rot, caused by a complex of soil microbes, is one of the most important and pervasive alfalfa diseases across the United States. Crown rot occurs to some extent in every alfalfa stand that is over one year old and is a significant cause of stand decline resulting in yield loss. Breeding for resistance has been successfully implemented to manage several alfalfa diseases, but the necessary broad-spectrum resistance has not been identified for developing crown rot resistant cultivars. Inadequate cultural and chemical management practices for this disease complex severely limits alfalfa production. Hence, there is an immediate need for the development of innovative methods to manage crown rot. We took a transgenic approach to alfalfa crown rot control. Candidate transgenes, antimicrobial peptides called plant defensins, were evaluated for activity against the crown rot pathogens. Defensins have broad antimicrobial activity and constitute one of the first lines of defense against pathogen invasion in plants, vertebrates, invertebrates, and fungi. The amount of defensin needed to inhibit growth of crown rot pathogen strains by 50% ( $IC_{50}$ ) was calculated. A bi-domain defensin from *Medicago truncatula* (MtDef5) displayed high activity against both bacterial and fungal crown rot pathogens *in vitro*. MtDef5 had  $IC_{50}$  values against *Pseudomonas syringae* pv. *syringae* of 0.198  $\mu$ M and 1.50  $\mu$ M against *Phoma medicaginis*. *Agrobacterium*-mediated transformation was used to create transgenic lines of alfalfa (cultivar Regen SY27x) constitutively expressing MtDef5. Transgene expression was confirmed by qRT-PCR and by Western blots using the polyclonal anti-MtDef5 antibody. Disease bioassays demonstrated increased resistance in the transgenic lines expressing MtDef5 against fungal crown rot pathogens, especially against *P. medicaginis*. Transgenic lines with greater levels of MtDef5 expression corresponded to increased fungal resistance. Transgenic expression of defensins could be utilized to implement an eco-friendly, protein-based strategy that could provide alfalfa with enhanced resistance against crown rot and the correlative increases in alfalfa yield.