Expression of a fungal medicarpin detoxification gene in alfalfa increases susceptibility to *Phoma medicaginis*.

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Upon pathogen infection, the biosynthesis of antifungal phytoalexins such as medicarpin is induced in alfalfa and other legumes, and this is believed by many to play an essential role in pathogen resistance. To demonstrate the significance of medicarpin in disease resistance, we reduced medicarpin accumulation in alfalfa by expressing the Makl gene from the fungal pathogen *Nectria haematococca*. Makl encodes a monooxygenase, which hydroxylates the pterocarpan phytoalexins medicarpin and maackiain, rendering them less toxic to pathogens (Figure 1). Previous studies in tobacco indicated that the functionally expressed Makl gene product can metabolize infiltrated medicarpin into the less toxic compound la-hydroxymedicarpin in transgenic plant cells (1).

The Mak1 cDNA, regulated by a CaMV-35S dual enhancer promoter, was introduced into alfalfa, and the transformants were screened for high Mak1 mRNA levels. Medicarpin synthesis was induced in transgenic alfalfa leaves by infection with fungal pathogen *Phoma medicaginis*. Endogenous medicarpin was readily metabolized into 1a-hydroxymedicarpin derivatives, which indicated high levels of Mak1 enzyme activity. In wound-inoculation experiments (2), transgenic leaves allowed increased hyphal growth and increased development of pycnidia relative to non-transformed leaves, which indicated enhanced disease susceptibility. Correlation between reduced medicarpin levels and enhanced disease symptoms in transgenic leaves provides direct evidence for the role of medicarpin in disease resistance in alfalfa.





(1) Mundodi, S.R., Watson, B.S., Lopez-Meyer, M. and Paiva, N.L. (2001) Functional expression and subcellular localization of the *Nectria haematococca* Mak1 phytoalexin detoxification enzyme in transgenic tobacco. Plant Molecular Biology **46**: 421-432.

(2) Hipskind, J.D. and Paiva, N.L. (2000) Constitutive accumulation of a resveratrol glucoside in transgenic alfalfa increases resistance to *Phoma* medicaginis. Molecular Plant-Microbe Interactions **13**: 551-62.