

IN VITRO AND IN PLANTA ANTIFUNGAL AND ANTIVIRAL ACTIVITIES OF THE RIBOSOME INACTIVATING PROTEIN I FROM PHYTOLACCA HETEROTEPALA

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Plant Ribosome Inactivating Proteins (RIP) are potentially useful for plant defence considering their broad spectrum antiviral and antimicrobial activities. However, their use in plant biotechnology is limited by their cytotoxic activity.

A new gene (PhRIP I) coding for a RIP isolated from leaves of *Phytolacca heterotepala*, was expressed in tobacco under the control of the wound-inducible promoter, as the purified protein isolated from *P. heterotepala* showed direct antifungal and antiviral activities. These properties were conserved in total protein extracts from tobacco leaves transiently expressing the PhRIP I gene. Furthermore, Agro-infiltrated leaves show some phenotypic abnormalities. The use of an inducible promoter for plant transformation allowed the regeneration of phenotypically normal transgenic lines that expressed the RIP transgene upon wounding and pathogen attack and showed a significant reduction of leaf damage after infection with *Alternaria alternata* and *Botrytis cinerea*. Furthermore, when induced, the PhRIP I protein conferred to the transgenic plants resistance against PVX. This work demonstrates that use of a wound-inducible promoter is useful to limit the accumulation of antimicrobial phytotoxic proteins only in infected areas and that the controlled expression of the PhRIP I gene can be very effective to control virus and fungal pathogens with different phytopathogenic actions.