Agrobacterium-mediated Transformation of Chitinase Gene from the Actinorhizal Tree, *Casuarina equisetifolia* in *Nicotiana tabacum*

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Abstract

Genetic transformation of plants offers the possibility of testing hypotheses about the function of individual genes as well as the exploitation of transgenes for targeted trait improvement. The cloning of a full-length class I chitinase (*CeChi1*) the *Casuarina equisetifolia* was reported earlier. In the present study, tobacco was used as a model system to functionally evaluate the potential of *CeChi1* driven by *Ubi* promoter. The pUH-*CeChi1* construct was introduced into tobacco by *Agrobacterium* – mediated transformation and the putative transformants were confirmed for stable gene integration, transcript expression and recombinant protein production using PCR, RT-qPCR, antifungal assays and *in planta* analysis. The expression of chitinase gene in putative transgenic line T2 was found to be 3.8-fold greater than the untransformed tobacco. The result of RT-qPCR also denoted the correct and stable expression of chimeric *CeChi1* in transgenic tobacco plants without silencing phenomena and growth retardation. The *in vitro* antifungal bioassay using the total proteins from transformed plantlets revealed the lysis of hyphal tips of targeted pathogenic fungi *viz. Trichosporium vesiculosum*, *Fusarium oxysporum* and *Rhizoctonia solani*, characteristic of class I chitinase enzyme. The *in planta* bioassay of transformed tobacco revealed reduced symptoms when compared to untransformed tobacco plants which showed marked diseased symptoms. The study reveals that the class I chitinase isolated from *C. equisetifolia* can act as a new gene resource in future transformation programs for incorporating disease tolerance caused by fungal pathogens.