Expression of a cysteine proteinase inhibitor (oryzacystatin-I) in transgenic tobacco plants

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Expression of cysteine proteinase inhibitors (cystatins) in tobacco or other plants has the potential for improving resistance against pathogens and insects that possess cysteine proteinases. A chimeric gene containing a cDNA clone of rice cystatin (oryzacystatin-I; OC-I), the cauliflower mosaic virus 35S promoter, and the nopaline synthase 3 region was introduced into tobacco plants by Agrobacterium tumefaciens. The presence of the chimeric gene in transgenic plants was detected by a polymerase chain reaction-amplified assay, and transcriptional activity was shown by RNA blot analysis. Heated extracts from transgenic tobacco plants, as well as from progeny which were obtained by selfing a primary transformant, contained protein bands that corresponded in molecular mass to OC-I and reacted with antibodies raised against rOC, a recombinant OC-I protein produced by Escherichia coli. Similar bands were absent in extracts from untransformed control plants. OC-I levels reached 0.5% and 0.6% of the total soluble proteins in leaves and roots, respectively, of some progeny. On a fresh weight basis, the OC-I content was higher in leaves (50 microg/g) than in roots (30microg/g). OC-I was partially purified from protein extracts of rice seeds and from transgenic tobacco leaves by affinity to anti-rOC antibodies. OC-I from both sources was active against papain.