Expression of a corn bifunctional inhibitor of serine proteinases and insect α-amylases in transgenic tobacco plants

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Abstract

Corn seed endosperm contains a putative defense protein (14K-CI) that inhibits both trypsin-like serine proteinases and insect α-amylases. A cDNA clone encoding 14K-CI under control of the cauliflower mosaic virus 35S promoter and the nopaline synthase polyadenylation region was introduced into tobacco plants by Agrobacterium-mediated transformation. The presence and expression of the chimeric gene in regenerated (R0) and progeny (R1) plants was confirmed by Southern, polymerase chain reaction, and Northern analyses. Protein extracts of selected transformed plants, but not control plants, reacted positively to corn seed 14K-CI antiserum. Comparison of 14K-CI mobilities from transgenic leaves and corn seeds after denaturing polyacrylamide gel electrophoresis indicated that recognition and cleavage of the 14K-CI signal peptide sequence occurred in tobacco leaves. Immunological assays showed that the inhibitor was expressed in amounts up to 0.05% of the total protein in young leaves of R1 plants. Lesser accumulation was generally detected in older leaves. Protein extracts from transgenic plants were more inhibitory than were control plant extracts to bovine trypsin activity. Four tobacco plants with a gene lacking the 14K-CI signal peptide region accumulated 3–5-fold less inhibitor than did the highest-expressing plant with the full cDNA clone. Use of a double 35S promoter did not enhance 14K-CI accumulation. Post-transcriptional events appear to be a major factor in the low accumulation of 14K-CI in tobacco.