Analysis of Plant Self-defense Mechanism in Tobacco Mosaic Virus Infected Tobacco Leaves

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Abstract

Identification of functions of gene by overexpression or suppression of particular gene is a common procedure in modern molecular biology. However, the expression level of foreign gene in a expression vector used in common was sometimes insufficient in plants. To solve the problem, a series of chimeric promoters for high-level expression of foreign genes in plants were constructed as fusion genes with ß-glucuronidase (*GUS*) gene and the activity of these promoters was assayed in tobacco and rice. As parts of these promoters, the CaMV *35S* core promoter, three different 5'-upstream sequences of the *35S* promoter, the first intron of a gene for phaseolin, and a 5'-untranslated sequence of tobacco mosaic virus (*Ω* sequence) were used in various combinations. Some chimeric promoters showed the levels of GUS activity 20- and 70-fold higher than those obtained with the *35S* promoter in pBI221 in tobacco and rice, respectively.

When the representative promoter El2Ω was introduced into tobacco with a gene for luciferase, the autofluorescence of the detached leaves was great and was easily detectable by the naked eye in a dark room. However, latter generation of the transgenic tobacco plants introduced with El2Ω::luc chimeric gene frequently exhibit post-transcriptional gene silencing (PTGS) of the *luc* transgene. As PTGS can suppress both transgene and endogenous gene with considerable homology to the transgene, the promoter may useful not only for overexpression but also for suppression of a particular gene by single transformation of the construct which overdrive the gene by the promoter. Indeed, ACC oxidase (*ACO*) gene could be overexpressed and suppressed by the introduction of the improved *promoter::ACO* fusion gene.

In plants, programmed cell death (PCD)-like events have been reported, although little is known of the mechanism at the molecular level. To access whether cell death

mechanism is conserved between animal and plant, Bcl-xL and Ced-9, suppressor of PCD in human and nematode, were overproduced in tobacco plants using the strong promoter El2Ω. In Bcl-xL and Ced-9 expressers, cell death induced by UV-B irradiation, paraquat treatment and the N gene-dependent hypersensitive reaction (HR) upon tobacco mosaic virus infection was suppressed depending on the amount of foreign protein. Caspase like activity, which is known as cell death-executing protease, was transiently induced upon HR and the induction was suppressed in Bcl-xL expresser. Furthermore, the expression of *P35*, which suppress animal cell death by inhibiting caspase activity, also suppressed plant cell death induced by UV and paraquat treatment. These results may indicate that signaling pathways of cell death in plant and animal kingdoms are conserved in several part.