354(1pG09)

ISOLATION AND CHARACTERIZATION OF AN *Arabidopsis* STAY-GREEN MUTANT

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Leaf senescence is a genetically controlled process, but the mechanism controlling the process is poorly understood. Genetic approach is a very powerful tool to dissect this sort of complicated processes. We screened *Arabidopsis* T-DNA tag lines, and isolated a mutant line, T47, which had stay-green cotyledons after green seedlings were kept in the dark for a prolonged period. Genetic analysis revealed T47 is a monogenic recessive mutation and the mutation is cosegregated with the inserted T-DNA. In the T47 mutant, leaf senescence progresses more slowly than wild type plants when plants were grown under natural light conditions. These observations suggested that the gene at the T47 locus plays a fundamental role in both age-dependent and dark-induced leaf senescence. The seedling of T47 mutant showed normal response to exogenous ethylene, indicating that the stay-green phenotype of the T47 mutant is not related to the action of ethylene.

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AtDAD-1/-2 EXPRESSION LEVELS AND CELL DEATH DURING SENESCENSE OF *ARABIDOPSIS* COTYLEDONS

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In order to understand the function of the two defender against apoptotic cell death (*DAD*) genes in *Arabidopsis thaliana*, we have examined the antiapoptotic activity of *AtDAD-1/-2*, and the relationship between dead cell number and gene expression level in senescing cotyledons of wt and *etr1-1* mutant. *AtDAD-1* suppressed more efficiently DNA fragmentation in mutant tsBN7 hamster cells undergoing apoptosis than *AtDAD-2*. The number of dead cells stained by trypan-blue in wt was more than in *etr1-1*. In wt, *AtDAD-1/-2* mRNA levels increased at the early stage of senescence but started to decrease at the late stage where the levels in *etr1-1* did not. Together with these results, yellow cotyledons of T1 young seedlings overexpressing antisense *AtDAD-1* suggest that *AtDAD-1/-2* are involved suppressively in the cell death process.

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PLANT BAX INHIBITOR-1 (BI-1) GENE INHIBITS BAX -INDUCED CELL DEATH IN YEAST

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The mammalian Bax Inhibitor-1 (BI-1) gene was isolated as a novel regulator of cell death using yeast system (Xu and Reed, 1998). During searching of EST clones, we identified one cDNA clone showed similarity to the product of the mammalian BI-1 gene. The partial EST clone was used for a probe to screen libraries for full-length cDNAs containing the entire coding region. After successive screening, we isolated plant homologue cDNAs of BI-1 from *Arabidopsis* and rice.

The amino acid sequences of the predicted proteins were well conserved in both animal and plant (45% in aa), and contained six or seven membrane-spanning segments. The plant BI-1 genes were able to suppress the cell death induced by mammalian Bax in yeast, suggesting functional conservation of BI-1 homologues in the plant kingdom.

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Identification of the suppressors against Bax-induced cell death with *Arabidopsis* cDNA library by functional screening in yeast

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The mammalian proapoptotic protein Bax confers a lethal phenotype when expressed in yeast. We have identified dozens of inhibitors of Bax-induced cell death with *Arabidopsis* cDNA library by exploiting this phenotype. These suppressors inhibited the Bax-induced cell death to a different extent. We found that some suppressors didn't interfere with the production of the Bax protein in yeast, while others did, as determined by immunoblot analysis.

Among these suppressors, one was the plant homologue of mammalian Bax Inhibitor-1 (BI-1)--AtBI-1; some were inter/intra-membrane proteins; others included some stress-related proteins. Interestingly, AtEBP (Ethylene-responsive element-binding protein) turned out to be most abundant. The investigation of the biological functions of AtEBP and other suppressors are ongoing.