Injury/Gravity

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Difference in expression patterns of ethylene biosynthesis enzymes in ozone-treated and sulfur dioxide-treated tomato leaves

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Ozone (O_3) and sulfur dioxide (SO_2) are typical air pollutants. As components of acid rain and photochemical oxidants they cause visible damage of leaves of many plant species. It has been reported that O_3 and SO_2 elevate a rate of ethylene evolution from leaves and that the rate of ethylene evolution is related to extent of leaf injury. However, it is not clear whether SO_2 and O_3 induce ethylene evolution by the same mechanism. Complementary DNAs encoding 3 isozymes (*LE-ACS1A*, *LE-ACS2* and *LE-ACS6*) of ACC synthase and that of an ACC oxidase (*LE-ACO1*) were isolated from both O_3-treated and SO_2-treated tomato leaves. Levels of mRNA for all of these enzymes increased in leaves exposed to these gases, but the patterns of induction were different in O_3- and SO_2-treated leaves. These results suggest that different reactions are involved in the induction of ethylene synthesis in O_3- and SO_2-treated leaves.

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The analysis of tobacco stable transformant constitutively expressing LBM1 which was Myb-related protein and was induced by wounding and elicitor treatment

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The transcriptional activation of Tobacco retrotransposon, *Tto1* is dependent on the enhancer/promoter region located in long terminal repeats (LTRs), and the 13-bp repeated motif in LTR has recently been identified as a *cis*-regulatory element involved in the activation by these stresses. We cloned the 13-bp motif/L-box binding factors, which belong to the MYB-related transcription factors. We characterized one of these factors, LBM1 (LTR-Binding MYB1), whose expression is induced by wounding and elicitor treatment. Specific binding activity of LBM1 to the 13-bp motif/L-box can be detected in nuclear fractions from leaves after wounding or treated with an elicitor derived from *Trichoderma viride* fungal extracts. Treatment with cycloheximide which is one of the protein synthesis inhibitor, inhibited the LBM1 protein synthesis and DNA binding activity depending on LBM1. These evidences suggest that LBM1 functions as an important regulator of transcription of *Tto1* and the *PAL* genes in response to defense related stresses. In order to know whether LBM1 can work *in vivo* and also regulated by transcriptional levels, over-expresser of LBM1 was made and analyzed. The results show that depending on the LBM1 expression from the transgene which was driven by 35S promoter, not only reporter gene but also PAL and Tto1 expression were detected.

We also analyzed the mechanisms of LBM1 expression regulation. Mutational analysis of LBM1 promoter showed that AG-motif (AGATCC) was a sufficient *cis*-element. A zinc finger protein named LBP1 could bind to AG-motif and was phosphorylated by 46kDa kinase *in vitro* depending on wounding. In vitro analysis suggested that NTF3 which is a MAPK of tobacco may be a putative LBP1 kinase. Further analysis is under way.

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WOUND-INDUCTION OF AGROPINE SYNTHASE GENE PROMOTER OF Ri PLASMID: PROMOTER ANALYSIS. II

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Agropine synthase gene (Ri-*ags*) is a kind of opine synthase genes located on the T-DNA of Ri plasmids. The activity of Ri-*ags* promoter (Ri-P*ags*) in transgenic plants was shown to be specific to root and callus tissues and to be induced by wounding in leaf and stem tissues. 5' deletions in the Ri-P*ags* revealed that all the characteristics of the Ri-P*ags* was regulated by a 36 bp region designated as the wound-responsive (WR) region. In this study, we detected a nuclear factor interacts with the Ri-P*ags* and identifed its binding site by a gel shift assay.

Nuclear extracts prepared from tobacco suspension cells were assayed for binding to a distal fragment of the Ri-P*ags* (KE1) which did not contein the TATA box but the WR region. As a result, one major complex could be observed. When segments of KE1 (KE1-1 to 6) were added to the reaction, only KE1-3 corresponding to the WR region prevented the formation of this complex. On the other hand, when the WR region was removed from KE1 (KE1Δ3), no complex was observed. These results were consistent with the result of the 5' deletion experiment. The binding site was further localized using smaller competitor fragments. Then transversion mutations introduced into the competitor finally revealed that the factor recognized a GC rich sequense of ten base pairs.

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Studies on Regulatory Mechanism of Wound-Inducible Expression of ERF3 Gene in Tobacco Plants.

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We have been investigating the wound-inducible expression of genes encoding ethylene-responsive transcription factors (ERFs) in tobacco plants. Rapid increase of mRNA levels of ERF2, 3, and 4 genes occurred not only in wounded leaves but also in distal unwounded leaves. Results of pharmacological studies suggested that early induction of expression of ERF3 and 4 genes in wounded leaves was controlled by JA- and ethylene-independent signaling pathways. We analyzed wound-responsive transcription of genes for ERF2, 3 and 4 in transgenic tobacco plants carrying the ERF2, 3 and 4 promoter::β-glucuronidase (GUS) fusions. The results showed that rapid activation of transcription in response to wounding is involved in immediate early increase of mRNA levels of ERF genes in both wounded and unwounded distal leaves. Furthermore, we investigated the regulatory mechanism of wound-responsive transcriptional activation of ERF3 gene which shows the most significant responsiveness among the ERF genes. The experiments with 5' deletion of the promoter region of ERF3 gene showed a -224/-164 region from translational initiation sites is important for the wound-inducible expression of this gene. Electrophoretic mobility shift assays revealed that the binding activity of nuclear proteins in leaves, which specifically interact with 22 bp DNA fragment derived from the -224/-164 region, was induced by wounding within 10 min.