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Ethylene-Independent Signal Transduction by Xylanase in Tobacco Suspension Cultured Cells

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Xylanase from *Trichoderma viridae* is a strong elicitor inducing synthesis of pathogenesis - related proteins. It is known that signal transduction by xylanase is independent of ethylene, calcium, and state of protein phosphorylation, but the intermediator in this pathway is not yet known. When xylanase was treated to tobacco suspension cultured cells, β-1,3 glucanase activity and lignin biosynthesis increased markedly. H_2O_2 and peroxidase have been thought to be related to lignin biosynthesis, we thought about the possibility of active oxygen species (AOS)'s mediation in this system. Pretreatment of n-propylgallate and mannitol, known as antioxidants, for 24 hrs, caused inhibition of β-1,3 glucanase activity about 85% and 50%, respectively. On the contrary, H_2O_2 increased activity of the enzyme about 80% at 24 hrs earlier than xylanase. Using lucigenin and luminol-dependent chemiluminescence, we detected AOS as O_2^- at 40, 52 hrs and H_2O_2 at 44, 56 hrs respectively, after xylanase treatment. Also, through the enzyme assays related to AOS synthesis, the detected H_2O_2 in this pathway was mainly caused by transition of O_2^- to H_2O_2 by superoxide dismutase.

E202

cDNA Cloning and Expression Study of 1-Aminocyclopropane-1-Carboxylic Acid Oxidase Genes from Tobacco.

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ACC oxidase that catalyzes the final step of ethylene biosynthesis has been known to regulate ethylene production at various conditions along with ACC synthase which converts AdoMet to ACC. To study the expression pattern of this gene, we have cloned cDNAs encoding tobacco ACC oxidase. By comparing the amino acid sequences of other reported ACC oxidases, we constructed two degenerated oligonucleotide primers, corresponding to the conserved sequences of DACENWG (sense primer) and KFQAKEP (antisense primer). Polymerase Chain Reaction (PCR)-based amplification was carried out using the first strand cDNA prepared from the total RNA of tobacco suspension cultured cells as the template. The PCR products of about 660 bp in size were sequenced to confirm their homology to ACC oxidase, and used to screen a tobacco leaf cDNA library. We were able to isolate two different full length clones and confirmed their identity as an ACC oxidase cDNA by sequence analysis with the previously known sequences. Using these cDNA clones, we investigated the genomic structure of ACC oxidase genes by genomic Southern blot analysis. The experession of ACC oxidase mRNAs in tobacco plants and suspension cultured cells will be discussed.