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Wound-induced Gene Expression of Ferulate 5-hydroxylase in Leaves of *Camptotheca acuminata*

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Ferulate 5-hydroxylase (F5H) is a cytochrome p450-dependent monooxygenase that catalyses the irreversible hydroxylation of ferulaic acid, coniferaldehyde and coniferyl alcohol in the pathway leading to sinapic acid and syringyl lignin biosynthesis. In a effortto demonstrate the signal molecules in wound response on the F5H gene, we have detected the effects of ethylene, methyljasmonate (MJ) and hydrogen peroxide on the F5H gene expression. When ethylene, MJ and hydrogen peroxide were exogenously applied for 24 hrs, the transcripts level of F5H gene increased in *C. acuminata* detached leaves. By the way, when these chemicals were applied for 1, 2, 4 and 6 hrs, the transcripts level of F5H gene did not increased in detached leaves, but increased in leaf discs. To demonstrate the regulatory mechanism of wound response on the F5H gene, a 2.15 kb F5H promoter was isolated for studying the wound-induced signal transduction. A ACGT core sequence and a ERE-like sequence (ATTTCAAT) were found in this promoter, but a GCC core-like sequence was not found in this promoter. We propose that the stimulation of F5H gene expression by wound is mediated by ethylene, MJ and hydrogen peroxide in *C. acuminata* leaves, and in these processes of signal transduction, wound signal is absolutely necessary.

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Activities of Intracelluar and Extracellular Antioxidant Enzymes of Cultured Sweetpotato Cells

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생체는 대사과정과 환경스트레스에 의해 발생되는 활성산소종(ROS)으로부터 자신을 보호하기 위하여 각각의 스트레스원에 대한 개별의 신호전달체계를 구축하고 있다. 동식물의 세포내(intracellular)에서 활성산소제거시스템에 관여하는 항산화효소로는 superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), glutathione peroxidase (GSH-Px)등이 있다. 세포외(extracellular)에 존재하는 항산화효소들은 고등포유류의 여러 조직의 extracellular space와 많은cell types의 cultured media에서 발견되었으며, 현재 그들의 기능을 밝히는 연구가 진행되면서 중요성이 부각되고 있다. 반면, 식물체 및 식물배양세포에서는 이들에 관한 연구가 전무하다. 본 연구에서는 POD 고생산주로 선발된 고구마 (*Ipomoea babatas*) 세포주를 이용하여 세포내 외에 존재하는 항산화효소를 배양시기별로 살펴 보았다. 세포내와 마찬가지로 cultured medium도 POD, SOD, GSH-Px의 활성이 검출되었는데, SOD, GSH-Px, POD는 배양 5일 째 세포 외로 가장 많이 분비하고 점차로 감소된 반면, 세포 내의 항산화효소의 활성은 높은 비율로 나타났다.또한 세포 내 SOD와 GSH-Px는 배양 15일과 20일 째 각각 높은 활성을 보였다. 앞으로, 이러한 항산화효소 활성의 변화가 transcription level과 같이 수반되어 일어나는지 항산화효소의 mRNA level을확인함으로써 밝힐 수 있을 것이다.

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Isolation and Characterization of Chloroplast ABC Protein Gene in Soybean

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In the course of a study concerning the molecular mechanisms of hypocotyl elongation during soybean seedlings which were grown in darkness, we generated a lot of ESTs from a cDNA library prepared from the dark grown soybean seedlings. One of the ESTs has a high similarity with a Arabidopsis thailana plastidic ATP-binding-cassette (atABC) protein in amino acid level. The putative soybean ABC protein contains an N-terminal transit peptide which targets it into chloroplast same as atABC1 protein. The transcription level of putative soybean ABC gene has investigated under the continuous red light, continuous far-red light, and complete dark condition. The main function of atABC1 protein is the transport of protoporphyrinogen IX which is the precursorof chlorophyll from the cytoplasm to the chloroplast. The soybean ABC gene was transferred into tobacco plant under the CaMV 35S promoter. The chlorophyll level of this transgenic tobacco plants will be compared with the chlorophyll level of the wild type tobacco plant. (This research is supported by grants from ARPC)

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Cloning and Characterization of the cDNA for β-carotene Hydroxylase from *Daucus carota*

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Plant carotenoids are isoprenoid-derived pigments that are synthesized and localized in plastids. The β-carotene hydroxylase catalyzes hydroxylation of the -rings of -carotene and β-carotene, and is thus necessary for synthesis of all xanthophylls of higher plant chloroplasts. We have isolated a full length cDNA of β-carotene hydroxylase from *Daucus carota* leaf by polymerase chain reaction. Sequence analysis indicated that cDNA contains an open reading frame encoding 309 amino acids. The predicted amino acid sequence for this enzyme revealed 67%, 65%, and 69% identity to *Lycopersicon*, *Capsicum* and *Citrus* β-carotene hydroxylase, respectively. Southern blot analysis showed that β-carotene hydroxylase gene exists in genome as multi copy. In northern blot analysis, it revealed that its expression is equivalent in leaves, stems and roots. When detached leaves were treated with H2O2 for 24 hours and exposed to moderate light after incubation in dark condition for 3 days, the β-carotene hydroxylase mRNA levels were decreased and increased, respectively. Now we study the effects of various stresses on the expression of β-carotene hydroxylase gene and the change of mRNA level of β-carotene hydroxylase and carotenoid contents at different developmental stages in *Daucus carota*.