Canavalia lineata

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Genomic structure of canaline-dependent ornithine carbamoyltransferase (OCT) gene from *Canavalia lineata* leaves was determined. We found the necleotide sequences of the canaline-dependent OCT gene containing 3,902 bp 3′ region and 2,737 bp promoter region. Canaline-dependent OCT gene consists of 5 exons and 4 introns. The exons range in size from 132 bp to 474 bp, while the introns range in size from 83 bp to the relatively large size of 1525 bp. Genomic structure of canaline-dependent OCT gene was compared to ornithine-dependent OCT gene from *Canavalia lineata*. Two genes consist of 5 exons and 4 introns, all the splicing junctions followed the conserved GT/AG rule. The size of exons of the two genes was similiar, but the size of introns showed great difference. The size of intron 1 of canaline-dependent OCT gene was 652 bp, while that of OCT gene was 338 bp. Also intron 4 of the canaline-dependent OCT gene 83 bp only, but that of OCT gene was 762 bp. The transcription initiation site of canaline-dependent OCT gene and ornithine-dependent OCT gene is located 66 bp and 12 bp upstream of the ATG translation initiation site, respectively. The 1,000 bp 5′ upstream region of canaline-dependent OCT gene contains many regulatory elements such as GT-1, I-BOXCORE, AT1BOX, NIT2 and GATAMOTIFCAMV etc. Also a single canaline-dependent OCT gene exists in the *Canavalia lineata* genome.

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Purification and Characterization of Ornithine Cabamoyltransferase from *Glycine max* Leaves

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Ornithine Cabamoyltransferase (OCT, EC 2.1.3.3) has been purified from *Glycine max* leaves. OCT was purified 121-fold with a yiled of 15.9% by dialysis, DEAE-Sephacel ion-exchange chromatography, Sephacryl S-200 gel filtration and Procion red-dye chromatography. The molecular weight of the native enzyme was approximately 114 kDa as estimated by Sephacryl S-200 gel filtration chromatography. The subunit molecular weight of the enzyme was 40 KDa based on SDS-PAGE. These result suggest that the native enzyme is a trimer. The effect of pH is significantly influenced by ornithine concentration ; optimal activity is at pH 7.5 when ornithine is saturating. At pH 7.5, the *km* values for the substrates are 0.36 and 0.12 mM for ornithine and cabamyl phosphate, respectively. Canaline competitively inhibited OCT activity enzyme. S-carbamoyl-L-cystein and L-cysteine were very strong inhibitors for the enzyme activity. OCT activity was approximately inhibited by 55% with 2 mM Zn^{2+} and Cd^{2+}. When tested from the three organs of *Glycine max*, leaves, shoots and roots, OCT activity of shoots is 2-fold higher than that of leaves. OCT activation energy was 13.8 kcal/mole as calculated from an Arrhenius plot.

E212

폴리아민 함량이 증가된 형질전환 담배 식물체에서의 생물적 스트레스에 대한 저항성 조사

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식물은 대부분 변화가 많은 환경조건에서 살게 되는데, 특히 식물의 생장과 발달에 지장

을 초래하는 환경조건에 처하게 되는 경우가 많다. 폴리아민은 모든 식물체에 존재하는 다가양이온으로서 세포분열과 세포 생장을 촉진하는 물질로 알려져 있다. 폴리아민 생합성의 rate limiting step에 관여하는 *S*-adenosylmethionine decarboxylase (SAMDC) 유전자를 sense 방향으로 도입한 식물체를 제조하였다. 또한 SAM과 경쟁적으로 작용하면서 에틸렌 생합성에 관여하는 1-aminocyclopropane-1-carboxylic acid synthase (ACC synthase) 및 ACC oxidase의 유전자를 antisense 방향으로 담배에 형질전환시킨 식물체를 제조하였다. 이들 T_1 세대의 식물체 잎에 fungal pathogen인 *Phytophthora parasitica* var.*nicotianae*를 감염시켰을 때 야생형 식물체와 positive control 식물체는 줄기가 검은색으로 변하면서 7 일후 부터 서서히 죽어가면서 10 일째에는 거의 죽었지만 폴리아민 생합성량이 비교적 크게 증가한 SAMDC 과다발현 식물체, ACC synthase 발현저해 식물체와 ACC oxidase 발현저해 식물체들은 10 일이 지나더라도 아주 건강한 상태를 유지하였다. 또한 bacterial pathogen인 *Pseudonomas syringae* pv.*tabaci* 를 감염시킨 후 5 일째 결과를 보면 야생형 식물체는 박테리아 생장이 1.2 배 증가함에 비해 SAMDC 과다발현 식물체에서는 박테리아 생장이 크게 저해되어 박테리아의 colony forming unit (CFU)가 1/16로 줄어들었다. 따라서 생물적 스트레스에 상당한 저항성을 갖고 있는 형질전환 식물체들의 스트레스에 의한 손상이 폴리아민 특히 spermidine에 의해 크게 완화된 것으로 생각된다.

E213

Differential Gene Expressions during Early Development Stages of Pear (*Pyrus pyrifolia* Nitaka) Leaf Necrosis Disease

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Plants react to environmental changes by altering their gene expression to meet the imposed conditions. Most genes involved in plant defense mechanism against invading pathogens are expressed in response to necrosis. To elucidate plausible causes of Pear leaf necrotic disease, total soluble proteins from wild and diseased plants were isolated. The protein patterns were resolved by one and two dimensional gel electrophoresis. SDS-PAGE of crude extracts of wild and diseased leaves of pear revealed differences in several protein patterns. Synthesis of new proteins was observed, while other proteins were not found on the gel in response to necrosis. Analysis by two-dimensional electrophoresis detected 6 distinct spots (pH 5.5-6.0, pH 6.7-7.0) in infected leaves. These suggest that distinct proteins could be originated from the virus. The proteins are being characterized to figure out their amino acid compositions. Based upon the amino acid sequences, DNA sequences of the gene will be deduced and DNA probes will be synthesized to clone the gene responsible for the disease.

E214

Activation of Rubisco by GA3 in Soybean

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Our experiments were studied the effect of application of exogenous GA3 upon rubisco activation in soybean leaves. Rubisco activity at 0.1 μM GA3 was significantly greater than that at no treatment. Rubisco content showed patterns of change similar to rubisco activity. These data suggest that rubisco activity was associated with an amount of rubisco protein, and that the activation of rubisco is promoted by GA3. The degree of intensity of 50 and 14.5 kD polypeptides identified as the large and small subunit of rubisco by SDS-PAGE