orientation into the tomato genome. We have studied the protection of plant against paraquat and H_2O_2 treatments in transgenic tomato plant.

E224

Proteolytic Processing of E. coli-Expressed P1/HC-pro Complex in Soybean Mosaic Virus

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Soybean mosaic virus (SMV) proteinases P1 and helper component (HC-Pro) participate in the proteolytic processing of viral polyproteins. The P1 proteinase catalyzes proteolysis between itself and HC-Pro. The each gene product and fused P1/HC-Pro complex were synthesized by reverse transcription-polymerase chain reaction (RT-PCR) using the combination of primers derived from 5′ and 3′ ends of P1 and HC-Pro, respectively, and their sequences have been determined. To define the junction region between the two partners responsible for processing, single amino acid replacement of serine by phenylalanine was introduced into the junction site by RT-PCR using a primer derived from the mutated 3′ end of P1. We inserted each fused gene construct containing a conserved and a mutated cleavage site into pET vector. After production, the corresponding protein was cleaved in vivo by the proteinase, that is encoded by the plasmid harboured by a specialized E.coli host. The mutation at the junction site resulted in no proteolytic processing of the fused recombinant protein. The released protein remains soluble and can be purified from cell extracts by means of an affinity tag (a poly His group). Cleavage at the junction between P1 and HC-Pro occurs during expression, respectively producing 30 kDa and 45 kDa on the SDS-PAGE. We purified the P1 and HC-Pro by detergent extraction of cell lysate and FPLC. The purified each protein was active in a mildly-denaturing gelatin SDS-PAGE. We examined the dependence of proteinase activity on pH, temperature and presence of inhibitors, using proteinase assay by gelatin or casein as a substrate.

E225

Enhanced resistance to dehydration in trehalose-producing transgenic tobacco plants

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Six lines of transgenic tobacco plants harboring *E. coli* TPS (trehalose-6-phosphate synthase) gene (*ots* A) were generated by leaf disc transformation. Transgenic plants were identified by PCR, Northern hybridization, and trehalose synthesis. All lines of transgenic plants manifested stunted growth and extended generation time in varying degree. In addition, transgenic plants typically exhibited some morphological changes including altered leaf morphology and increased number of leaves with more vestigial branches along the main stem. Leaves became longer and thinner to form lancet-shaped and some leaves were severely irregular-shaped with curls and wrinkles. The stunted growth was not accompanied with early senescence or necrosis.

Homozygous plants obtained in F_2 generation were used to test their responses against dehydration. All transgenic plants showed enhanced tolerance to dehydration as shown by improved retention in fresh weight upon air-drying both in detached leaves and whole plants. In addition,

transgenic plants respondea equally well to dehydration by 10% PEG-treatment. After dehydration by PEG-treatment, transgenic plants stayed in much less withered state than nontrasformants. Subsequent rehydration fully restored the turgor and revived the transgenic plants, but not the nontransformants. Water potential of leaves in all lines of transgenic plants was same or higher compared with nontransformants indicating no accumulation of solutes acting as an osmoprotectant. Quantification of trehalose in transgenic plants revealed no significant accumulation of trehalose to be effective as osmoprotectant (less than mg trehalose per g fresh wt). Decrease in water potential by dehydration through PEG-treatment was in parallel phase in both nontransformants and transgenic plants implying that trehalose confers resistance to dehydration, not through the maintenance of osmotic potential in the leaves. These results suggest that enhanced resistance to water stress in transgenic plants through trehalose production is not through its action as an osmoprotectant, but elsewhere. Futhermore, our preliminary results indicated that trehalose-producing plants exhibited enhanced tolerance against high salt or chilling. Trehalose production may activate a common signalling pathway to cope better against various abiotic stresses involving dehydration.

E301

Isolation and Purification of Pneumococcal Surface Protein A (PspA) of *Streptococcus pneumoniae* A43 Isolated in Korea

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As a basic study for the rapid detection and identification of pneumococcal disease and preventive vaccine developent, this study researched on the isolation, purification and characteristics of PspaA protein. *Streptococcus pneumoniae* A43 was chosen as selected strains through the former study of serotype, genotupe, and antimicrobial resistance pattern. PspA protein was isolated and purified by using the binding characteristics to human lactosferrin in *Streptococcus pnemoniae* A43. Also, when PspA protein was isolated after *Streptococcus pneumoniae* A43 strain had been cultivated in the general media, there were a lot of problems in the isolation process and in the acquired rate. So in this study, PspA was isolated and purified after *Streptococcus pneumoniae* A43 strain had been cultivated in the CDM-ET media, and purified through affinity column. This experiment, it was identified that pure isolation and purification of PspA protein could be acquired simply with high rate. In order to confirm the immunity of PspA protein purely isolated and purified from *Streptococcus pneumoniae* A43 strain, clinical test was done with mice. According to this test, it was observed that the isolated and purified PspA protein had immunity and protective force.

E302

Cloning and the Nucletide Sequence of PspA Gene from *Streptococcus pneumoniae* A43 in Korea

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In order to analyze the characteristics of PspA (pneumococcal surface protein A) protein in the domestic pathogenic *Streptococcus pneumoniae, S. pneunoniae* A43 showing immunity and protective force among the pathogenic strains collected was chosen in formerstudy. So, the nucleotide sequences of PspA gene in *S. pneunoniae* A43