Proteomic analysis of soybean seeds and sprouting seeds

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We observed a comparative proteomic analysis of wild and cultivated genotypes of soybean seeds. A high-throughput proteomic approach was employed to determine the expression profile and identity of proteins using two-dimensional gel electrophoresis and tandem mass spectrometry. The study was conducted to elucidate the effects of timing and duration of soaking on the germination of soybean. Comparative proteomics of the soybean seed have been used to identify the specific tissue-expressed proteins. These tissues have the significantly different levels of soaking. The concentrations of most of the proteins were increased through the soaking period. At 4 days after the soaking, the concentrations of some proteins were increased even though those of other proteins were decreased. Over 200 protein spots were reproducibly resolved in the two-dimensional gels from seeds. The different gel patterns and little correlation among the proteins were observed, respectively. Some proteins that were expressed only in the seeds were chosen for the identification by the mass spectrometry. We identified the proteins using MALDI-TOF mass spectrometry and peptide mass fingerprint database searching. Some selected protein spots were identified as follows: allergen Gly m Bd 28K, Glycinin A1aBx, p24 oleosin isoform B.

Proliferative effect of cholangiocarcinoma-associated fibroblasts on biliary cancer cells

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The analysis of stress-responsiveness in plant is an important route to the discovery of genes conferring stress tolerance and their use in breeding programs. This study was conducted to determine the effects of timing and duration of soaking on the germination of soybean. Proteomic analysis of soybean seeds and sprouting seeds was performed to determine the expression profile and identity of proteins using two-dimensional gel electrophoresis and matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF). The overall distribution pattern of proteins is similar in wild and cultivated soybeans following separation with broad range pH 3.0-10.0 immobilized pH gradient and narrow pH 4.0-7.0 immobilized pH gradient for the first dimension. The proteins were identified by comparing the masses of their tryptic peptides with those of all known proteins using MALDI-TOF MS and the NCBI database. Of the 1000 proteins detected, 41 differentially expressed. Two major storage proteins, β-coryphulin and glycine were included in the proteins identified. In addition, differentially expressed proteins during seed sprouting in Glycine Max were analyzed at various days after soaking. Database searching with the spectra resulted in the identification of 33 differentially expressed proteins. As far as we are aware, this is the first study reporting the comparison of protein profiles during soybean seed sprouting using proteomic tools.

Photochemical and biochemical characterization of the PYP-phytochrome, Ppr from Rhodosporillum centenum

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A phytochrome-like protein Ppr in the purple photosynthetic bacterium Rhodosporillum centenum has a photosensitive yellow protein domain at the amino-terminus that contains the conventional blue light absorption chromophore, p-coumaric acid. In addition, Ppr includes a central domain homologous to phytochrome and a carboxyl-terminal histidine kinase domain. We have isolated apo-Ppr and subsequently reconstructed holo-Ppr by covalent attachment of p-coumaric acid and biliverdin. Absorption spectrum of dark-adapted recombinant holo-Ppr displays 395 nm and 700 nm peaks corresponding to phytochrome containing biliverdin and a 432 nm peak corresponding to absorbance of p-coumaric acid. The absorption spectrum of dark-adapted recombinant Ppr containing both chromophores is very similar to that of S-tagged Ppr that is purified from R. centenum indicating that Ppr in vivo contains both p-coumaric acid and biliverdin. Most phytochromes show photoconvertibility by switching between a red light absorbing form (Pr) and a far-red light absorbing form (Pfr). However, recombinant Ppr containing either two chromophores or only the biliverdin chromophore does not exhibit red and far-red photoconvertibility. Instead, holo-Ppr is photo-bleached by illumination of red (700 nm) and blue (430 nm) light with this bleaching not recovered by irradiation of either red or blue light. Bleaching is, however, recovered by more than 5 hrs of dark incubation. To answer the question about the activity of histidine kinase of Ppr, we undertook autophosphorylation experiments after irradiation of different wavelengths using holo-Ppr with both chromophores.

Pathogen-inducible CaUGT1 gene is involved in resistance response against TMV infection by controlling salicylic acid biosynthesis and glycolysis

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Capucin annuus L. Bukan exhibits a hypersensitive response (HR), efficient forms of defense reaction against pathogen infection, by Tobacco mosaic virus (TMV) pathotype P0. The microarray analysis (5 K chip) was carried out to isolate genes involved in defense mechanism against TMV and/or Xanthomonas campestris pv. vesicatoria (Xcv). Of differentially regulated clones, UDP-glucosyltransferase (CaUGT1) gene was up-regulated during resistance response by TMV and Xcv infection. CaUGT1 was also induced in response to salicylic acid (SA), ethephon, methyl viologen (MV), and local wounding but not by methyl jasmonate (MeJA), ABA, and systemic wounding. When CaUGT1 was knocked-down by virus-induced gene silencing (VIGS) in hot pepper, ion conductivity of TMV-inoculated leaves was reduced, showed delayed HR and TMV proliferation was increased. In addition, SA-induced expression of CaUGT1-knockdown hot pepper plant were decreased than those of control plants. These results suggest that pathogen-inducible CaUGT1 gene might be involved in resistance response against TMV infection by controlling SA biosynthesis and glycolysis as well.

Isolation of drought tolerance determinant genes by screening of Arabidopsis activation tagged lines

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Environmental or abiotic stresses are major limiting factors of crop productivity. It has been estimated that the crop losses by various abiotic stresses are approximately ten times higher than those resulting from biotic stresses. Plants have the ability to perceive and respond adaptively to abiotic stresses. We are interested in identifying key regulatory components of the adaptive responses and set out to isolate novel stress signal transduction components by screening activation tagged lines. Toward the end, we generated an Arabidopsis activation-tagged seed pool and screened their progeny for enhanced stress tolerance phenotypes. From the screen of approximately 70,000 T1 plants, we isolated a pathogen-inducible gene expressed in tolerant or drought-sensitive lines. After subsequent rounds of screens to confirm their phenotypes, we established several tagged lines with altered drought tolerance. The identity of the corresponding genes was then determined by conventional methods, such as TAIL-PCR and plasmid rescue. Experiments to determine the role of the genes in drought tolerance are in progress, and some of the results will be presented in the meeting.