

## 324(F425)

PROMOTER ANALYSIS OF AREB GENES IN THE REGULATION OF DEHYDRATION- AND ABA-RESPONSIVE GENE EXPRESSION OF *rd29B* IN *ARABIDOPSIS*

Mohammad Masud PARVEZ<sup>1</sup>, Takashi FURIHATA<sup>1</sup>, Kazuo Shinozaki<sup>2</sup> & Kazuko Yamaguchi-Shinozaki<sup>1</sup>. <sup>1</sup>Japan International Research Center for Agricultural Sciences (JIRCAS), 1-2 Ohwashi, Tsukuba City, 305-8686, Japan, <sup>2</sup>RIKEN Tsukuba Institute, 3-1-1 Koyadai, Tsukuba City 305-0074, Japan.

The induction of dehydration-responsive gene *rd29B* is mediated by abscisic acid (ABA) in *Arabidopsis*. Previously we have reported that AREB1 and AREB2 proteins function as transcriptional activators in the ABA-inducible expression of the *rd29B* gene under dehydration condition. In this study, we isolated *Arabidopsis* genomic DNAs of AREB1 and AREB2, and carried out promoter analysis of these genes. We found an intron having 321bp in the genomic DNA sequence of 5' upstream promoter region of AREB1. Under dry condition, AREB1 construct containing the 321bp intron had a significantly higher GUS activity than that of control in transgenic tobacco. High GUS activity was also found in transgenic *Arabidopsis* seedlings with this construct while were treated with dry, exogenous ABA and NaCl. Histochemical analysis of *Arabidopsis* seedlings showed a high correlation with its GUS activity. In *Arabidopsis* plants having AREB1 construct containing the 321bp intron showed that GUS stained strongly in leaf, stem and root under dry, exogenous ABA and NaCl conditions. Detention analysis of AREB1 construct is underway and will be discussed.

## 325(F426)

PROPERTIES OF THE ABUNDANT PROTEINS IN CHLOROPLASTS OF SPORES OF *OSMUNDA JAPONICA*

Hiroshi INOUE, Hiroyuki KAMACHI, Kohzo NAKAYAMA<sup>1</sup>, Dept. Environ. Biol. Chem., Fac. Sci., Toyama Univ., Toyama 930-8555, <sup>1</sup>Dept. Anatomy, School Medicine, Shinshu Univ. Matsumoto 390-0802

we reported that three polypeptides in the thylakoid membranes of chloroplasts from green spores of the fern *Osmunda japonica* decreased during spore germination. The 22-kDa protein had been purified from thylakoid membranes. We have attempted to determine a gene of the 22-kDa protein by PCR using the information of amino-terminal sequences of proteolytic products. The cDNA obtained from the mRNA shows that the precursor protein is composed of the signal peptide having 16 amino acid residues and the mature protein having 180 amino acid residues. A computer search of the protein database revealed similar features between the 22-kDa protein and LEA 3 homologs of *Deinococcus radiodurans*, *Drosophila melanogaster*, *Caenorhabditis elegans*, and cDNA corresponding to cold hardening-induced *Chlorella* genes.

## 326(F427)

Hypertonic treatment induces a transient  $\text{Ca}^{2+}$ -dependent cessation of cytoplasmic streaming in *Vallisneria gigantea* mesophyll cells

Teruyuki HAYASHI and Shingo TAKAGI; Dept. Biol., Grad. Sch. Sci., Osaka Univ., Toyonaka 560-0043, Japan

Plant cells respond to various kinds of mechanical stimuli, including touch and extracellular changes in osmotic pressure. In mesophyll cells of *Vallisneria gigantea*, an aquatic angiosperm, actin-dependent rotational streaming of the cytoplasm is observed in light. We found that hypertonic treatment with 0.5M sorbitol induced, within a few minutes, a transient cessation of the cytoplasmic streaming. The response depends on the presence of extracellular  $\text{Ca}^{2+}$ , and was substantially inhibited, even in the presence of  $\text{Ca}^{2+}$ , after treatment of cells with  $\text{Ca}^{2+}$ -channel blockers, such as  $\text{LaCl}_3$ ,  $\text{GdCl}_3$ , and nifedipine. Hypertonic treatment seems to induce  $\text{Ca}^{2+}$  influx across the plasma membrane through  $\text{Ca}^{2+}$  channels to produce a transient cessation of the cytoplasmic streaming. Once cells were plasmolyzed, those cells hardly responded to the second hypertonic treatment until 12 to 24 hours after deplasmolysis. We used Arg-Gly-Asp (RGD) hexapeptide, which is known to inhibit a binding of animal cells to extracellular matrices, to examine whether the intact adhesion of plasma membrane to cell wall is necessary to respond to hypertonic treatment. RGD peptide markedly disturbed the arrangement of bundles of actin filaments, however, it had no effect on the response to hypertonic treatment. This result indicated that an RGD-sensitive adhesion is probably not involved in the response. Monitoring of  $[\text{Ca}^{2+}]_{\text{cyt}}$  level during the response to hypertonic treatment in mesophyll cells loaded with a ratiometric  $\text{Ca}^{2+}$ -sensitive fluorescent dye fura-2 is under investigation.

## 327(F428)

Detection of submergence stress-induced genes in submergence tolerant rice cultivar by differential display method and expression mechanism of their genes

Masaharu Osako, Naoyoshi Kawano, Osamu Ito<sup>1</sup>, Yasuo Yamauchi, Kiyoshi Tanaka (Fac. Agr., Tottori Univ., <sup>1</sup>JIRCAS, Ministry of Agr., For. Fish.)

It has been observed that plants exposed to submergence stress survive a period of imposed anoxia, only to die on reexposure to air. It is expected that submergence tolerant plants have developed the tolerance mechanism in well-devised manners. To analyze the gene expression mechanism under submergence stress, detection and isolation of submergence stress-induced genes were attempted using submergence-tolerant rice cultivar (*Oryza sativa* IND FR-13A). In the differential display between the rice cultivar submerged for 7 days and then reexposed to air for 2 days, and the control one, the inductions of a lot of genes under the stress were observed. Using Northern blotting method, gene expression mechanism were minutely examined.