#### Symposium 5. Application of gene technology to agriculture

## S26(S5-01)

SALT INDUCIBLE GENES FROM BARLEY

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We isolated a cDNA clone, *Bnuc1*, encoding a nuclease I from leaves of salt-stressed barley by differential display method. Northern blot analysis revealed that the transcript of *Bnuc1* gene was increased dramatically in barley leaves under salt stress. The expression of the gene was also increased by exogenously applied abscisic acid in leaves, but not by gibberellic acid during seed germination. *Bnuc1* gene was expressed more in old leaves than in young leaves during salt stress. We suggest that during adaptation to salt stress, nutrient recycling system for nucleic acid from old leaves to young leaves is induced and Bnuc1 probably functions in the system. We also report all salt-inducible genes obtained from barley by differential display method using 480 primers.

We also report unique inducers such as oxidative damage for glycinebetaine accumulation in barley leaves under salt stress.

#### S27(S5-02)

DIVERSITY OF PROTEINS EXPRESSED IN OVERWINTERING PLANTS UNDER THE LOW TEMPERATURE ENVIRONMENT Daisuke TAKEZAWA, Keita ARAKAWA, Shizuo YOSHIDA, Seizo FUJIKAWA, Inst. Low Temp. Sci. Hokkaido Univ., Sapporo 060-0819

Many perennial plants grown in the temperate zone and northward have an ability to acclimate to cold temperatures and exhibit the maximal freezing tolerance in midwinter. Freezing injury is thought to be caused by irreversible damage to the plasma membrane. However, the temperatures that cause freezing injury and the mechanisms that lead to the membrane lesion are diverse in different plant species, organs and tissues, as revealed by Cryo-SEM observation of the cell ultrastructures. The damage to the plasma membrane is thought to be alleviated by the function of proteins newly synthesized in the cold-acclimated plants. Fractions were obtained from plasma membrane, cell wall, and ER that developed near the plasma membrane, from plant tissues that exhibit high freezing tolerance, and proteins specifically accumulated during the cold acclimation process were analyzed. The result indicated that proteins with diverse functions such as heat-shock proteins, highly hydrophilic proteins, and thaumatin-like protein are expressed under the low temperature environment.

## S28(S5-03)

TRIENOIC FATTY ACIDS AND PLANT TOLERANCE OF HIGH TEMPERATURE Koh IBA, Dept. Biol., Kyushu Univ., Fukuoka 812-8581

The membrane lipids of plant cells consist of a high proportion of polyunsaturated fatty acids. In particular, the chloroplast membrane of higher plants contains an unusually high concentration of trienoic fatty acids. Although it is widely known that plants that grow in colder temperatures have a higher content of trienoic fatty acids, the physiological significance of the level of trienoic fatty acids has not been clearly elucidated. In this study, we produced transgenic tobacco plants in which the gene encoding chloroplast ω-3 fatty acid desaturase, the enzyme which catalyzes the conversion of dienoic fatty acids to trienoic fatty acids, was knocked out by the gene-silencing technique. The chloroplast membranes of the knock-out plants contained a substantially lower level of trienoic fatty acids than the chloroplast membranes of the wild-type plants. Acclimation of the knock-out plants to higher temperature was significantly enhanced. This enhancement of acclimation to higher temperatures was not transient, but was highly preserved during plant growth and inheritance. These results suggest that a wide variety of higher plants can be adapted to hightemperature environments using gene-silencing and antisense techniques.

## S29(S5-04)

# PLANT CELL WALL RECOGNIZES AND RESPONDS TO SIGNALS FROM PHYTOPATHOGENS.

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By using a model system with pea and an elicitor and suppresor from a pea pathogen, Mycosphaerella pinodes, we found that ATPase activity and superoxide generation in cell wall were regulated by the fungal signals. The elicitor enhanced both activities nonspecifically but the suppressor inhibited them in a species-specific manner, suggesting that the receptors for these signals exist in the cell walls. In the protein fraction bound to an ATP-conjugated column, a Mr 60 kDa protein recognized these fungal signals specifically. The sequence analysis of two cDNAs of the fungal signalbinding protein showed similarity to pea nuclear-NTPase and potato apyrase. The recombinant proein revealed the ATPase activity responding to the fungal signals. Moreover, the elicitor and suppressor were bound to the recombinant protein specifically. These results strongly suggest that i) cell wall-bound ATPase is the receptor for these fungal signals, ii) the cell wall equips with inependent defense systems and iii) the plant specific organelle, cell wall, with the specific suppressor may play a crucial role in determination of plant-pathogen specificity.