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Mitochondrial Uncoupling Proteins (UCPs) of Higher Plants: Comparative Study on *UCP* genes of Arabidopsis and Rice

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Uncoupling proteins (UCPs) are a heat-generating protein which is located in the inner membrane of mitochondria. In mammals, they dissipate proton gradient formed through respiratory chain without ATP synthesis. The freed energy is converted to heat, helping the animals maintain their body temperature under cold environments. Interestingly, recent reports described that higher plants also have the heat-generating protein in mitochondria, and our previous study revealed that the *UCP* gene constitutes a small multigene family in Arabidopsis, consisting of at least two members (Watanabe *et al. Plant Cell Physiol.* 40: 1160-1166, 1999). A Northern analysis suggested that these two *UCP* genes are differently regulated in cold-treated Arabidopsis.

Also, analysis of the UCP genes of rice, which is of tropic origin and thus sensitive to low temperature, is being carried out in our laboratory. The data obtained have shown the occurrence of two isoforms of the protein in rice, as was the case for Arabidopsis. However, no significant enhancement of the UCP genes was observed in rice under cold environments. Furthermore, both of the rice genes appeared to have defects in processing their transcripts. In this meeting, we will present the comparative study on the *UCP* genes of Arabidopsis and rice.

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TRANSCRIPTIONAL ANALYSIS OF WHEAT *cox2* UNDER LOW TEMPERATURE CONDITION

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Mitochondrial genome might have an important role for a cold response in higher plants, as well as nuclear genome. We have investigated the effects of low temperature on the transcriptional patterns of various mitochondrial genes in wheat, and have revealed that the transcriptional pattern of *cox2* gene is affected by low temperature. *cox2* is a mitochondrial gene encoding the subunit II of cytochrome *c* oxidase, and has an intron. Northern blot analysis has identified two *cox2* transcripts, corresponding to a precursor with an intron and a mature transcript without intron. After the cold treatment at 2°C for one week, the precursor transcripts clearly increase while the mature transcripts show the same level as those of control plants. These data suggest that the initial transcription is increased or the splicing activity is decreased by low temperature.

Wheat *cox2* intron belongs to group II. For the splicing of group II intron, the complementary base pairing between the specific intron and exon sites is one of the important features. RNA editing event is reported on the intron binding site 1 (IBS1) of wheat *cox2* first exon. We will discuss the possibility that low temperature could affect the splicing activity through the RNA editing event on the IBS1.

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Gene expression of *WAP20* and *WAP27*

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We have shown that small-heat shock protein homolog, WAP20, and group III LEA protein homolog, WAP27, were accumulated in ER of cortical parenchyma cells in mulberry tree during winter. In this study, expression of *WAP20* and *WAP27* was determined by Northern blot analysis. *WAP20* and *WAP27* were induced in the process of seasonal cold acclimation in cortical tissue of mulberry. In addition, *WAP20* and *WAP27* were also induced by low temperature treatment in cortical tissue. Expression of *WAP20* in the process of seasonal cold acclimation was most abundant in cortical and less in xylem and winter bud tissues. On the other hand, expression of *WAP27* in the process of seasonal cold acclimation was almost same levels in each tissue. Expression of *WAP20* and *WAP27* by heat shock, ABA and dehydration was also determined.

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Characterization of cDNA clones of wheat chitinase induced during hardening

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Winter wheat plants acquire both freezing tolerance and snow-mold resistance during cold acclimation. It has been reported that pathogenesis-related(PR) proteins were induced in winter crops by low temperature. It was also reported that glucanase-, chitinase- and thaumatin-like proteins that accumulated in the leaf apoplast of winter cereals in response to low temperature exhibited antifreeze activity and that these cold-induced PR proteins might be isoforms of PR proteins induced by pathogens. These proteins are involved in one trait of cross-adaptation to freezing stress and disease stress in a winter environment. The objectives of this study were to isolate and characterize wheat chitinase cDNA induced during hardening. Three types of chitinase cDNA homologes (*Chi 1, 7* and *10*) were cloned from hardened winter wheat c.v. PI 173438. According to each deduced amino acid sequence, *Chi1* was a class II chitinase, and *Chi 7* and *Chi10* were class I chitinase. *Chi 7* possessed a hydrophobic C-terminal domain similar to the vacuole targeting sequences of class I chitinase of otherplants. Transcriptional levels of these three cDNAs were enhanced in both leaf and crown tissues of field-grown wheat during hardening. All of the enzymatic assays of each of the proteins produced by methylotrophic yeast, *Pichia pastoris*, transformed with each of the three cDNA showed end-chitinase activities.