35. Proteomic Analysis of Cold-Responsive Proteins in Rice Leaf Tissue by Cold Stress

Dong-Gi Lee, Sang-Hoon Lee and Byung-Hyun Lee
(College of Agriculture and Life Science, Gyeongsang National University, Jinju 660-701, Korea)

벼에서 저온 스트레스 반응 단백질의 프로테옴 분석

이동기 · 이상훈 · 이병현
(경상대학교, 동물자원과학부)

Key words : Cold, 2-DE, Proteomics, MALDI-TOF-MS, RT-PCR.

<Objectives>

Cold stress is one of the major environmental stresses that can delay plant growth and development, reduce productitivy and in the extreme can cause the plant to die. To examine the response of rice to cold stress, changes in protein expression were analyzed using a proteomic approach.

<Materials and Methods>

1. Materials

Rice *(Oryza sative* L. cv. Dongjinbyeo) was used in this study. Fourth- and fifth-leaf stage rice seedlings grown under natural light in a greenhouse (20 ℃ / 30 ℃) were used for stress treatment.

2. Methods

For cold treatment, rice plants were transferred to growth chamber set at 4 ℃ or 10 ℃ and incubated for various time periods. Total soluble proteins were extracted from leaf tissue. The soluble proteins were fractionated with 15 % PEG. Protein profiles after cold treatment were analyzed by two-dimensional gel electrophoresis (2-DE). Protein spots were visualized by silver staining. Differentially expressed spots were identified by peptide mass fingerprinting using matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF- MS).

<Results and Discussion>

More than 1,000 protein spots were reproducibly detected on 2-DE gel including 43 that were up-regulated and 4 were down-regulated under 4 ℃ treatment, 47 that were up-regulated and 7 were down-regulated under 10 ℃ treatment, respectively(Fig. 1 and 2). All of differentially expressed proteins spots were subjected to MALDI-TOF mass spectrometry followed by database searching, which allowed the identification of 34 protein spots at 4 ℃ treatment, 27 protein spots at 10 ℃ treatment, respectively. These proteins are involved in redox regulation, reactive oxygen species scavenging, signal transduction, and nitrogen and amino acid metabolism-related proteins. Proteins involved in transcription factor or in plant defense mechanism were also identified and will be discussed. The expression profile of the GS, APX, and NDPK1 gene upon exposure to cold stress, was analyzed by RT-PCR using gene-specific primers. The expression level of above genes increased in response to cold stress. These results confirm that the expression patterns of the three proteins identified from 2-DE were consistent with RT-PCR analysis.

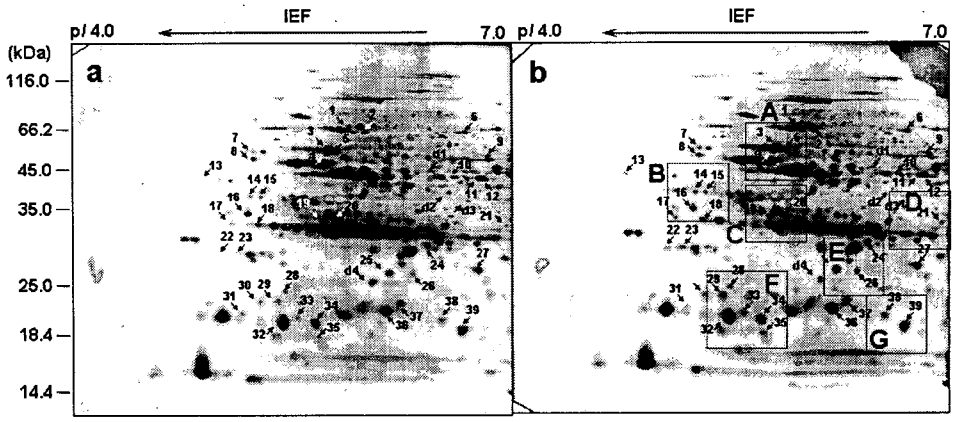

Fig. 1. 2-DE analysis of PEG-fractionated proteins induced by cold treatment in rice leaves. Protein samples (170 μg) in 15 % w / v PEG supernatant fractions were separated on 2-DE gels (pI 4-7), and silver-stained. Arr-ow indicated up-or down-regulated proteins after cold treatment. Cold response proteins both 4 ℃ and 10 ℃ were located in the boxed areas (A, B, C, D, E, F and G). The relative Mr are indicated on the left side in kDa. a, control (30 ℃), b, 4 ℃ for 36 hours.

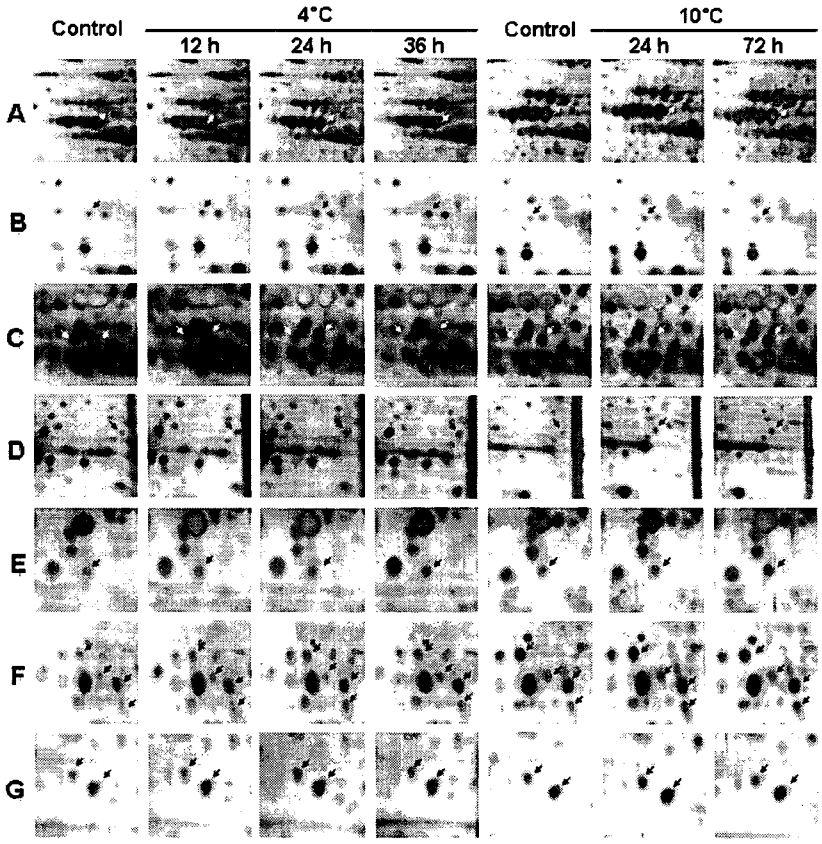

Fig. 2. 2-DE analysis of PEG-fractionated proteins induced by cold treatment. High magnification views of cold response proteins showing significant differences are shown. Whole Dongjin rice plants were used to monitor the accumulation of cold-induced proteins in response to cold treatment. Fourth- and fifth-leaf stage rice seedlings were subjected to cold treatment, kept in a humidity chamber at 4 ℃ or 10 ℃, and harvested at 12, 24 and 36 hours after 4 ℃ treatment, 1 and 3 days after 10 ℃ treatment, respectively. Protein sample (170 μg) in PEG supernatant fractions were separated on 2-D-gel (pI 4-7), and silver-stained. Cold response proteins both 4 ℃ and 10 ℃ are indicated in the gels (A, B, C, D, E, F and G). A total of twelve proteins were induced by cold treatment. Data analysis was performed using PDQuest software program.