

High throughput metabolome and proteome analysis of transgenic rice plants (*Oryza sativa* L.)

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Abstract We profiled metabolic patterns of transgenic rice organs and tissue by Fourier-transform ion cyclotron mass spectrometry (FT-MS) to reveal effects of the over-expression of *YK1* possessing high homology with maize HC-toxin reductase gene. Comparison of metabolic patterns revealed that compositions of organ- or tissue-specific metabolites were not significantly varied between the control and the *YK1* rice, where expression levels of some metabolites were altered. Proteome analysis of cultured cells over-expressing *YK1* showed the up-regulation of several stress-related proteins such as osmotin-like protein and *osr40cl*. Thus, alteration of metabolites as well as proteins may contribute to multiple stress tolerances in transgenic *YK1* rice.

Key words: Metabolome, proteome, Fourier-transform ion cyclotron mass spectrometry (FT-MS), HC-toxin reductase like rice gene (*YK1*), rice (*Oryza sativa* L.).

To elucidate metabolic networks of plants, metabolome studies consisting of multitarget profiling is essential (Fiehn et al. 2000). Nevertheless, a simultaneous detection of a great number of intracellular metabolites is a bottleneck of such studies in terms of sensitivity and selectivity of metabolites. The high throughput technology such as Fourier-transform ion cyclotron mass spectrometry (FT-MS) technique has been developed to detect a number of intracellular metabolites. FT-MS as an ultra-high-resolution mass spectrometry is capable of detecting complexes of oligosaccharides in *Arabidopsis* (Penn et al. 1997) as well as thousands of metabolites in strawberry (Aharoni et al. 2002). Hirai et al. (2004) showed that several metabolic pathways in *Arabidopsis* leaves and roots were altered by nutritional stress such as a deficiency of sulfur or nitrogen. Furthermore, the improved methods such as a nuclear magnetic resonance (NMR) and gas chromatography mass spectrometry (GC/MS) have been contributed to analyze plant metabolism (Roessner et al. 2000; Sriram et al. 2004). The enormous amounts of data from these powerful analyzers enabled us to create the fingerprinting of plant metabolites. Thus, information obtained is highly effective in characterization of plant genes (Mele et al. 2003).

Recently, various proteome approaches such as high-resolution two-dimensional gel electrophoresis (2-DE), protein sequencing, and mass spectrometry have been

used for identifying the protein expression patterns. In the case of rice, Rakwal and Komatsu (2000) reported the results of proteome analysis of jasmonate-treated plants.

Here we performed metabolome analysis of transgenic rice by FT-MS as well as proteome analysis by 2-DE. We used transgenic rice plants and calli over-expressing *YK1* gene, the homolog of maize HC-toxin reductase (*HCTR*) in rice (Uchimiya et al. 2002).

Materials and methods

Plant materials and FT-MS analysis

Transgenic rice plants (*Oryza sativa* L. cv. Nipponbare) harboring empty vector (C) or *YK1* (L-1) were grown at 25°C in a green house. Leaf and panicle of two-month-old plants were collected and freeze-dried. Suspension cultured cells were grown in MS medium containing 3% sucrose, 1 mg/L 2,4-dichlorophenoxyacetic acid, B5 vitamin, and 50 µg/ml hygromycin at 27°C in dark. After 5 days, calli were washed twice with 40 ml of Milli-Q water and freeze-dried. Sample preparation and FT-MS analysis were basically performed according to Aharoni et al. (2002).

Proteome analysis

Protein extraction, 2-DE, and internal amino acid sequence analysis were performed as described by

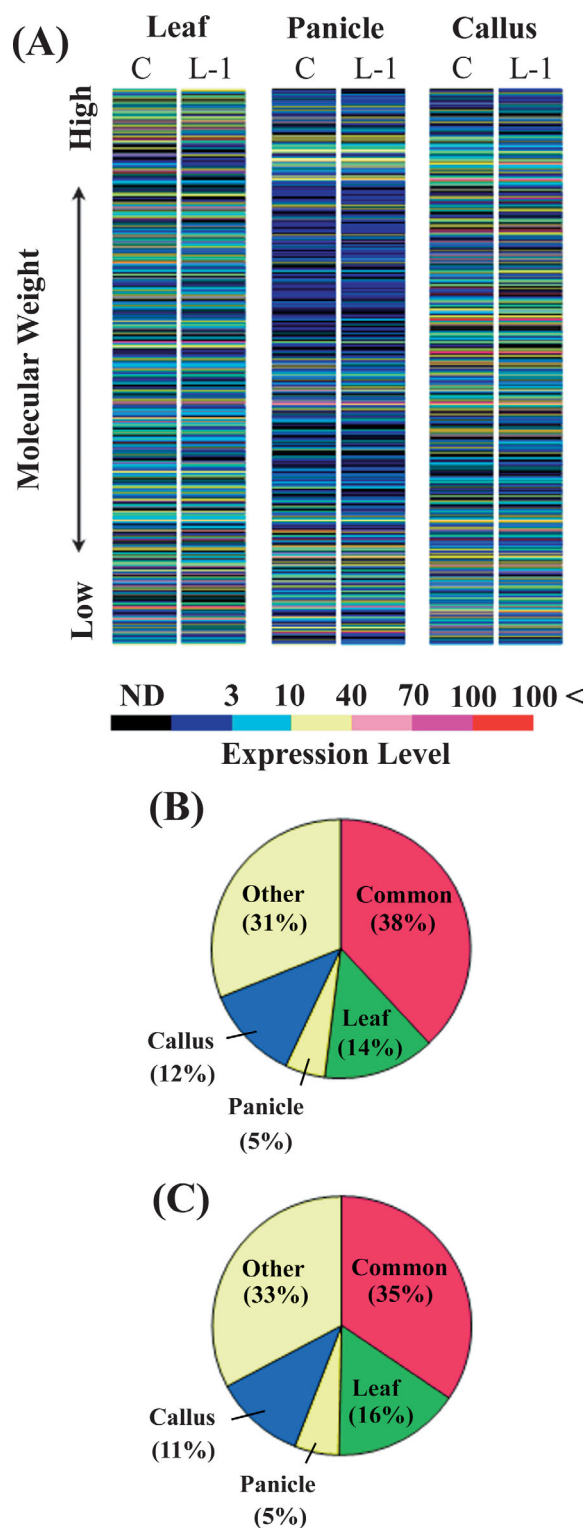


Figure 1. Comparison of metabolic fingerprinting in different organs between control (denoted by C) and *YKI* (L-1) rice. (A) Metabolites (866) detected were compared in leaf, panicle, and callus, respectively. Expression levels of metabolites were distinguished by seven different colors correlating to each signal to noise (S/N) ratio. Molecular weight range was approximately 1500 (high) to 120 (low). All data are average from three independent replicates. ND, not detected. (B) and (C) Comparison of organ-specific metabolites in control (B) and *YKI* (C) rice. Common means metabolites detected in all organs and tissues. Leaf, Panicle, and Callus indicate organ-specific metabolites.

Rakwal and Komatsu (2000). For measurements of approximate volume of spots, coomassie brilliant blue (CBB)-stained gels were scanned and the data were analyzed by 2D-Elite (Amersham Bioscience). Collected protein spots were subjected to amino acid sequencing and the homology search was carried out using the Rice BLAST database of National Institute of Agrobiological Resources, Japan.

Western blot analysis was performed as described in Uchimiya et al. (2002).

Results and discussion

Metabolome and proteome analyses were conducted to elucidate the effects of over-expression of *YKI* gene on

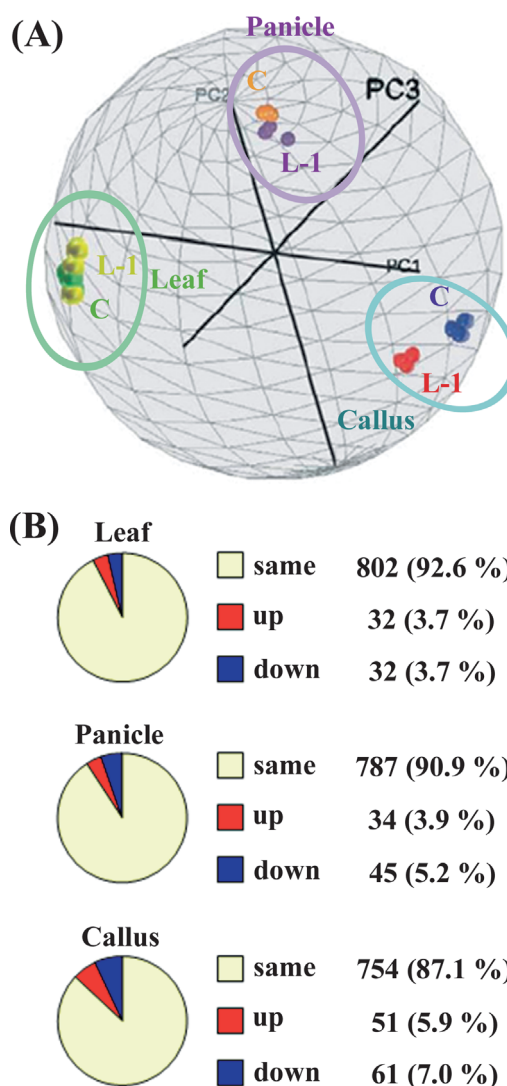


Figure 2. Metabolic alteration in *YKI* transgenic rice. (A) Principal component analysis in different organs of control (C) and *YKI* (L-1) rice. (B) Comparison of altered metabolite levels in individual tissues between the control and L-1 line. Number and percentage of metabolites were denoted. Up (red box) indicates up to 2-fold-increased metabolites against control and down (blue box) represents metabolites decreased by less than half level of control.

numerous metabolites and polypeptides. In respective organs, 866 metabolites were determined by FT-MS. The metabolic fingerprintings in callus, leaf, and panicle were significantly different from one another. Highly-expressed metabolites ($S/N > 100$) were also apparent in several organs. However, the difference between control and the *YK1* rice was not distinct (Figure 1A). The compositions of organ-specific metabolites in *YK1* line were similar to the control (Figures 1B, C).

Figure 2A shows the differences of main metabolites between control and *YK1* rice by a principal component analysis. The metabolic differences between control and *YK1* line were three-dimensionally visualized (Hirai *et al.* 2004). Principal components were slightly different between control and *YK1* callus, but those in leaf and panicle were nearly identical. Up-regulated metabolites were 5.9%, 3.7%, and 3.9% in callus, leaf, and panicle, respectively. On the other hand, down-regulated metabolites were 7.0%, 3.7%, and 5.2% in callus, leaf, and panicle, respectively (Figure 2B).

These results suggested that the compositions of metabolites were obviously different, but the over-expression of *YK1* had little effect on plant metabolism. However, since *YK1* rice conferred several stress tolerances (Uchimiya *et al.* 2002), there was a possibility that less than 10% of the metabolic alterations contributed to stress tolerances.

We also carried out proteomic analysis on cultured cells of *YK1* rice. Total polypeptides (668 spots) were detected on the gel (pH 3–10) and stained by coomassie brilliant blue. The images of the immobilized pH gradient (IPG, pH 6–10) gels are shown in Figure 3. Five spots in L-1 cells (a–e) were up-regulated relative to the control. Each spot was then subjected to amino acid sequence analysis. These spots coincided with the 40s ribosomal protein S1 (a), the osmotin-like protein (b and d), the *osr40c1* (c), and fructose-bisphosphate aldolase (e), respectively.

The cDNA clone *osr40c1*, encoding ABA-responsive protein associated with salt tolerance was isolated from

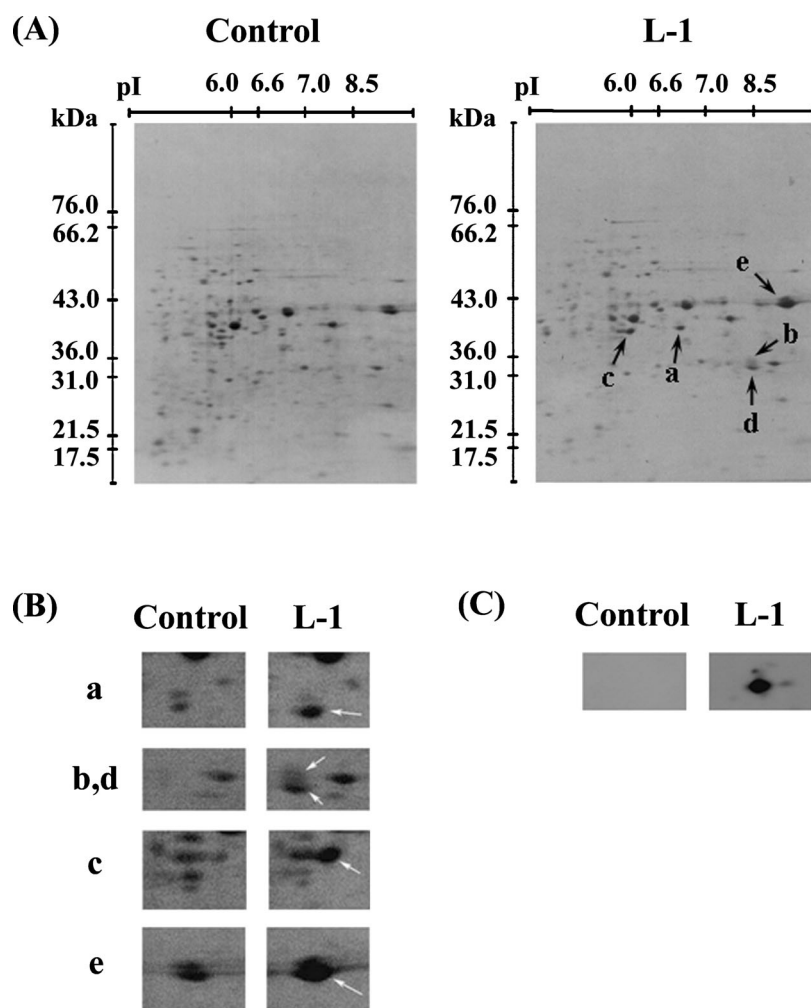


Figure 3. Proteome analysis of polypeptides differentially expressed in cultured cells of rice. (A) 2-DE images of L-1 sample separated by IPG gel (pH 6–10). (B) Upregulated (a–e) protein spots in L-1 cells (*YK1* line) over the control (5-day-old). The 40s ribosomal protein S1 (a), osmotin-like protein (b and d), the *osr40c1* (c) and fructose bisphosphate aldolase (e) were identified by amino acid sequences and rice BLAST database. (C) Immunological detection of *YK1* protein of control and L-1 callus.

roots of rice seedlings. Exogenously applied ABA and salt induced a marked increase of the *osr40cl* transcript level in roots, whereas constant *osr40cl* mRNA levels were found in the shoots (Moons et al. 1997). Furthermore, osmotin is a member of pathogen-related (PR) protein and known to be involved in plant defense responses. It has been reported that genes encoding osmotin-like proteins were induced by abiotic stresses including ABA and NaCl in potato (Zhu et al. 1995). Several stresses such as UV-irradiation and heavy metal also induced osmotin-like protein in rice (Rakwal et al. 1999). In *YK1* line, a clear appearance of *osr40cl* and osmotin-like protein though neither the salt nor the osmotic pressure were enforced was confirmed.

As a conclusion, our present results suggested that ectopic over-expression of a single gene (*YK1*) in rice cells might affect the expression of unrelated proteins and metabolites. This evidence may be important to extend our knowledge to the genetic engineering of plants with a novel foreign gene transfer.

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