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BRIEF COMMUNICATION

Protein analysis of dwarfed transgenic rice plants overexpressing GA2-oxidase gene

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Abstract

Using 2-D electrophoresis, we analyzed proteins from transgenic rice overexpressing gibberellin acid (GA) catabolic enzyme, GA2-oxidase. These results indicate eight specific proteins differentially expressed in the transformed rice stems of T_1 generation, but non in case of T_2 generation. Proteins isolated from different stages of leaves of T_1 generation showed no significant differences, except one-month-old leaf, where five differentially expressed proteins are visible.

Additional key words: dwarf plants, gene silencing, *Oryza sativa*, protein analysis, 2-D electrophoresis.

Gibberellins (GA) promotes various growth and developmental processes in higher plants. In recent years, cDNA clones encoding GA biosynthetic enzymes have been isolated from various plant species (Chiang *et al.* 1995, Lester *et al.* 1999, Martin *et al.* 1999). A number of GA-responsive mutants have also been isolated from maize, pea, tomato, *Arabidopsis* and rice (Hedden *et al.* 1997). Phenotypes caused by the reduced GA production in spontaneous mutants of *Arabidopsis* imply a role of GAs in stem elongation and flowering. The rice dwarf mutants in which GA is deficient have considerable agricultural significance (Sasaki *et al.* 2002). The overexpression of GA degradation enzyme genes, such as GA2-oxidase (EC1.14.11.13) could regulate the active GA content.

The aim of this work is to analyze proteins of transgenic rice expressing GA2-oxidase gene in comparison to those of non-transgenic rice. For this purpose, high-resolution two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) was applied, which is very useful for separating complex protein mixtures (O'Farrell 1979).

Previously we have isolated and characterized a cDNA, designated *Oryza sativa* GA2-oxidase 1 (*OsGA2ox1*) from rice (*Oryza sativa* L. cv. Nipponbare) that encodes a GA2-oxidase. Ectopic expression of the *OsGA2ox1* cDNA in transgenic rice inhibited stem elongation and the development of reproductive organs. These transgenic plants were deficient in endogenous GA1 (Sakamoto *et al.* 2001). From T_0 generation three transgenic plants (2-β 6,

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Abbreviations: GA - gibberellin; IEF - isoelectric focusing; PAGE - polyacrylamide gel electrophoresis.
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2-β 7 and 2-β 8) showing medium dwarf phenotype were selected and their seeds were sown in a greenhouse.

For protein extraction, 200 mg of leaf segments were homogenized in 0.5 cm^3 of lysis buffer containing 9.5 M urea, 2 % NP-40, 2 % ampholine (pH 3.5 - 10 and pH 5 - 8; equal amounts), 5 % mercaptoethanol, and 0.05 % polyvinylpyrrolidone (PVP-40), using a chilled glass mortar and pestle. The homogenates were transferred to a 1.5 cm^3 microtube, and centrifuged at 18 500 *g* once for 5 min. The supernatant was transferred and then centrifuged for 10 min, and the resulting supernatant was used as the crude protein extract. All the above steps were carried out at 4 °C. Fifty mm^3 of the supernatant was subjected to 2-D polyacryamide gel electrophoresis (PAGE).

For the 2-D PAGE, isoelectric focusing (IEF) in the first dimension was carried out in a capillary glass tube of 13 cm length and 3 mm diameter. Briefly, the gel solution consisted of 10 % NP-40, 30 % (m/v) acrylamide, 9.5 M urea and an equal mixture of 2 % carrier ampholytes (pH 3.5 - 10 and 5 - 8). The sample overlay buffer consisted of 20 cm^3 of half strength lysis buffer. Electrophoresis was carried out at 200 V for 30 min, followed by 400 V for 16 h and 600 V for 1 h. SDS-PAGE in the second dimension was performed using 15 % polyacrylamide gel and 5 % stacking gel at a constant current of 35 mA. The gels were stained with Coomassie Brilliant Blue. The 2D-PAGE experiments were repeated several times to confirm reproducibility, an important factor in this study.

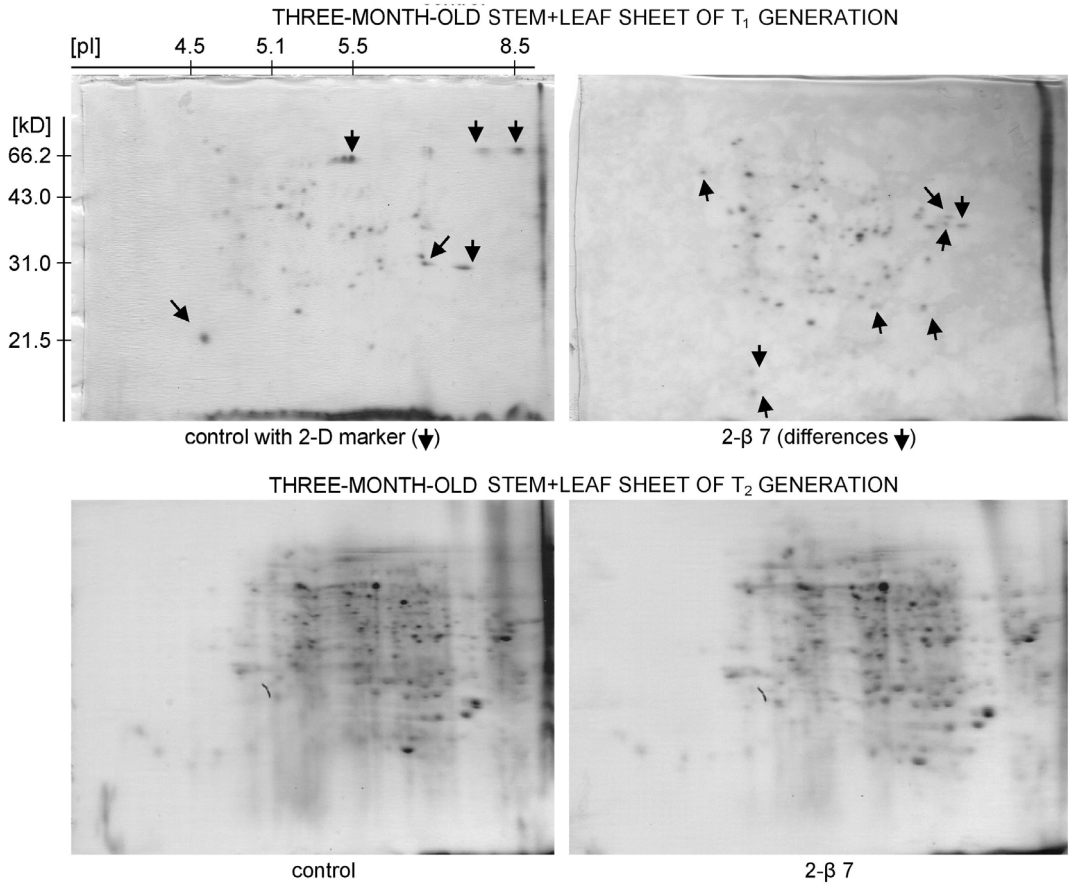

Fig. 1. Coomassie blue stained protein profiles of 2-DE. On upper two profiles, stem proteins of T_1 generation three-month-old control and 2-β 7 plants were loaded. On lower two profiles, stem and leaf sheath proteins of T_2 generation three-month-old control and 2-β 7 plants were loaded.

In case of T_1 generation plants, the stem proteins isolated from three-month-old 2-β 7 and control (cv. Nipponbare) plants were used for 2-D PAGE (Fig. 1). The control 2-D PAGE was performed with 2-D PAGE marker. These results indicate eight specific proteins differentially expressed in the transformed rice stems. We analyzed the T_2 generation plants also. The T_2 generation seeds of 2-β 7 plants were sown to obtain the plant material. As the growth of the T_2 generation plants were not so good, it was hard to obtain enough amount of stem tissues for 2-D PAGE analysis. Therefore, we used the leaf sheath together with the stems to obtain enough amounts of proteins for analysis. The stems plus leaf sheaths were isolated from three-month-old plants of T_2 generation

2-β 7 and from control plants. Even though the results obtained on T_1 generation indicated differentially expressed proteins in transgenic plants, the results shown in Fig. 1 did not indicate significant differences between control and 2-β 7 plants in T_2 generation.

Proteins isolated from different stages of leaf blades of control and transformed dwarf plants (2-β 7) of T_1 generation at three different developmental stages (one-month-old, two-month-old and three-month-old) showed no significant differences, except one-month-old leaf, where five differentially expressed proteins are visible (Fig. 2).

This work was carried out to analyze differences in protein expression between transformed rice plants and control. The stems and leaf sheaths isolated from three-month-old transformed plants were selected as main tissues for analysis, because most differences were predicted to appear in stems and probably in leaf sheaths as well. The overexpression of GA2-oxidase gene reduces GA content inducing dwarf phenotype of transformed

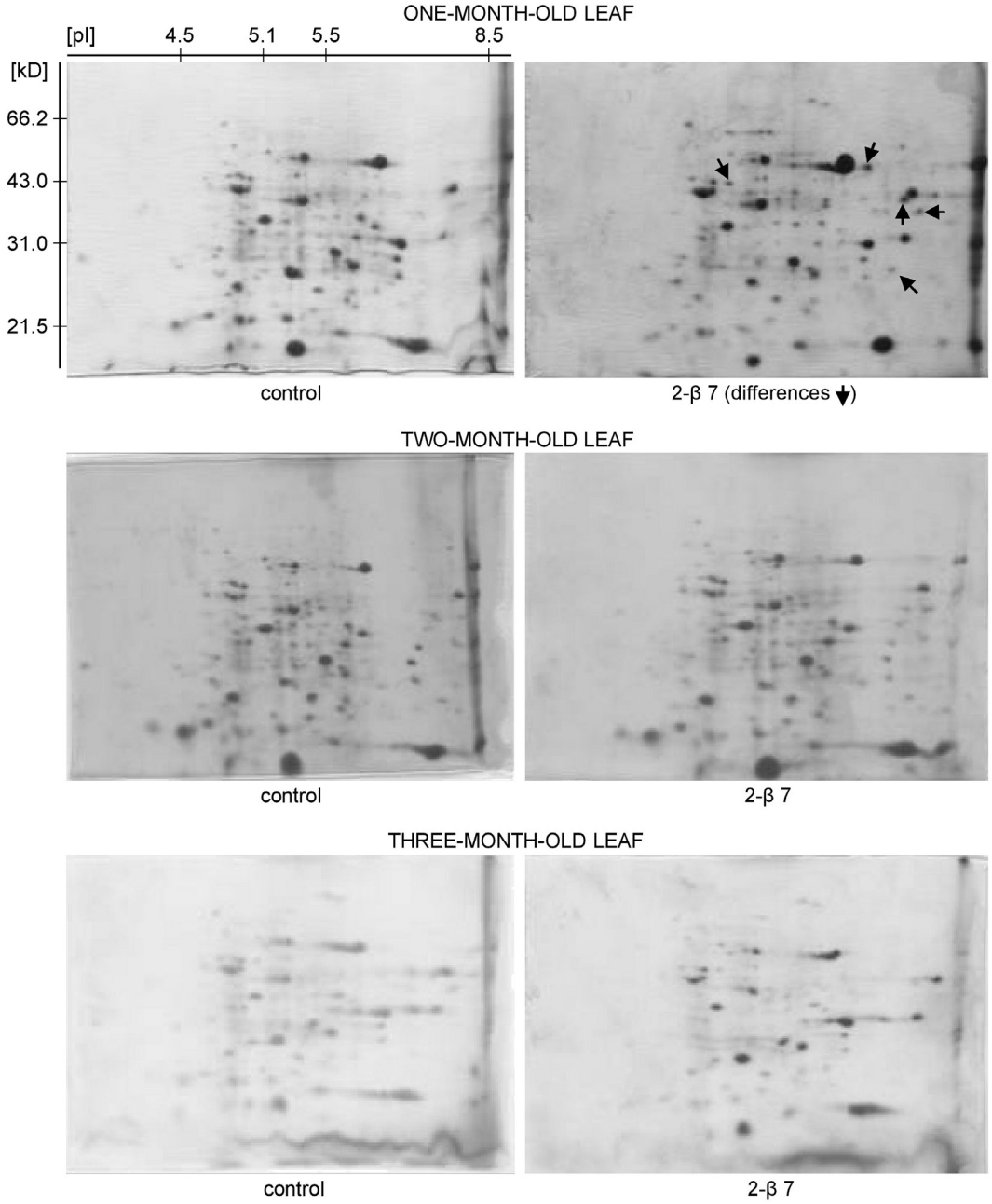

Fig. 2. Coomassie blue stained protein profiles of 2-DE. Leaf blade proteins of three different developmental stage (one-month-old, two-month-old and three-month-old) plants were loaded. Left side is control and right side is 2-β 7 plants of T_1 generation.

plants (Sakamoto *et al.* 2001). The leaves of one-month-, two-month-, three-month- and three-week-old plants were analyzed in this study as well. In total, three independent plant lines were selected from T_0 generation of transformed rice plants with GA2-oxidase gene.

The analysis of T_1 generation of 2-β 7 plants indicated some differentially expressed proteins in three-month-old stem and one-month-old leaf when compared to control. However, further analysis of T_2 generation of 2-β 7 (Fig. 1), did not reveal any visible changes in protein expression when 2-D PAGE was stained with CBB.

The transgenic plants in this paper are harboring the GA2-oxidase (molecular mass 40.6 kDa and pI 6.68) and hygromycin phosphotransferase genes (molecular mass 30 kDa and pI 4.77). The presence of inserted genes in the genome of analyzed plants was verified by PCR analysis using specific primers for the hygromycin resistance gene (data not shown). However, we were not able to detect the introduced two proteins on our 2-D PAGE profile. It means, they were not present in such amounts that allow detection by CBB staining. The introduced foreign gene did not express at such level that is possible to detect by CBB staining, even in case when the transgenic plant had dwarf phenotype.

Based on this study it is possible to conclude, that in our case, the transformation of rice plant did not cause conspicuous changes in gene expression of proteins when analyzed using the 2-D PAGE method stained with CBB. However, this is only one part of the story, because the characterization of differentially expressed proteins needs to be done in the future.

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