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Differential Expression of *C4H* and *F5H* Genes in Rice (*Oryza sativa* L.) after Gamma-irradiation

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Abstract - To reveal effects of gamma-irradiation with various doses on the expressions of *C4H* and *F5H* genes, the transcription levels of *OsC4HL* and *OsF5HL* were investigated in leaves and stems of two rice cultivars, Ilpoombyeo and IR-29, after the irradiation with 5, 10, 50, or 100 Gy for 4 h. In overall pattern of 24 h after the irradiation, the transcription levels of the two genes increased with the increasing doses of radiation in the leaves of both cultivars, except that of *OsC4HL* in IR-29. However, in the stems, the transcription level of *OsF5HL* increased in Ilpoombyeo and decreased in IR-29 dose-dependently, while that of *OsC4HL* decreased in Ilpoombyeo with the increasing doses of radiation and remained constant in IR-29. When the expressions of *OsC4HL* and *OsF5HL* were investigated in a time-course after the irradiation with 100 Gy, they reached their highest levels in the leaves of both cultivars 5 h and 72 h after the irradiation, respectively. Therefore, we suggest that the expressions of *OsC4HL* and *OsF5HL*, which involved in the same phenylpropanoid pathway, are differentially regulated during the post-irradiation period, showing different cultivar and tissue specificity. Furthermore, the dose dependency of the gene expressions is also discussed immediately after the irradiation.

Key words : *OsC4HL*, *OsF5HL*, Ilpoombyeo, IR-29, gamma-radiation

INTRODUCTION

The secondary metabolites, e.g., lignin, suberin, flavonoids, and other phenolic compounds, of the phenylpropanoid pathway, are involved in the differentiation and protection of plant tissues against environmental stresses (Hahlbrock and Scheel 1989; Frank *et al.* 1996; Mizutani *et al.* 1997; Gravot *et al.* 2004). Especially, their UV-B-absorbing nature and primary accumulation in the epidermal layers of leaves support their protective roles against UV-B in plants (Chapple *et al.* 1992; Day 1993).

Cinnamate-4-hydroxylase (C4H) is a key enzyme in the core phenylpropanoid pathway and catalyzes the *para*-hydroxylation of *trans*-cinnamic acid derived from phenylalanine (Fig. 1A). In contrast, ferulate-5-hydroxylase (F5H) catalyzes an irreversible hydroxylation step in the phenylpropanoid pathway that diverts ferulic acid away from guaiacyl lignin biosynthesis and toward sinapic acid and syringyl lignin (Chapple 1998; Meyer *et al.* 1998) (Fig. 1B). The expressions of *C4H* and *F5H* genes are induced by wounding and UV-B irradiation (Teutsch *et al.* 1993; Frank *et al.* 1996). Recently, we isolated and characterized cDNAs of rice *C4H* and *F5H* genes (Yang *et al.* 2005; Kim *et al.* 2006). Both the genes were differentially expressed in tissues, being inducible by the wounding treatment.

As an environmental stress factor, ionizing radiation affects cellular macromolecules, e.g., protein, DNA, and

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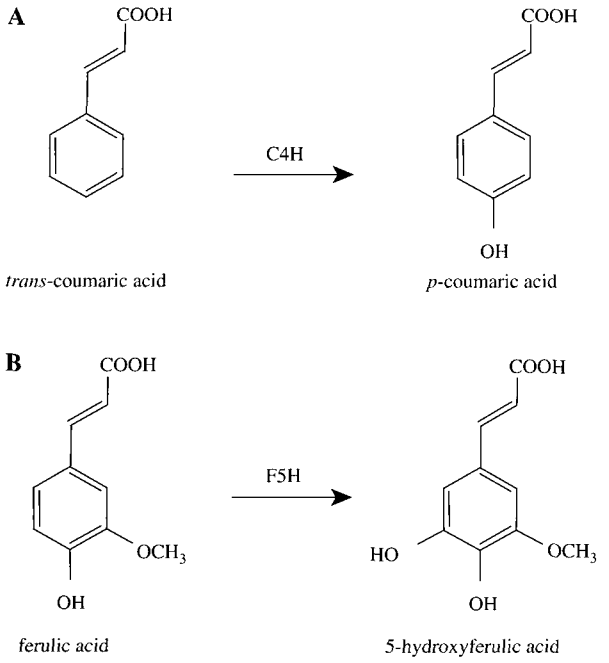

Fig. 1. Enzymatic action of *C4H* and *F5H*. A, The conversion of *trans*-cinnamic acid to *p*-coumaric acid catalyzed by cinnamate-4-hydroxylase; B, The conversion of ferulic acid to 5-hydroxyferulic acid by ferulate-5-hydroxylase.

lipid, causing loss of cellular functions (Casarett 1968; Luckey 1980; McLennan 1988; Wi *et al.* 2005).

However, it also shows a dose-response phenomenon which is characterized by a counterintuitive switchover from low-dose stimulation to high-dose inhibition (Calabrese 2002). Actually, our previous papers demonstrated that gamma-irradiation at the levels of several grays could improve the resistance of plants to environmental stress factors such as photoinhibition and UV-B (Lee *et al.* 2002, 2003; Kim *et al.* 2004).

In the present study, therefore, we attempted to reveal differential effects of gamma-irradiation with low to high doses on the expressions of *C4H* and *F5H* genes in rice plants. Accordingly, the transcription levels of *C4H* and *F5H* genes were investigated in leaves and stems of two rice cultivars, Ilpoombyeo (ssp. japonica) and IR-29 (ssp. indica), after the gamma-irradiation.

MATERIALS AND METHODS

1. Plant materials and gamma-irradiation

Seeds of two rice cultivars, *Oryza sativa* L. ssp. Japonica cv. Ilpoombyeo and ssp. Indica cv. IR-29, were used. The seeds of IR-29 were obtained from the IRRI (International Rice Research Institute, Los Baños, Philippines). Rice plants were hydroponically cultivated in a half-strength Murashige and Skoog (MS) nutrient solution for three months. The growth chamber was maintained at 28/20°C (D/N), under a 14-h photoperiod. Photosynthetic photon flux density (PPFD) at pot level was 330 μmol m^{-2} s^{-1}, supplied from two natrium lamps and six fluorescence lamps. Then, three-month-old plants were irradiated with various doses of gamma-radiation (0, 5, 10, 50, or 100 Gy). Radiation was generated by a gamma irradiator (^{60}Co, *ca.* 150 TBq of capacity, Atomic Energy of Canada Limited) at the Korea Atomic Energy Research Institute. The control and irradiated plants were transferred and maintained under the growth condition and then leaf and stem fragments (0.1 g) were collected 5 to 72 h after the gamma-irradiation. For the total RNA extraction, they were immediately frozen in liquid nitrogen and stored at −70°C until used.

2. RNA extraction and Reverse Transcription (RT) -PCR

Total RNA was isolated using the Trizol (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instruction. One microgram total RNA was reverse-transcribed in an RT system, AccuPower RT Premix (Bioneer, Daejeon, Korea) for 60 min at 42°C using 0.5 μg anchored oligo $(dT)_{18}$ V primers. Products of the first-strand cDNA synthesis reaction were amplified by PCR using *OsC4HL* specific primers forward (5′-AGTTTAGGCCGGAGAGGTTC-3′) and reverse (5′-TGTCCACCTTGTCCATCCC-3′) and *OsF5HL* specific primers forward (5′-ATCTCAACGGCATCGGTAAG-3′) and reverse (5′-TCTCTGGTCAGGTGGAGGTC-3′), which were designed from cDNA sequences of *OsC4HL* and *OsF5HL* (Yang *et al.* 2005; Kim *et al.* 2006). Amplification of *OsAct1* (Yamanouchi *et al.* 2002) as an internal control using forward (5′-TCCATCTTGGCATCTCTCAG-3′) and reverse (5′-GTACCCTCATCAGGCATCTG-3′) primers was performed to ensure that equal amounts of cDNA were added to each PCR reaction. The subsequent PCR was carried out with 1 μL of a total 20 μL RT reaction mixture in a PCR system, Perfect Premix ver. 2.1 (Takara Korea Biomedical Inc., Seoul, Korea) as follows: denaturation at 94°C for 5 min, 30 cycles of 94°C (30 s) −53°C (30 s) −72°C (30 s), and an extension at 72°C

for 7 min except for *OsAct1*, which was subjected to denaturation at 95°C for 5 min, 30 cycles of 95°C (30 s) −54°C (30 s) −72°C (40 s), and an extension at 72°C for 7 min. The resultant RT-PCR products were electrophoresed and analyzed on a 1.0% (w/v) agarose gel after staining with ethidium bromide (EtBr).

RESULTS AND DISCUSSION

Recently, we isolated and characterized rice *C4H* and *F5H* genes, *OsC4HL* and *OsF5HL*, which involve in the phenylpropanoid pathway (Yang *et al.* 2005a, b). Both the genes were differentially expressed in various rice tissues, being noticeably inducible by the wounding treatment. Similar results are also available in pea and Jerusalem artichoke (Teutsch *et al.* 1993; Frank *et al.* 1996). However, the expressions of Arabidopsis *C4H, phenylalanine ammonia lyase-1* (*PAL1*) and *4-coumarate CoA ligase* (*4CL*) genes were not tissue-specific (Mizutani *et al.* 1997). The induction of these genes in Arabidopsis followed the same time course after the onset of the light period or the wounding treatment, implicating a common mechanism for their transcriptional activation. For further study of *OsC4HL* and *OsF5HL*, therefore, we investigated differential effects of gamma-irradiation at from low to high doses on the expressions of *OsC4HL* and *OsF5HL* in leaves and stems of two rice cultivars, Ilpoombyeo (ssp. japonica) and IR-29 (ssp. indica).

Three-month-old plants of Ilpoombyeo and IR-29 were gamma-irradiated for 4 h with different doses of 5, 10, 50, and 100 Gy, and the expression levels of *OsC4HL* and *OsF5HL* in their leaves were evaluated in time-course experiments after the irradiation. Twenty four hours after the irradiation, both cultivars had higher transcription levels of *OsC4HL* and *OsF5HL* in the irradiation groups than in the control, showing dose dependence (Fig. 2). In overall pattern, the expression levels of the two genes were increased with 50 and 100 Gy of gamma-radiation except that of *OsC4HL* in the leaves of IR-29. However, in the stems of both cultivars, the expression patterns of *OsC4HL* and *OsF5HL* were more complex. The transcription level of *OsF5HL* increased in Ilpoombyeo and decreased in IR-29 dose-dependently 24 h after the irradiation, while that of *OsC4HL* decreased in Ilpoombyeo with the increasing doses of gamma-radiation and remained constant in IR-29 (Fig. 3). These results indicate that the expressions of *OsC4HL* and *OsF5HL* were affected by differences in the cultivar, tissue, and radiation dose. Especially, the existence of the cultivar and tissue specificity to the gamma-irradiation is suggested.

In relation to the dose dependence of the gene expression, it was also reported that expressions of diverse genes were differentially controlled responding to the gamma-irradiation with two different doses (Cho *et al.* 2000). Interestingly, in the present study, we observed a unique exp-

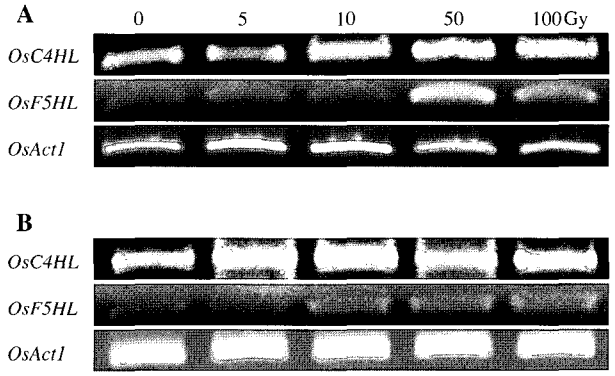

Fig. 2. Dose-dependent expressions of *OsC4HL* and *OsF5HL* in the leaves of Ilpoombyeo and IR-29 24 h after the irradiation. All data were generated by RT-PCR analysis using the leaf samples harvested at the indicated times as described in 'Materials and Methods'. *OsAct1* was amplified as an internal control to ensure that equal amounts of cDNA were added to each PCR reaction. A, Ilpoombyeo; B, IR-29.

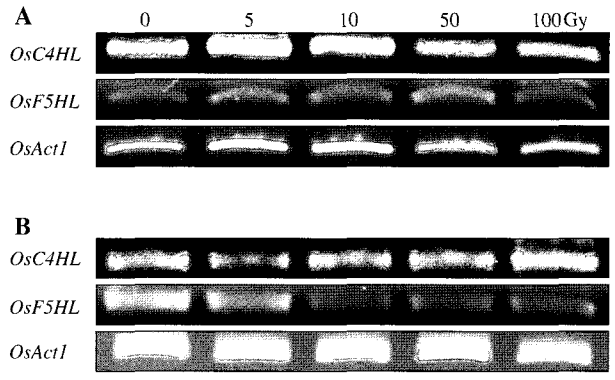

Fig. 3. Dose-dependent expressions of *OsC4HL* and *OsF5HL* in the stems of Ilpoombyeo and IR-29 24 h after the irradiation. All data were generated by RT-PCR analysis using the leaf samples harvested at the indicated times as described in 'Materials and Methods'. *OsAct1* was amplified as an internal control to ensure that equal amounts of cDNA were added to each PCR reaction. A, Ilpoombyeo; B, IR-29.

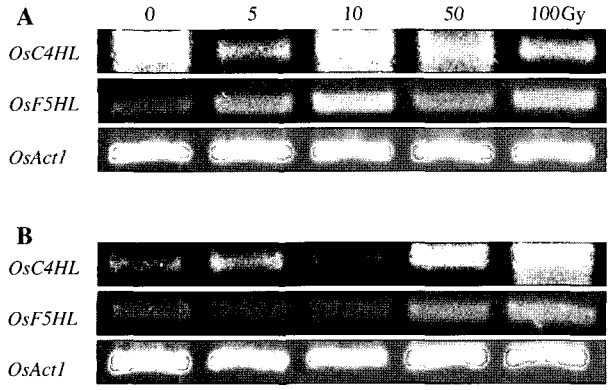

Fig. 4. Dose-specific expressions of *OsC4HL* and *OsF5HL* in the leaves and stems of Ilpoombyeo immediately after the irradiation. All data were generated by RT-PCR analysis using the leaf samples harvested at the indicated times as described in 'Materials and Methods'. *OsAct1* was amplified as an internal control to ensure that equal amounts of cDNA were added to each PCR reaction. A, leaves; B, stems.

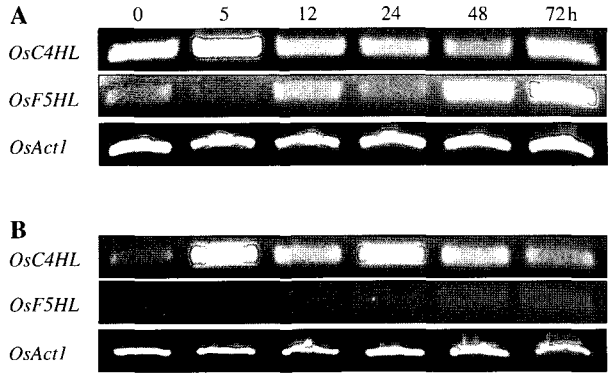

Fig. 5. Time-course expressions of *OsC4HL* and *OsF5HL* in the leaves of Ilpoombyeo and IR-29 after the irradiation with 100 Gy. All data were generated by RT-PCR analysis using the leaf samples harvested at the indicated times as described in 'Materials and Methods'. *OsAct1* was amplified as an internal control to ensure that equal amounts of cDNA were added to each PCR reaction. A, Ilpoombyeo; B, IR-29.

ression pattern of *OsC4HL* and *OsF5HL* in Ilpoombyeo immediately after the irradiation with various doses. The expressions of the two genes in leaves were most inducible by the 10-Gy irradiation, while those in stems were most noticeable after the 100-Gy irradiation (Fig. 4). This pattern was confirmed through a couple of independent experiments. These results may imply the possible existence of the dose dependency of the gene expression immediately after the irradiation rather than the linear dose dependence. Unlike Ilpoombyeo, the highest expressions of the two genes in IR-29 were observed in the 100-Gy groups of both the leaves and stems (data not shown). This supports the existence of the cultivar specificity to the gamma-irradiation as stated above.

In Fig. 5, the time-course expressions of *OsC4HL* and *OsF5HL* after the irradiation with 100 Gy were investigated in the leaves of Ilpoombyeo and IR-29. The *OsC4HL* and *OsF5HL* reached their highest expression levels in both the cultivars 5 h and 72 h after the irradiation, respectively. This pattern was also observable in the stems of the two cultivars (data not shown). Accordingly, it is suggested that the expressions of *OsC4HL* and *OsF5HL* in rice plants, although they involve in the same phenylpropanoid pathway, are differentially controlled after the irradiation at least in the long-term level.

In the present study, we analyzed the expression patterns of two phenylpropanoid pathway genes, *OsC4HL* and *OsF5HL*, in the irradiated rice plants, specifically leaves and stems. Although the RT-PCR analysis adopted in this study have many technical limitations, it can be useful for study of rarely expressed genes, e.g., *OsF5HL*. Therefore, through two or more independent or replicate experiments to obtain the reproducibility, we suggest that the expressions of *OsC4HL* and *OsF5HL*, which involve in the same phenylpropanoid pathway, are differentially regulated during the post-irradiation period, showing different cultivar and tissue specificity. Moreover, the dose dependency of the gene expressions observed immediately after the irradiation needs to be further analyzed in terms of enhanced physiological effects of specific radiation dose.

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