Isolation of the Novel Genes, *OsC4HL* and *OsF5HL*, Induced by Irradiation of Gamma-Ray in Rice (*Oriza sativa* L cv. Ilpoombyeo) Plants

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1. Introduction

Cytochrome P450-dependent monooxygenases (P450s) catalyze reactions of the general phenylpropanoid pathway [1]. The phenylpropanoid pathway provides a variety of secondary metabolites that are involved in differentiation and the protection of plant issues against environmental stresses. Among the P450s, C4H and F5H give rise to the important secondary metabolites (Figure 1) [2-3]. In the present study, we dealt with two P450s, C4H and F5H, in rice plants because they would be dose-dependent by gamma-radiation.

2. Materials and Methods

2.1 Plant Materials and Gamma-irradiation

Rice (*Oryza sativa* L. cv. Ilpoombyeo) plants were hydroponically cultivated in one half strength Murashige and Skoog (MS) nutrient solution in growth chamber. The growth chamber was maintained at 28/20°C (D/N) with a 14 h photoperiod. For gamma-radiation treatment, 3-month-old plants were exposed to 0, 10, 50, and 100 Gy gamma-ray, and then stem and leaf fragments (0.1g) were collected and frozen in liquid nitrogen for the total RNA extraction. The gamma-radiation was generated by a gamma irradiator (^{60}Co, *ca*. 150 TBq of capacity, AECL) in Korea Atomic Energy Research Institute.

2.2 Reverse Transcription (RT)-PCR Analysis and Rapid Amplification of cDNA Ends (RACE)

Total RNA was extracted using the Trizol (Invitrogen, Carlsbad, CA, USA). For RT-PCR analysis, Aliquot (5 μg) of total RNA was reverse transcribed using the RevertAim™ H Minus First Strand cDNA Synthesis Kit, according to the manufacturer's instruction. The products of the first-strand cDNA synthesis reaction were amplified by PCR. For 5′ RACE, oligo(dT)-primed cDNA was tailed and amplified using a 5′/3′-RACE kit (Roche Diagnostics, Indianapolis, IN, USA) and four additional *OsC4HL, OsF5HL* specific primers. *OsC4HL and OsF5HL* specific primers designed by comparing sequences of several C4H and F5H genes from *Arabidopsis thaliana*, *Camptotheca acuminate. Populus kitakamiensis* and *Sorghum bicolor*. Amplified fragments were cloned into the pGEM-T plasmid vector (Promega, Madison, WI, USA) and sequenced.

2.3 Southern Blot Analysis

Genomic DNA was extracted from young rice leaf material digested with restriction endonucleases (*Bam*HI, *Eco*RV and *Hind*III), electrophoretically separated, transferred to Hybond N^+ membrane, and hybridized with a ^{32}P-labeled cDNA fragment as a probe.

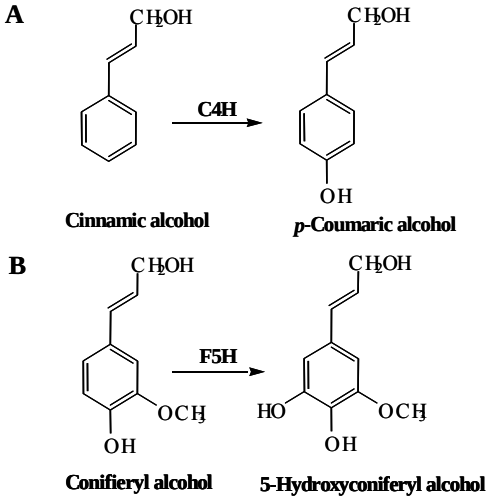

Figure 1. Activation sites of C4H and F5H. A. The conversion of cinnamic alcohol to *p*-coumaric alcohol is catalyzed by cinnamate-4-hydroxylase. B. The conversion of coniferyl alcohol to 5-hydroxyconiferyl alcohol is catalyzed by ferulate-5-hydroxylase.

3. Results and Discussion

3.1 Isolation of the Novel Genes Encoding OsC4HL and OsF5HL genes from Rice

The cDNA clones from rice were isolated using the RT-PCR and 5'/3' RACE method [4] and analyzed the DNA sequences. The sequences of the *OsC4HL* and *OsF5HL* cDNA clones revealed that these have open reading frame about 1.5kb. To gain insight for the function of the clones, we performed sequence homology

search. The BLAST searches revealed that the clones share high sequence homology with C4H and F5H from several plants such as *Sorghum bicolor*, *Citrus sinensis* and *Arabidopsis thaliana*.

These results suggested that the isolated cDNA clones represent *OsC4H*-like gene and *OsF5H*-like gene from *O. sativa* L., thus we named these genes *OsC4HL* (*Oryza sativa* L. *C4H*-like gene) and *OsF5HL* (*Oryza sativa* L. *F5H*-like gene).

3.2 Southern Blot Analysis

Southern blot analysis was performed to estimate the number of the genes such as *OsC4HL* and *OsF5HL*, in the whole rice genome. Genomic DNA was digested with three restriction enzymes, *Bam*HI, *Eco*RV and *Hind*III. As a probe for blotting, cDNA fragments of the *OsC4HL* gene and *OsF5H* gene were used. Only one band in each lane implied that these two genes exist as a single copy gene in the rice genome (Fig. 2).

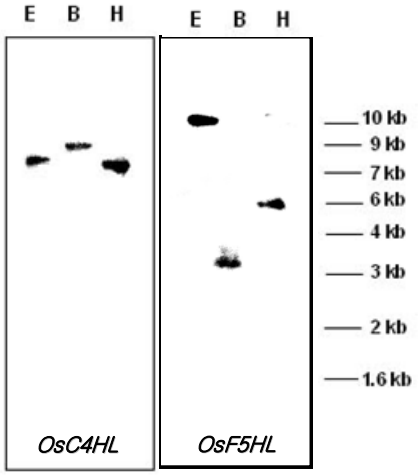

Figure 2. Genomic Southern blot analysis of the *OsC4HL* and *OsF5HL* in rice. E: *Eco*RV, B: *Bam*HI, H: *Hind*III

3.3 Analysis of Gene Expression by Gamma-irradiation

To determine whether the expression of the *OsC4HL* and *OsF5HL* affected *by Gamma-irradiation*, we performed RT-PCR. RT-PCR was prepared from these leaves indicated a induction, when gamma-radiation was 100 Gy. On the other hand, RT-PCR was prepared from these stems indicated a induction, when gamma-radiation was 10 Gy (Fig. 3). The results showed that *OsC4HL* and *OsF5HL* expression were dose-dependent expressed by g*amma-radiation* treatments.

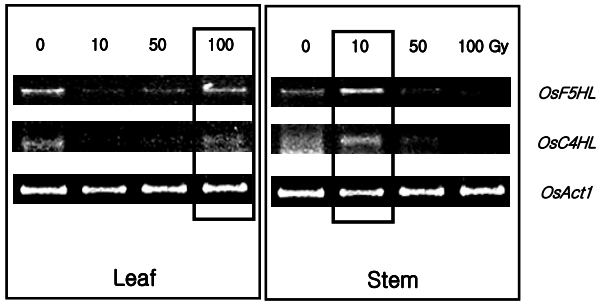

Figure 3. RT-PCR analysis of two gene. The different in the response patterns between the control and irradiated rice leaves and stem.

4. Conclusion

In conclusion, we found the two candidate rice P450s genes. They are each conserved in the most C4H and F5H genes. Base on these results, the effect of ionizing radiation would be stimulated the plant P450s gene expression such as cinnamate-4-hydroxylase (C4H) and ferulate-5-hydroxylase (F5H).

REFERENCES

[1] Chapple C, Molecular genetic analysis of plant cytochrome P450-dependent monooxygenases. Annu Rev Plant Physiol Plant Mol Biol 49 (1998) 311–343.

[2] Mizutani M, Ohta D, Sato R, Isolation of a cDNA and a geneomic clone encoding cinnamate-4-hydroxylase from Arabidopsis and its expression manner in planta. Plant Physiol 113 (1997) 755-763.

[3] Meyer K, Cusumano J, Somerville C, Chapple C, Ferulate-5-hydroxylase from Arabidopsis thaliana defines a new family of cytochrome P450-dependent monooxygenases. Proc Natl Acad Sci USA 93 (1996) 6869-6874.

[4] Frohman MA, Dush MK, Martin GR, Rapid production of full-length cDNAs from rare transcripts: amplification using a single gene specific oligonucleotide primer. Proc Natl Acad Sci USA 85 (1988) 8998-9002