Functional Characteristics of Genome-wide Rice Transcriptomes Responded to Gamma Radiation

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Abstract - Genome-wide transcriptomic profiles disclose physiomes or phenomes associated with diverse phenotypes in plants. Some transcriptomes associated with DNA damage response (DDR) in *Arabidopsis* are used for phytodosimetry of ionizing radiation (IR). This study reveals functional characteristics of genome-wide rice transcriptomes after gamma irradiation. Biological pathway analysis demonstrated that some genes responsible for histone modification and chromatin remodeling were notably affected in association with transcriptional regulation in rice after gamma irradiation. Gene ontology (GO) enrichment analysis further showed that the GO terms representing stimulus or stress were enriched commonly in both *Arabidopsis* and rice after gamma irradiation, while the cell cycle was more conspicuous in rice. In addition, the transcriptomic profile in rice after gamma irradiation revealed a substantial difference in expression of DDR genes compared to that in *Arabidopsis*, having about two times more genes downregulated than those upregulated. These results suggest that rice transcriptomes responded to gamma radiation should be functionally verified before being used for phytodosimetry of IR or correlated with diverse phenotypes after gamma irradiation.

Key words : Differentially expressed gene, Gamma radiation, Gene ontology, Rice, Transcriptome

INTRODUCTION

Ionizing radiation (IR) is a potent mutagen and sterilant for plant mutation breeding and agricultural produce quarantine (Kim *et al*. 2019). Genome-wide profiling studies of *Arabidopsis* transcriptomes responded to IR have helped to reveal many DNA damage response (DDR) genes in plants (Kim *et al*. 2013). A gene ontology (GO) analysis of genome-wide transcriptomes discovers potential impacts on biological pathways to determine a physiome or a phenome. Therefore, biomonitoring using transcriptomes can visualize nutrient status dynamics of rice plants under field conditions and is able to evaluate genotoxicity in *Arabidopsis* plants induced by IR (Ryu *et al*. 2018; Takehisa and Sato 2019). The latter application proved to be effective in biological dosimetry or phytodosimetry to monitor environmental risks of IR using the dicot model plant, *Arabidopsis* (Ryu *et al*. 2018).

Rice is a monocot model plant and is a representative crop cultivated in East Asia. Many improved rice cultivars have been developed using radiation-induced mutagenesis. However, there is a lack of data exploring the genome-wide rice transcriptomes responded to IR. Because rice has a bigger genome size and less DDR information compared to *Arabidopsis*, we need extensive transcriptome analysis and functional profiling study before effectively using rice transcriptomes for phytodosimetry. Recently, a transcriptome dynamics study using RNA-Seq data discovered the genes associated with different coleoptile elongation rates of six rice genotypes during submerged germination (Hsu and Tung 2017).

In this study, we performed GO enrichment analysis and biological pathway analysis for the genome-wide rice transcriptomes responded to gamma radiation using RNA-Seq

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data. The results will provide important insights into the genome-wide transcriptomic changes to determine diverse phenotypes in rice plants exposed to gamma irradiation.

MATERIALS AND METHODS

1. Plant materials and gamma irradiation

Japonica variety rice (*Oryza sativa* L. cv. Dongjin-byeo) seedlings were grown on Murashige and Skoog (MS) medium under a 16-h photoperiod at 25°C for 7 d. Seedlings were irradiated with gamma radiation at a dose rate of 60 $Gy \cdot h^{-1}$ for 5 h using a ^{60}Co gamma irradiator (IR-222, MDS Nordion Inc., Kanata, Canada) at the Advanced Radiation Technology Institute. Then, they were placed under the growth condition until harvested for RNA extraction 12 h after gamma irradiation.

2. RNA-Seq analysis

Total RNA was extracted from the whole rice seedlings using the RNeasy Plant Mini Kit (Qiagen, Stanford, CA, USA). An integrity check of total RNA was performed using an Agilent 2100 BioAnalyzer (Agilent Technologies, Santa Clara, CA, USA). mRNA sequencing libraries were prepared using the Illumina Truseq Stranded mRNA Library Prep Kit (Illumina, San Diego, CA, USA). Poly-A RNA containing mRNA was purified and fragmented from total RNA using poly-T oligo-attached magnetic beads. Complementary DNA (cDNA) was synthesized from cleaved RNA fragments using random hexamer primers, followed by a single "A" base addition and subsequent adapter ligation. The final strand-specific cDNA library was generated by PCR-based enrichment of cDNA fragments. Quality of the amplified libraries was verified by the Agilent 2100 BioAnalyzer. After qRT-PCR using the SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA), the index-tagged cDNA libraries were combined in equimolar amounts in the pool. Finally, RNA-Seq libraries were subjected to paired-end sequencing with a read length of 2×101 nucleotides on a HiSeq 2000 system (Illumina).

Quality assessment of the paired-end reads was conducted using the CLCAssemblyCell v4.2.0 (CLCbio-Qiagen, Aarhus, Denmark). After filtering, high-quality reads were mapped into the reference japonica Nipponbare genome IRGSP-1.0 using TopHat (Trapnell *et al*. 2009). The expression level of a gene from RNA-Seq reads was quantified using Cufflinks (Trapnell *et al*. 2012). Cufflinks assembled individual transcripts from RNA-Seq reads that were aligned to the genome sequences. Fragments per kilobase of exon per million reads (FPKM) were thenf calculated to quantify the expression level. After global normalization, principal component analysis (PCA) was applied to the obtained gene expression profiles using the GeneSpring GX software (ver. 12.5, Agilent Technologies).

3. Genome-wide transcriptome analysis

The differentially expressed genes (DEGs) with $\geq$2-fold change and $\leq$0.05 false discovery rate were considered significant and aligned to the MSU7 reference genome by the Rice Annotation Project Database ID Converter (http://rapdb.dna.affrc.go.jp/tools/coverter/). They were then subjected to biological pathway analysis in the MapMan software (ver. 3.6.0RC1) using Osa_MSU_v7 mapping files downloaded from the MapManStore server (http://mapman.gabipd.org/web/guest/mapmanstore/) (Usadel *et al*. 2009).

GO enrichment analysis was carried out on the above DEGs with the parametric analysis of gene set enrichment (PAGE) method using the web-based GO analysis toolkit "agriGo v2.0" (http://systemsbiology.cau.edu.cn/agriGOv2/) (Tian *et al*. 2017). To compare *Arabidopsis* and rice transcriptomes after gamma irradiation, the raw data of *Arabidopsis* transcriptome analysis was retrieved from the National Center for Biotechnology Information Gene Expression Omnibus database (http://www.ncbi.nlm.nih.gov/geo/; accession numbers GSE116647 and GSM3244781 to GSM3244790) (Ryu *et al*. 2019).

RESULTS AND DISCUSSION

1. Biological pathways of rice transcriptomes responded to gamma radiation

To get an overview of the genome-wide transcriptomic changes between control and irradiated rice plants, we performed biological pathway analysis using the MapMan software (Usadel *et al*. 2009). Genes associated with cell division, cell cycle, DNA synthesis, and cell organization were downregulated overall, while those involved in DNA repair and stress response were mostly upregulated (Fig. 1). Regulation of transcription was partly associated with DNA methylation, histone modification, and chromatin remodeling. For exam-

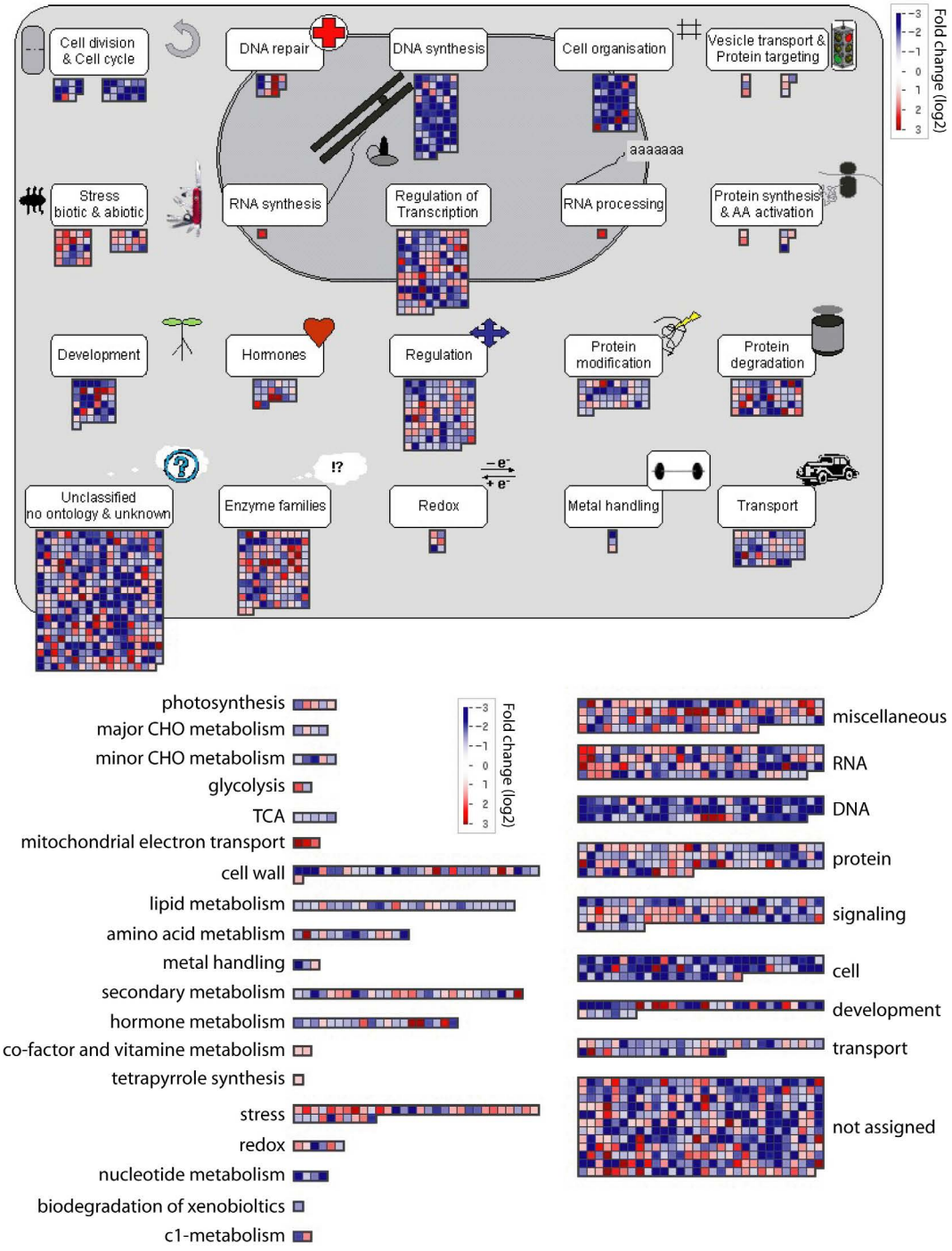

Fig. 1. Biological pathways that have responded to gamma radiation through transcriptional regulation. Overviews of biological pathways were produced by the MapMan software using differentially expressed rice transcriptomes after gamma irradiation as described in the "Materials and Methods" section. The $\log_2$ fold change between control and irradiated samples ranges from −3.5 (dark blue) to 3.5 (red).

ple, DNA methyltransferases MET1 (LOC_Os07g08500) and CMT3 (LOC_Os10g01570), histone deacetylase HDA1 (LOC_Os02g12350), and chromatin remodelers DDM1 (LOC_Os09g27060) and DRD1 (LOC_Os03g06920), were substantially affected by gamma irradiation, showing the $\log_2$ fold change values of −2.71, −3.63, 1.87, −2.42, and 1.57, respectively. DNA hypomethylation attributable to a decrease in transcription of *CMT3* after gamma irradiation was not associated with transcription levels of radiation-responsive genes in *Arabidopsis* (Kim *et al*. 2013a; Kim *et al*. 2017). In

contrast, histone H3 lysine 4 tri-methylation and H3 lysine 9 acetylation were associated with the transcriptional induction of DDR genes such as *AtCIPK11, AtRPA1E, AtGMI1*, and *AtRAD51* in *Arabidopsis* after gamma irradiation (Mondal *et al*. 2016). These results suggest that histone modification and chromatin remodeling may play an important role in regulating transcription in rice after gamma irradiation.

2. GO terms of rice transcriptomes responded to gamma radiation

Biological pathways responded to gamma radiation were further interpreted in terms of GO enrichment of genome-wide transcriptomes using the web-based GO analysis toolkit "agriGo v2.0" (Tian *et al*. 2017). The GO terms enriched after gamma irradiation were associated with biological process (BP) and molecular function (MF) in the GO category, but not with cellular component (CC) (Table 1). The GO terms representing response to stimulus or stress in BP were strongly enriched and upregulated, while those for DNA metabolic process or cell cycle were downregulated. The GO terms in MF were commonly downregulated. In addition, hierarchical structure analysis of the GO terms demonstrated that networks of metabolic process in BP and catalytic activity in MF as well as response to stimulus in BP were substantially affected by gamma irradiation (Fig. 2). Previous studies show that the GO terms associated with response to stimulus, nucleic acid metabolic process, and catalytic activity in *Arabidopsis* were strongly enriched after gamma irradiation (Kim *et al*. 2013b). Therefore, the GO terms representing response to stimulus or stress were enriched commonly in both *Arabidopsis* and rice after gamma irradiation, while the GO term for cell cycle indicated a more conspicuous cellular process of cell cycle arrest in rice. Interpretation of the GO enrichment reveals potential impacts on biological pathways associated with diverse phenotypes in rice after gamma irradiation.

3. Transcriptomes show differences in DDR to gamma radiation between *Arabidopsis* and rice

In *Arabidopsis*, the number of upregulated genes after gamma irradiation was similar or somewhat more than that of downregulated genes (Kim *et al*. 2013b). However, the genome-wide rice transcriptomic profile showed that the number of downregulated genes (901) was about two times more than that of upregulated genes (444) (Fig. 1). To investigate the differences in DDR between dicot and monocot plants, we therefore compared transcriptomic profiles obtained from *Arabidopsis* and rice after gamma irradiation. The results demonstrated that *RPA1* (*AtRPA1e* or *OsRPA1b*), *BRCA1*, and *CYCB1;1* genes were upregulated in *Arabidopsis* but downregulated in rice after gamma irradiation (Table 2). In addition, *AGO2, CIPK11*, and *PARP1* transcription was significantly increased only in *Arabidopsis*, while *PARP2, RAD51, NAC* (*AtNAC103* or *OsNAC75*), and *RAD17* genes were upregulated commonly in both *Arabidopsis* and rice. Although some of these DDR genes in *Arabidopsis* proved to be very effective in phytodosimetry to monitor the environmental risks of IR (Ryu *et al*. 2018), the corresponding genes in rice should be further verified before use in phytodosimetry of IR. In particular, functional differences between homologous genes need to be checked for *AtNAC103* and *OsNAC75* as well as for *AtRPA1e* and *OsRPA1b* (Eschbach and Kobbe 2014).

Table 1. GO enrichment analysis of genome-wide rice transcriptomes that have responded to gamma radiation. This analysis was performed using the web-based GO analysis toolkit "agriGo v2.0" as described in the "Materials and Methods" section. BP or MF represents biological process or molecular function, respectively. Z-score, standard score; and FDR, false discovery rate.

GO term	GO category	No. of genes	Description	Z-score	Mean	FDR
GO:0009607	BP	61	response to biotic stimulus	6.2	0.77	2.9e-08
GO:0006950	BP	183	response to stress	6	0.048	5.3e-08
GO:0009719	BP	64	response to endogenous stimulus	4.2	0.22	0.00032
GO:0019748	BP	32	secondary metabolic process	3	0.23	0.024
GO:0006259	BP	86	DNA metabolic process	−3.7	−1.7	0.0021
GO:0007049	BP	67	cell cycle	−4.3	−1.9	0.00022
GO:0030234	MF	29	enzyme regulator activity	−3	−2	0.0083
GO:0017111	MF	26	nucleoside-triphosphatase activity	−4.3	−2.5	0.0001
GO:0016818	MF	26	hydrolase activity, acting on acid anhydrides, in phosphorus-containing anhydrides	−4.3	−2.5	0.0001
GO:0016817	MF	26	hydrolase activity, acting on acid anhydrides	−4.3	−2.5	0.0001
GO:0016462	MF	26	pyrophosphatase activity	−4.3	−2.5	0.0001
GO:0003774	MF	26	motor activity	−4.3	−2.5	0.0001

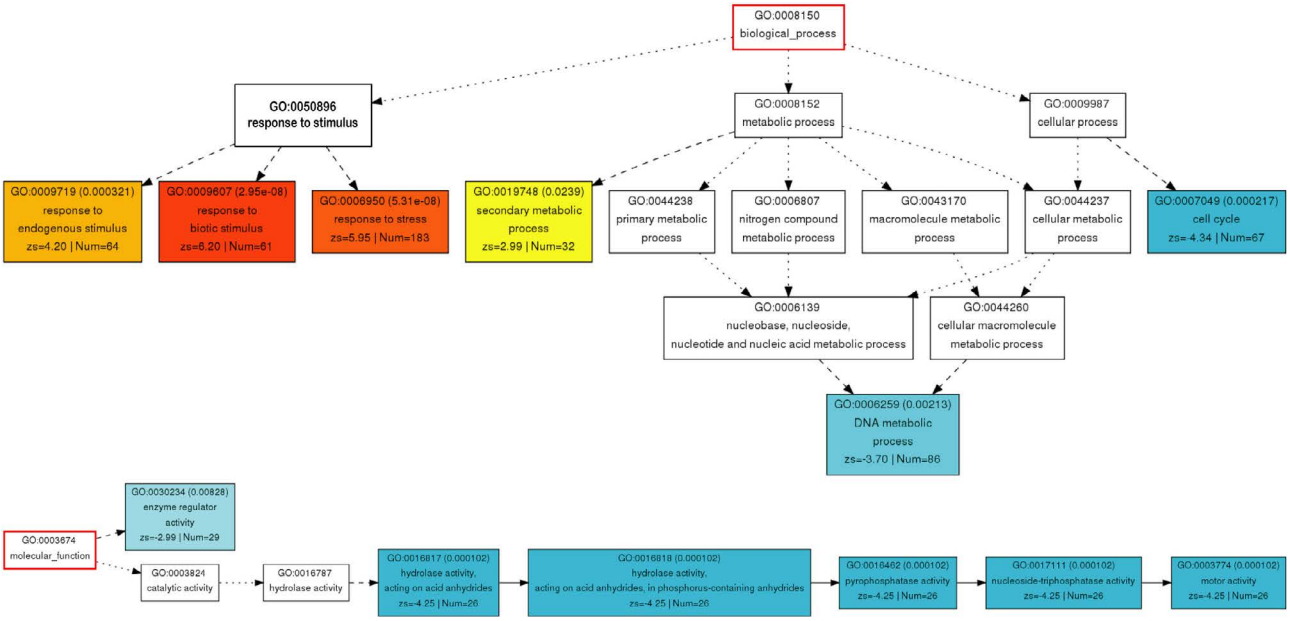

Fig. 2. Hierarchical structures of the GO terms that have responded to gamma radiation. The direct acyclic trees were produced by the web-based GO analysis toolkit "agriGo v2.0" as described in the "Materials and Methods" section. Numbers in parentheses represent false discovery rates. zs, Z-score; Num, number of genes.

Table 2. Differential expression of some DDR genes between *Arabidopsis* and rice after gamma irradiation. IDs represent *Arabidopsis* Genome Initiative or Rice Genome Annotation Project numbers, respectively. Transcription levels were expressed as a $\log_2$ fold change value between the respective control and irradiated samples. N.S., not significant due to $-1 < \log_2$ fold change < 1.

ID		Gene name	Description	Transcription	
Arabidopsis	Rice			*Arabidopsis*	Rice
At1g31280	LOC_Os04g52540	*AGO2*	Argonaute 2	1.17	N.S.
At2g30360	LOC_Os01g60910	*CIPK11*	CBL-interacting protein kinase 11	2.80	N.S.
At2g31320	LOC_Os07g23110	*PARP1*	Poly(ADP-ribose) polymerase 1	1.62	N.S.
At4g02390	LOC_Os01g24940	*PARP2*	Poly(ADP-ribose) polymerase 2	4.00	2.72
At4g19130	LOC_Os03g11540	*RPA1*	Replication protein A1 (AtRPA1e / OsRPA1b)	1.62	−3.23
At4g21070	LOC_Os05g43610	*BRCA1*	BRCT domain containing protein	3.29	−1.24
At4g37490	LOC_Os01g59120	*CYCB1;1*	G2/mitotic-specific cyclin B1;1	2.35	−4.72
At5g20850	LOC_Os12g31370	*RAD51*	Yeast RAD51 homolog	3.12	3.70
At5g64060	LOC_Os05g10620	*NAC*	NAC domain containing protein (AtNAC103 / OsNAC75)	3.73	2.95
At5g66130	LOC_Os03g13850	*RAD17*	Yeast RAD17 homolog	2.07	1.43

CONCLUSION

In this study, biological pathways and significant GO terms of rice affected by gamma irradiation were revealed from the genome-wide transcriptomic profile obtained using RNA-Seq analysis. The results will help characterize a physiome or a phenome associated with diverse phenotypes in rice plants exposed to gamma irradiation. However, the transcriptomic profile in rice after gamma radiation was shown to be considerably different from that in *Arabidopsis*. Therefore, rice transcriptomes responded to gamma radiation should first be verified by additional functional genomics studies before being used for phytodosimetry of IR or correlated with diverse phenotypes after gamma irradiation.

ACKNOWLEDGMENT

This study was supported by the Ministry of Science and ICT, Republic of Korea.

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Received: 2 February 2019
Revised: 27 February 2019
Revision accepted: 14 March 2019