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Epigenetic changes of *Arabidopsis* genome associated with altered DNA methyltransferase and demethylase expressions after gamma irradiation

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1. Introduction

DNA methylation at carbon 5 of cytosines is a hall mark of epigenetic inactivation and heterochromatin in both plants and mammals [1]. In *Arabidopsis*, DNA methylation has two roles that protect the genome from selfish DNA elements and regulate gene expression [2]. Plant genome has three types of DNA methyltransferase, METHYLTRANSFERASE 1 (MET1), DOMAIN-REARRANGED METHYLASE (DRM) and CHROMOMETHYLASE 3 (CMT3) that are capable of methylating CG, CHG (where H is A, T, or C) and CHH sites, respectively [2,3]. MET1 is a maintenance DNA methyltransferase that controls CG methylation [4]. Two members of the DRM family, DRM1 and DRM2, are responsible for *de novo* methylation of CG, CHG, and CHH sites but show a preference for CHH sites. Finally, CMT3 principally carries out CHG methylation and is involved in both *de novo* methylation and maintenance [3,4]. Alternatively, active DNA demethylation may occur through the glycosylase activity by removing the methylcytosines from DNA [5]. It may have essential roles in preventing transcriptional silencing of transgenes and endogenous genes and in activating the expression of imprinted genes. DNA demetylation in *Arabidopsis* is mediated by the DEMETER (DME) family of bifunctional DNA glycosylase. Three targets of DME are *MEA* (*MEDEA*), *FWA* (*FLOWERING WAGENINGEN*), and *FIS2* (*FERTILIZATION INDEPENDENT SEED 2*) [5]. The DME family contains DEMETER-LIKE 2 (DML2), DML3, and REPRESSOR OF SILENING 1 (ROS1). DNA demetylation by ROS1, DML2, and DML3 protect the hypermethylation of specific genome loci. ROS1 is necessary to suppress the promoter methylation and the silencing of endogenous genes. In contrast, the function of DML2 and DML3 has not been reported.

Several recent studies have suggested that epigenetic alterations such as change in DNA methylation and histone modification should be caused in plant genomes upon exposure to ionizing radiation. However, there is a lack of data exploring the underlying mechanisms. Therefore, the present study aims to characterize and explain the epigenetic modification of *Arabidopsis* genome after gamma irradiation.

2. Methods and Results

2.1 Plant materials and gamma-ray treatment

The wild type (WT) and *cmt3-11* mutant (SALK_148381) of *Arabidopsis thaliana* (ecotype Columbia) were grown in a growth chamber with a 16-h photoperiod at 22/18°C (day/night) for 4 weeks. Plants were irradiated with gamma rays at a dose rate of 1.25, 12.5, or 50 Gy h^{-1} for 4 h using a ^{60}Co gamma irradiator (IR-222, MDS Nordion Inc., Kanata, Canada) at the Advanced Radiation Technology Institute (ARTI). Then, they were placed under the growth condition and their rosette leaves were harvested for analysis at 1 and 5 d after the irradiation.

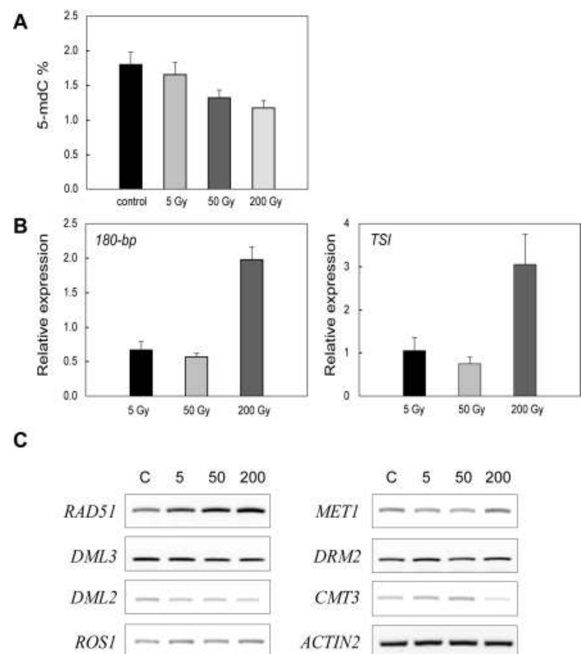

Fig. 1. Comparison of DNA methylation and gene expression depending on dose rates of gamma radiation.
(A) The amount of methylated DNA is proportional to the fluorescence intensity measured in the WT after gamma irradiation. (B) Expression analysis using transcriptional gene silencing markers *180-bp centromeric repeats* and *TSI* of the WT after gamma irradiation. Relative RNA levels were measured by quantitative real-time PCR and the values are normalized to *ACTIN2* expression. (C) The transcript level of DNA methyltransferase genes and demethyltransferase genes after treatment gamma-ray in the WT *Arabidopsis*. *RAD51*, *RAS ASSOCIATED WITH DIABETES PROTEIN 51*. *ACTIN2* was used as control.

2.2 Comparison of DNA methylation and gene expression depending on dose rates of gamma radiation

For quantification of DNA methylation, genomic DNA was digested into fragments of 100-600 bp using the dsDNA shearase™ and then purified. Global DNA methylation was estimated using the MethylFlash™ Methylated DNA Quantification Kit (Epigentek, Brooklyn, NY). For RT-PCR, the first strand cDNA was produced by Maxime RT PreMix Kit (iNtRON Biotechnology, Seongnam, Korea), and then used in PCR with Maxime PCR PreMix Kit (iNtRON). Alternatively, real-time quantitative PCR analysis of the same RT products was performed using the SYBR® Premix Ex Taq™ (Takara Bio Inc, Otsu, Japan) in a quantitative thermal cycler, ABI7300 (Applied Biosystems Inc., Foster City, CA). The relative transcript level was expressed as an expression ratio using the comparative ($2^{-\Delta\Delta Ct}$) method [6].

A quantification assay of methylated DNA revealed that genome-wide DNA methylation had substantially decreased after the irradiation of 200 Gy (Fig. 1A). In contrast, the expression levels of 180-bp centromeric repeats and transcriptionally silent information (*TSI)* were significantly increased after the irradiation (Fig. 1B). Among DNA methyltransferase genes, the transcript level of *CMT3* largely decreased after the 200-Gy gamma-irradiation, while that of *MET1* increased lightly. The transcript level of *DRM2* was not affected. In contrast, the transcript levels of DNA demethylase genes generally decreased after the irradiation, except for that of *ROS1* at 200 Gy (Fig. 1C).

2.3 Comparison of DNA methylation and gene expression between WT and cmt3 after gamma irradiation

As shown in Fig. 1, the decrease in the DNA methylation of the *Arabidopsis* genome after gamma-irradiation of 200 Gy can be associated with the decreased expression of *CMT3*, or the increased expression of *ROS1*. Therefore, when WT *Arabidopsis* and a *cmt3* mutant were irradiated with 200-Gy gamma rays, they showed significant differences in the DNA methylation status, the expression level of *180-bp centromeric repeats*, and the transcript levels of DNA methylase and demethylase genes (Fig. 2). Interestingly, deficiency of CMT3 in the *cmt3* mutant rather increased the DNA methylation of the genome and the expression level of *180-bp centromeric repeats* after gamma-irradiation (Fig. 2A and B).

3. Conclusions

In this study, the data revealed that the DNA methylation of *Arabidopsis* genome was decreased by gamma irradiation and that DNA methylation-mediated transcriptional gene silencing of pericentromeric repeats *180-bp* and *TSI* was substantially released by the 200-Gy gamma rays. Moreover, meaningful changes in the transcript levels of DNA methyltransferase and demethyltransferase genes were compared between the WT and the *cmt3* after gamma irradiation. Accordingly, we suggest that CMT3, ROS1 and MET1 should be associated with the change of the genome-wide DNA methylation in *Arabidopsis* after gamma-irradiation.

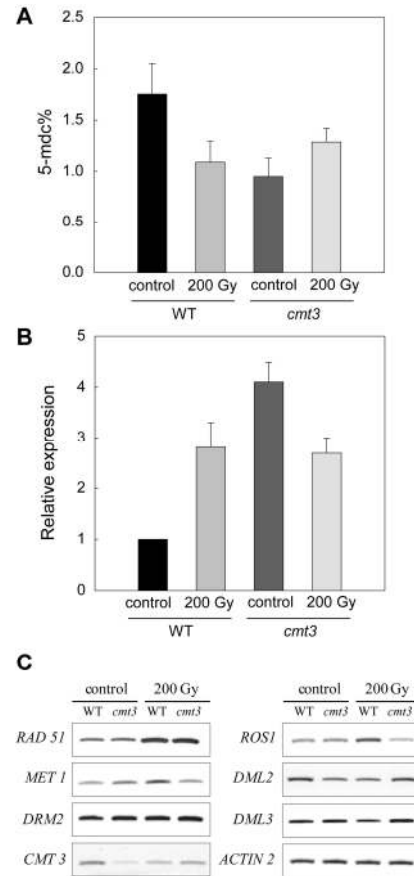

Fig. 2. Comparison of DNA methylation and gene expression between WT and *cmt3* after gamma irradiation.
(A) Quantification of methylated genomic DNA. (B) Relative expression level of *180-bp repeats*. (C) The transcript level of DNA methyltransferase genes and dimethyl-transferase genes after treatment gamma-ray.

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