Identification of γ-Radiation-responsive Proteins in *Arabidopsis thaliana*

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Abstract - Due to an increase in public concern about radiation after the Fukushima nuclear accident, an array of studies have been executed to understand the biological effects of radiation using model organisms such as *Arabidopsis* rather than humans. Although a large amount of information about the biological effect of radiation has been accumulated, most of the studies were performed at the transcriptional level. To systematically understand the biological effects of radiation, here, we performed proteome analysis using *A. thaliana* in the two vegetative stages [early (10-day-old) and late (21-day-old) vegetative stages] and identified radiation-responsive proteins. This analysis revealed that the proteins involved in the oxidative stress, molecular chaperone, and general stress responses showed an abundant change post γ-radiation exposure. Notably, we identified four proteins (acryl carrier protein 4, glycine cleavage system H protein 1, peptidyl-prolyl cis-trans isomerase CYP20-3, and calreticulin-1) that showed protein expression changes in both early and late vegetative stages after γ-radiation exposure. Furthermore, the Gene Ontology (GO) analysis revealed that proteins changed post γ-radiation exposure were mainly involved in protein binding activities and responses to stress. Collectively, we reported that proteins involved in oxidative stress and molecular chaperones showed an abundant change in response to radiation exposure in the vegetative stage of the *A. thaliana*.

Key words : *Arabidopsis thaliana*, Proteome, Radiation-response

INTRODUCTION

Radiation, which is the emission of energy in the form of electromagnetic waves from natural or man-made radioactive materials, affects living organisms in direct or indirect manners (Gutteridge 1999). Ionizing radiation directly interacts with the sugar backbone or the purine/pyrimidine base of DNA, and as a result, destroys the DNA structure (Close *et al*. 2013). In the indirect manner, ionizing radiation produces reactive oxygen species (ROS), including the hydroxyl radical through the radiolysis of water. Subsequently, the oxygen species has a fatal effect on cellular molecules, including DNA, proteins, and lipids, thereby leading cells to death (Gutteridge 1999; Close *et al*. 2013).

Due to the increased need for radiation breeding and environmental monitoring of radioactive sites (Kovalchuk *et al*. 1998), a plethora of studies have been conducted to understand the biological effects of radiation using *Arabidopsis thaliana* as a model plant. A high level of radiation (more than 100 Gy) induces growth inhibition, suppresses silique, radially expand root cells, elongates root hairs, and delays flowering in *A. thaliana* (Nagata *et al*. 2004; Kim *et al*. 2011;

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Hwang *et al*. 2016). To counteract these detrimental effects imposed by radiation exposure, plants increase the synthesis of ascorbic acid and anthocyanin (Nagata *et al*. 2003). In addition to the physiological changes in plants against radiation exposure, genome-wide transcriptome analyses have revealed that expression levels of genes involved in the ROS scavenging system, signal transduction pathway, nucleic acid metabolic process and secondary metabolism of isoprenoids are changed (Nagata *et al*. 2005; Kim *et al*. 2007; Kim *et al*. 2011; Kim *et al*. 2013; Hwang *et al*. 2016; van de Walle *et al*. 2016).

Although a plethora of transcriptome analyses have been performed to identify radiation-responsive markers and elucidate radiation responses, the transcriptome analyses are not sufficient to understand the biological processes in response to radiation exposure of *A. thaliana*. First, given that the post-translational modification process affects the half-life and activity of proteins, the transcription of a target gene does not always result in the protein synthesis of the gene in eukaryotes (Gygi *et al*. 1999; Griffin *et al*. 2002). Furthermore, the alternative splicing of mRNA often results in the production of protein isoforms with different functions (Kelemen *et al*. 2013). Therefore, the analysis of proteomes needs to understand biological phenomenon and cellular processes in response to γ-radiation.

In this present study, we performed proteomic analysis to identify radiation-responsive proteins depending on the growth stages [early (10-day-old) and late (21-day-old) vegetative stages]. We found that the production of proteins involved in the oxidative stress, molecular chaperones, metabolic pathways, and stress responses changed. This study is helpful for enhancing understanding of biological phenomenon in *A. thaliana* after radiation exposure.

MATERIALS AND METHODS

1. Plant growth and gamma radiation

The *Arabidopsis thaliana* (ecotype Columbia; Col-0) used in this study was grown in a growth room with 23°C with 16 h light/8 h dark cycles. For the early stage sample, the seeds were sterilized with ethanol and the bleach solution and then grown on a 1/2 Murashige and Skoog medium with 1.5% sucrose and 0.65% phyto agar for 10 days. For the late stage sample, the plants were grown on soil for 21 days. For ionizing the radiation, the plants were irradiated with gamma radiation at the indicated dose rate (12.5, 50, and 100 $Gy \cdot h^{-1}$ for 4 h) using a ^{60}Co source (IR-222, MDS Nordion Inc., Kanata, Canada) in the radiation facility housed in the Advanced Radiation Technology Institute (ARTI). After irradiation, the plants were incubated in the growth room to recover for 1 day. Then the samples were harvested, immediately frozen in liquid nitrogen, and kept at −80°C until analysis.

2. Total protein extraction

Total proteins were extracted with an extraction buffer (50 mM Tris-HCl (pH 7.2), 200 mM NaCl, and 0.25% Triton X-100) containing a protease inhibitor cocktail (Roche), 2 mM phenylmethanesulfonyl fluoride, 10 mM 2-chloroacetamide, 10 mM sodium metabisulfite, and 1 mM N-ethylmaleimide added just before use. The homogenate was filtered through two layers of Miracloth and one layer of cheesecloth and clarified at 12,000 × g for 20 min.

3. Mass spectrometry

To break and inhibit the formation of disulfide bonds, proteins (200 μg) were incubated with 10 mM dithiothreitol for 1 h and then alkylated with 50 mM iodoacetamide in the dark for 1 h followed by quenching the reaction with 200 mM dithiothreitol for 10 min. Samples were diluted 10-fold with 25 mM ammonium bicarbonate and digested using 2 μg of trypsin (Sequencing grade trypsin, Promega) for 12 hr at 37°C, followed by a second digestion for 6 hr with an additional 2 μg of trypsin. The digested samples were desalted using a C_{18} solid-phase extraction pipette tip (SPEC PT C_{18}, Varian), vacuum-dried, and reconstituted in 10 μl 5% acetronitrile and 0.1% formic acid in water for mass spectrometry analysis.

LC-MS/MS was performed with a microflow liquid chromatography system (Waters) coupled to an electrospray ionization FT/ion-trap mass spectrophotometer (LTQ Orbitrap Velos; Thermo Scientific). The LC separation was performed using a fused silica microcapillary column (15 cm × 365 μm o.d.) that was packed with a 3 μm diameter, 100 Å pore size and C_{18} beads (Magic C_{18}; Bruker). Peptides were separated on the C_{18} column with a gradient of 2 to 30% acetonitrile in 0.1% formic acid for over 150 min with a gradient of 2 to 30% acetonitrile in 0.1% formic acid at a flow rate of 200 $\mu l \cdot min^{-1}$. The samples were analyzed in the data-dependent positive acquisition mode using the normal scan rate for the precursor ion analysis, and dynamic exclusion was enabled. Following each full scan (300~1,500 m/z at a resolution of

60,000), data-dependent triggered MS/MS scans were collected for the 10 most intense parent ions.

4. Data analysis

RAW files were searched against the *A. thaliana* protein database (http://www.uniprot.org) using the MASCOT (version 2.4, Matrixscience) search algorithms through the Proteome Discoverer platform (version 2.1, Thermo Scientific) that assigns peptides for protein identification. The MS and MS/MS tolerance was set at 10 p.p.m and 0.8 Da, respectively. Cysteine carbamidomethylation and methionine oxidation were considered as fixed and variable modifications, respectively. Two missed trypsin cleavages were allowed to identify the peptide. Peptide identification was filtered by a 1% false discovery rate cut-off. A minimum of two unique peptides were used as a cut-off for protein assignment. Peptide spectrum matches were used to estimate the relative abundance between the control and treatment samples. Three independent biological samples were used for proteome analysis. Among these, one independent sample was excluded as an outlier.

RESULTS AND DISCUSSION

1. Proteome profiling of *A. thaliana* post γ-radiation exposure

To obtain information about radiation-responsive proteins in the early and late vegetative stages of *A. thaliana*, we analyzed changes in protein production after radiation exposure. The proteome data showed that the response to γ-radiation is distinct depending on the growth stages and radiation dose, which is similar to the transcriptome data performed in the vegetative and developmental (reproductive) stages (Kim *et al*. 2011). We classified the γ-radiation-responsive proteins depending on the change in the rations of more or less than 0.5-fold at least on the indicated dose of radiation compared to the non-exposure sample (Tables 1 and 2).

The ROS scavenging process reduces ROS such as superoxide radicals and hydroxyl radicals generated by radiohydrolysis. Similar to previous studies, the proteins involved in the ROS scavenging process changed post radiation exposure. The proteome revealed that the protein production levels of 2-Cys peroxiredoxin (AT3G11630; 2-Cys Prx A and AT5G06290; 2-Cys Prx B), peroxiredoxin II B (AT1G65980), and peroxiredoxin Q (AT3G26060) gradually decreased in response to radiation in the early stages, not in the late stages (Table 1). The data indicate that responses to γ-radiation in *A. thaliana* were distinct depending on the growth stage. Similarly, the enzyme activities of proteins such as peroxiredoxin and catalase were different between stages post-radiation exposure (Kim *et al*. 2011). Similar to peroxiredoxin, the protein levels of glutathoione S-transferases (AT1G78380 and AT2G47730) were decreased in the early stage. These phenomenon that proteins related to oxidative stress were down-regulated post γ-radiation exposure is also observed in the proteome data of rice (Hayashi *et al*. 2015). They found that peroxidase (Os02g0240300) and 2-cys peroxiredoxin BAS1 proteins significantly decreased in the rice. Therefore, the decrease in oxidative stress-related proteins, including peroxiredoxin is one of the defense mechanisms to ionizing radiation.

In contrast to peroxiredoxin and glutathione S-transferase proteins, the protein abundance of L-ascorbate peroxidase S increased (Table 1). Then ascorbate peroxidase mainly controls ROS levels in the different subcellular compartments, including the chloroplast, cytosol, and mitochondria (Caverzan *et al*. 2012). Supporting this notion, the expression levels of diverse types of L-ascorbate peroxidase genes (AT4G09010, AT1G77490, AT4G08390, and AT1G07890) changed after γ-radiation exposure (Kim *et al*. 2007). In addition to the oxidative stress response, the ascorbate peroxidase is involved in diverse abiotic stresses such as salt stress, high temperature stress, drought stress, and heavy metal stress (Caverzan *et al*. 2012). Therefore, not only the oxidative stress, but also other abiotic stresses caused by radiation exposure can lead to an increased ascorbate peroxidase.

Similar to the oxidative stress response-related proteins, the expression levels of molecular chaperone proteins changed post-radiation exposure (Tables 1 and 2). The chaperones prevent newly synthesized proteins from aggregating and assisted the unfolded proteins to be properly folded. The proteome data revealed that ERD14 (AT1G76180) and HSP70 (AT5G49910) proteins were increased post radiation exposure. This phenomenon is similar to the induction of heat shock proteins in rice after γ-radiation exposure (Hayashi *et al*. 2015). ERD14 is one of seven dehydrin proteins and is also known as a stress inducible protein in *A. thaliana* (Nylander *et al*. 2001). The HSP70 (AT5G49910) is already denoted as cpHsc70-2, which contributes to thermotolerance (Su and Li 2008; Latijnhouwers *et al*. 2010). These data indicate that chaperone proteins are needed to handle misfolded

Table 1. Identification of γ-radiation-responsive proteins in the early vegetative stage of *A. thaliana*

Accession ID[a]	Gene ID	Description	PSM[b] ratio			
			0 Gy/ 0 Gy	50 Gy/ 0 Gy	200 Gy/ 0 Gy	400 Gy/ 0 Gy
O03042	ATCG00490	Ribose diphosphate carboxylase large chain	1.00	0.53	0.34	0.43
Q8LCE1	AT1G66200	Glutamine synthetase cytosolic ribozyme 1-2	1.00	0.68	0.63	0.47
P36210	AT3G27830	50S ribosomal protein L12-1, chloroplast	1.00	0.64	0.73	0.28
Q9SW21	AT4G25050	Acyl carrier protein 4, chloroplast	1.00	0.88	0.59	0.41
O80860	AT2G30950	ATP-dependent zinc metalloprotease FTSH 2, chloroplast	1.00	0.69	0.58	0.42
P46283	AT3G55800	Sedoheptulose-1,7-bisphosphatase, chloroplast	1.00	0.72	0.67	0.47
P25697	AT1G32060	Phosphoribulokinase, chloroplast	1.00	0.66	0.63	0.43
Q96291	AT3G11630	2-Cys peroxiredoxin BAS1, chloroplast	1.00	0.79	0.45	0.50
P10795	AT1G67090	Ribose diphosphate carboxylase small chain 1A, chloroplast	1.00	0.69	0.43	0.45
Q9S726	AT3G04790	Probable ribose-5-phosphate isomerase 3, chloroplast	1.00	0.84	0.41	0.44
P22953	AT5G02500	Probable mediator of RNA polymerase II transcription subunit 37e	1.00	0.75	0.59	0.48
Q9C5R8	AT5G06290	2-Cys peroxiredoxin BAS1-like, chloroplast	1.00	0.85	0.45	0.48
P42737	AT5G14740	Beta carbonic anhydrase 2, chloroplast	1.00	0.62	0.42	0.50
P34791	AT3G62030	Peptidyl-prolyl cis-trans isomerase CYP20-3, chloroplast	1.00	0.59	0.48	0.45
P42644	AT5G38480	14-3-3-like protein GF14 psi OS = Arabidopsis thaliana	1.00	0.83	0.65	0.43
Q9XEX2	AT1G65980	Peroxiredoxin-2B OS = Arabidopsis thaliana	1.00	0.90	0.71	0.38
Q9XI01	AT1G21750	Protein disulfide isomerase-like 1-1	1.00	0.92	1.00	0.32
P56779	ATCG00580	Cytochrome b559 subunit alpha	1.00	0.63	0.42	0.53
Q9FKW6	AT5G66190	Ferredoxin-NADP reductase, leaf ribozyme 1, chloroplast	1.00	0.77	0.54	0.46
P25855	AT2G35370	Glycine cleavage system H protein 1, mitochondrial	1.00	1.00	0.79	0.42
P27140	AT3G01500	Beta carbonic anhydrase 1, chloroplast	1.00	0.65	0.43	0.52
O65581	AT4G26530	Fructose-diphosphate aldolase 5, cytosolic	1.00	0.71	0.67	0.43
Q9LU86	AT3G26060	Peroxiredoxin Q, chloroplast	1.00	0.73	0.96	0.46
Q9FKA5	AT5G39570	Uncharacterized protein At5g39570	1.00	1.10	0.86	0.43
P24101	AT3G49110	Peroxidase 33	1.00	1.11	1.56	1.33
Q93YR3	AT4G22670	FAM10 family protein At4g22670	1.00	0.86	0.62	0.48
Q9M158	AT4G01050	Rhodanese-like domain-containing protein 4, chloroplast	1.00	0.76	0.59	0.29
P25819	AT4G35090	Catalase-2	1.00	0.61	0.56	0.39
P31169	AT5G15970	Stress-induced protein KIN2	1.00	1.45	2.00	1.45
Q9ASV6	AT3G15190	30S ribosomal protein S20, chloroplast	1.00	1.30	1.40	0.40
P16127	AT4G18480	Magnesium-chelatase subunit ChlI-1, chloroplast	1.00	0.76	0.65	0.41
O04151	AT1G56340	Calreticulin-1	1.00	0.79	0.57	0.36
Q9ZRW8	AT1G78380	Glutathione S-transferase U19	1.00	0.67	0.42	0.42
Q96266	AT2G47730	Glutathione S-transferase F8, chloroplast	1.00	0.67	0.58	0.33
P42763	AT1G76180	Dehydrin ERD14	1.00	1.43	2.00	2.14
Q29Q26	AT2G17390	Ankyrin repeat domain-containing protein 2B	1.00	1.50	1.70	1.00
Q42592	AT4G08390	L-ascorbate peroxidase S, chloroplast/mitochondrial	1.00	1.43	1.71	1.29
Q9SJ12	AT2G21780	Probable ATP synthase 24 kDa subunit, mitochondrial	1.00	2.25	1.50	1.63

[a]Accession ID was obtained from Uniprot database (http://www.uniprot).
[b]PSM: the number of peptide spectrum matches

or unfolded proteins induced by radiation. Protein disulfide isomerases (PDIs) are involved in diverse cellular processes, including protein folding and redox signaling through formation or breakage of disulfide bonds with unfolded proteins as substrates (Wilkinson and Gilbert 2004; Ali Khan and Mutus 2014). Notably, the production of PDIs (AT1G21750 and AT2G47470) in 50 Gy and 200 Gy exposures were indistinguishable from non-radiation treatment. However, upon 400 Gy exposure, these proteins were significantly reduced in early and late vegetative stages, respectively. It implies that the suppression of PDIs appears to be required for dealing with stress responses imposed by a high dose of radiation exposure (e.g., more than 400 Gy). Moreover, the proteome analysis revealed that CYP20-3, which is also designated as ROC4 and has peptidyl-prolyl cis-trans isomerase activity (Romano *et al*. 2004), decreased in the early vegetative stage while it was elevated in the late vegetative stage (Fig. 1). The folding activity of CYP20-3 increased through a redox-dependent disulfide bond formation mediated by thioredoxin under oxidative stress (Motohashi *et al*. 2003). The impairment of CYP20-3 leads to hypersensitivity in response to oxidative stresses such as high light condition and salt stress (Dominguez-Solis *et al*. 2008), which suggests that CYP20-3 controls cellular resistance in response to abiotic stress. In

Table 2. Identification of γ-radiation-responsive proteins in the late vegetative stage of *A. thaliana*

Accession ID[a]	Gene ID	Description	PSM[b] ratio			
			0 Gy/ 0 Gy	50 Gy/ 0 Gy	200 Gy/ 0 Gy	400 Gy/ 0 Gy
Q9S841	AT3G50820	Oxygen-evolving enhancer protein 1-2, chloroplastic	1.00	0.56	0.48	0.40
Q41932	AT4G05180	Oxygen-evolving enhancer protein 3-2, chloroplastic	1.00	1.70	2.37	2.22
Q9FZ47	AT1G16880	ACT domain-containing protein ACR11	1.00	1.74	1.11	1.19
Q9LTX9	AT5G49910	Heat shock 70 kDa protein 7, chloroplastic	1.00	1.60	1.40	1.48
Q9SW21	AT4G25050	Acyl carrier protein 4, chloroplastic	1.00	0.71	0.59	0.35
O22160	AT2G44920	Thylakoid lumenal 15 kDa protein 1, chloroplastic	1.00	2.00	1.43	1.43
Q03250	AT2G21660	Glycine-rich RNA-binding protein 7	1.00	0.88	0.41	0.88
Q9LXC9	AT5G09650	Soluble inorganic pyrophosphatase 6, chloroplastic	1.00	0.44	0.88	0.56
Q9SEU6	AT3G15360	Thioredoxin M4, chloroplastic	1.00	1.44	1.56	1.22
Q9LQL0	AT1G32470	Glycine cleavage system H protein 3, mitochondrial	1.00	1.71	1.43	1.86
P25855	AT2G35370	Glycine cleavage system H protein 1, mitochondrial	1.00	1.22	1.22	1.56
O22263	AT2G47470	Protein disulfide-isomerase like 2-1	1.00	0.93	0.93	0.36
Q9SZY1	AT4G10480	Nascent polypeptide-associated complex subunit alpha-like protein 4	1.00	0.38	0.92	1.00
P57752	AT1G31812	Acyl-CoA-binding domain-containing protein 6	1.00	1.63	1.25	1.13
Q42418	AT4G29350	Profilin-2	1.00	0.40	0.20	0.24
P34791	AT3G62030	Peptidyl-prolyl cis-trans isomerase CYP20-3, chloroplastic	1.00	1.00	1.33	1.83
Q43127	AT5G35630	Glutamine synthetase, chloroplastic/mitochondrial	1.00	0.47	0.40	0.33
P56780	ATCG00710	Photosystem II reaction center protein H	1.00	1.67	1.33	1.50
O04151	AT1G56340	Calreticulin-1	1.00	3.00	1.50	2.25

[a]Accession ID was obtained from Uniprot database (http://www.uniprot).
[b]PSM: the number of peptide spectrum matches

conclusion, molecular chaperones appear to be required for damaged proteins imposed by ionizing radiation.

One interesting finding in this analysis is that the production of proteins related to metabolic pathways changed. Of them, glutamine synthetases (AT1G66200 and AT5G35630), which are involved in the nitrogen acquisition and amino acid metabolism, were reduced in the early and late vegetative stages, respectively (Tables 1 and 2). Similar to this result, the transcript levels of genes related to nitrogen acquisition and amino acid metabolism were generally downregulated in post radiation exposure in eukaryotes (Robertson *et al*. 2012; Jung *et al*. 2016). The glutamine synthetase plays a role in converting glutamate with ammonium, which is a nitrogen source and assimilated from the soil, into glutamine by hydrolyzing ATP. Among the six glutamine synthetase genes in the *A. thaliana* genome, *GLN1;2* (AT1G66200) plays major roles in assimilating ammonium (Lothier *et al*. 2011; Guan *et al*. 2016). Other than nitrogen metabolism, the roles of glutamine synthetases are involved in abiotic stresses, including salt and cold shock (Hoshida *et al*. 2000; Cai *et al*. 2009), which suggest that nitrogen metabolism mediated by glutamine synthetase contributes to resistance to external diverse stresses. Therefore, the change in the nitrogen metabolism mediated by glutamine synthetase might be a defense mechanism in response to stress responses caused by radiation exposure in plants.

Previous studies have reported that high levels of radiation can damage the cellular structure of plants and reduce the chlorophyll contents (Angelini *et al*. 2001). In accordance with the previous study, transcripts and proteins related to photosynthetic process had decreased in rice post radiation exposure (Hayashi *et al*. 2014; Hayashi *et al*. 2015). Supporting this notion, the proteome data showed that the proteins related to the photosynthesis process such as ribulose bisphosphate carboxylase large chains (ATCG00490) and small chains (AT1G67090), which are components of Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase), decreased in the early vegetative stage (Table 1). The expression level of ACP4, which is responsible for regulating the biosynthesis of fatty acids in the chloroplast membrane lipids, is known to be correlated with the photosynthetic status (Branen *et al*. 2003). Our study revealed that ACP4 (acryl carrier protein 4) production decreased in both the early and late vegetative stages (Fig. 1). Photorespiration is the initiated uptake of O_2, which is a byproduct of photosynthesis, and ultimately produces H_2O and CO_2 by consuming ATP and NAD (P)H. As the radiation reduces the photosynthesis activity, the proteins related to the photorespiration system decreased (Hayashi *et al*. 2014; Hayashi *et al*. 2015). The glycine cleavage system (GCS) plays a role in converting glycine to serine in the mi-

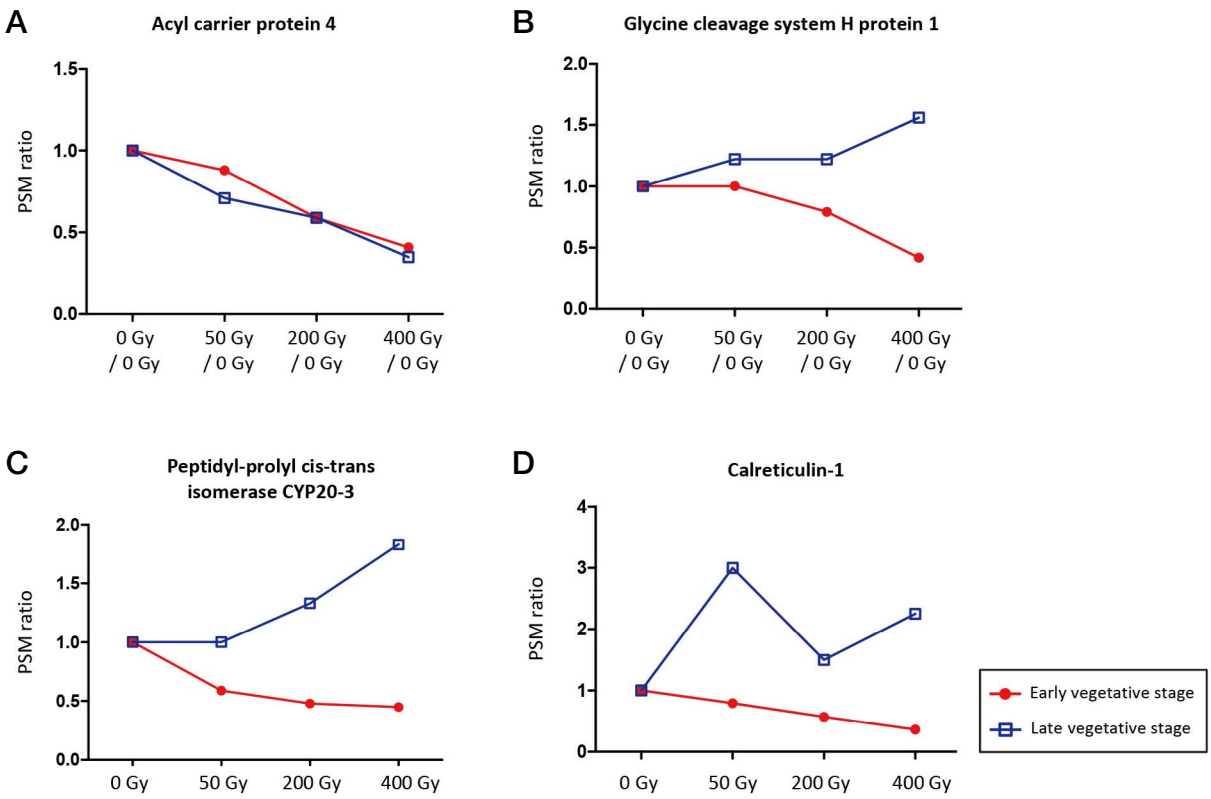

Fig. 1. Identification of γ-radiation-responsive proteins in both early and late vegetative stages. PSM indicates the number of peptide spectrum matches.

tochondria, which is involved in the photorespiration (Hanson and Roje 2001). This proteome revealed that the glycine cleavage system H protein 1, which is one component of the GCS, was downregulated in the early vegetative stage (Fig. 1). Taken together, the decreased levels of ACP4 and glycine cleavage system H appear to be derived from reducing the photosynthesis caused by radiation exposure.

Given that radiation affects the living organisms through both direct and indirect manners, diverse defense mechanisms coincide to alleviate these stresses. Supporting this notion, we found that proteins involved in the stress response showed changes in expressions. Intracellular and extracellular signals induce the remodeling of the actin structure, which affects diverse cellular processes in plants. In particular, the actin depolymerization is involved in the enhancement of ROS in the root under NaCl treatment (Liu *et al*. 2012). Our proteome results showed that the protein level of profilin-2 (AT4G29350), which regulates the polymerization and depolymerization of actin, tends to decrease post radiation exposure (Table 2). It has been known that a low intracellular level of profilin enhances the depolymerization of actin. It implies that a reduced level of profilin can prevent the increase of ROS by depolymerizing actin. In response to the external stress, cellular signal cascades are activated by intracellular signaling mole cules. The concentration of calcium, which is regarded as a second messenger, can change to activate defense systems. In harmony with this notion, the calcium-mediated responses post radiation exposure have been observed in other eukaryotic systems (Hallahan *et al*. 1994; Yi *et al*. 2017). The calcium is indispensable for activating protein kinase C following radiation exposure in humans. In addition, calreticulin (CRT), which is an ER-localized calcium-binding protein that regulates ER homeostasis through the calreticulin/calnexin cycle, is up-regulated in mouse liver tissues post the low dose of radiation exposure (Hallahan *et al*. 1994; Yi *et al*. 2017). *A. thaliana* has three isoforms of calreticulin (CRT1, CRT2, and CRT3) that play roles in regulating calcium and water stress (Kim *et al*. 2013). Our proteome showed that CRT1 (AT1G 56340) decreased in the early vegetative stage whereas it increased in the late vegetative stage, suggesting that the calreticulin-mediated response plays a role in the defense mechanisms in response to stress caused by radiation exposure.

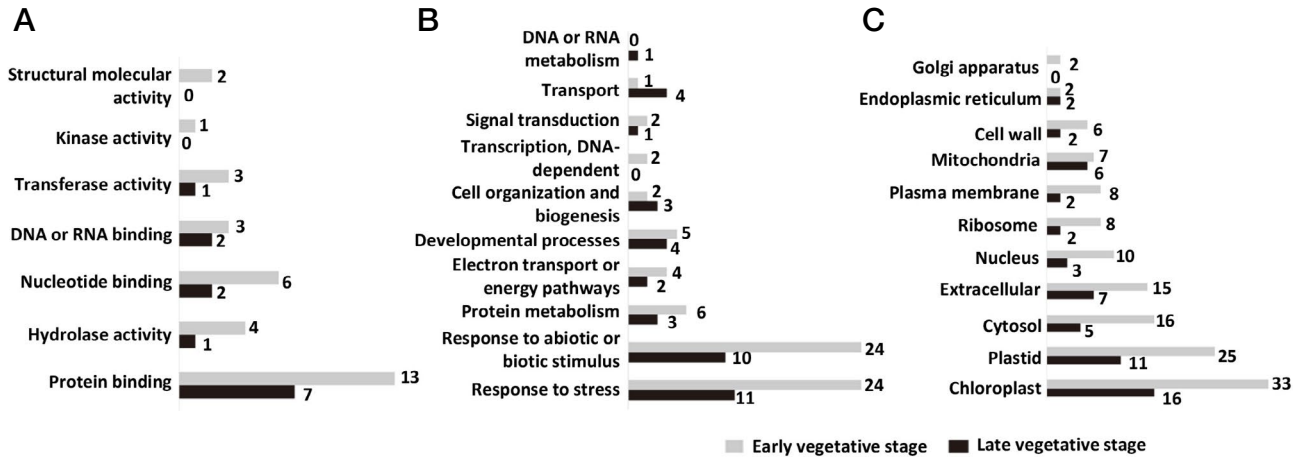

Fig. 2. Gene ontology classification of γ-radiation-responsive proteins according to the molecular function (A), biological process (B), and cellular component (C).

2. GO analysis of γ-radiation-responsive proteins

To further characterize changes in the *Arabidopsis* proteome in response to the radiation exposure, we categorized these proteins using Gene Ontology (GO) annotation. This analysis revealed that the proteins changed in both the early and late vegetative stages were mainly associated with the protein binding activity based on the molecular function (Fig. 2A). This analysis showed that the proteins involved in the transferase activity, nucleotide binding, and hydrolase activity changed abundantly in both the early and late vegetative stages. In addition, the proteins identified in the early stage, not the late stage, were related to structural molecular and kinase activities. Based on the proteins classified by the biological process, a majority of proteins involved in responses to an abiotic or biotic stimulus and responses to stress changed abundantly in both the early and late stages (Fig. 2B). These data indicate that γ-radiation exposure induces diverse cellular stresses, and *A. thaliana* activates defense signal networks to counteract these stresses. We found that a majority of proteins that changed in the expression were localized in the chloroplast (Fig. 2C). This result appears to be consistent with the fact that γ-radiation exposure to plants affects the photosynthetic activity (Kim *et al*. 2011). The facts that radiation-responsive proteins identified in this study were localized in the nucleus and have DNA or RNA binding and nucleotide binding activity support that DNA damage is one of detrimental effects caused by γ-radiation. However, we could not identify the proteins involved in the DNA repair system in this proteome analysis. This result may come from differences in the recovery time post γ-radiation exposure.

CONCLUSION

In this study, we performed proteome analysis to identify radiation-responsive proteins in the two vegetative stages. This analysis revealed that the proteins involved in the oxidative stress, molecular chaperones, and general stress responses showed abundant changes post γ-radiation exposure. Notably, we identified four proteins (acryl carrier protein 4, glycine cleavage system H protein 1, peptidyl-prolyl cis-trans isomerase CYP20-3, and calreticulin-1) that showed abundant changes in both the early and late vegetative stages after γ-radiation exposure.

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