Identification of γ-radiation-responsive proteins in *Arabidopsis thaliana*

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1. Introduction

Due to the increased need for radiation breeding and environmental monitoring of radioactive sites [1], a plethora of studies have been conducted to understand biological effect of radiation using *Arabidopsis thaliana* as a model plant. High levels of radiation exposure to *A. thaliana* induce physiological changes including growth inhibition and delayed flowering [2,3]. In addition to physiological changes, transcriptome analyses in respond to γ-radiation reveal 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 [2-5].

Although a plethora of transcriptome analyses were performed to identify radiation-responsive marker and elucidate radiation response, the transcriptome analyses are not sufficient to understand for biological process in response to radiation exposure of *A. thaliana*. Transcript level of target gene may not accurately correlate with protein production, given that post-translational modification process affects half-life and activity of proteins in eukaryotes [6,7]. Therefore, direct measurement of protein levels is helpful to increase understanding biological process in response to γ-radiation. In this study, we performed proteomic analysis to identify radiation-responsive proteins.

2. Methods and Results

2.1 Proteome profiling of A. thaliana post γ-radiation exposure

To obtain information about radiation-responsive proteins in *A. thaliana,* plants were irradiated with gamma radiation at the indicated dose rate (12.5, 50, and 100 Gy/h for 4 h) using a ^{60}Co source (IR-222, MDS Nordion Inc., Kanata, Cannada) in the radiation facility of the Advanced Radiation Technology Institute (ARTI). After irradiation, the plants were incubated in the growth room to recover for 1 day, and then samples were harvested.

Total proteins were extracted from the samples with extraction buffer (50 mM Tris-HCl (pH 7.2), 200 mM NaCl, and 0.25% Triton X-100) containing protease inhibitor cocktail (Roche), 2 mM phenylmethanesulfonyl fluoride, 10 mM 2-chloroacetamide, 10 mM sodium metabisulfite, and 1 mM N-ethylmaleimide.

To identify radiation-responsive proteins, 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). RAW data from LC-MS/MS 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) for assigning peptides for protein identification. We found that proteins involved in the oxidative stress, molecular chaperone, and amino acid metabolism exhibited abundance change compared to those of non-exposure samples.

Our proteome revealed that 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) were gradually decreased in response to high dose of radiation. Similar to peroxiredoxin, protein level of glutathoione S-transferase (AT1G78380 and AT2G47730) was downregulated. In contrast to peroxiredoxin and glutathione S-transferase proteins, protein abundance of L-ascorbate peroxidase S (AT4G08390) was increased (Table 1).

In addition to oxidative stress response-related proteins, protein levels of molecular chaperone protein were changed post radiation exposure. Our proteome data revealed that ERD14 (AT1G76180) and HSP70 (AT5G49910) proteins were increased post radiation exposure. Notably, production of protein disulfide isomerases (PDIs) (AT1G21750 and AT2G47470) was significantly reduced post radiation exposure (Table 1). Furthermore, our proteome analysis revealed that CYP20-3 (AT3G62030), which is also designated as ROC4 and has peptidyl-prolyl cis-trans isomerase activity, was downregulated in the early vegetative stage whereas it was increased in the late vegetative stage (Fig. 1).

One interesting finding in this analyses is that production of proteins related to metabolic pathways were changed. Of them, glutamine synthetases (AT1G66200 and AT5G35630), which is involved in the nitrogen acquisition and amino acid metabolism,

were reduced in both early and late vegetative stages. Similar to this result, transcript levels of genes related to nitrogen acquisition and amino acid metabolism are generally downregulated post radiation exposure in eukaryote.

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

Gene ID	Description	PSM ratio			
		0 Gy/ 0 Gy	50 Gy/ 0 Gy	200Gy/ 0 Gy	400 y/ 0 Gy
Early vegetative stage					
ATCG00490	Ribose diphosphate carboxylase large chain	1.00	0.53	0.34	0.43
AT1G66200	Glutamine synthetase cytosolic ribozyme 1-2	1.00	0.68	0.63	0.47
AT4G25050	Acyl carrier protein 4	1.00	0.88	0.59	0.41
AT3G11630	2-Cys peroxiredoxin BAS1	1.00	0.79	0.45	0.50
AT1G67090	Ribose diphosphate carboxylase small chain 1A	1.00	0.69	0.43	0.45
AT5G06290	2-Cys peroxiredoxin BAS1-like	1.00	0.85	0.45	0.48
AT3G62030	Peptidyl-prolyl cis-trans isomerase CYP20-3	1.00	0.59	0.48	0.45
AT1G65980	Peroxiredoxin-2B	1.00	0.90	0.71	0.38
AT1G21750	Protein disulfide isomerase-like 1-1	1.00	0.92	1.00	0.32
AT2G35370	Glycine cleavage system H protein	1.00	1.00	0.79	0.42
AT3G26060	Peroxiredoxin Q	1.00	0.73	0.96	0.46
AT1G56340	Calreticulin-1	1.00	0.79	0.57	0.36
AT1G78380	Glutathione S-transferase U19	1.00	0.67	0.42	0.42
AT2G47730	Glutathione S-transferase F8	1.00	0.67	0.58	0.33
AT1G76180	Dehydrin ERD14	1.00	1.43	2.00	2.14
AT4G08390	L-ascorbate peroxidase S	1.00	1.43	1.71	1.29
Late vegetative stage					
AT5G49910	Heat shock 70 kDa protein 7	1.00	1.60	1.40	1.48
AT4G25050	Acyl carrier protein 4	1.00	0.71	0.59	0.35
AT2G35370	Glycine cleavage system H protein 1	1.00	1.22	1.22	1.56
AT2G47470	Protein disulfide-isomerase like 2-1	1.00	0.93	0.93	0.36
AT4G29350	Profilin-2	1.00	0.40	0.20	0.24
AT3G62030	Peptidyl-prolyl cis-trans isomerase CYP20-3	1.00	1.00	1.33	1.83
AT5G35630	Glutamine synthetase	1.00	0.47	0.40	0.33
AT1G56340	Calreticulin-1	1.00	3.00	1.50	2.25

In addition to amino acid metabolism, proteins related to photosynthesis process such as ribulose bisphosphate carboxylase large chain (ATCG00490) and small chain (AT1G67090), which are components of Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase), were decreased in the early vegetative stage (Table 1). In agreement with our data, transcripts and proteins related to photosynthetic process had decreased in rice. It has been known that high levels of radiation damages cellular structure of plant and reduces chlorophyll content. The expression level of ACP4 (AT4G25050), which is responsible for regulating biosynthesis of fatty acid in the chloroplast membrane lipids, is known to be correlated with photosynthetic status. Our study revealed that ACP4 production was decreased in both early and late vegetative stages (Fig. 2). Similarly, glycine cleavage system H protein 1 (AT2G35370), which is one component of glycine cleavage system (GCS), was downregulated in the early vegetative stage (Fig. 2). The GCS plays roles in the conversion of glycine to serine in the mitochondria, which is involved in the photorespiration. Photorespiration is initiated uptake of O2, which is a byproduct of photosynthesis, and ultimately produces H_2O and CO_2 by consuming ATP and NAD(P)H. As radiation reduces photosynthesis activity, proteins related to photorespiration system would be decreased. Therefore, decreased levels of ACP4 and glycine cleavage system H appear to be originated from reduction of photosynthesis caused by radiation exposure.

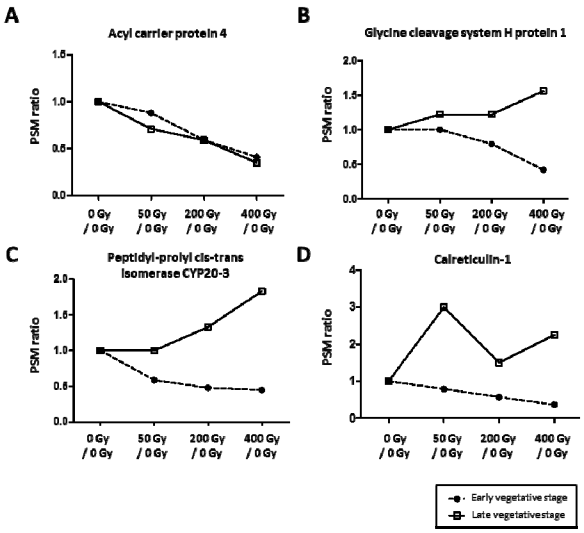

Fig. 1. Identification of γ-radiation responsive proteins in both early and late vegetative stages.

We found that diverse proteins involved in stress response showed abundance change. Intracellular and extracellular signals induce remodeling of actin structure, which affects diverse cellular process in plant. Especially, actin depolymerization is involved in the enhancement of ROS in the root under NaCl treatment. Our proteome result revealed that protein level of profilin-2 (AT4G29350), which regulates polymerization and depolymerization of actin depending on the protein abundance, tends to decrease post radiation exposure (Table 1). It has been known that low intracellular level of profilin enhances the polymerization of actin. It implies that reduced level of profilin would prevent from the increment of ROS by polymerizing actin. The concentration of calcium, which is regarded as a second messenger, is changed to activate defense systems in response to external signals. Calreticulin (CRT) is an ER-localized calcium-binding protein that regulates ER homeostasis through the calreticulin/calnexin cycle in response to environmental

stress. *A. thaliana* has three isoforms of calreticulin (CRT1, CRT2, and CRT3) that play roles in regulating calcium and water stress. Our proteome showed that CRT1 (AT1G56340) was decreased in the early vegetative stage whereas it was increased in the late vegetative stage. The calcium-mediated responses post radiation exposure were also observed in other eukaryotic systems. The Calcium is indispensable for activation of protein kinase C following radiation exposure in humans and calreticulin is up-regulated in mouse liver tissues after low dose of radiation exposure, suggesting that calreticulin-mediated response appears to be one of the defense mechanisms in response to stress caused by radiation exposure.

2.2 GO analysis *of γ-radiation-responsive proteins*

To further characterize changes in Arabidopsis proteome in response to radiation exposure, we categorized these proteins using Gene Ontology (GO) annotation. This analysis revealed that proteins changed in both early and late vegetative stages were mainly associated with protein binding activity based on the molecular function (Fig. 2). This analysis showed that proteins involved in the transferase activity, nucleotide binding, and hydrolase activity were abundantly changed in both early and late vegetative stages. In addition, proteins identified in the early stage, not the late stage, were related to structural molecular activity and kinase activity. When proteins were classified according to biological process, a majority of proteins involved in response to abiotic or biotic stimulus and response to stress were abundantly changed in the both the early and late stages (Fig. 2). This data indicates that γ-radiation exposure induces diverse cellular stresses and *A. thaliana* activates defense signal network to counteract these stresses.

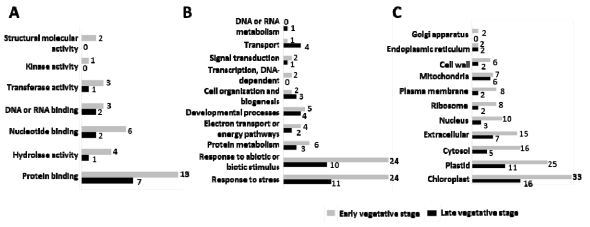

Fig. 2. Gene ontology classification of γ-radiation responsive proteins according to the molecular function (A), biological process (B), and cellular component (C).

We found that majority of proteins changed in the abundance were localized in the chloroplast (Fig. 2). This result appears to be consistent with the fact that γ-radiation exposure to plants affect photosynthetic activity. Radiation-responsive proteins identified in this study are localized in the nucleus and have DNA or RNA binding and nucleotide binding activity seems to be apparent as one of detrimental effects caused by γ-radiation is DNA damage. However, we could not identified proteins involved in the DNA repair system in this proteome analysis. This result may come from difference in the recovery time post γ-radiation exposure.

3. Conclusions

In this study, we performed proteome analysis to identify radiation-responsive proteins in the two vegetative stages. This analysis revealed that proteins involved in the oxidative stress, molecular chaperone, and general stress response showed abundance 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 abundance change in both early and late vegetative stages after γ-radiation exposure.

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