Gamma Irradiation Induced Transcriptional Repression of the Gibberellin Acid Regulating Genes in Arabidopsis Plants

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Abstract - The model plant, *Arabidopsis thaliana* is the subject of an international genome research project. Massive doses of ionizing radiation have been shown to induce physiological changes in plants. The wild-type (Ler) Arabidopsis plants were irradiated with 100 Gy and 800 Gy of gamma-ray. Gibberellin (GA) affects developmental processes and responses according to the various environment conditions in diverse plant. The 13 GA isomers were analyzed at vegetative (VE) and reproductive (RE) stages by HPLC. Total GA contents were reduced with the increase in radiation doses at VE and RE stages. Specifically, levels of GA3, GA4, GA12, and GA34 were significantly reduced with the increase of radiation doses. Oligonucleotide microarrays analysis was performed with Arabidopsis plants at different developmental stages and doses of gamma-ray. Through the microarray data, we isolated 41 genes related to GA biosynthesis and signaling transduction. Expression of these genes was also decreased as the reduction of GA contents. Interestingly, in GA signaling related gene expression, gibberellin-responsive protein, putative (At2g18420) was down-regulated at VE and RE stages. Myb21 (At3g27810), Myb24 (At5g40350), and Myb57 (At3g01530) was down-regulated at RE stage. In GA biosynthesis related gene expression, YAP169 (At5g07200) and GA20ox2 (At5g51810) were down-regulated at 100 Gy treatment of VE stage and 800 Gy treatment of RE stage in cytoplasm, respectively. However, exceptively, GA3ox2 (At1g80340) was up-regulated at 100 Gy treatment of RE stage in cytoplasm. In this study, the wild type (Ler) Arabidopsis plants showed differences in response with development stage at the various doses of gamma-rays. GA contents change was reported in gamma irradiated plant.

Key words : *Arabidopsis thaliana*, DELLA protein, Gamma-rays, Gibberellin, Transcriptome

INTRODUCTION

The model plant, *Arabidopsis thaliana* is the subject of an international genome research project. Arabidopsis plant provides a useful system for the development and exploitation of mutagenesis (Van Haaren and Ow 1993; Vizir *et al.* 1994, 1996; Kilby *et al.* 1995).

Plants presumably have few sensors and signal transduction system for gamma-irradiation because there are no high levels of gamma-irradiation that generate mutagenesis in the natural environment. In general, ionizing radiation effects on plant growth and development. Irradiation mutants of Arabidopsis supports the general theory that irradiation causes single- and double-strand breaks which result in chromosomal rearrangements. Low doses of gamma-ray irradiation have been used for mutant isolation in conventional plant

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breeding (Chopra 2005). According to the knowledge, low dosage effects stimulate plant growth, while high radiation levels have increasingly harmful effects for vegetative growth at intermediate levels, and pronounced decrease in reproductive fitness and yields (Britt 1999, Gorbunova and Levy 1999; Sachs *et al.* 2000; Lieber *et al.* 2003; Kovalchuk *et al.* 2003, 2004).

Massive doses of ionizing radiation have been shown to induce physiological changes in plants (Nagata *et al.* 1999). Gibberellin helps in developmental processes and responses to the environment in diverse plant species. Gibberellin deficiency causes male sterility due to accumulation of DELLA proteins, and GA causes DELLA degradation to promote stamen development (Cheng *et al.* 2009). GAs are not only required for stem elongation but indeed participate in almost all the stages of plant development. Furthermore, they mediate between certain environmental signals such as light quality and photoperiod, and the induced physiological responses for instance stem extension and flowering (Hedden and Phillips 2000).

Microarray is an important tool for gene expression analysis. To know the gene expression profiles after gamma irradiation and for the analysis of signal transduction, we used the microarray system. Recently, microarray technology has developed a useful tool for the analysis of genome-scale gene expression (Schena *et al.* 1995; Eisen and Brown 1999). Large arrays have been used to identify novel genes involved in nutrient response and seed formation in plant (Girke *et al.* 2000; Wang *et al.* 2000).

In this study, the wild type (Ler) Arabidopsis plants showed differences in response with development stage at the various doses of gamma-rays. GA contents change was reported in plant. An informative transcriptomic profile of the responses of Arabidopsis genes were generated to gamma irradiation. Using the microarray result, Oligonucleotide DNA chip is an effective technology which potentially allows for a rapid and cost-effective screening for all possible radiation-responsive genes.

MATERIALS AND METHODS

1. Plant materials and gamma irradiation

Arabidopsis thaliana Landsberg plants were maintained in the controlled rooms at 24°C with a photoperiod of 16/8 hrs light (5000 lx) and 70% humidity. To identify the radiation sensitivity at different development stages, vegetative (VE, 21 Day After Germination, DAG) and reproductive (RE, 28 DAG) stages were taken into consideration). Vegetative stage was when the rosette leaves were completely developed, and reproductive stage was when stem elongation process continues to elongate after emergence of floral axis. The wild-type (Ler) Arabidopsis plants were irradiated with 100, 200, 400, 800, 1200, 1600 and 2000 Gy of gamma-rays for 24 hrs using a ^{60}Co gamma-irradiator (150 TBq of capacity; ACEL, Canada) at Korea Atomic Energy Research Institute.

2. GA analysis

Extraction of GAs was conducted as described by Foster and Morgan (1995). Freeze-dried seed samples were extracted by successively adding 80% and 100% methanol. The extract was filtered through Whatman No. 5 filter paper. Filtrate was brought to 60% methanol with distilled water and stored at −70°C for 1-2 h before filtration through GF/A filter. Each 20 ng internal standard ($2H_2$ GA_1, $2H_2$ GA_4, $2H_2$ GA_7, $2H_2$ GA_8, $2H_2$ GA_9, $2H_2$ GA_{12}, $2H_2$ GA_{19}, $2H_2$ GA_{20}, $2H_2$ GA_{24}, $2H_2$ GA_{34} and $2H_2$ GA_{53}) were added in the filtrate. The filtrate was adjusted to pH 8.0-8.3 with 2 N NaOH, filtered through Davisil C18 column (90-130 micron, 60Å, Alltech), and concentrated by a rotary evaporator under vacuum.

The residue dried using celite (3 g) was loaded on a SiO column (ICN Silica 32-100, Aktiv 60Å, Alltech) and eluted with EtOAc : hexane (95 : 5, v/v) saturated with formic acid. The filtrate was concentrated by a rotary evaporator under vacuum. The residue was dissolved in phosphate buffer (pH 8.0) adjusted to pH 8.0-9.0 with 2 N NaOH, and fractionated three times with 4 ml EtOAc. The phosphate fraction, added with PVPP (1 g) and agitated for 1 h, was filtered through Whatman No. 5 filter paper. Filtrate after being adjusted to pH 2.5 using 6 N HCl was fractionated with EtOAc for three times. The EtOAc fraction washed three times with distilled water at pH 2.5 was concentrated *in vacuo*. The residue was dissolved in 100% methanol added with [1,2 ^{3}H] GA_{20} and [1,2 ^{3}H] GA_9 and fractionated by HPLC.

HPLC system was consisted of a gradient controller (Waters model 680), injector (Waters model U6K), pump (Waters model 510), and a fraction collector (Isco). Sample solutions

were fractionated through a reverse phased column (mBondapak C18, 3.9 × 300 mm) using elution gradient formed with 28% and 100% methanol containing 1% acetic acid. Up to forty fraction were taken, and radioactivity was measured with liquid scintillation spectrometry (Beckman, LS 6000CC) to determine accurate retention time of each GA based upon the elution of ^{3}H-GA standards. The fractions were dried on a savant speedvac and combined according to the retention times of ^{3}H standards and previously determined retention times of the deuterated GAs standards. Each dried GA fraction was redissolved in 100% methanol, transferred to a 1 ml reaction vial and dried under N_2 gas and drivertized using ethereal diazomethane. GAs were quantified by GC-selected ion monitoring-MS based on peak area ratios of endogenous (non-deuterated) to deuterated GAs, after correcting for any contribution from the deuterated standard to the non-deuterated GA (Beall *et al.* 1991).

3. Microarray data analysis

Total RNAs were isolated from the control and 100 and 800 Gy gamma-irradiated plants at VE and RE stages. Total RNA was assessed quantitatively and qualitatively by gel electrophoresis, respectively. Labeled cRNAs were prepared by Cy3-labeled and Cy5-labeled fluorescent dyes. Microarray experiments were done using the Agilent Arabidopsis 2 Oligo (60 mers) Microarray Kit (V2) (Agilent Technologies, Palo Alto, CA) representing 21,500 Arabidopsis genes and transcripts. 1 μg of total RNA was labeled by using the Agilent Fluorescent Linear Amplification kit (Agilent Technologies). The cRNA was hybridized to an agilent in situ Hybridization Kit Plus (Agilent Technologies). The microarray was scanned using an Axon 4000B scanner (Axon Instru-

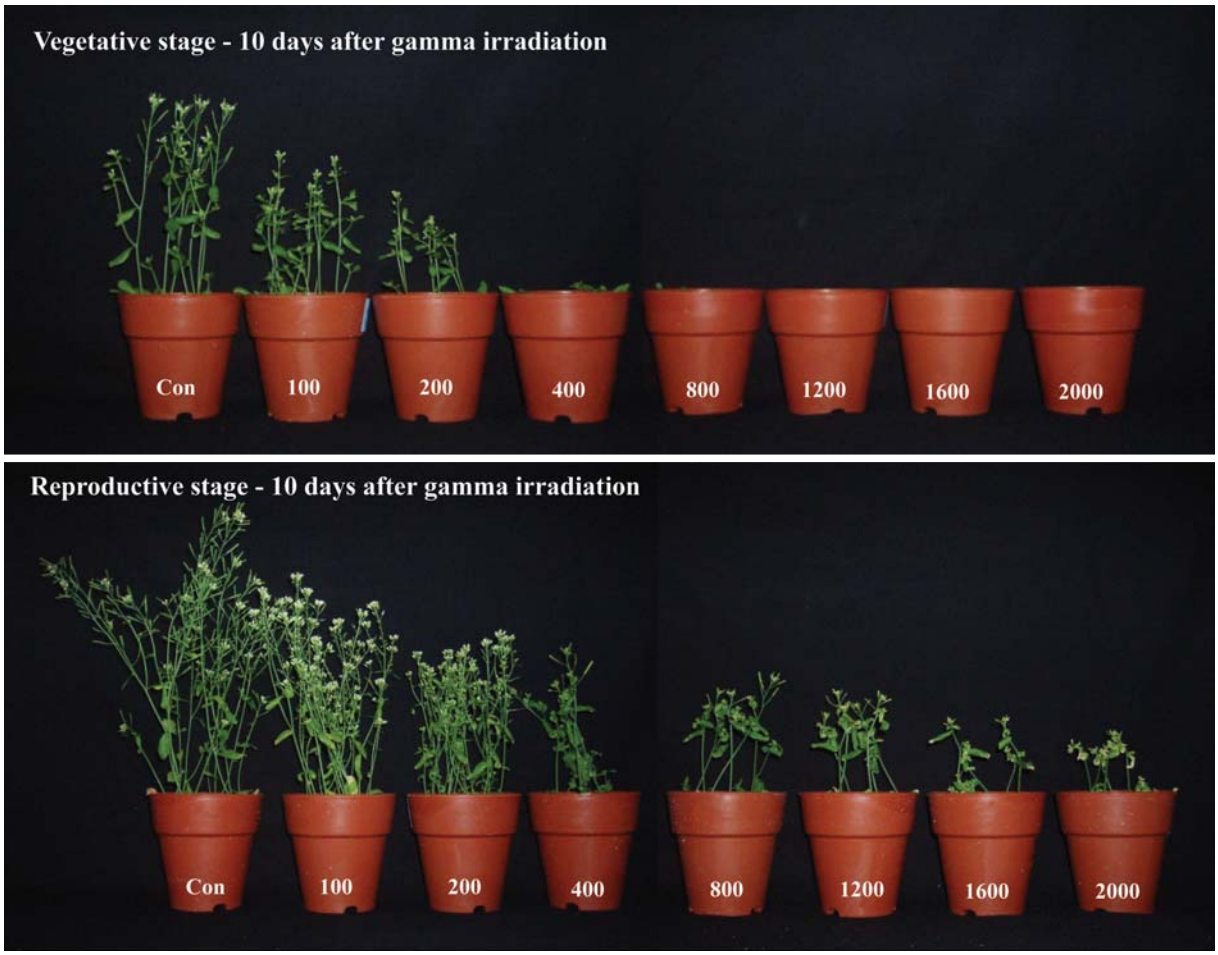

Fig. 1. Growth of *Arabidopsis* irradiated with gamma rays. Gamma rays were irradiated to the plants in two different developing stages. The VE stage is defined as the stage at which the rosette leaves were fully grown, and the RE stage was designated as the stage during which stem elongation continues after the emergence of the floral axis.

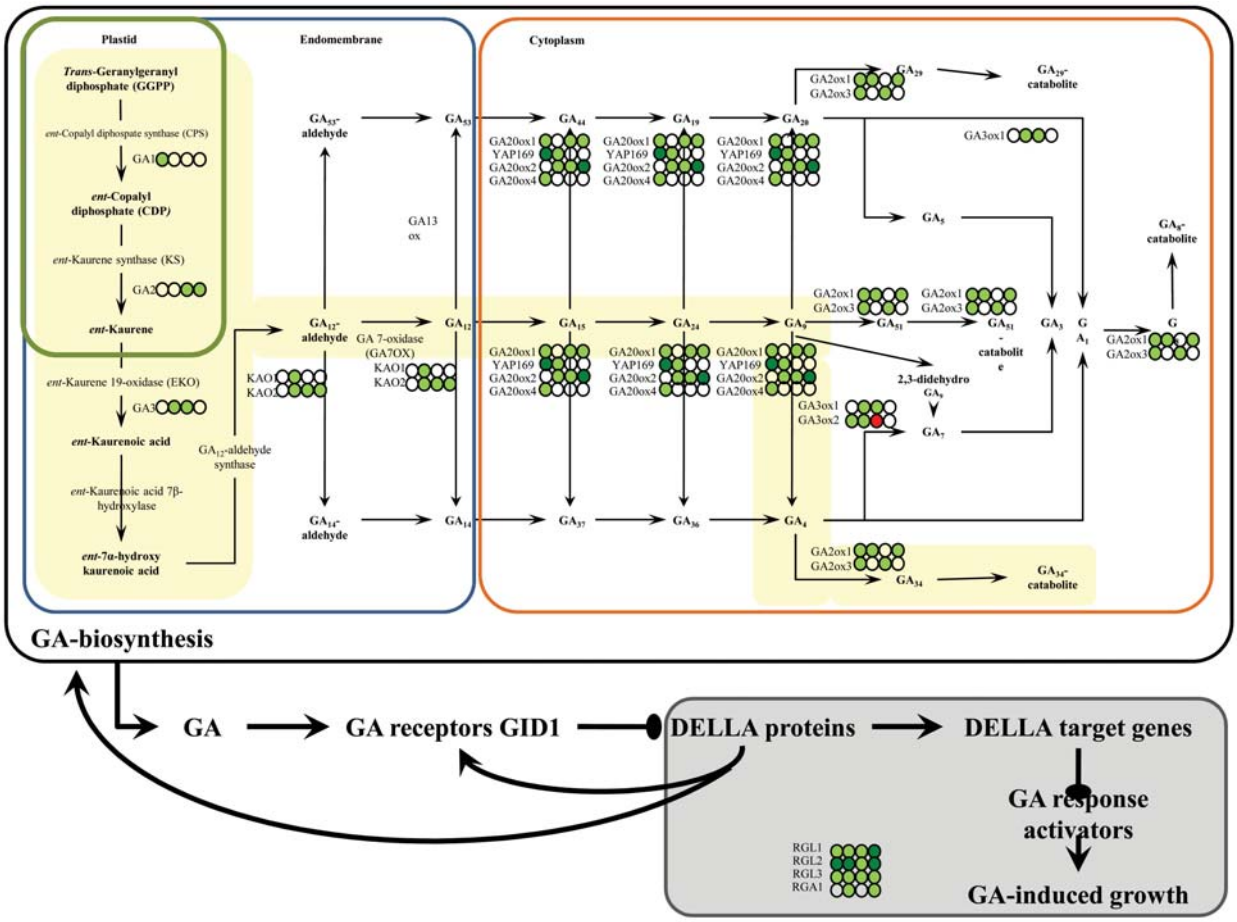

Fig. 2. Regulation mechanisms of GA biosynthesis and signaling. The biosynthesis and catabolic pathway of GA in plant species. GAs are biosynthetic intermediates or catabolites of bioactive GAs. GA_9, GA_{20}, GA_4, and GA_1 are converted to GA_{51}, GA_{29}, GA_{34}, and GA_8 by GA2ox. GA action through DELLA protein degradation. The GID1 receptor binds GA, then interacts with the DELLA protein. GA action is repressed by DELLA protein. Red circles, 2x or more up-regulated gene; Green circles; 2x or more down-regulated gene; Light green circles; more down-regulated gene compared to control plant.

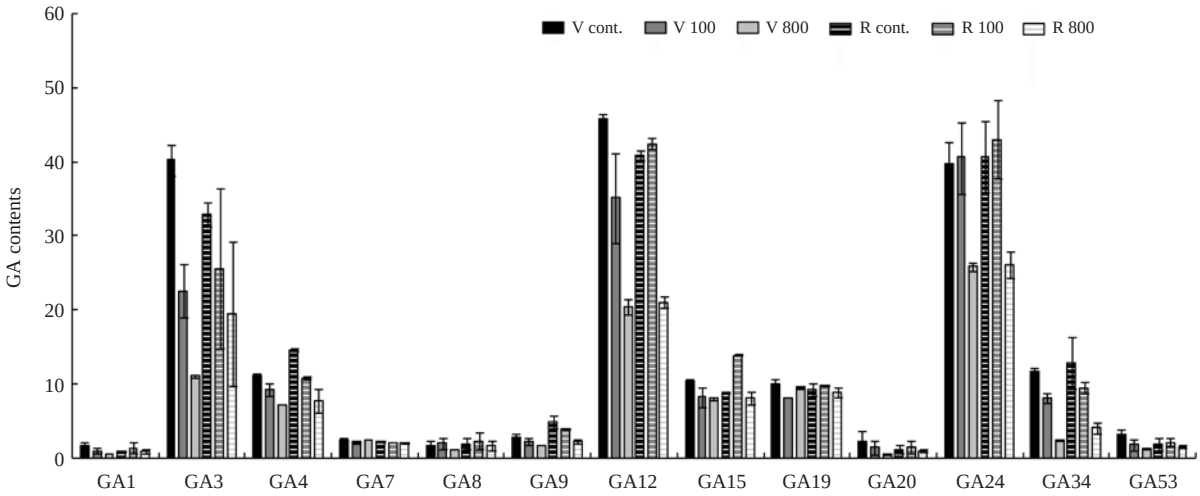

Fig. 3. GA levels in different developing stages and dose rates. Vegetative (VE, 21 Day After Germination, DAG) and reproductive (RE, 28 DAG) stages. GA levels were measured by GC-selected ion-monitoring MS using deuterated internal standards. Mean ± S.E. n=3.

ments, Whipple Road union city, CA). Gene clustering analysis was performed using GeneSpring GX 7.3 (Silicon Genetics, San Carlos, CA). The *t*-test was performed at a *p* value of 0.05 to explain the genes that were expressed differently as compared to the control plants.

RESULTS AND DISCUSSION

1. Growth changes by a gamma-irradiation

To identify the radiosensitivity, the wild-type (*Ler*) Arabidopsis plants were irradiated with gamma-rays of 100, 200, 300, 400, 800, 1200, 1600 and 2000 Gy doses at the VE and RE stages. All the doses consistently reduced plant height compared to the control. The higher the dosage, the more effect that plant height reduced. Above 400 Gy the length decreased with 5.2% and 11.7% at VE and RE stages, respectively (Fig. 1). Bae *et al.* (2009) reported the effects of gamma ray irradiation in seed germination, survival plants and unbolting plants. Various doses of gamma-rays (0, 10, 25, 50, 75 and 100 Gy) was irradiated in *Zoysia*. Seed germination, survival and unbolting rate were decreased with increas-

Table 1. Gene expression changes by gamma irradiation in the GA biosynthesis of Arabidopsis

AGI code	Gene name	Description	Average Log_2 (FC)			
			VE (Gy)		RE (Gy)	
			100	800	100	800
At1g14920	GA1	gibberellin response modulator	0.43	−0.24	0.29	0.11
At4g02780	GA1	copalyl diphosphate synthase / CPS / ent-kaurene synthetase A	−0.57	0.17	0.62	0.04
At5g25900	GA3	ent-kaurene oxidase, putative	0.12	−0.69	−0.10	0.02
At1g79460	GA2	ent-kaurene synthase / ent-kaurene synthetase B (KS) (GA2)	0.39	0.03	−0.06	−0.53
At1g05160	KAO1	ent-kaurenoic acid hydroxylase	0.08	−0.27	0.06	0.16
At2g32440	KAO2	ent-kaurenoic acid hydroxylase, putative	0.03	−0.80	−0.04	−0.45
At4g25420	GA20OX1	gibberellin 20-oxidase	−0.63	−0.31	0.22	−0.24
At5g51810	GA20OX2	gibberellin 20-oxidase, putative	0.09	−0.24	−0.52	**−1.15**
At1g60980	GA20OX4	gibberellin 20-oxidase, putative	−0.79	0.10	0.25	0.34
At5g07200	YAP169	gibberellin 20-oxidase	**−1.15**	−0.19	0.08	0.04
At1g44090	GA20OX5	gibberellin 20-oxidase family protein	−0.13	0.29	−0.18	0.39
At1g78440	GA2OX1	gibberellin 2-oxidase	−0.40	−0.38	−0.01	−0.68
At1g30040	GA2OX2	gibberellin 2-oxidase	0.13	0.04	−0.37	−0.30
At2g34555	GA2OX3	gibberellin 2-oxidase	−0.56	0.24	−0.14	0.14
At1g47990	GA2OX4	gibberellin 2-oxidase, putative	−0.68	−0.23	−0.23	0.12
At1g02400	GA2OX6	gibberellin 2-oxidase, putative	0.15	0.15	−0.41	−0.58
At1g50960	GA2OX7	gibberellin 20-oxidase-related	−0.10	0.50	0.61	0.33
At5g51310		gibberellin 20-oxidase-related	−0.45	−0.27	−0.12	0.26
At1g15550	GA3OX1	gibberellin 3-beta-dioxygenase	0.35	−0.64	−0.02	0.20
At1g80340	GA3OX2	gibberellin 3-beta-dioxygenase / gibberellin 3 beta-hydroxylase	−0.29	−0.17	**1.06**	0.63
At4g21690	GA3OX3	gibberellin 3 beta-hydroxylase family protein	**−1.35**	−0.09	0.25	0.02
At1g80330	GA3OX4	gibberellin 3-beta-dioxygenase, putative	0.17	0.21	−0.17	−0.28
At1g75750	GASA1	gibberellin-regulated protein 1	0.73	0.85	−0.01	−0.53
At4g09610	GASA2	gibberellin-regulated protein 2	−0.04	−0.22	−0.10	0.19
At4g09600	GASA3	gibberellin-regulated protein 3	−0.22	−0.05	−0.72	0.23
At5g15230	GASA4	gibberellin-regulated protein 4	−0.19	−0.75	−0.13	−0.58
At2g14900		gibberellin-regulated family protein	**−1.22**	−0.16	**−1.35**	−0.77
At2g39540		gibberellin-regulated family protein	−0.93	−0.02	**−1.01**	−0.98
At2g30810		gibberellin-regulated family protein	−0.89	−0.08	−0.90	**−2.23**
At1g66350	RGL1	gibberellin regulatory protein	−0.22	−0.68	−0.65	**−1.14**
At5g14920	GAI	gibberellin-regulated family protein	0.65	0.98	0.00	0.31
At3g03450	RGL2	gibberellin response modulator, putative	**−1.19**	**−1.26**	−0.64	**−1.18**
At5g17490	RGL3	gibberellin response modulator, putative	−0.14	−0.13	−0.82	−0.84
At2g01570	RGA1	gibberellin response modulator	0.16	−0.27	0.05	−0.29
At1g22690		gibberellin-responsive protein, putative	−0.08	−0.28	0.71	**1.02**
At1g74670		gibberellin-responsive protein, putative	−0.99	**−2.02**	−0.01	**−1.06**
At2g18420		gibberellin-responsive protein, putative	**−1.55**	**−1.32**	**−2.15**	**−3.45**
At3g11540	SPINDLY	gibberellin signal transduction protein	0.68	0.30	0.30	0.01
At3g27810	Myb21	myb family transcription factor	0.74	0.64	**−3.32**	**−3.64**
At5g40350	Myb24	myb family transcription factor	−0.14	0.29	**−1.89**	**−2.18**
At3g01530	Myb57	myb family transcription factor	−0.81	0.04	**−2.56**	**−3.06**

ing doses of irradiation. Dry rice seeds were irradiated to 100, 200, 300, 400, and 500 Gy of gamma rays from ^{60}Co source. With the increase in radiation dose a decrease in germination, seedling height, and root length were observed (Minn *et al.* 2008). In general, mutagenic treatment caused aborted growth in the higher dosages.

2. GA changes by a gamma-irradiation

Fig. 2 was shown the biosynthesis of GA in most plant species. The 13 GA isomer were analyzed, especially levels of GA_3, GA_4, GA_{12}, and GA_{34} were reduced with the increase in radiation dose. GA_8 (2.02 μg and 2.39 μg, VE and RE, respectively) and GA_{24} (40.60 μg and 43.14 μg, VE and RE, respectively) were the highest level at 100 Gy in both stages but, there is no difference between 100 Gy and 800 Gy in GA level. Total GA contents were reduced with the increase in radiation dose at both stages, respectively (Fig. 3). The highest level of GA isomer was found in GA_3, GA_{12}, and GA_{24}. GA biosynthesis inhibitors are known to block conversion of GA_{20} to GA_1 (Nakayama *et al.* 1990; Grigg *et al.* 1991; Kamiya *et al.* 1992). The isomers inhibit the conversion of GA_{20} to GA_1 and growth, while markly promoting floral initiation and development, appear unique and suggests the possibility of a direct florigenic effect of these compounds (Foster *et al.* 1997). GA_3 promoted stem elongation (Evans *et al.* 1994).

Among the identified VE and RE genes, we found GA-related 38 genes. Among these genes, gibberellins-responsive protein, putative (At2g18420) was down-regulated by over 2-fold at both stages. Additionally, gibberellins-regulated family protein (At2g14900) was down-regulated at 100 Gy in both stages. RGL2 (At3g03450) and gibberellins-responsive protein, putative (At1g74670) were down-regulated at a dose rate of 800 Gy in both stages (Table 1). DELLA proteins are negative regulators of gibberellins (GA) signaling. DELLA proteins act as a repressors of plant growth function (Richards *et al.* 2001). Binding of GA to GID1, the GID1-GA complex then interacts with DELLA proteins (Hirano *et al.* 2008). The Arabidopsis genome encodes five DELLA proteins (Lee *et al.* 2002). DELLA proteins GAI, RGA, RGA-like (RGL) functions have shown in the repression of plant growth. RGA, RGL1 and RGL2 act to repress stamen and anther development in GA-deficient plants (Cheng *et al.* 2004). GAI and RGA regulated plant stem elongation growth in response to GA (Peng *et al.* 1999; Ikeda *et al.* 2001; Chandler *et al.* 2002).

Our results show that GA de-represses its signaling pathway by inducing the degradation of RGA and GAI. Therefore, the reason for GA reduction in gamma-irradiated plants was that expression of coding DELLA protein repressed in order to synthesize the GA.

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