

Phenotypic and Molecular Responses of Wheat (*Triticum aestivum* L.) to Chronic Gamma Irradiation

M. J. Hong¹, Y. H. Yoon¹, D. S. Kim², S. H. Kim¹, S. Y. Kang¹, D. Y. Kim³, Y. W. Seo³,
and J. B. Kim^{1*}

ABSTRACT

The objectives of this study were to determine the effects of chronic gamma irradiation on growth and biochemical characteristics of wheat. Wheat plants were exposed to a ⁶⁰Co gamma rays at doses ranging from 10 to 150 Gy for 3 weeks. Our results indicate that irradiation at 10–15 Gy enhanced plant growth as compared to non-irradiated wheat, while at high doses (>20 Gy) a significant decrease in wheat height was recorded. *APX* and *CAT* transcript levels were higher in plant irradiated at 12.5 Gy than in the controls. Also, the enzyme activities of *APX* and *CAT* and *POD* were increased by 12.5 Gy gamma irradiation. Chronic irradiation caused an increase in the total anthocyanin content. To assess whether anthocyanin biosynthesis-related genes were involved in the response to chronic gamma irradiation in wheat plants, we examined their expression under different doses of gamma rays. Levels of *F3H*, *DFR*, *ANS* transcripts increased due to chronic gamma irradiation, whereas *CHS* and *CHI* expression decreased. Total anthocyanin contents significantly increased after chronic irradiation. Furthermore, Ultra Performance Liquid Chromatography (UPLC) revealed that cyanidin 3-glucoside, one of the anthocyanin compounds, rapidly increased in wheat plants after chronic gamma irradiation. This study demonstrated that the growth of wheat plants and markers of biochemical activity were negatively influenced by chronic gamma irradiation in a dose-dependent manner, although low-dose radiation showed stimulatory effects. Results from this study are very useful for future chronic gamma irradiation studies for the improvement of wheat varieties.

Keywords: Anthocyanin, Antioxidant, Biochemical activity, Reactive oxygen species, Cyanidin 3-glucoside.

INTRODUCTION

Radiations, such as alpha particles, beta particles, gamma rays, X-rays, and neutrons, have the potential to induce ionization leading to subsequent cell damage. Exposure to ionizing radiation can generate oxidative stress through the formation of Reactive Oxygen Species (ROS). ROS, including superoxide anions, hydrogen peroxide, and hydroxyl radicals, have high reactivity with a variety of cellular macromolecules such as

DNA, lipids, and proteins. Upon exposure to ionizing radiation, the free radicals cause negative biological effects when interacting with various cell components. Increases in ROS and free radicals affect their DNA stability. The increase in ROS and free radicals following ionizing radiation causes random changes in the nuclear DNA or in cytoplasmic organelles, resulting in gene, chromosomal, or genomic mutation. If the free radicals and abnormal molecules

¹Advanced Radiation Technology Institute, Korea Atomic Energy Research Institute, 29 Geumgu, Jeongeup 580-185, Republic of Korea.

²NJ Biopia Co. Ltd, Haseo-ro 672, Gwangju 500-260, Republic of Korea.

³Division of Biotechnology, Korea University, Seongbuk-Gu, Seoul 136-713, Republic of Korea.

* Corresponding author; e-mail: jbkim74@kaeri.re.kr



accumulate in cells, the cells may be detrimentally injured.

Conversely, ionizing radiations can be used as a tool to induce mutagenesis in plants. Gamma rays are commonly used to generate genetic variability owing to their availability and ease of penetration compared with other types of ionizing radiation. Many breeders have successfully developed new plant varieties using gamma rays to improve the qualitative and quantitative characters of many crops. Different colors of French bean (*Phaseolus vulgaris* L.) flowers, *indica* rice mutant population, and dwarf mutants of turf-type Bermuda grass were developed by gamma irradiation (Mahamune and Kothekar; 2011, Lu et al., 2009; Wu et al., 2005). Alterations in plant growth and development by inducing genetic, cytological, structural, biochemical, physiological, and morphological changes depend on the intensity and duration of gamma ray exposure. In *Pisum sativum*, seedlings exposed to 6 Gy of gamma rays exhibited significant inhibition of growth and productivity (Zaka et al., 2004). Furthermore, *P. sativum* plants exposed to doses above 40 Gy of gamma rays were not viable (Zaka et al., 2004). The height of *Arabidopsis* seedlings increased slightly following exposure to low-dose gamma irradiation (1 or 2 Gy) while exposure to 50 Gy significantly decreased seedling height (Wi et al., 2007). Nishiguchi et al. (2012) reported no alteration in growth of *Lombardy poplar* after gamma irradiation up to 20 Gy, although growth and morphological changes were detected with exposure to 50–300 Gy (Nishiguchi et al., 2012). Although radiosensitivity may be exhibited differently depending on plant variety, high-dose radiation was generally shown to have a negative effect on plant growth and development (Kovalchuk et al., 2003). High doses of gamma irradiation disrupt protein synthesis, hormone balance, leaf gas exchange, water exchange, and enzyme activity (El-Beltagi et al., 2011).

The patterns of exposure ionizing radiations can be divided into two types, acute exposure to high dosage, and chronic exposure to low dosage. The majority of studies on the effects of gamma irradiation submitted to date, have investigated the short-term effects of acute irradiation (El-Beltagi et al., 2011; Moghaddam et al., 2011). After the Chernobyl nuclear power plant accident, more in-depth studies on the effects of chronic exposure began. An increased incident of DNA strand breaks was observed in *Arabidopsis* after chronic exposure to ionizing radiations (Syomov et al., 1992). Kovalchuk et al. (2000) reported that Homologous Recombination (HR) was more highly detected in response to chronic irradiation compared to acute irradiation. Vandenhove et al. (2010) observed that exposure of *Arabidopsis* to chronic irradiation had different responses according to development stage and dosage used. However, the effect of chronic irradiation on plant growth and physiological responses remain largely unknown compared to those in response to acute irradiation.

The purpose of this study was to investigate the effect of chronic irradiation on the expression of antioxidant-related genes and anthocyanin biosynthesis-related genes in wheat plants. Wheat plants were chronically irradiated with different doses of gamma rays for 3 weeks. To evaluate physiological and molecular responses of wheat plants to chronic gamma irradiation, we measured plant height antioxidant-related genes expression contents and antioxidant enzyme activity. After chronic irradiation, a deep purple color accumulated in wheat leaves. Especially, anthocyanins have a decisive effect on the red, purple, and blue colors of many flowers, fruits, vegetables, and cereal grains (Welch et al., 2008). Anthocyanin, as a powerful antioxidant, can effectively eliminate free radicals generated under stress conditions. We also measured the anthocyanin content and the expression of anthocyanin biosynthesis-related genes to determine the physiological role of anthocyanin by

exposure to different doses of chronic gamma-irradiation in wheat plants. Furthermore, cyanidin, the major anthocyanin, was measured and quantified by UPLC using an external standard.

MATERIALS AND METHODS

Plant Materials

Purple grain color of wheat developed by Korea University (accession no. K4191) was used in this study. K4191 seeds were germinated with distilled water at 4°C in the dark for vernalization treatment. After 6 weeks, germinated seeds were transferred to pots, and filled with Sunshine Mix #1 (Sun Gro Horticulture, Agawam, MA, USA). The wheat plants were grown in growth rooms under 18–22°C and 16/8 hours (day/night). After 4 weeks, plants (Zadoks scale of Z23–25) were irradiated for 3 weeks with gamma rays at different doses generated by a ⁶⁰Co gamma irradiator (20 TBq of capacity, Nordion, Ottawa, ON, Canada) in gamma phytotron room at the Korea Atomic Energy Research Institute. Dose rate for each chronic irradiation were 66.6 (10 Gy), 83.3 (12.5 Gy), 100 (15 Gy), 133.3 (20 Gy), 166.6 (25 Gy), 233.3 (35 Gy), 333.3 (50 Gy), 416.6 (62.5 Gy), 500 (75 Gy), 666.6 (100 Gy), 833.3 (125 Gy), and 1,000 mGy h⁻¹ (150 Gy). After irradiation, the irradiated wheat plants were incubated for 1 week under normal growth conditions. Whole plant tissues were harvested and stored at -80 °C until further use.

Total Anthocyanin Contents

Anthocyanin was extracted using methanol-hydrochloric acid (HCl) (1% HCl, w/v) and quantified according to the method described by Mita *et al.* (1997). One gram of frozen homogenized wheat leaves were used to extract for 24 hours in 5 mL of the extraction solvent at 4°C. After incubation, homogenized samples were centrifuged at

9,000×g for 20 minutes and supernatant was filtered through a 0.2 µm filter. The absorbance of the supernatant was recorded at 530 and 657 nm using a UV-VIS spectrophotometer (Jenway, Keison products, Chelmsford, UK). Anthocyanin content was determined using the following formula:

$$Q = (A_{530} - 0.25A_{657}) \times M^{-1}$$

Where, *Q*: Anthocyanin yield, *A*₅₃₀ and *A*₆₅₇: Absorptions at the indicated wavelengths, *M*: Mass of the plant (Mancinelli *et al.*, 1991).

UPLC Analysis

Anthocyanins were extracted by methanol containing 1N HCl (85:15, v/v) at 4°C for 24 hours, and the extract was centrifuged for 20 minutes at 7,000×g. The extracts were filtered through 0.45 µm filters, and then anthocyanins were separated by UPLC equipped with a Nexera X2 (SHIMADZU, Kyoto, Japan). The separation conditions were as follows: solvent A (water – 0.1% TFA); solvent B (Acetonitrile – 0.1% TFA); flow rate, 0.5 mL min⁻¹; column, COSMOSIL 2.5 Cholesterol Packed column 2.0 mm ID X50 mm; column temperature, 40°C. Using the UPLC detector, Nexera X2 SPD-M30A (SHIMADZU, Kyoto, Japan), 520 nm wave length was investigated.

RNA Extraction, RT-PCR, and qRT-PCR

Total RNA was extracted from whole plants using TRIzol reagent (Invitrogen, Grand Island, NY, USA). First strand cDNA was synthesized from approximately 1 µg of total RNA using a Power cDNA synthesis kit (Intron Biotechnology, Gyeonggi-do, Korea) according to the manufacturer's protocol. RT-PCR primers (Hong *et al.*, 2014) for the target genes were designed using Primer3Plus (<http://www.primer3plus.com/>) according to annealing temperature (58–63°C)



specifications. Primer sets were designed from wheat sequences deposited in GenBank (<http://www.ncbi.nlm.nih.gov>; *CHS*: Chalcone synthase, AB187025; *CHI*: Chalcone isomerase, AB187026; *F3H*: Flavanone 3-hydroxylase, AB187027; *DFR*: dihydroflavonol 4-reductase, AB187028, and *ANS*: Anthocyanin synthase, AB247919) (Hong et al., 2014). The housekeeping gene, *actin* (AB181991) was used as an internal control. The RT-PCR conditions were as follows: 95°C for 10 minutes followed by 35 cycles of 95°C for 30 seconds, annealing at a gene specific temperature for 30 seconds, and 72°C for 30 seconds, with a final extension of 72°C for 4 minutes. The RT-PCR products were separated using 1% agarose gel electrophoresis and stained with ethidium bromide to visualize the DNA. The gels were viewed under UV transillumination and quantitated using a gel Documentation System (i-MAX™ Gel Image Analysis system, Core-Bio, USA). The qRT-PCR was performed using the SYBR premix Ex Taq II (Takara, Tokyo, Japan) and the Eco Real-Time PCR system (Illumina, San Diego, CA, USA). Gene-specific primers are presented in Table 1. The qRT-PCR programs were as follows: the 2-step thermal cycling profile consisted

of incubation for 10 seconds at 95°C and 30 seconds at 65°C. The reactions were performed in biological triplicates using RNA samples extracted from independent plants.

Measurement of Antioxidant Enzyme Activity

For all enzymatic assay, 0.5 g of plant tissues was homogenized in extraction buffer of 200 mM ice-cold potassium phosphate buffer (pH 7.0) containing 0.1 mM Ethylene Diamine Tetra Acetic acid (EDTA). The homogenate was transferred to tubes and was centrifuged at 4°C for 20 minutes. The supernatants were used for enzyme activity assay. The protein contents of the enzyme extracts were determined according to Bradford (1976) assay using Bovine Serum Albumin (BSA) as a standard. The activity of catalase (EC 1.11.1.6) was measured by following Aebi's method (Aebi, 1984). The CAT activity was determined by monitoring the rate of disappearance of H₂O₂ in absorbance at 240 nm of a reaction mixture containing 50 mM potassium phosphate buffer (pH 7.0), 15 mM H₂O₂, and 10 μL protein extract. SOD

Table 1. Lists of genes and primers for RT-PCR and qRT-PCR amplification.

	Gene	Forward	Reverse
Anthocyanin-related genes	<i>CHS</i>	<i>CTCATGATGTATCAGCAGGG</i>	<i>ACATCCTTGAGGTGGAA</i>
	<i>CHI</i>	<i>GCAGTACTCGGACAAGGTGA</i>	<i>GTTCTGTTACACCCGAAACC</i>
	<i>F3H</i>	<i>CCTACTTCTCGTACCCGGTG</i>	<i>GAACGTCGCGATCGACAG</i>
	<i>DFR</i>	<i>TGCTGGAGCTTCCCGGAGC</i>	<i>CGTGGGGATGATGCTGATGA</i>
	<i>ANS</i>	<i>GTCTCCGCGCTCTCCTTC</i>	<i>TCCTTCTCCTCCTTGTGAGC</i>
	<i>Actin</i>	<i>GCCACACTGTTCCAATCTATGA</i>	<i>TGATGGAATTGTATGTCGCCTTC</i>
Antioxidant-related genes	<i>APX</i>	<i>GCAGCTGCTGAAGGAGAAGT</i>	<i>CACTGGGGCCACTCACTAAT</i>
	<i>CAT</i>	<i>CCATGAGATCAAGGCCATCT</i>	<i>ATCTTACATGCTCGGCTTGG</i>
	<i>DHAR</i>	<i>GACCAAGGAGAACCTGATCG</i>	<i>CGTCGCTACTCTCACACGAC</i>
	<i>GPX</i>	<i>CCCCCTGTACAAGTTCCTGA</i>	<i>GTCAACAACGTGACCCTCCT</i>
	<i>GR</i>	<i>TGCGTCCCGAAGAAGATACT</i>	<i>GTTGATGTCCCCGTTGATCT</i>
	<i>MDAR</i>	<i>GCTCCTCGACCATAAAGCTC</i>	<i>CATAGCTGCGACCAACTTGT</i>
	<i>MnSOD</i>	<i>CAGAGGGTGTGCTTTACAA</i>	<i>GGTACAAGAGGGTCTCTGAT</i>
	<i>ZnCuSOD</i>	<i>CGCTCAGAGCCTCCTCTTT</i>	<i>CTCCTGGGGTGGGAGACAAT</i>
	<i>Actin</i>	<i>GCCACACTGTTCCAATCTATGA</i>	<i>AGCGGTTGTTGTGAGGGAGTA</i>

(EC 1.15.1.1) activity was assayed by measuring the inhibition of the photochemical reduction of NBT using Giannopolitis and Ries (1977) method. The reaction mixture containing 50 mM phosphate buffer (pH 7.8), 13 mM methionine, 75 μ M NBT, 2 μ M riboflavin, 0.1 mM EDTA and 60 μ L of enzyme extract was placed under strong light conditions for 30 min. The absorbance by the reaction mixture was recorded at 560 nm. Ascorbate peroxidase (EC 1.11.1.11) activity was determined by following the procedure of Nakano and Asada (1981) with some modification. The reaction mixture consisted of 50 mM potassium phosphate buffer (pH 7.0) containing 50 mM (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 0.1 mM EDTA, 1 mM H₂O₂, and 0.6 mM ascorbate. The APX activity was assayed by measuring the decrease in absorbance at 290 nm for 90 seconds using the extinction coefficient of ascorbate ($\epsilon = 13.7 \text{ mM}^{-1} \text{ cm}^{-1}$). Peroxidase (EC 1.11.1.7) activity was determined by following Chance and Maehly (1955) with some modification. The reaction mixture consisted of 100 mM potassium phosphate buffer (pH 6.0), 147 mM H₂O₂, 5% pyrogallol, and 30 μ L of enzyme extract. POD activity was recorded by measuring the absorbance changes at 420 nm for 20 seconds according to the oxidation of pyrogallol.

Statistical Analysis

Means and Standard Deviation (SD) were calculated from at least three different

samples. The Anderson-Darling test was used to test formally for statistical test. The statistical analyses were performed using MINITAB16 software.

RESULTS

Plant Growth Responses

The effect of gamma rays was investigated by studying plant development and physiological changes (Figure 1). After chronic gamma irradiation, plant height was significantly inhibited at higher doses (Table 2). With an increase in radiation dose (25-150 Gy), the average height of wheat plant was significantly reduced. However, at an absorbed dose of 10, 12.5, and 15 Gy, the plant height increased by 15, 19, and 11%, respectively.

Expression of Antioxidant-Related Genes and Antioxidant Enzyme Activity

To examine the expression of antioxidant-related genes in chronic irradiated wheat plants, qRT-PCR was performed (Figure 2). Those genes encoding antioxidant enzymes exhibited similar expression patterns in response to chronic gamma irradiation. Transcription levels of antioxidant-related genes were significantly decreased by chronic gamma irradiation. But, *APX* and *CAT* expression levels were higher in chronic gamma irradiation dose at 12.5 Gy than in control (Figures 2-A and -B). The *Cu/ZnSOD*, *MnSOD*, *GR* and *GPX*

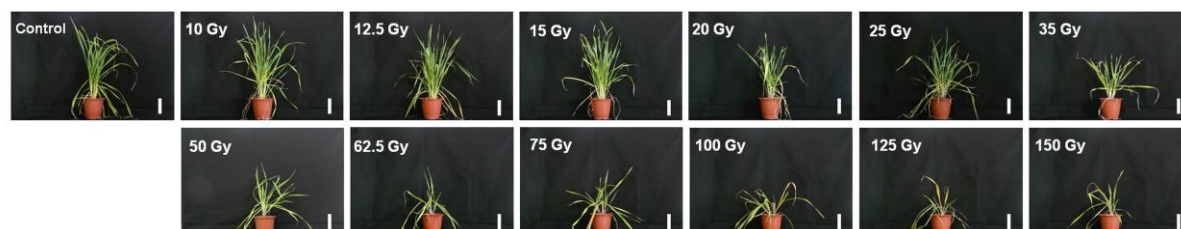


Figure 1. Effect of chronic gamma irradiation at increasing doses on plant growth. Wheat plants were exposed to the indicated doses of gamma rays for 3 weeks. The effects of radiation at doses of 0, 10, 12.5, 15, 20, 25, 35, 50, 62.5, 75, 100, 125, and 150 Gy, respectively, are shown. Scale bar indicates 10 cm.

**Table 2.** Wheat plant height after chronic gamma irradiation (n= 3).

Dose (Gy)	Plant height (cm) (Mean±SD)
0	41.3 ± 1.53
10	47.7 ± 1.15
12.5	49.3 ± 2.08
15	46.0 ± 1.00
20	42.7 ± 1.53
25	40.7 ± 3.06
35	36.0 ± 1.00
50	32.7 ± 1.53
62.5	34.7 ± 2.52
75	33.0 ± 1.00
100	33.3 ± 1.15
125	32.7 ± 0.58
150	32.7 ± 3.21

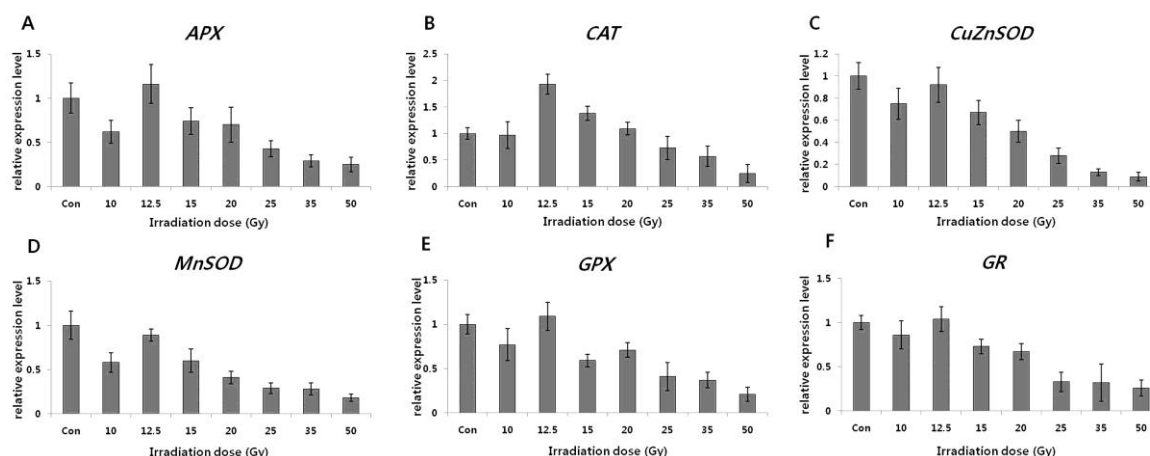
transcripts in 12.5 Gy of chronic gamma rays showed similar level compared to control (Figure 2). Also, in order to determine the activities of the antioxidant enzyme of different dosages of chronic gamma irradiation on wheat plants, we measured the enzymatic activity of APX, CAT, SOD, and POD (Figure 3). APX, CAT, and POD showed highest enzyme activity at the dosage of 12.5 Gy. The maximum CAT activity was recorded at irradiation dose of 12.5 Gy compared with control. After irradiation dose 12.5 Gy, the activity of CAT declined. The highest SOD

activity was observed at 10 Gy. The activities of APX, SOD, and POD were increased at 10, 12.5, and 15 Gy, respectively. APX activity increased in all dosage under chronic gamma irradiation. Higher doses of gamma irradiation (> 20 Gy) led to decrease in the activities of CAT and SOD. POD activities exhibited a slight increase under high dose of gamma irradiation (> 20 Gy).

Anthocyanin Contents and Expression of Anthocyanin Biosynthesis-Related Genes

After chronic irradiation, wheat leaves showed accumulation of purple-colored pigments (Figure 4-A). To confirm the accumulation of anthocyanin following chronic gamma irradiation in wheat plants, we measured anthocyanin content. The total anthocyanin content gradually increased following chronic exposure to 25 Gy gamma irradiation compared to non-irradiated plants (Figure 4-B). This result indicates that color change of wheat leaf following chronic gamma irradiation is positively correlated with the increase in anthocyanin content.

We analyzed UPLC to identify the anthocyanin pigment in chronic gamma irradiated wheat plants, and RT-PCR to determine the expression of anthocyanin

**Figure 2.** Effect of chronic gamma irradiation on expression of antioxidant-related genes: (A) APX; (B) CAT; (C) CuZnSOD; (D) MnSOD; (E) GPX, and (F) GR. Each bar represents mean±SD for average n= 3 independent experiments.

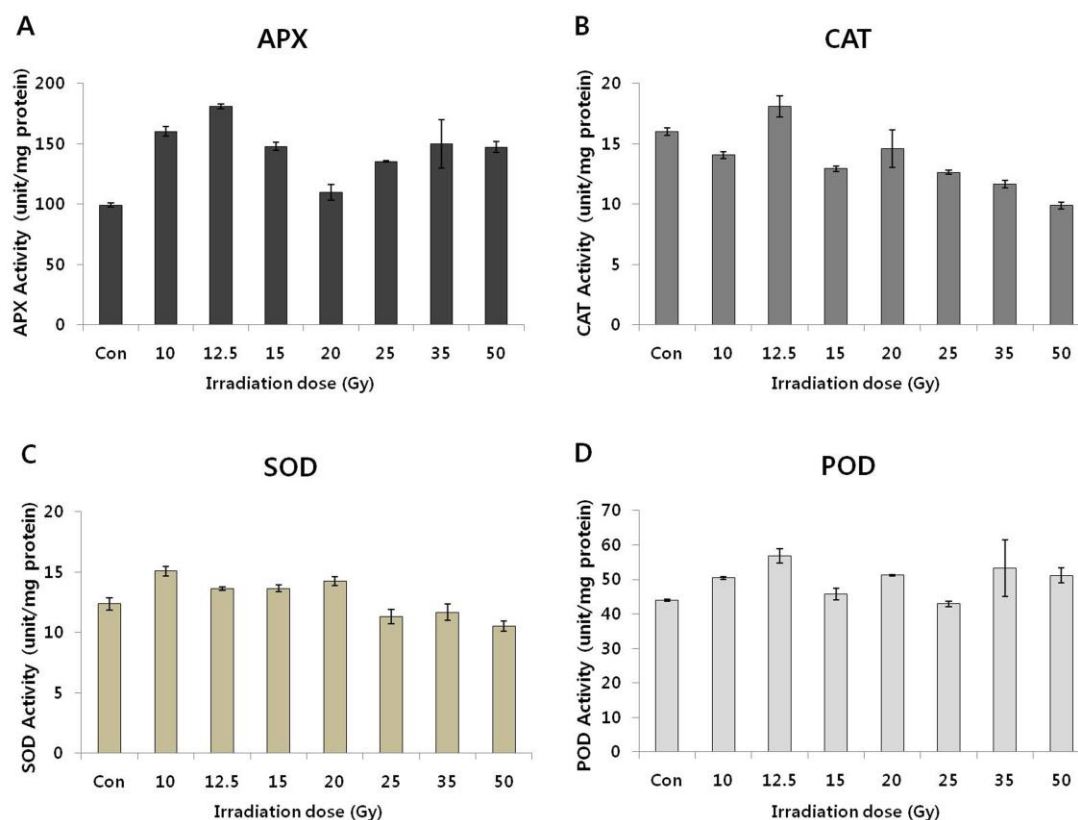


Figure 3. Antioxidant enzyme activities of: (A) APX; (B) CAT; (C) SOD, and (D) POD in wheat plants according to chronic gamma irradiation dose. Each bar represents mean \pm SD for average $n=3$ independent experiments.

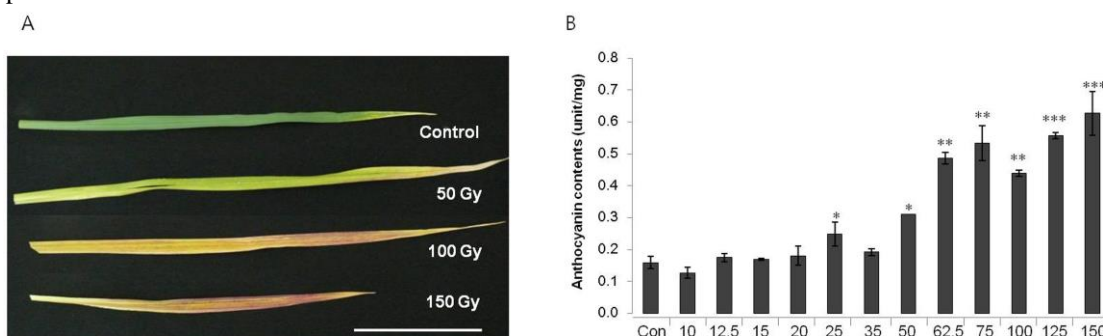


Figure 4. Anthocyanin accumulation patterns after chronic gamma irradiation: (A) Changes in wheat leaf color according to different gamma radiation doses, (B) Effect of chronic gamma irradiation on total anthocyanin levels. Each bar represents mean \pm SD for average $n=3$ independent experiments. Scale bar indicates 10 cm. * $P < 0.05$; ** $0.01 < P < 0.05$; *** $P < 0.001$.

biosynthesis-related genes. Some reports have shown that the major anthocyanin pigments of the black rice are Cyanidin-3-Glucoside (C3G) and Peonidin-3-Glucoside (P3G) (Chen *et al.*, 2012; Hou *et al.*, 2013). The C3G significantly increased following

chronic gamma irradiation (Table 3). The C3G content decreased following exposure to 150 Gy, but the C3G content of irradiated wheat plants was ten-fold higher than that of non-irradiated plants. Although total anthocyanin contents increase according to

**Table 3.** Content of cyanidin-3-glucoside in purple colored-wheat leaf (n = 3).

Cyanidin-3-glucoside	Mean±SD (mg 100 g ⁻¹)
Con	4.45 ± 0.6
50 Gy	97.05 ± 8.8
100 Gy	96.07 ± 9.5
150 Gy	56.80 ± 5.7

chronic gamma irradiation of dose range, further study is needed to confirm whether degradation/synthesis of each anthocyanin pigments is changed at each dose range of gamma irradiation.

To determine the expression of anthocyanin biosynthesis-related genes in irradiated wheat plants, semi-quantitative RT-PCR analysis was performed (Figure 5). Transcript levels of the *CHS* gene decreased as the gamma dose increased. *CHI* transcripts were detected from 10 to 20 Gy, and then declined to almost non-detectable levels after 25 Gy in irradiated wheat. *F3H*, *DFR*, and *ANS* transcripts were detected between 20 and 150 Gy. Depending on the radiation dose, anthocyanin biosynthesis related genes steadily increased unlike the *CHS* gene. Expression of *F3H*, *DFR*, and *ANS* genes and the total anthocyanin graph were found to be similar, suggesting that

anthocyanin content was influenced by expression levels of *F3H*, *DFR*, and *ANS*.

DISCUSSION

Numerous researchers suggested that exposure to low-dose ionizing radiation enhances plant reproduction, growth, maturation, and development (El-Beltagi *et al.*, 2011; Melki and Marouani, 2010). This phenomenon, called hormesis, has been shown to stimulate accelerated cellular proliferation, germination rate, enzyme activity, stress resistance, and crop yield following low-dose ionizing irradiation (Calabrese, 2002). The biomass and seed yield of soybean exposed to low-dose gamma irradiation (20 Gy) was significantly increased compared with that of the non-irradiation (Moussa, 2011). The growth of *Arabidopsis* seedlings was markedly suppressed by high-dose irradiation with 50 Gy, while seedling growth was slightly increased compared with that in non-irradiated plants (Wi *et al.*, 2007). Stimulatory effects by low-dose gamma irradiation may be affected by altering the hormonal signaling network in the plant cells or by increasing the antioxidative capacity of the cells (Wi *et al.*, 2007). Low dose radiations as stress factor induce the growth stimulation by developing defense

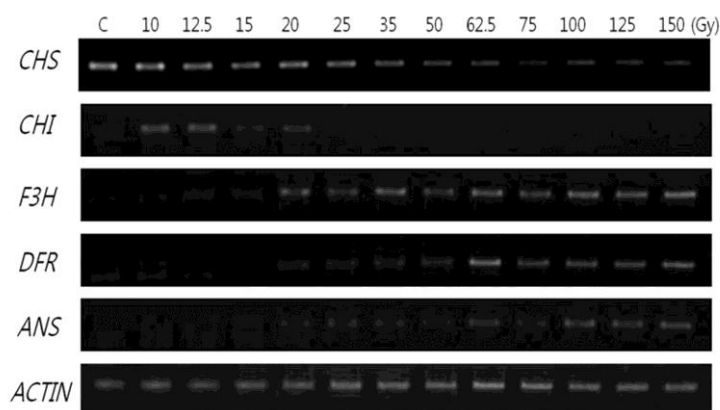


Figure 5. The differentially expressed genes of anthocyanin biosynthesis-related genes (*CHS*, *CHI*, *F3H*, *DFR*, and *ANS*) after chronic gamma irradiation. Relative transcripts were normalized to actin (GeneBank ID: AB181991) transcripts.

mechanisms and increasing the antioxidative capacity of the cells. Low dose of chronic irradiation may be responsible for induction in plant growth and survival by regulation of genes involved in metabolic and physiological functions and changes the homeostasis of plants (Kim *et al.*, 2004). On the other hand, high-dose gamma irradiation leads to growth inhibition because cell cycle arrest occurs in the G₂/M phase during somatic cell division or by damage throughout the entire genome (Preuss and Britta, 2003). We demonstrated that inhibition of wheat growth was regulated in a dose-dependent manner following chronic irradiation. And also, this study indicated that low dose of chronic gamma irradiation may stimulate plant growth in wheat.

Higher levels of antioxidant-related gene expression and antioxidant enzyme activity may be in correlation with plant growth. Gamma irradiation is known to be ROS generator, causing disturbances to cellular metabolism. High concentration of ROS can cause oxidative stress leading to damage of proteins, lipids, carbohydrates, and DNA (Gill and Tuteja, 2010). The ROS scavenging by a protective system of enzymes such as SOD, APX, and CAT maintain the cell in oxidative balance (Mittler, 2002). The scavenging of ROS by increased activation of antioxidant enzymes can induce cell proliferation, germination rate, and cell growth (Chakravarty and Sen, 2001; Kim *et al.*, 2004). The activity of CAT showed a positive correlation with transcript levels of the genes encoding these proteins (Figures 2 and 3). But, the activities of APX and SOD were slightly induced by low-dose of chronic gamma irradiation despite decrease of mRNA expression. The discrepancy between gene expression and enzyme activity seen in chronic gamma irradiation indicates that enzyme activity alterations were not caused by transcript levels but were regulated at post-transcription levels which they might partly be translation/post-translation or degradation/synthetic processes (Nie *et al.*, 2006). Although no clear correlation between increased gene expression and enzyme

activity was definite tendency in whole dose of chronic gamma irradiation, the increased levels of antioxidant-related gene transcripts at low dose of chronic gamma irradiation may be a cause of up-regulated activation of these proteins. These results demonstrate that ROS scavenging through increased enzyme activity by low dose of chronic gamma irradiation may help in triggering the wheat growth.

Anthocyanin performs major roles in defense against pathogens and pests, and accumulates in response to various stresses such as desiccation, low temperature, and during UV exposure (Petrucci *et al.*, 2013). Anthocyanin is a member of the flavonoid class of compounds that produce plant colors ranging from orange to purple and blue (Shoji *et al.*, 2010). According to differences in their transcriptional regulation, anthocyanin biosynthesis genes were classified into two groups: Early Biosynthesis Genes (EBGs), and Late Biosynthesis Genes (LBGs) (Nesi *et al.*, 2000). EBGs include *CHS* and *CHI* genes are involved in anthocyanin and flavones and/or flavonol biosynthesis (Nesi *et al.*, 2000). *DFR* and *ANS* genes are classified into the LBGs pathways for proanthocyanin and anthocyanin biosyntheses (Nesi *et al.*, 2000). The expression of LBGs, starting at *F3H*, is regulated by EBGs (Mol *et al.*, 1998; Nesi *et al.*, 2000). Previous studies indicate that anthocyanin molecules are produced by signal cascades of enzyme activity (Gou *et al.*, 2011). *CHS* is the key enzyme at the beginning of the flavonoid pathway. And subsequent *CHI* converts the naringenin-chalcone to naringenin-flavanone (Dao *et al.*, 2011). Although *CHS*, *CHI* transcripts was not directly correlated with anthocyanin accumulation, *CHS*, *CHI* expression may affect subsequent steps for synthesis of anthocyanin pigments in chronic gamma irradiation. These experiments suggested that *F3H*, *DFR*, *ANS* transcripts were directly and positively correlated with anthocyanin accumulation, because total anthocyanin content increased according to gene expression of their genes (Figure 5, Table 3). We found that the rapid accumulation of



anthocyanins was correlated with an increase in *DFR* expression at 62.5 Gy of chronic gamma irradiation. Expression of anthocyanin biosynthesis-related genes following chronic gamma irradiation exposure showed a positive correlation with anthocyanin accumulation.

Anthocyanin has protective effects as a ROS scavenger and protects plants against damage caused by environmental stress. This study investigated the regulation of anthocyanin accumulation and anthocyanin biosynthesis-related genes in anthocyanin biosynthesis following chronic gamma irradiation. Anthocyanin accumulation as a consequence of chronic irradiation can affect normal plant growth and development through the elimination of ROS.

We irradiated various doses of chronic gamma ray on wheat plants to study its effects on plant response. We suggest that effective stimulatory dose for plant development is 12.5 Gy for wheat plants under chronic gamma irradiation. This study establishes the preliminary parameters of radiosensitivity in wheat plants under chronic gamma irradiation and provides important data on gamma dose range for chronic radiation-induced mutagenesis of wheat varieties.

ACKNOWLEDGEMENTS

This work was supported by grants from Nuclear R&D Program by the Ministry of Science and ICT (MSIT), and the research program of KAERI, Republic of Korea.

REFERENCES

1. Aebi, H. 1984. Catalase *In Vitro*. *Method. Enzymol.*, **105**: 121-126.
2. Bradford, M. M. 1976. A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein Dye Binding. *Anal. Biochem.*, **72**: 248-254.
3. Calabrese, E. J. 2002. Hormesis: Changing View of the Dose-Response, a Personal Account of the History and Current Status. *Mutat. Res. Rev. Mutat. Res.*, **511**: 181-189.
4. Chakravarty, B. and Sen, S. 2001. Enhancement of Regeneration Potential and Variability by γ -Irradiation in Cultured Cells of *Scilla indica*. *Biol. Plant.*, **44**:189-193.
5. Chance, B. and Maehly, A. C. 1955. Assay of catalase and peroxidase. *Method. Enzymol.*, **2**: 764-775.
6. Chen, X. Q., Nagao, N., Itani, T. and Irifune, K. 2012. Anti-Oxidative Analysis, and Identification and Quantification of Anthocyanin Pigments in Different Colored Rice. *Food Chem.*, **135**: 2783-2788.
7. Dao, T., Linthorst, H. and Verpoorte, R. 2011. Chalcone Synthase and Its Functions in Plant Resistance. *Phytochem. Rev.*, **10**: 397-412.
8. El-Beltagi, H. S., Ahmed, O. K. and El-Desouky, W. 2011. Effect of Low Doses γ -Irradiation on Oxidative Stress and Secondary Metabolites Production of Rosemary (*Rosmarinus officinalis* L.) Callus Culture. *Radiat. Phys. Chem.*, **80**: 968-976.
9. Giannopolitis, C. N. and Ries, S. K. 1977. Superoxide Dismutase I. Occurrence in Higher Plants. *Plant Physiol.*, **59**: 309-314.
10. Gill, S. S. and Tuteja, N. 2010. Reactive Oxygen Species and Antioxidant Machinery in Abiotic Stress Tolerance in Crop Plants. *Plant Physiol. Biochem.*, **48**:909-930.
11. Gou, J. Y., Felippes, F. F., Liu, C. J., Weigel, D. and Wang, J. W. 2011. Negative Regulation of Anthocyanin Biosynthesis in *Arabidopsis* by a miR156-Targeted SPL Transcription Factor. *Plant Cell*, **23**: 1512-1522.
12. Hong, M. J., Kim, J., Yoon, Y. H., Kim, S. H., Ahn, J., Jeong, I. Y., Kang, S., Seo, Y. W. and Kim, D. S. 2014. The Effects of Chronic Gamma Irradiation on Oxidative Stress Response and the Expression of Anthocyanin Biosynthesis-Related Genes in Wheat (*Triticum aestivum*). *Int. J. Radiat. Biol.*, **90**:1218-1228.
13. Hou, Z., Qin, P., Zhang, Y., Cui, S. and Ren, G. 2013. Identification of Anthocyanins Isolated from Black Rice (*Oryza sativa* L.) and Their Degradation Kinetics. *Food Res. Int.*, **50**: 691-697.
14. Kim, J., Baek, M., Chung, B. Y., Wi, S. G. and Kim, J. 2004. Alterations in the Photosynthetic Pigments and Antioxidant Machineries of Red Pepper (*Capsicum*

- annuum* L.) Seedlings from Gamma-Irradiated Seeds. *J. Plant Biol.*, **47**: 314-321.
15. Kovalchuk, O., Arkhipov, A., Barylyak, I., Karachov, I., Titov, V., Hohn, B. and Kovalchuk, I. 2000. Plants Experiencing Chronic Internal Exposure to Ionizing Radiation Exhibit Higher Frequency of Homologous Recombination than Acutely Irradiated Plants. *Mutat. Res. Fund. Mol. M.*, **449**: 47-56.
 16. Kovalchuk, O., Burke, P., Arkhipov, A., Kuchma, N., James, S. J. and Kovalchuk, I. and Pogribny, I. 2003. Genome Hypermethylation in *Pinus silvestris* of Chernobyl: A Mechanism for Radiation Adaptation? *Mutat. Res. Fund. Mol. M.*, **529**: 13-20.
 17. Lu, S., Wang, Z., Niu, Y., Chen, Y., Chen, H., Fan, Z., Lin, J., Yan, K., Guo, Z. and Li, H. 2009. Gamma-Ray Radiation Induced Dwarf Mutants of Turf-Type Bermudagrass. *Plant Breed.*, **128**: 205-209.
 18. Mahamune, S. and Kothekar, V. 2011. Gamma Ray Induced Flower Colour and Seed Mutants in French Bean (*Phaseolus vulgaris* L.). *Recent Res. Sci. Technol.*, **3**: 33-35.
 19. Mancinelli, A. L., Rossi, F. and Moroni, A. 1991. Cryptochrome, Phytochrome, and Anthocyanin Production. *Plant Physiol.*, **96**:1079-1085.
 20. Melki, M. and Marouani, A. 2010. Effects of Gamma Rays Irradiation on Seed Germination and Growth of Hard Wheat. *Environ. Chem. Lett.*, **8**:307-310.
 21. Mita, S., Murano, N., Akaike, M. and Nakamura, K. 1997. Mutants of *Arabidopsis thaliana* with Pleiotropic Effects on the Expression of the Gene for β -Amylase and on the Accumulation of Anthocyanin that Are Inducible by Sugars. *Plant J.*, **11**: 841-851.
 22. Mittler, R. 2002. Oxidative Stress, Antioxidants and Stress Tolerance. *Trend. Plant Sci.*, **7**: 405-410.
 23. Moghaddam, S. S., Jaafar, H., Ibrahim, R., Rahmat, A., Aziz, M. A. and Philip, E. 2011. Effects of Acute Gamma Irradiation on Physiological Traits and Flavonoid Accumulation of *Centella asiatica*. *Mol.*, **16**: 4994-5007.
 24. Mol, J., Grotewold, E. and Koes, R. 1998. How Genes Paint Flowers and Seeds. *Trend. Plant Sci.*, **3**: 212-217.
 25. Moussa, H. 2011. Low Dose of Gamma Irradiation Enhanced Drought Tolerance in Soybean. *Acta Agronomica Hung.*, **59**: 1-12.
 26. Nakano, Y. and Asada, K. 1981. Hydrogen Peroxide Is Scavenged by Ascorbate-Specific Peroxidase in Spinach Chloroplasts. *Plant Cell Physiol.*, **22**: 867-880.
 27. Nesi, N., Debeaujon, I., Jond, C., Pelletier, G., Caboche, M. and Lepiniec, L. 2000. The *TT8* Gene Encodes a Basic Helix-Loop-Helix Domain Protein Required for Expression of DFR and BAN Genes in *Arabidopsis siliques*. *Plant Cell*, **12**:1863-1878.
 28. Nie, L., Wu, G. and Zhang, W. 2006. Correlations of mRNA Expression and Protein Abundance Affected by Multiple Sequence Features Related to Translational Efficiency in *Desulfovibro vulgaris*: A Quantitative Analysis. *Genet.*, **174**: 2229-2243.
 29. Nishiguchi, M., Nanjo, T. and Yoshida, K. 2012. The Effects of Gamma Irradiation on Growth and Expression of Genes Encoding DNA Repair-Related Proteins in Lombardy Poplar (*Populus nigra* var. *italica*). *J. Environ. Radioact.*, **109**: 19-28.
 30. Petrucci, E., Braidot, E., Zancani, M., Peresson, C., Bertolini, A., Patui, S. and Vianello, A. 2013. Plant Flavonoids—Biosynthesis, Transport and Involvement in Stress Responses. *Int. J. Mol. Sci.*, **14**: 14950-14973.
 31. Preuss, S. B. and Britta, A. B. 2003. A DNA-Damage-Induced Cell Cycle Checkpoint in *Arabidopsis*. *Genet.*, **164**: 323-334.
 32. Shoji, K., Momonoi, K. and Tsuji, T. 2010. Alternative Expression of Vacuolar Iron Transporter and Ferritin Genes Leads to Blue/Purple Coloration of Flowers in Tulip cv. 'Murasakizuisho'. *Plant Cell Physiol.*, **51**: 215-224.
 33. Syomov, A. B., Ptitsyna, S. N. and Sergeeva, S. A. 1992. Analysis of DNA Strand Break Induction and Repair in Plants from the Vicinity of Chernobyl. *Sci. Total Environ.*, **112**: 1-8.
 34. Vandenhove, H., Vanhoudt, N., Cuypers, A., Van Hees, M., Wannijn, J. and Horemans, N. 2010. Life-Cycle Chronic Gamma Exposure of *Arabidopsis thaliana* Induces Growth Effects but No Discernable Effects on Oxidative Stress Pathways. *Plant Physiol. Biochem.*, **48**:778-786.
 35. Welch, C. R., Wu, Q. and Simon, J. E. 2008. Recent Advances in Anthocyanin Analysis



- and Characterization. *Curr. Anal. Chem.*, **4**:75-101.
36. Wi, S. G., Chung, B. Y., Kim, J., Kim, J., Baek, M., Lee, J. and Kim, Y. S. 2007. Effects of Gamma Irradiation on Morphological Changes and Biological Responses in Plants. *Micron*, **38**:553-564.
37. Wu, J., Wu, C., Lei, C., Baraoidan, M., Bordeos, A., Madamba, M., Suzette, R., Ramos-Pamplona, M., Mauleon, R. and Portugal, A. 2005. Chemical- and Irradiation-Induced Mutants of Indica Rice IR64 for Forward and Reverse Genetics. *Plant Mol. Biol.*, **59**: 85-97.
38. Zaka, R., Chenal, C. and Misset, M. 2004. Effects of Low Doses of Short-Term Gamma Irradiation on Growth and Development through Two Generations of *Pisum sativum*. *Sci. Total Environ.*, **320**:121-129.

واکنش های فنوتیپیک و مولکولی گندم (*Triticum aestivum*) به تابش مزمن اشعه گاما

م. ج. هوتنگ، ی. ه. یون، د. س. کیم، س. ه. کیم، س. ی. کانگ، د. ی. کیم، ی. و. سو، و. ج. ب. کیم

چکیده

هدف پژوهش حاضر تعیین اثر تابش مزمن اشعه گاما روی رشد و ویژگی های زیست-شیمیایی گندم بود. بوته های گندم به مدت ۳ هفته در معرض اشعه گاما ^{60}Co در دز های بین ۱۰-۱۵۰ Gy قرار داده شدند. نتایج نشان داد که تابش 10-15 Gy موجب افزایش رشد گندم در مقایسه با بوته های تابش ندیده شد در حالیکه در دز های بالا تر (بیش از 20 Gy) کاهش معناداری در طول گیاه ثبت شد. سطح رونوشت های (transcript level) *APX* و *CAT* در بوته های تابش داده شده با اشعه 12.5 Gy بیش از بوته های شاهد بود. همچنین، فعالیت های آنزیمی *APX* و *CAT* و *POD* بر اثر تابش 12.5 Gy افزایش یافت. تابش مزمن موجب افزایش آنتوسیانین کل شد. برای ارزیابی مداخله ژن های مربوط به زیست ساخت (*biosynthesis*) آنتوسیانین در واکنش گندم به تابش مزمن گاما، بیان آن ها در در دز های مختلف اشعه گاما بررسی شد. سطوح رونوشت های *DFR*، *F3H*، و *ANS* در اثر تابش مزمن اشعه گاما افزایش یافت در حالیکه بیان *CHI* و *CHS* کم شد. در پی تابش مزمن اشعه مزبور، آنتوسیانین کل به طور معناداری زیاد شد. افزون بر این، کروماتوگرافی مایع با کارایی فوق العاده (*UPLC*) آشکار ساخت که در پی تابش مزمن اشعه گاما، یکی از مواد آنتوسیانین، یعنی *cyanidin 3-glucoside*، به سرعت در گیاه گندم افزایش یافت. این پژوهش نشان داد که رشد گیاه گندم و مارکرها فعالیت بیوشیمیایی تحت تابش اشعه مزمن گاما در فرآیندی وابسته به دز، به طور منفی تحت تاثیر قرار گرفت، هر چند که تابش با دز های کم تا حدودی اثر محرک داشت. نتایج این پژوهش برای مطالعات آینده استفاده از تابش مزمن اشعه گاما در اصلاح رقم های گندم بسیار مفید است.