Plant Omics Journal POJ

POJ 7(5):382-391 (2014) ISSN:1836-3644

Response of soybean plants to gamma radiation: Biochemical analyses and expression patterns of trichome development

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Abstract

This is the first report on soybean with the aim to show the effects of gamma radiation on trichome metabolim. Soybean seeds were subjected to 300 Gy gamma radiation at a dose rate of 10 Gy/min using a Cs-137 gamma source. The photosynthetic pigment, total protein content and ascorbate peroxidase activity were studied. The results showed that the chlorophyll a content was decreased by 80% on day 14 and by 77% on day 21 of irradiation. The chlorophyll b content was reduced by 58.6% and 62.06% on day 14 and 21 after irradiation, respectively. The total carotenoid concentration was reduced by 81.14% on the 14th day after irradiation and by 91% on the 21st day of irradiation, compared to control. The total protein concentration was found to have decreased significantly at 14 and 21 day after treatment. High level of ascorbate peroxidase (APX) activity was recorded in the leaves developed from irradiated soybean seeds, compared to the non-irradiated group. The trichome densities were 6.76 fold increased at 21 day of irradiation, while the stomatal densities were decreased, compared to control. We also performed a qRT-PCR analysis to detect the transcription levels of the soybean trichome developmental genes. The *GL2* and *CPC* genes were up-regulated (P≤0.05). The results of this study pointed out that the *CPC* transcription factor has to be study in further studies to provide an insight on its exact role in regulation of trichome development in soybean under radiation stress.

Keywords: Gamma radiation; *Glycine max*; trichome; ortholog genes; qRT-PCR.
Abbreviations: APX_Ascorbate peroxidase, ROS_Reactive oxygen species, GSH_Glutathione, GL1_GLABROUS 1, GL2_ GLABROUS 2, TTG1_TRANSPARENT TESTA GLABRA 1, WER_WEREWOLF, CPC_CAPRICE, SEM_Scanning electron microscopy, IS_irradiated seeds, NIS_non-irradiated seeds.

Introduction

Leaf surface is covered by various types of epidermal hairy outgrowths called trichomes. Trichomes can be glandular or non-glandular and can vary with regard to their presence across species, location on plant organs, density and functionality. Trichomes have a protective role against external factors, such as herbivores, pathogens, high temperatures and excessive water loss (Roy et al., 1999; Klich 2000; Steinite and Ievinsh, 2003; Valkama et al., 2003). Stomata, another structure on plant leaves, allows the plant to adapt to environmental and physiological conditions through the opening and closing of the stomatal pore (Schlüter et al., 2003). It is well understood that trichomes and stomata are adaptive features to extraordinary conditions (Gutschick, 1999). Furthermore, several studies have shown that glandular and non-glandular trichome numbers are induced after different abiotic stress treatments (Roy et al., 1999; Gonzáles et al., 2008; Mehri et al., 2009; Karray-Bouraoui et al., 2009).

Radiation is an important environmental stress that affects all living organisms. Ionizing radiation affects cellular components; thus, potentially inducing physiological changes in plants. Evaluation of these biological changes is very important as they occur through such physicochemical changes. Ionization, dissociation and excitation are the primary effects of ionizing radiation and result from energy deposition in the irradiated material. Ionizing radiation produces ·OH and O_2·- (Sun et al., 1998; Tian et al., 2007; Roldán-Arjona and Ariza 2009; Esnault et al., 2010; Vanhoudt et al,. 2010) and induces damage in cellular membranes (Mitsuhashi et al., 1998; Atak et al., 2004; Shuryak and Brenner 2009), changes in plastid ultrastructure (Kovács and Keresztes, 2002) and fragmentation of the endoplasmic reticulum, Golgi apparatus, DNA and proteins by breaking chemical bonds (Somosy, 2010). Free radicals interact with biomolecules, which affects enlargement of the thylakoid membranes, changes in photosynthesis and the accumulation of phenolic compounds (Wi et al., 2007; Al-Rumaih and Al-Rumaih, 2008). Plants limit ROS production by excess excitation energy through carotenoids and photorespiration (Esnault et al., 2010).

Ascorbate and glutathione (GSH) are important molecules that have roles in the ascorbate–glutathione cycle in many processes in plant cells. Abiotic and biotic stresses lead to the production of these molecules, which function to protect the cells from oxidative stress and in stress responses (Latowski et al., 2010).

The molecular mechanism of trichome formation in the leaf epidermis is under the control of different genes. However, the molecular mechanisms and developmental processes of trichomes in soybean plants have not been clearly described. In Arabidopsis, some genes have been identified for trichome initiation and development. The *GLABROUS 1 (GL1)* and

TRANSPARENT TESTA GLABRA 1 (TTG1) genes are the major genes in trichome initiation and leaf trichome spacing. *TTG1* encodes a WD-40 repeat-containing protein (Szymanski et al. 1998; Hauser et al. 2001; Gonzáles et al., 2008; Kryvych et al., 2008; Yan et al., 2012), and the *GLABRA2 (GL2)* gene has similarity to homeodomain proteins. *GL2* is an environmental change-sensitive gene and is regulated by the *TTG1* and *GL1* and *WEREWOLF (WER)* genes (Rerie et al. 1994; Masucci et al. 1996; Marks 1997; Szymanski et al. 1998). *CAPRICE (CPC)* encodes a small Myb protein; its transcription is promoted by *TTG1* and *GL1*, and it is known to be a negative regulator in *Arabidopsis* (Walker et al., 1999; Kirik et al., 2004).

In the present study, we irradiated soybean seeds to identify the physical harmful effects of radiation on M_1 generation. We also determined the effects of radiation on photosynthetic pigments and proteins. Developmental effects and differences were evaluated according to the leaf area, ultrastructural components of the leaves and the relations between soybean trichome densities and expression levels of the genes which have role in trichome-metabolism.

Results

Photosynthetic and biochemical results to gamma radiation

To determine the effects of gamma radiation on soybean leaves, we investigated the photosynthetic pigment concentrations, protein content, ascorbate peroxidase enzyme activity and trichome and stomatal densities on the abaxial and adaxial surfaces of the leaves of irradiated and non-irradiated soybean groups. The soybean plants showed a significant decrease in leaf size after the gamma radiation treatment, compared to control (Table 1). The leaf areas of the irradiated group were reduced by an average of 98.86% and 98.00%, compared to control, at 14 and 21 days after irradiation.

The chlorophyll a, chlorophyll b, total chlorophyll and chl a/b contents of the leaves collected from the germinated seeds of irradiated and non-irradiated groups are presented in Table 2. All the data were evaluated at 14 and 21 days after irradiation. In comparison to the control group, the total chlorophyll content was significantly decreased in the irradiated samples, and the chlorophyll a content was decreased by 80% on day 14 and by 77% on day 21. The chlorophyll b content was reduced by 58.6% on day 14 and by 62.06% on day 21 ($P \leq 0.05$). The total carotenoid concentration was reduced by 81.14% on the 14th day after irradiation and by 91% on the 21st day, compared to control ($P \leq 0.05$) (Table 2). Alterations in the chl a/b ratio were also observed (Table 2).

The biochemical studies demonstrated a high level of ascorbate peroxidase activity in the leaves generated from irradited soybean seeds, compared to the non-irradiated group. The APX activity increased in the non-irradiated group over the experimental period and was increased by 3.6-fold at 14 days and 3.8-fold at 21 days after irradiation ($P \leq 0.05$) (Figure 1).

MALDI-MS results to gamma radiation

The total soluble protein concentration was measured at 14 and 21 days after the irradiation of soybean seeds with gamma radiation. Comparing the relative changes of the irradiated and non-irradiated samples, the total protein concentration was found to have decreased significantly by 44.47% at day 14 and 88.85% at day 21 ($P \leq 0.05$) (Fig 1).

The total proteins extracted from the leaves generated from IS (Irradiated Seeds) and NIS (Non-Irradiated Seeds) were analyzed by SDS-PAGE in the range of 10-250 kDa (Fig 2). Similar protein bands were observed in the control and the treated samples after 14 and 21 days, but the intensities of the bands appeared different. A protein band with molecular weight of approximately 60-65 kDa was different between the control and the treated samples. We focused on identifying this protein band using 2 spots from this band for protein characterization by MALDI-TOF.

The MALDI-TOF analysis showed that both spots included the same protein. The spots showed 79% homology with the DEAD-box ATP-dependent RNA helicase S1 of *A. thaliana*, with a 0.88 expect rate. This protein has a predicted molecular weight of 63.777 Da.

Ultrastructural responses to gamma radiation

We determined the number of trichomes and stomata on the adaxial and abaxial surfaces of the leaves of non-irradiated and gamma-irradiated samples per mm^2 using SEM, as provided in Table 3. In the control group, the trichome and stomatal densities were higher on the abaxial surface in comparison to those on the adaxial surface of the leaves. On the 14th day after treatment, the number of trichomes on the abaxial and adaxial surfaces was increased compared to control. This was also found on the 21st day after treatment. The trichome densities were 39.63 on the abaxial surface and 42.52 on the adaxial surface of the leaves on the 21st day of treatment with respect to the control. The effect of irradiation on the trichome density of soybean leaves is shown in Fig 3.

The stomatal density on the leaves of the gamma-irradiated group showed a 70.39% decrease on the abaxial surface and a 32.76% decrease on the adaxial surface on the 14th day after treatment compared to the leaves of non-irradiated group. At 21 days after treatment, the number of stomata were decreased by 16.82% on the abaxial surface and 11.10% on the adaxial surface, compared to control. The comparison of stomatal density on the leaf surfaces of soybean (Üstün-1 cv.) on the 14th and 21st days after 300 Gy gamma radiation is shown in Fig 4.

The number of trichomes and stomata on the surface of the leaves of irradiated soybean weas evaluated. I was observed that the number of trichomes and stomata were decreased on both leaf surfaces. According to the data presented in Table 4, the trichome number on the abaxial and the adaxial surfaces of the leaves of irradiated groups exhibited decreases of 86.5% and 82.54% on day 21, respectively, compared to non-irradiated. The number of stomata was also reduced on the abaxial and adaxial surfaces (Table 4).

Trichome-related gene expression response to gamma radiation

In real-time PCR studies we observed up-regulation of all trichome-related genes after 14 and 21 days of irradiation. The highest increase rate was observed at *CPC* gene at 14 and 21 days of irradiation. All the expression levels changes (fold) in trichome-related genes were found statistically significant ($P \leq 0.05$). While, 0.32, 0.57 and 0.87 fold increases were observed at expression level of *TTG1*, *GL2* and *CPC* genes at 14 days of irradiation, compared to control.

Table 1. Leaf areas of control and gamma radiated plants (Mean values ± SD).

	Total Leaf Area (cm^2)	
	Control	300 Gy
Day 14	11.09 ± 0.10^{a}	0.126 ± 0.01^{c}
Day 21	12.53 ± 0.04^{b}	0.25 ± 0.01^{d}

*The results belong to the analysis done after 14 and 21 days of gamma irradiation. Letters above each column indicate statistical significance at the 5% probability level. Different letters imply significant difference between treatments.

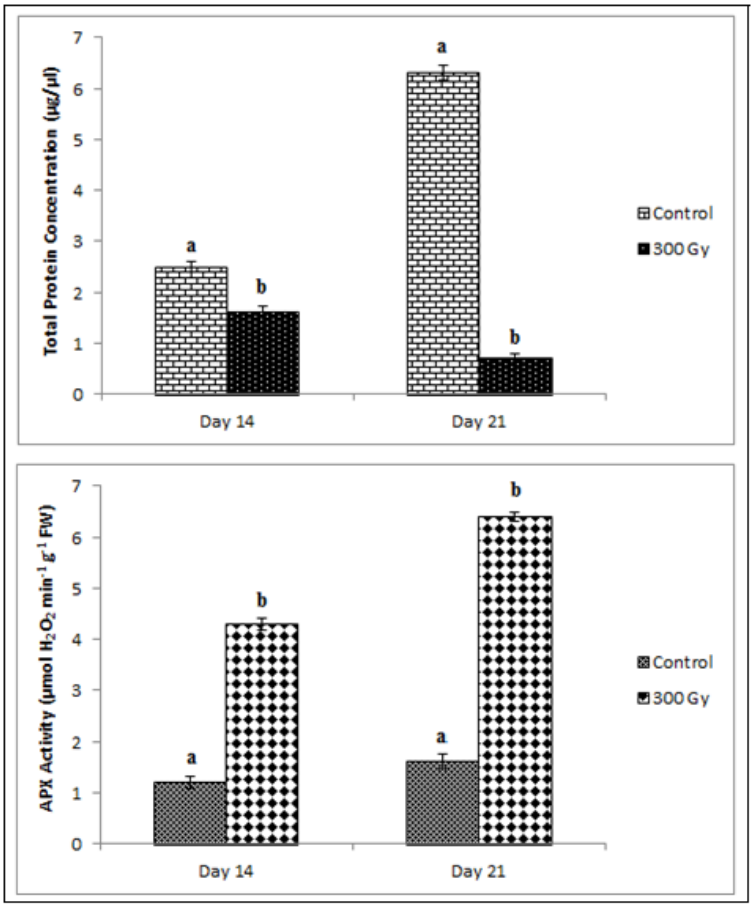

Fig 1. Effects of gamma radiation on the total protein concentration and on the APX activities of the leaves of Üstün-1 variety. Results are given as the means of three replicative experiments with standard error. Mean values in columns with different letters are significant at 5% level according to Duncan's Multiple Range Test.

Expression level of *TTG1* gene showed less up-regulation at 21 day than 14 day of irradiation. The increment rates were found as 0.14, 0.64, 0.93 fold in *TTG1*, *GL2* and *CPC* levels with respect to non-irradiated groups, respectively.

Discussıon

In our study, we observed a significant inhibitory effect of a 300 Gy gamma radiation dose on the growth of soybean leaves at 14 and 21 days after irradiation.

It is known that chloroplasts are extremely sensitive organelles (Wi et al., 2007; Borzouei et al., 2010). In the first stage of radiation stress, gamma radiation-induced free radicals. The free radicals can damage photosynthetic activity through thylakoid membrane extension, causing changes in photosynthesis (Shereen et al., 2009). Wi et al. (2007) observed that the structure of the chloroplasts were altered after gamma irradiation, with thylakoids that bulged and lacked structure.

In our study, the chlorophyll a, chlorophyll b and total chlorophyll concentrations were decreased at 14 and 21 days after irradiation compared to the non-irradiated group. The total carotenoid concentration showed a significant decrease compared to the control plants ($P \leq 0.05$).

Kovács and Keresztes (2002) reported that light-stressed cells contain four times more carotenoids than non-stressed cells. These authors also found that the accumulation of carotenoids is time-dependent and chloroplasts were modified into chromoplast-like organelles with higher levels of carotenoids after a long stress period. Carotenoids have been shown to be potential reactive oxygen species scavengers (Polyakov et al., 2001; Tian et al., 2007). We observed a decrease in the carotenoid concentration in the 300 Gy irradiated group compared to the non-irradiated control ($P \leq 0.05$). Although the irradiation dose was sufficiently high to affect carotenoid metabolism, we did not

Table 2. Contents of photosynthetic pigments chlorophyll a, b, total chlorophyll, carotenoid and chl a/b rate in leaves of 14 and 21 day old seedlings from the control and 300 Gy irradiated soybean seeds (Mean values expressed in μg g^{-1} FW ± SD).

	Chl a		Chl b		T Chl		Carotenoid		Chl a/Chl b	
	Control	300 Gy	Control	300 Gy	Control	300 Gy	Control	300 Gy	Control	300 Gy
Day 14	64.73±0.21^{a}	12.87±0.23^{e}	26.26±0.28^{b}	10.86±0.22^{f}	90.96±0.22^{c}	23.73±0.43^{g}	15.43±0.71^{d}	2.92±0.05^{h}	2.46±0.03^{a}	1.18±0.01^{b}
Day 21	48.34±0.22^{a}	10.96±0.05^{e}	25.24±0.03^{b}	9.59±0.22^{f}	73.58±0.20^{c}	20.54±0.17^{g}	17.90±0.37^{d}	1.62±0.12^{h}	1.92±0.01^{c}	1.14±0.03^{d}

* Letters above each column indicate statistical significance at the 5% probability level. Different letters imply significant difference between treatments.

Table 3. Trichome and stomatal density (number per mm^2) on the abaxial and adaxial surfaces of the leaves of control and 300 Gy gamma irradiated plants on the 14th and 21st day of treatment (Mean values ± SD).

	Control				300 Gy			
	Trichome Density		Stomatal Density		Trichome Density		Stomatal Density	
	Abaxial Surface	Adaxial Surface	Abaxial Surface	Adaxial Surface	Abaxial Surface	Adaxial Surface	Abaxial Surface	Adaxial Surface
Day 14	12.05±3.76^{a}	8.45±2.33^{c}	337.78±29.75^{e}	198.28±40.75^{g}	71.40±23.76^{b}	63.27±25.73^{d}	100±11.80^{f}	133.33±22.36^{h}
Day 21	5.86±1.13^{a}	4.86±1.66^{c}	331.11±33.32^{e}	91.24±13.29^{g}	39.63±10.82^{b}	42.52±19.18^{d}	275.40±40.70^{f}	81.11±29.98^{h}

* Letters above each column indicate statistical significance at the 5% probability level. Different letters imply significant difference between treatments.

Table 4. Trichome and stomata numbers of control and gamma irradiated plants determined due to total leaf area (TLA) on the 14th and 21st day of treatment (Mean values ± SD).

	Control				300 Gy			
	Trichome Number /TLA		Stomata Number/TLA		Trichome Number/TLA		Stomata Number/TLA	
	Abaxial Surface	Adaxial Surface	Abaxial Surface	Adaxial Surface	Abaxial Surface	Adaxial Surface	Abaxial Surface	Adaxial Surface
Day 14	13363.4±342.7^{a}	9371.0±147.9^{d}	374598.0±2166.1^{h}	219892.5±22325.9^{l}	899.6±232.5^{c}	797.2±162.4^{f}	1260.0±333.8^{j}	1679.9±467.2^{n}
Day 21	7342.5±133.8^{b}	6089.5±135.7^{e}	414880.8±3222.8^{i}	114323.7±35158.3^{m}	990.7±347.3^{c}	1063.0±367.4^{g}	6885.0±356.9^{k}	2027.7±438.0^{p}

*Letters above each column indicate statistical significance at the 5% probability level. Different letters imply significant difference between treatments.

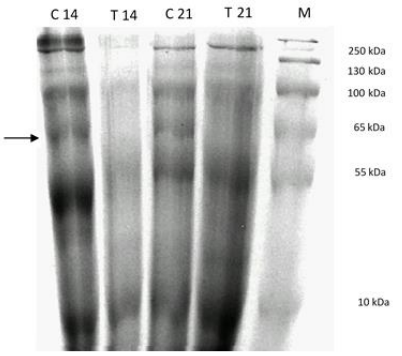

Fig 2. SDS-PAGE analysis of leaves of control and 300 Gy gamma irradiated plants on 14th and 21st day, separated on 12.5% Bis-Tris gel, stained with Coomassie Brillant Blue. Lane 1 and 2 represent proteins extracted from the leaves of control and treated plants after 14 days of irradiation, respectively; Lane 3 and 4 represent proteins extracted from the leaves of control and treated plants after 21 days of irradiation, respectively; Lane 6 represents the molecular weight marker. The arrow shows the protein band used in MALDI-TOF analysis.

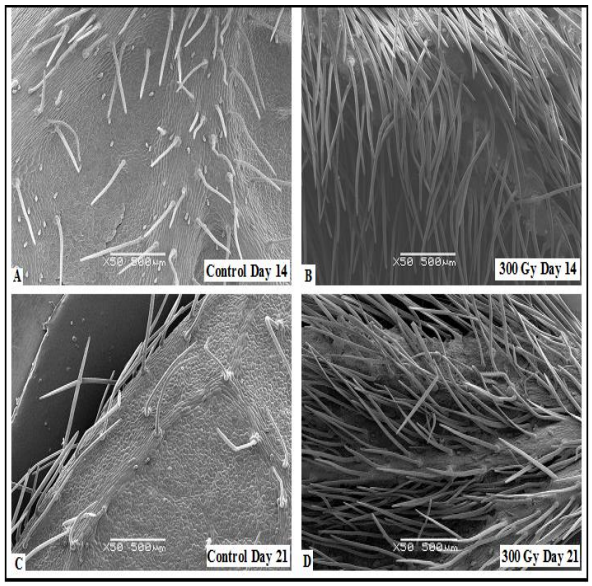

Fig 3. Increased trichome densities after 300 Gy radiation treatment screened by scanning electron microscopy analysis. The images belong to the abaxial surfaces of the leaves of control and 300 Gy gamma irradiated of soybean Üstün-1 cv. (x50). The images show the trichome density changes at 14 and 21 day after gamma radiation treatment. (A) Control on Day 14, (B) 300 Gy on Day 14, (C) Control on Day 21, (D) 300 Gy on Day 21.

observe any renewable effect on carotenoids after radiation-induced stress.

The total protein content of the soybean leaves was also affected by gamma radiation. The total protein concentration was higher in the control group on the 14th day, but the protein content in the leaves of the seedlings developed from irradiated seeds was decreased. We noted that the decrease was greater on the 21st day, which was consistent with the accumulation of radiation damage.

Changes in protein metabolism are the functional aspects of the effects of radiation. Kiong et al. (2008) and Maity et al. (2010) observed a radiation dose-dependent depletion of the total protein content and also reported that the soluble free amino acids of the seeds were increased in response to increasing doses of radiation. In contrast, Aziz and Mahrous (2004) reported that 5 kGy gamma-irradiated wheat and bean seeds showed no change in their protein, lipid or carbohydrate contents.

Singh and Datta, (2010) used a Cobalt-60 gamma source to irradiate wheat seeds at 0.01, 0.03, 0.05 and 0.10 kGy doses and observed an increase in the protein content and qualitative changes in the protein profiles of the irradiated grains.

According to the MALDI-TOF analysis results, an affected protein showed the highest similarity with DEAD-box ATP-dependent RNA helicase. DEAD-box proteins are present in organisms from all kingdoms (Jenks and Hasegawa, 2005), and DNA/RNA helicases are named according to their conserved amino acid sequence D-E-A-D (Asp-Glu-Ala-Asp). The DEAD-box proteins are involved in many processes, including RNA synthesis, RNA modification, RNA splicing, initiation of translation, organ maturation, cellular growth and differentiation (Aubourg et al. 1999; Cordin et al. 2006; Matthes et al. 2007; Linder and Owttrim 2009). Helicases are classified according to their main groups as superfamily (SF) 1 to 5. The SF1 and SF2 are the largest groups, and these helicases display DNA-dependent ATPase activities. Moreover, it has been reported that DEAD-box helicases are induced under different type of stresses (Kim et al. 2008; Linder and Owttrim 2009), and that helicases might play a key role in stress signaling (Kim et al., 2008). Luo et al. (2009) expressed the DEAD-box-containing cDNA sequence of *Medicago sativa* (MH1) in *Arabidopsis thaliana* and observed increases in the activities of antioxidative system enzymes.

Despite the increases in antioxidant enzyme activities, a developmental decline of the plants developed from irradiated soybean seeds was observed, which is in contrast to the expected stabilization of growth and reduction of some stress-induced pathways by the over-expression of DEAD-box RNA helicase protein.

Photosynthesis restriction through radiation stress causes an increase in oxygen radical-induced cellular damage. Plants respond to such abiotic stress through antioxidative enzymes that function in different cellular compartments. Abiotic stress enhances ROS production by the chloroplasts and mitochondria via the induction of the APX and CAT enzymes (Mittler 2002; Jenks and Hasegawa, 2005).

The APX activities in pepper was increased after low doses (2-8 Gy) of gamma irradiation (Kim et al., 2005).

Alcalá et al. (2000) indicated an increased GSH biosynthetic enzyme level in *Arabidopsis* leaf trichomes under salt stress. Harada et al. (2010) reported that trichomes have GSH-based defense mechanisms as a response to oxidative stresses to control the redox potential of the cells.

In plants, the main route of ascorbate regeneration is the ascorbate–glutathione pathway. Ascorbate is generated by GSH via the reduction of dehydroascorbate (DHA), and ascorbate and GSH are influenced by different abiotic stresses (Noctor and Foyer 1998; Alcalá et al. 2000; Foyer and Noctor 2011). In this study, we investigated the ascorbate level as a marker of the stress caused by 300 Gy gamma radiation.

The present results indicated that increases in the APX activities continued until the 21st day after treatment. An important leaf surface characteristic under abiotic stress conditions is trichome formation (Roy et al. 1999).

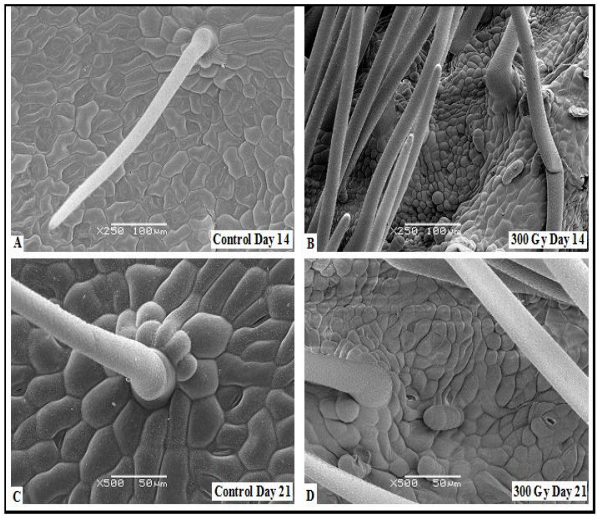

Fig 4. Effect of gamma irradiation on stomatal density on the abaxial surfaces of the leaves of soybean (Üstün-1 variety) (x50). (A) Control on Day 14, (B) 300 Gy on Day 14, (C) Control on Day 21, (D) 300 Gy on Day 21.

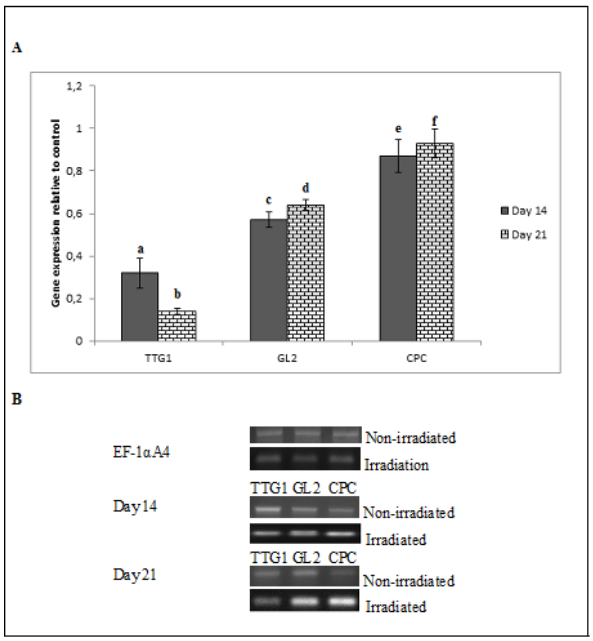

Fig 5. Expression of soybean orthologs of TTG1, GL2 and CPC genes. (A) The relative changes in expression profiles after 14 and 21 days of gamma radiation treatment (Mean ± SD) (B) Expression in the gamma irradiated soybean leaves were detected by RT-PCR. Gene specific fragments were amplified by RT-PCR with 35 cycles. EF-1αA4 was used a a housekeeping gene in response to γ-radiation.

Trichomes may serve as mechanical barriers against various environmental stresses (Liakoura et al., 1997; Valkama et al., 2003; Banowetz et al., 2008; Du et al., 2009). The density of leaf trichomes is an important parameter that reflects the adaptive responses of soybean plants to environmental conditions (Du et al., 2009).

Shahzad et al. (2005) subjected three different genotypes of chickpea (*Cicer arietinum*) plants to 100, 200, 300 and 400 Gy of gamma radiation, showing a positive correlation between radiation and trichome density. In our research, we found higher trichome densities on the abaxial surface than

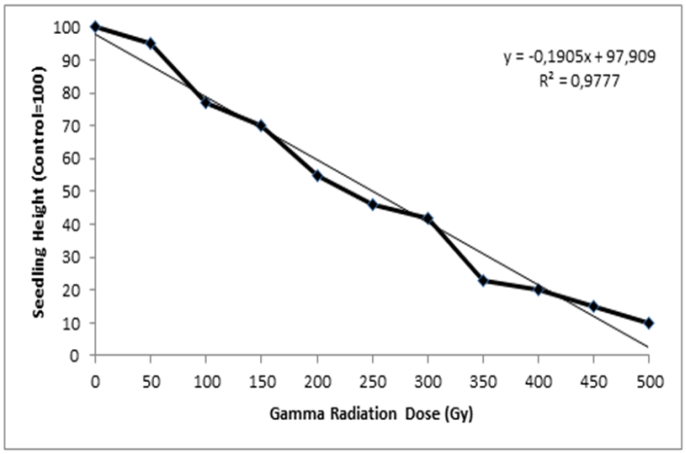

Fig 6. Standart curve illustrating the effect of increasing gamma radiation dose on seedling height.

the adaxial surface of the leaves of control plants, after 14th and 21st days of irradiation. At 14 days, the density of trichomes was increased on the abaxial surface of the leaves, an increase that was higher than the increase on the adaxial surface. In contrast, the increase in trichome density was higher on the adaxial surface than on the abaxial surface at 21 days.

Stomata responded quickly to environmental changes by reducing their dimensions and areas (Mehri et al., 2009). However, some studies have shown that abiotic stress treatments have no effect on stomatal density (Rodiyati, 2004; Inamullah and Isoda, 2005).

In our study, soybean plant leaves developed from gamma-irradiated seeds showed reductions in stomatal density and stomatal number compared to the control plants. The trichome and stomatal parameters were evaluated in accordance with their densities (number per mm^2). We also determined the number of trichomes and stomata in the total leaf area. Although the trichome density increased with gamma radiation, there was a decrease in the total number of trichomes. We demonstrated the detrimental effects of gamma radiation on the chlorophyll, carotenoid and total protein contents and ascorbate peroxidase enzyme activities. These effects included a decrease in the photosynthetic pigment concentration and total protein content, increases in the activity of ascorbate peroxidase enzyme and an inhibition of DEAD-box ATP-dependent RNA helicase protein.

Because the trichome density increased with gamma radiation, we examined the expression of soybean orthologs of genes that showed roles in trichome development. Our qRT-PCR analysis demonstrated an induction of the soybean orthologs of *TTG1* and *GL2* in soybean plants after radiation treatment, and these increases in expression were correlated with increased trichome density. The time-dependent increase in *GL2* expression also supported these results. As shown in the SEM, the 300 Gy gamma radiation treatments caused increases in the trichome length, compared to control (Fig 3 and 4). As the *GL2* gene is expressed in developing trichomes, we suggest that the up-regulation of the *GL2* gene is responsible for the elongation of trichomes, observed at 14 and 21 days of irradiation. The relative increase in the expression of the soybean ortholog of *CPC* due to 300 Gy gamma radiation was higher than the other genes. Walker et al. (1999) reported that the over-expression of the *CPC* gene caused a reduction in leaf trichomes. In conclusion, our results showed that the number of trichomes on both surfaces

of soybean leaves were decreased after gamma irradiation. The increased expression level of the CPC transcription factor suggests that it could have a potential to evaluate in futher studies as a key element in the regulation of trichome formation in soybean plants under radiation stress.

Materıals and Methods

Plant material

Seeds of the Üstün-1 soybean variety (*Glycine max* L. Merrill) were obtained from the Black Sea Agricultural Research Institute in Samsun, Turkey.

Irradiation of seeds

The radiosensitivity of Üstün-1 soybean variety was ascertained on the basis of seedling height. To determine the irradiation dose, the soybean seeds were irradiated with 0, 50, 100, 150, 200, 250, 300, 400 and 500 Gy gamma rays using a Cs-137 source at Leukemia Children Foundation at a dose rate of 10 Gy/min. The GR_{50} dose resulted in a 50% reduction in seedling height. The seedling height of the control was measured after 14 days, a time when the first leaf had stopped growing. The heights of the seedlings germinated from the irradiated seeds (IS) were measured at the same time point (Atak et al., 2004). The GR_{50} gamma radiation dose was determined to be 225 Gy on the basis of a fitted curve and a linear function (Fig 6). According to these results, Üstün-1 seeds irradiated with 300 Gy gamma rays inhibited plant growth by 69%, and this dose appeared to generate the maximum physiological damage in this soybean variety. The irradiated seeds (IS) and non-irradiated seeds (NIS) were sown in pots, and their leaves were harvested at 14 and 21 days after irradiation. All the experiments were conducted in a random plot design with 3 replicates. Leaves at the same physiological age and at the same positions were used for the biochemical analysis and ultrastructural and expression analyses.

Chlorophyll and carotenoid content determination

The photosynthetic pigment was extracted and calculated from the leaves of 14- and 21-day-old plants according to the Arnon (1949) using an Amersham Spectrophotometer, USA. The pigment concentrations were expressed as $\mu g\ g^{-1}$ FW.

Enzyme extraction and assays

All experiments were performed at 4 °C. For the protein and enzyme extraction, 0.5 g leaf sample was homogenized in ice-cold 50 mM sodium phosphate buffer (pH 7.8) containing 1 mM disodium EDTA and 2% (w/v) polyvinylpoly-pyrrolidone (PVPP). The total soluble protein content was determined according to Bradford (1976) using bovine serum albumin as a standard.

The ascorbate peroxidase (APX, EC 1.11.1.11) activity was determined according to Nakano and Asada, (1981). The absorption coefficient was 2.8 $mM^{-1}\ cm^{-1}$. One unit of APX was defined as 1 mmol ml^{-1} ascorbate oxidized min^{-1}.

SDS-PAGE

Protein samples of the 14- and 21-day-old leaves, developed from non-irradiated and 300 Gy gamma-irradiated seeds, were diluted 1:4 with 4X loading buffer and heated at 100 °C for 5 min and cooled to room temperature. The samples were applied at equal concentrations onto 12.5% (w/v) gels to avoid volume effects. Electrophoresis was performed at 50 mA using a vertical gel electrophoresis system (Mini Protean 3 Cell, Bio-Rad, USA). The gels were stained with 0.05% (w/v) Coomassie blue R-250, and the protein bands were evaluated using a gel imaging system (Molecular Imager Gel Doc^{TM} XR, Bio-Rad, USA).

In-gel digestion

After SDS-PAGE and protein staining, the bands that showed differences were excised from the gel and subjected to in-gel trypsin digestion (Shevchenko et al., 2006). The gel slices of interest were placed in Eppendorf tubes, and 50 µl of trypsin buffer was added to cover the dry gel pieces; more trypsin was added after 30 minutes. After an additional 90 minutes, 10-20 µl of ammonium bicarbonate buffer was added, and the samples were then stored at 37 °C overnight.

MALDI-MS sample preparation

The dried samples were centrifuged after being equilibrated to room temperature. The supernatants were transferred to new tubes, and the sample preparation was performed according to Shevchenko et al. (2006). Samples of 1 µl were loaded onto the sample plate and were left to dry at room temperature. Glufibrinopeptide and alcohol dehydrogenase were used as the calibrators.

Mass spectrometry and protein identification

The peptide mass fingerprint spectra were measured using a MALDI-TOF Mass Spectrometer (Micromass, Waters, USA) in the Bioengineering Department of Marmara University. The combined dataset was submitted to MASCOT (Matrix Science, London, UK, http://www.matrixscience.com) for protein identification (Liska and Shevchenko 2003). We executed the search against the SwissProt database using Viridiplantae (green plants) as the taxonomy. The other search parameters were tryptic digestion, one missed cleavage (Montford et al. 2002). Only significant hits, as defined by the MASCOT probability analysis ($P \leq 0.05$), were accepted.

Scanning electron microscopy

Fresh leaves were fixed for 24 h in 2.5% glutaraldehyde (pH 7.2, phosphate buffered) and then rinsed at least twice with distilled water. This was followed by dehydration in a graded series of ethanol (from 70% to 100%), and leaves were then passed through Amyl acetate. The samples were dried to the critical point with CO_2 (Polaron, CPD 7501) and mounted with double-sided tape on SEM stubs. The samples were coated with gold using a Polaron SC 502 Sputter coater and examined with a Jeol JSM 6060 Scanning Electron Microscope at 0.5-20 kV. The measurements and counting of trichomes and stomata on the adaxial and abaxial surfaces of the leaves were performed at 100 X and 500 X magnification with SEM. Photomicrographs were obtained digitally.

For some specimens, the calculation of trichomes can be difficult due to their density. To solve this problem, the trichomes were removed with tweezers in the buffer solution after the fixation step. After removal, the cells around the base of each trichome remained intact and were easily counted. However, when trichomes were removed from dried or fresh specimens, the results were not adequately reliable due to the breakage of the base structure.

The adaxial and abaxial surfaces of the growing leaves of germinated irradiated and non-irradiated seeds were examined by SEM at 14 and 21 days after treatment. The leaf areas were measured using the Scion Image software. Individual leaves were photographed with a Panasonic Lumix DMC FZ 30, and the images were converted to grayscale. The final versions were saved as TIFF files without LZW compression. We used utilized public domain software (Image 4.0.2 for Windows, National Institute of Health, Bethesda, MD) to estimate the areas of the samples. The operating system was downloaded from http://www.scioncorp.com.

We opened each TIFF file to be analyzed within the Image Software and selected the "set scale" option from the "analyze" menu. We selected a unit (cm) to convert pixels to a unit of measurement. We applied a ruler within the image for calibrating the pixel conversion. When the object was outlined, the surface area was calculated by selecting the "measure" option from the "analyze" menu (O'Neal et al., 2002). These measurements were replicated 5 times.

RNA isolation

The leaves collected from the plants generated from IS and NIS were used for total RNA extraction at 14 and 21 days after treatment. RNA extraction was performed with the MoBio UltraClean Plant RNA Isolation Kit (USA). The first-strand cDNA synthesis was performed in a total volume of 20 µl with the iScript cDNA synthesis kit (Bio-Rad, USA). Each reaction mixture contained 1 µg of total RNA and 4 µl iScript reaction mix. The mixtures were incubated at 25 °C for 5 minutes, 42 °C for 30 minutes and 85 °C for 5 minutes and were stored at 4 °C.

Expression analyses using quantitative RT-PCR

The synthesized cDNA was subjected to quantitative RT-PCR analysis using SYBR Green Supermix (Bio-Rad, USA) in an Mini Opticon™ Real-Time PCR System (Bio-Rad, USA).

The similarities between *Arabidopsis* trichome genes and soybean orthologs Hunt et al. (2011) were found to be higher than 60%, and the identical rate of particular domains were found to be higher than 92%. Therefore, the design of the primers was guided by the known conserved genomic sequences of the genes from *Arabidopsis.* The *GL2* (GenBank accession no. NM001198514.1), *TTG1* (GenBank accession no. NM180738.2), and *CAPRICE (CPC)* (GenBank accession no. NM130205.1). These genes were amplified using these following primers: *TTG1* F (5'-GCTGGACCCAATGGGATTGA-3') and *TTG1* R (5'-CAA GTGTGAGACCTGTCGCT-3'), *GL2* F (5'-CCGAGCTC GATAAGCTTCGT-3') and *GL2* R (5'-GCGCGTTACACG TACACATC-3') (Rerie et al., 1994) and *CPC* F (5'-CGTTGGCGACAGGTTAGAGA-3') and *CPC* R (5'-GATCCTTCCGGCGATCAACT-3'). The PCR conditions were as follows: a denaturation step at 94 °C for 3 min was followed by 35 cycles of 55 °C for 15 s, 72 °C 1.5 min and 94 °C for 15 s. The elongation factor *EF-1αA4* was used as an internal control to estimate the relative transcript level of the genes tested. Each PCR reaction was carried out in triplicate. A melting curve analysis was performed at the end of the PCR run over the range 30-85^{o}C, increasing the temperature stepwise by 0.5^{o}C every 10 s.

Relative quantification

Baseline and quantification cycles were determined. The raw C_t values obtained fom the CFX Manager™ Software were converted ino relative quantities via the delta-delta Ct method (Pfaffl et al. 2003) and the different expression levels between the irradiated and non-irradiated soybean samples were determined.

Statistical analysis

The data were presented as the mean ± SD of three independent experiments or 3 replicates of biochemical tests. All the statistical analyses were performed using an ANOVA, and we applied Duncan's multiple range test to compare the results of the experiments ($P \leq 0.05$).

Conclusıon

Ionizing radiation is an important environmental stress that effect living organisms. In this study, we aimed to identify the harmful effects of higher dose of radiation (300 Gy) on M_1 generation of soybean. Radiation caused decreases in photosynthetic pigment concentrations and protein content. According to the oxidative stress caused by irradiation, increased ascorbate peroxidase activity was recorded. Trichomes are known as a general adaptive responses of plants to survive under biotic and abiotic stresses We found higher trichome densities on the abaxial surface than the adaxial surface of the leaves of the control plants on the 14th and 21st days after irradiation. Soybean leaves developed from gamma-irradiated seeds showed reductions in stomatal density and stomatal number, compared to the control plants. The molecular mechanism of trichome formation has been just described for Arabidopsis. However, for soybean, it is no clear yet. To supplement fundamental studies on radiation stress as an environmental stress effect, we also analysed the expression levels of soybean orthologs of the genes with role in trichome formation. All the transcript levels of the genes were increased but the highest increase rate was observed at CPC transcription factor. CPC transcription factor should be examined to define the exact mechanism of the trichome formation as a scavenging mechanism against radiation stress in soybean plant.

References

Alcalá GG, Gotor C, Meyer AJ, Fricker M, Vega JM, Romero LC (2000) Glutathione biosynthesis in *Arabidopsis* trichome cells. Proc Natl Acad Sci USA. 97:1108-11113.

Al-Rumaih MM, Al-Rumaih MM (2008) Influence of ionizing radiation on antioxidant enzymes in the species of Trigonella. Am J Environ Sci. 4:151-156.

Arnon DI (1949) Copper enzymes in isolated chloroplasts polyphenoloxidase in *Beta vulgaris*. Plant Physiol. 4:1-15.

Atak Ç, Alikamanoğlu S, Açık L, Canbolat Y (2004) Induced of plastid mutations in soybean plant (*Glycine max* L. Merrill) with gamma radiations and determination with RAPD. Mutat Res. 556:35-44.

Aubourg S, Kreis M, Lecharny A (1999) The DEAD box RNA helicase family in *Arabidopsis thaliana*. Nucleic Acids Res. 27:628-636.

Aziz NH, Mahrous SR (2004) Effects of gamma irradiation on aflotoxin B1 production by *Aspergillus flavus* and chemical composition of three crop seeds. Nahrung. 48:234-238.

Banowetz GM, Azevedo MD, Stout R (2008) Morphological adaptations of hot springs panic grass (*Dichanthelium lanigunosum* var sericeum (Schmoll)) to thermal stress. J Therm Biol. 33:106-116.

Bharti AK, Khurana JP (2003) Molecular characterization of *transparent testa (tt)* mutants of *Arabidopsis thaliana* (ecotype Estland) impaired in flavonoid biosynthetic pathway. Plant Sci. 165:1321-1332.

Borzouei A, Kafi M, Khazaei H, Naseriyan B, Majdabadi A (2010) Effects of gamma radiation on germination and physiological aspects of wheat (*Triticum aestivum* L.) seedlings. Pak J Bot. 42:2281-2290.

Bradford MM (1976) A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 72:248-254.

Broin M, Cuiné S, Peltier G, Rey P (2000) Involvement of CDSP 32, a drought-induced thioredoxin, in the response to oxidative stress in potato plants. FEBS Lett. 467:245-248.

Cordin O, Banrouques J, Taner NK, Linder P (2006) The DEAD-box protein family of RNA helicases. Gene. 367:17-37.

Dai Q, Peng S, Chavez AQ, Vergara BS (1995) Effects of UVB radiation on stomatal density and opening in rice (*Oryza sativa* L.). Ann Bot. 76:65-70.

Esnault MA, Legue F, Chenal C (2010) Ionizing radiation: advances in plant response. Environ Exp Bot. 68:231-237.

Foyer CH, Noctor G (2011) Ascorbate and glutathione: the heart of the redox hub. Plant Physiol. 155:2-18

Gonzáles WL, Negritto MA, Suárez LH, Gianoli E (2008) Induction of glandular and non-glandular trichomes by damage in leaves of *Madia sativa* under contrasting water regimes. Acta Oecol. 33:128-132.

Gravano E., Tanı C, Bennici A, Gucci R (1998) The ultrastructure of glandular trichomes of *Phillyrea latifolia* L. (Oleaceae) leaves. Ann Bot. 81:327-335.

Gutschick VP (1999) Biotic and abiotic consequences of differences in leaf structure. New Phytol. 143:3-18.

Hameed A, Shah TM, Atta BM, Haq MA, Sayed H (2008) Gamma irradiation effects on seed germination and growth, protein content, peroxidase and protease activity, lipid peroxidation in desi and kabuli chickpea. Pak J Bot. 40:1033-1041.

Harada E, Kim JA, Meyer AJ, Hell R, Clemens S, Choi YE (2010) Expression profiling of tobacco leaf trichomes identifies genes for biotic and abiotic stresses. Plant Cell Physiol. 51(10):1627-1637.

Hauser MT, Harr B, Bettina A, Schlötterer C (2001) Trichome distribution in *Arabidopsis thaliana* and its close relative *Arabidopsis lyrat*: Molecular analysis of the candidate gene Glabrous1. Mol Biol Evol. 18(9):1754-1763.

Hunt M, Kaur N, Stromvik M, Vodkin L (2011) Transcript profiling reveals expression differences in wild-type and glabrous soybean lines. BMC Plant Biol. 11:145-160.

Inamullah A, Isoda A (2005) Adaptive responses of soybean and cotton to water stress. I. transcription changes in relation to stomatal area and stomatal conductance. Plant Prod Sci. 8:16-26.

Jenks MA, Hasegawa PM (2005) Plant Abiotic Stress, Roberts J.A. (Eds), Blackwell Publishing, Oxford.

Karray-Bouraoui N, Rabhi M, Neffati M, Baldan B, Ranieri A, Marzouk B, Lachaâl M, Smaoui A (2009) Salt effect on yield and composition of shoot essential oil and trichome morphology and density on leaves of *Mentha pulegium*. Indust Crops Prod. 30:338-343.

Kim JH, Chung BY, Kim JS, Wi SG (2005) Effects of in planta gamma-irradiation on growth, photosynthesis, and antioxidative capacity of red pepper (*Capsicum annuum* L.) plants. J Plant Biol. 48:47-56.

Kim JS, Kim KA, Oh TR, Park CM, Kang H (2008) Functional characterization of DEAD-box RNA helicases in *Arabidopsis thaliana* under abiotic stress conditions. Plant Cell Physiol. 49:1563-1571.

Kiong ALP, Lai AG, Hussein S, Harun AR (2008) Physiological responses of *Orthosiphon stamineus* plantlets to gamma irradiation. Am-Eur J Sust Agric. 2:135-149.

Kirik V, Simon M, Huelskamp M, Schiefelbein J (2004) The enhancer of TRY and CPC1 gene acts redundantly with TRIPTYCHON and CAPRICE in trichome and root hair cell patterning in *Arabidopsis*. Dev Biol. 268:506-513.

Klich MG (2000) Leaf variations in *Elaeagnus angustifolia* related to environmental heterogeneity. Environ Exp Bot. 44:171-183.

Kovács E, Keresztes Á (2002) Effect of gamma and UV-B/C radiation on plant cells. Micron. 33:199-210.

Kryvych S, Nikiforova V, Herzog M, Perazza D, Fisahn J (2008) Gene expression profiling of the different stages of *Arabidopsis thaliana* trichojme development on the single cell level. Plant Physiol Biochem. 46:160-173.

Latowski D, Surowka E, Strzalka K (2010) Regulatory role of components of ascorbate-glutathione pathway in plant stress tolerance, In: Anjum, N.A., Umar, S., Chan M.T. (Eds.), Ascorbate-glutathione pathway and stress tolerance in plants, Springer, The Netherlands, pp. 1-53.

Liakoura V, Stefanou M, Manetas Y, Cholevas C, Karabourniotis G (1997) Trichome density and its UV-B protective potential are affected by shading and leaf position on the canopy. Environ Exp Bot. 38:223-229.

Linder P, Owttrim GW (2009) Plant RNA helicases: linking aberrant and silencing RNA. Trends Plant Sci. 14:344-352.

Ling APK, Chia JY, Hussein S, Harun AR (2008) Physiological responses of *Citrus sinensis* to gamma irradiation. World App Sci J. 5:12-19.

Liska AJ, Shevchenko A (2003) Combining mass spectrometry with database interrogation strategies in proteomics. Trends Anal Chem. 22:291-298.

Luo Y, Liu YB, Dong YX, Gao XQ, Zhang XS (2009) Expression of a putative alfalfa helicase increases tolerance to abiotic stress in Arabidopsis by enhancing the capacities for ROS scavenging and osmotic adjustment. J Plant Physiol. 166:385-394.

Maity JP, Chakraborty S, Kar S, Panja S, Jean JS, Samal AC, Chakraborty A, Santra SC (2009) Effects of gamma irradiation on edible seed protein, amino acids and genomic DNA during sterilization. Food Chem. 114:237-1244.

Marks MD (1997) Molecular genetic analysis of trichome development in *Arabidopsis.* Ann Rev Plant Physiol Plant Mol Biol. 48:137-163.

Masucci JD, Rerie WG, Foreman DR, Zhang M, Galway ME, Marks MD, Schiefelbein JW (1996) The homebox gene GLABRA2 is required for position-dependent cell differentiation in the root epidermis of *Arabidopsis thaliana*. Dev. 122: 1253-1260.

Matthes A, Schmidt-Gattung S, Köhler D, Forner J, Wildum S, Raabe M, Urlaub H, Binder S (2007) Two DEAD-box proteins may be part of RNA-dependent high-molecular-mass protein complexes in Arabidopsis mitochondria. Plant Physiol. 145:1637-1646.

Mehri N, Fotovat R, Saba J, Jabbari F (2009) Variation of stomata dimensions and densities in tolerant and susceptible wheat cultivars under drought stress. J Food

Agric Environ. 7:167-170.
Mitsuhashi N, Koshiba T, Satô M (1998) Effect of γ-radiation on the plasma and vacuolar membranes of cultured spinach cells. Phytochem. 48(8):1281-1286.
Mittler R (2002) Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci. 7:405-410.
Montfort BA, Doeven MK, Canas B, Veenhoff LM, Poolmani B, Robillard GT (2002) Combined in-gel tryptic digestion and CNBr cleavage for the generation of peptide maps of an integral membrane protein with MALDI-TOF mass spectrometry. Biochim Biophys Acta. 1555:111-115.
Nagata T, Todoriki S, Hayashi T, Shibata Y, Mori M, Kanegae H, Kikuchi S (1999) γ-Radiation induces leaf trichome formation in Arabidopsis. Plant Physiol. 120:113-116.
Nakano Y, Asada K (1981) Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant Cell Physiol. 22:867-880.
Noctor G, Foyer CH (1998) Ascorbate and glutathione: keeping active oxygen under control. Ann Rev Plant Physiol Plant Mol Biol. 49:249-279.
O'Neal ME, Landis DA, Isaac R (2002) An inexpensive, accurate method for measuring leaf area and defoliation through digital image analysis. J Econ Entomol. 95:1190-1194.
Pfaffl MW, Gerstmayer B, Basio A, Windisch W (2003) Effect of zinc deficiency on the mRNA expression pattern in liver and jejenum of adult rats: Monitoring gene expression using cDNA microarrays combined with real-time RT-PCR. J Nutr Biochem. 14:691-702.
Polyakov NE, Leshina TV, Konovalova TA, Kispert LD (2001) Carotenoids as scavengers of free radicals in a fenton reaction: antioxidants or pro-oxidants?. Free Radic Biol Med. 31: 398-404.
Rerie WG, Feldmann KA, Marks MD (1994) The GLABRA2 gene encodes a homeo domain protein required for normal trichome development in Arabidopsis. Genes Dev. 8: 1388-1399.
Rodiyati A, Arisoesilaningsih E, Isagi Y, Nakagoshi N (2004) Responses of *Cyperus brevifolius* (Rottb.) Hassk. and *Cyperus kyllingia* Endl. to varying soil water availability. Environ Exp Bot. 53:259-269.
Roldán-Arjona T, Ariza RR (2009) Repair and tolerance of oxidative DNA damage in plants. Mutat Res/Rev Mutat Res. 68:169-179.
Roy BA, Stanton L, Eppley SM (1999) Effects of environmental stress on leaf hair density and consequence for selection. J Evol Biol. 12:1089-1103.
Salama HMH, Watban AAA, Al-Fughom AT (2011) Effect of ultraviolet radiation on chlorophyll, carotenoid protein and proline contents of some annual desert plants. Saudi J Biol Sci. 18:79-86.
Schlüter U, Muschak M, Berger D, Altmann T (2003) Photosynthetic performance of an Arabidopsis mutant with elevated stomatal density (sdd1-1) under different light regimes. J Exp Bot. 54:867-874.
Scion Image, http://scion-corporationsoftware
Shahzad K, Iqbal A, Khalil SK, Khattak S (2005) Response of different chickpea (*Cicer aritinum*) genotypes to the infestation of pod borer (*Helicoverpa armigera*) with relation to trichomes. Res J Agric Biol Sci. 1:120-124.
Shereen A, Ansari R, Mumtaz S, Bughio HR, Mujtaba SM, Shirazi MU, Khan MA (2009) Impact of gamma irradiation induced changes on growth and physiological response of rice under saline conditions. Pak J Bot. 41:2487-2495.
Shevchenko A, Tomas H, Havliš J, Olsen JV, Mann M (2006) In-gel digestion for mass spectrometric characterization of proteins and proteomes. J Nat Prod. 1:2856-2860.
Shuryak I, Brenner DJ (2009) A model of interactions between radiation-induced oxidative stress, protein and DNA damage in *Deinococcus radiodurans.* J Theor Biol. 261: 305-317.
Singh B, Datta PS (2010) Effect of low dose gamma irradiation on plant and grain nutrition of wheat. Rad Phys Chem. 79:819-825.
Somosy Z (2000) Radiation response of cell organelles. Micron. 31:165-181.
Štajner D, Popović BM, Taški K (2009) Effects of γ-irradiation on antioxidant activity in soybean seeds. Centr Europ J Biol. 4:381-386.
Steinite I, Ievinsh G (2003) Possible role of trichomes in resistance of strawberry cultivars against spider mite. Acta Univ Latv. 662:59-65.
Sun J, Chen Y, Li M, Ge Z (1998) Role of antioxidant enzymes on ionizing resistance. Free Radic Biol Med. 24:586-593.
Szymanski DB, Jilk RA, Pollock SM, Marks MD (1998) Control of *GL2* expression in *Arabidopsis* leaves and trichomes. Dev. 125:1161-1171.
Tian B, Xu Z, Sun Z, Lin J, Hua Y (2007) Evaluation of the antioxidant effects of carotenoids from *Deinococcus radiodurans* through targeted mutagenesis, chemiluminescence, and DNA damage analyses. Biochim Biophys Acta. 170:902-911.
Valkama E, Salminen JP, Koricheva J, Pihlaja K (2003) Comparative analysis of leaf trichome structure and composition of epicuticular flavonoids in Finnish birch species. Ann Bot-London. 91:643-665.
Vanhoudt N, Vandenhove H, Horemans N, Wannijn J, Hees MV, Vangronsveld J, Cuypers A (2010) The combined effect of uranium and gamma radiation on biological responses and oxidative stress induced in *Arabidopsis thaliana*. J Environ Radioact. 101:923-930.
Vashist AA, Tuteja N (2006) Stress responsive DEAD-box helicases: A new pathway to engineer plant stress tolerance. J Phytochem Phytobiol. 84:150-160.
Walker AR, Davison PA, Bolognesi-Winfield AC, James CM, Srinivasan N, Blundell TL, Esch JJ, Marks MD, Gray JC (1999) The *transparent testa glabra1* locus, which regulates trichome differentiation and anthocyanin biosynthesis in Arabidopsis, encodes a WD40 repeat protein. The Plant Cell. 11:1337-1349.
Du WJ, De-Yue Y, San-Xiong F (2009) Analysis of QTLs for the trichome density on the upper and downer surface of leaf blade in soybean *Glycine max* (L.) Merr. Agric Sci China. 8:529-537.
Wi SG, Chung BY, Kim JS, Kim JH, Baek MH, Lee H, Kim YS (2007) Effects of gamma irradiation on morphological changes and biological responses in plants. Micron. 38:553-564.
Yan A, Pan J, An L, Gan Y, Feng H (2012) The responses of trichome mutants to enhanced ultraviolet-B radiation in *Arabidopsis thaliana*. J Photochem Photobiol B. 113:29-35.