Pak. J. Bot., 42(4): 2281-2290, 2010.

EFFECTS OF GAMMA RADIATION ON GERMINATION AND PHYSIOLOGICAL ASPECTS OF WHEAT (*TRITICUM AESTIVUM* L.) SEEDLINGS

A. BORZOUEI[1, 2*], M. KAFI[2], H. KHAZAEI[2], B. NASERIYAN[1] AND A. MAJDABADI[1]

[1]*Agricultural, Medical and Industrial Research School, Nuclear Science and Technology Research Institute, P.O. Box: 31485/498, Karaj, Iran.*
[2]*Department of Agronomy, Ferdowsi University of Mashhad, P.O. Box 1163, Mashhad, Iran*
[*]*Corresponding author:aborzouei@nrcam.org*

Abstract

This investigation was carried out to determine the effects of gamma radiation on germination and physiological characteristics of wheat seedlings. Two wheat genotypes (Roshan and T-65-58-8) were irradiated with 100, 200, 300 and 400 Gy. The results showed that MGT (Mean Germination Time), root and shoot length, and seedling dry weight decreased with increasing radiation doses but final germination percentage was not significantly affected by radiation doses. Biochemical differences based on proline content revealed that seedling irradiated at 100 Gy contained highest amount of proline (1.71 mg/g FW), whereas only 0.92 mg/g FW of proline was detected in non-irradiated seedlings. The highest amount of total chlorophyll content was obtained in seedlings irradiated at 100 Gy. Furthermore, the concentration of chlorophyll *a* was higher than chlorophyll *b* in both irradiated and non-irradiated seedlings. Chlorophyll and proline contents, and root and shoot dry weights in cv. Roshan were higher than those in T-65-58-8 mutant. These results show that the up-regulation of some physiological characteristics and seedling growth of wheat following gamma radiation treatment may be used for aboitic control such as drought and salt stress.

Introduction

Gamma rays belong to ionizing radiation and are the most energetic form of such electromagnetic radiation, having the energy level from around 10 kilo electron volts (keV) to several hundred keV. Therefore, they are more penetrating than other types of radiation such as alpha and beta rays (Kova´cs & Keresztes, 2002).

There are several usages of nuclear techniques in agriculture. In plant improvement, the irradiation of seeds may cause genetic, variability that enable plant breeders to select new genotypes with improved characteristics such as precocity, salinity tolerance, grain yield and quality (Ashraf, 2003). Ionizing radiations are also used to sterilize some agricultural products in order to increase their conservation time or to reduce pathogen propagation when trading these products within the same country or from country to country (Melki & Salami, 2008).

A number of radiobiological parameters are commonly used in early assessment of effectiveness of radiation to induce mutations. Methods based on physiological changes such as inhibition of seed germination and shoot and root elongation have been reported for detection of irradiated cereal grains and legumes. Chaudhuri (2002) reported that the irradiation of wheat seeds reduced shoot and root lengths upon germination.

Gamma radiation can be useful for the alteration of physiological characters (Kiong *et al*., 2008). The biological effect of gamma-rays is based on the interaction with atoms or molecules in the cell, particularly water, to produce free radicals (Kova´cs & Keresztes

2002). These radicals can damage or modify important components of plant cells and have been reported to affect differentially the morphology, anatomy, biochemistry and physiology of plants depending on the radiation dose (Ashraf *et al*., 2003). These effects include changes in the plant cellular structure and metabolism e.g., dilation of thylakoid membranes, alteration in photosynthesis, modulation of the anti-oxidative system, and accumulation of phenolic compounds (Kova´cs & Keresztes 2002; Kim *et al*., 2004; Wi *et al*., 2007; Ashraf, 2009). From the ultra-structural observations of the irradiated plant cells, the prominent structural changes of chloroplasts after radiation with 50 Gy revealed that chloroplasts were more sensitive to a high dose of gamma rays than the other cell organelles. Similar results have been reported to be induced by other environmental stress factors such as UV, heavy metals, acidic rain and high light (Molas, 2002; Barbara *et al*., 2003; Quaggiotti *et al*., 2004). However, the low-dose irradiation did not cause these changes in the ultra-structure of chloroplasts. The irradiation of seeds with high doses of gamma rays disturbs the synthesis of protein, hormone balance, leaf gas-exchange, water exchange and enzyme activity (Hammed *et al*., 2008). Due to limited genetic variability among the existing wheat genotypes, Irfaq & Nawab (2001) opened a new era for crop improvement and now mutation induction has become an established tool in plant breeding that can supplement the existing germplasm and can improve cultivars in certain specific traits as well (Irfaq & Nawab 2001). Considering the effects of radiation on plants, the present study was conducted to determine the effects of radiation on wheat germination and some key physiological and biochemical characteristics of wheat seedlings.

Materials and Methods

Plant materials: Seeds of cvs. Roshan and T-65-58-8 (mutant line) of wheat were selected for irradiation. Moisture content of the seeds was adjusted at 13% before irradiation.

Gamma irradiation: Gamma irradiation was conducted using ^{60}Co gamma source at a dose rate of 0.864 kGy/h in, Agricultural, Medical and Industrial Research School, Nuclear Science and Technology Research Institute, Karaj, Iran.

Study of germination and seedling growth: Wheat seeds were irradiated with 100-400 Gy by 100 Gy intervals and non-irradiated seeds of each genotype served as control. After irradiation, 15 seeds from each treatment were sown in Petri dishes containing each 5 ml of distilled water. Petri dishes were placed in an incubator for 6 days at 25^{o}C. Number of germinated seeds was recorded during 6 days. The final germination percent (FGP) was calculated as follows:

$$(FGP) = \frac{\text{Number of germinated seeds after 6 days}}{\text{Total number of seeds}} \text{ X } 100$$

Two weeks after sowing, root and shoot length, root/shoot ratio and seedling dry weight were recorded. To assess the rate of germination, the mean germination time (MGT) was calculated as follows (Khaje-Hosseini *et al*., 2003):

$$MGT = \sum (f\chi)/\sum f$$

where MGT is mean germination time, f is the number of newly germinated seeds on each day and x in the day of counting.

Determination of chlorophyll content: For different biochemical estimation the irradiated and non-irradiated plantlets were frozen in liquid nitrogen, ground to a powder with a mortar and pestle under chilled condition and kept in a freezer (-25 ^{o}C) for further analyses.

Lyophilized leaf (0.1 g) powder were homogenized in 80% acetone and centrifuged at 10,000×*g* for 10 min. The supernatant was subjected to spectrophotometer determination of chlorophyll *a* and *b* at 646 and 663 nm, respectively. Chlorophyll *a* (C_a) and chlorophyll *b* (C_b) content was determined according to the following equation and expressed in milligram per gram fresh weight of plant material (Kiong, 2008):

Chlorophyll *a*, $C_a = 12.25\ (OD663) - 2.79\ (OD646)$

Chlorophyll *b*, $C_b = 21.50\ (OD646) - 5.10\ (OD663)$

Total chlorophyll, $C_a + C_b = 7.15\ (OD663) + 18.71\ (OD646)$

Determination of proline content: Free proline content in the leaves was determined following the method of Bates *et al*., (1973). Leaf samples (0.5 g) were homogenized in 5 mL of sulfosalycylic acid (3%) using mortar and pestle. About 2 mL of the extract were taken in a test tube and to it 2 mL of glacial acetic acid and 2 mL of ninhydrin reagent were added. The reaction mixture was boiled in a water bath at 100 ^{o}C for 30 min. After cooling the reaction mixture, 6 mL toluene were added and then transferred to a separating funnel. After thorough mixing, the chromophore containing toluene was separated and absorption was read at 520 nm on a spectrophotometer. Toluene was used as blank. Concentration of proline was estimated by referring to a standard curve of proline.

Statistical analysis: The experimental design was a completely randomized factorial. The factors were genotypes (Roshan and T-65-58-8) and gamma irradiation (4 levels) with three replications. Tukey Honestly Significant Difference (Tuky' s HSD) test ($P<0.05$) was used to determine the differences in average of all tested parameters between irradiated and non-irradiated plantlets. Statistical analysis was performed using Mstat-c software.

Results and Discussion

Effect of gamma irradiation on germination: Seed germination test after gamma radiation (100, 200, 300 and 400 Gy) revealed that mean germination time was decreased with increasing irradiation dose for both genotypes (Table 1). The delay in germination time was more pronounced in case of cv. Roshan genotype as compared to that in cv. T-65-58-8 (Table 2).

Gamma radiation had no significant effect on final germination percentage (Table 1). As illustrated in Table 1, irradiated wheat seeds kept their germination capacity compared to the control. Maximum decrease in germination percentage was observed with 300 Gy. These results were in accordance with the germination test done by Melki & Marouani (2009) whereby there was no significant difference in germination and survival percentage of irradiated and non-irradiated seedlings of hard wheat. The results of Koing *et al*., (2008) have shown that survival of plants to maturity depends on the nature and extent of chromosomal damage. Increasing frequency of chromosomal damage with increasing radiation dose may be responsible for less germinability and reduction in plant growth and survival.

Table 1. Effects of gamma radiation on mean germination time (MGT), final germination percentage (FGP), root and shoot length, root and shoot weight and proline content of wheat (*Triticum aestivum* L.) genotypes.

Parameter	Radiation dose (Gy)					
	0	100	200	300	400	SEM
MGT (day)	8.9 [a]	6.4 [b]	5.2 [b]	5.5 [b]	5.5 [b]	19.48
FGP (%)	100 [a]	100 [a]	98.7 [a]	98.7 [a]	97.7 [a]	1.17
Root length (cm)	9.8 [a]	9.5 [a]	3.7 [b]	3.4 [b]	1.9 [c]	81.37
Root weight (g)	24 [a]	25 [a]	12 [b]	13 [b]	9 [c]	334.13
Shoot length (cm)	3.5 [a]	3.8 [a]	2.5 [b]	2.4 [b]	2.4 [b]	2.81
Shoot weight (g)	30.5 [b]	38.1 [a]	26.1 [bc]	21.5 [c]	19.3 [c]	337.80
Proline content (mg/g FW)	0.93 [a]	1.36 [a]	1.71 [a]	1.52 [a]	-	0.65

Means in the same row with different letters (a-c) differ significantly ($p<0.01$)

Changes in the germination percentage were found to attribute to gamma rays treatments. The stimulating causes of gamma ray on germination may be certified to the activation of RNA or protein synthesis, which occurred during the early stage of germination after seeds irradiated (Abdel-Hady *et al*., 2008).

Chaudhuri (2002) reported that in higher radiation dose, germination percentage reduced in addition to root and shoot length, while, in lower dose i.e., 0.1 kGy the germination percentage was not significantly different from control. In another study by Kiong *et al*., (2008), it was found that radiation increases plant sensitivity to gamma rays and this may be caused by the reduced amount of endogenous growth regulators, especially the cytokines, as a result of breakdown, or lack of synthesis, due to radiation. These results are in agreement with the findings of Chaomei & Yanlin (1993) on wheat (*Triticum aestivum* L.), who noticed that treating seeds with high rates of gamma radiation reduced germination with a corresponding decline in growth of plants.

Effect of gamma radiation on root and shoot length: Gamma ray imposed a significant impact on the shoot length. The highest length of shoot (3.8cm) was observed at 100 Gy. By increasing radiation dose to 200, 300 and 400 Gy shoot length declined 40, 49 and 46 percent respectively compared to the control (Table 1).

Shoot length decreased in both Roshan and T-65-58-8 genotypes after all doses of gamma radiation. The maximum decrease in shoot length was observed, when wheat genotypes were exposed by gamma ray dose higher than 200 Gy (Table 2).

Results showed that radiation and interaction of radiation and genotypes imposed a significant effect on root length (Tables 1, 2). Maximum root length was measured for control (9.8 cm), while root length of 100 Gy seedlings (9.5 cm) was in statistically similar group (Table 1). Treatment of 200 and 300 Gy radiations also had no significant difference in root length and a minimum length of the root was found in 400 Gy (Table 1).

In both genotypes a significant effect ($p<0.01$) of the irradiation dose on the root length was observed. Root length decreased after all doses of irradiation as compared to non-irradiated control (Table 2). Maximum reduction in root length was observed after 200 Gy dose in both genotypes, but in Roshan the reduction of root length after 200 Gy was higher than that in the other genotype (Table 2).

Table 2. Mean germination time (MGT), shoot and root length, and weight, chlorophyll (Chl), and proline content of two wheat genotypes as affected by radiation dose.

Genotype	Irradiation dose (Gy)	MGT	Shoot length (cm)	Root length (cm)	Dry weight (g)		Proline content (mg/g FW)	Chlorophyll content (mg/g Fw)		
					Roots	Shoots		a	b	Total
T-65-58-8	0	8.8 ^{a}	3.5 ^{ab}	8.3 ^{b}	18.3 ^{b}	26.2 ^{bcd}	1 ^{a}	7.3 ^{abc}	3.5 ^{abc}	10.8 ^{bc}
	100	6.8 ^{b}	3.7 ^{a}	8.9 ^{b}	18.2 ^{b}	31.8 ^{bc}	1.3 ^{a}	11.6 ^{a}	7.2 ^{a}	18.9 ^{a}
	200	5.2 ^{b}	2.6 ^{bc}	4.1 ^{c}	11.9 ^{cd}	20.1 ^{de}	1 ^{a}	9.7 ^{ab}	4.3 ^{abc}	14.2 ^{ab}
	300	5.2 ^{b}	2.6 ^{c}	4.3 ^{c}	12.6 ^{c}	27.8 ^{bcd}	0.7 ^{a}	9.5 ^{ab}	4.5 ^{abc}	14 ^{abc}
	400	5.3 ^{b}	2.3 ^{c}	2.7 ^{cd}	9.9 ^{cd}	24.8 ^{bcd}	-	-	-	-
Roshan	0	9.1 ^{a}	3.5 ^{ab}	11.2 ^{a}	30.2 ^{a}	34.8 ^{ab}	0.9 ^{a}	6.1 ^{bc}	2.3 ^{bc}	8.8 ^{bcd}
	100	5.2 ^{b}	3.8 ^{a}	10.1 ^{ab}	31.0 ^{a}	44.1 ^{a}	1.4 ^{a}	7.6 ^{abc}	5.5 ^{ab}	13.4 ^{abc}
	200	6.0 ^{b}	2.3 ^{c}	3.4 ^{c}	12.6 ^{c}	23.0 ^{cde}	2.3 ^{a}	4.8 ^{c}	2.4 ^{bc}	7.3 ^{cd}
	300	5.8 ^{b}	2.2 ^{c}	2.6 ^{cd}	12.3 ^{cd}	24.2 ^{bcd}	2.3 ^{a}	3.1 ^{c}	0.5 ^{c}	3.6 ^{d}
	400	5.6 ^{b}	2.5 ^{c}	1.1 ^{d}	7.3 ^{d}	13.7 ^{e}	-	-	-	-

Means in the same row with different letters (a-c) differ ($p<0.01$)

The symptoms frequently observed in the low-or high-dose-irradiated plants are enhancement or inhibition of germination, seedling growth, and other biological responses (Kim *et al*., 2000; Wi *et al*., 2007). Although, no certain explanations for the stimulatory effects of low-dose gamma radiation are available until now, in accordance to the results obtained by Wi *et al*., (2007), there is a hypothesis that the low dose irradiation will induce the growth stimulation by changing the hormonal signaling network in plant cells or by increasing the anti oxidative capacity of the cells to easily overcome daily stress factors such as fluctuations of light intensity and temperature in the growth condition (Wi *et al*., 2007). In contrast, the high-dose irradiation that caused growth inhibition has been ascribed to the cell cycle arrest at G_2/M phase during somatic cell division and/or various damages in the entire genome (Preussa & Britta, 2003). In a study of the gamma radiation effects on chickpea seeds by Toker *et al*., (2005) seedlings irradiated at 200 Gy may have some significant increase in their shoot length, but at 400 Gy an obvious depression in shoot length was observed. Melki & Marouani (2009) also reported an improvement of 18 and 32% in root number and root length of hard wheat at the 20 Gy dose, respectively.

In the present study, the variability as measured by mean values of the root/shoot lengths decreased with increase in the radiation dose. Chaudhuri (2002) reported that when radiation is sufficient to reduce the rooting percentages, then the root lengths do not exceed a few millimeters in length. Due to metabolic disorders in the seeds after gamma irradiation, the seeds are unable to germinate.

Effect of gamma radiation on root and shoot weight: Data presented in Table 2 illustrate the effect of gamma irradiation on root weight of two different genotypes. The results showed highly significant effect on the root and shoot weights between Roshan and T-65-58-8 genotypes. However, there was a highly significant difference between all gamma ray doses as compared to the control. Root weight of 100 Gy with 24.6 g was statistically similar to control (Table 1). There were no significant differences in root weight between 200 and 300 Gy but they had significantly lower root weight compared to control (Table 1).

In cv. Roshan a noticeable reduction in root weight (60%) was observed after 200 Gy, however T-65-58-8 showed 30% decrease in root weight after irradiation with 200 Gy (Table 2). It seems that cv. Roshan is more sensitive to gamma stress than T-65-58-8 mutant.

Seedlings irradiated at 100 Gy showed the highest shoot dry weight (38.1g) and was 25% higher than that of the non-irradiated seedlings (Table 1). In addition, dry weight of seedling shoot which were subjected to 200, 300 and 400 Gy were also significantly lower than those of non-irradiated seedlings (Table 1). The results are contradictory with the results obtained by Melki & Salami (2008) in which they have shown that gamma rays (15 Gy) induced a significant improvement (nearly 20%) in plant dry weight in chickpea as compared with the 0 Gy dose.

Minimum shoot dry weight was observed after 400 Gy in cv. Roshan. The reduction in both shoot and root length as well as root and shoot dry weights are dependent upon the radiation dose. However Wi *et al*., (2007) reported no significant morphological aberrations in the phenotype of plants irradiated with relatively low doses of gamma rays, while high-dose irradiation inhibited seedling growth remarkably.

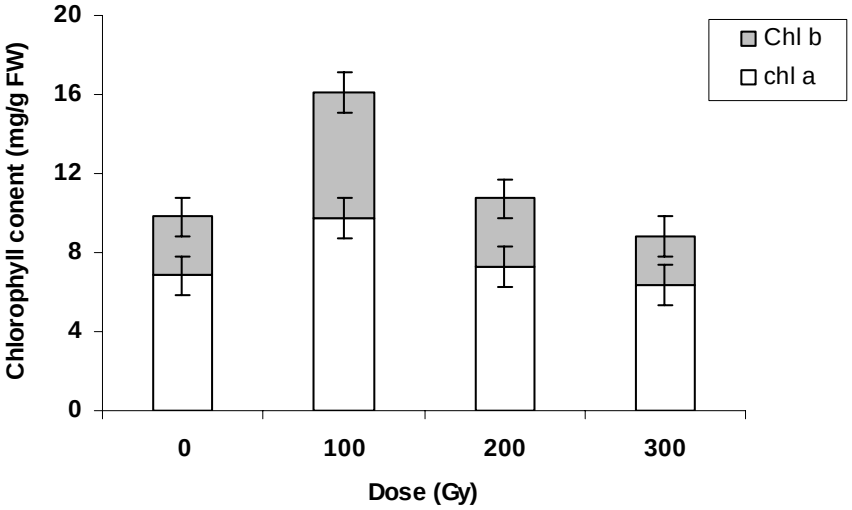

Fig. 1. Effects of gamma radiation on chlorophyll content of wheat (*Triticum aestivum* L.) genotypes. Means with different letter (s) are significantly different ($p<0.01$).

Effect of gamma irradiation on chlorophyll content: The results showed that seedlings were exposed to gamma irradiation (100 and 200 Gy) exhibited an increase in chlorophyll *a*, *b* and total chlorophyll levels (Fig. 1), when compared to non-irradiated treatment. Total chlorophyll increased 64.5% in both genotypes seedlings that were irradiated at 100 Gy.

As illustrated in Fig. 1, the concentration of chlorophyll *a* was higher than chlorophyll *b* in both seedling groups (irradiated and non-irradiated treatment). Seedlings exposed to 100 Gy recorded highly significant changes in chlorophyll *a*, *b* and total chlorophyll contents. In contrast, seedlings irradiated at 300 Gy showed a decrement of chlorophyll *a*, *b* and total chlorophyll, as compared to the non- irradiated seedlings. The lowest total chlorophyll content was obtained in seedlings irradiated at 300 Gy.

Chlorophyll content of seeds in both genotypes was affected by gamma radiation; T-65-58-8 mutant had higher chlorophyll content than cv. Roshan. According to the results of this study, it could be concluded that radiation above 200 Gy caused an obvious depression of chlorophyll *a*, *b* and total chlorophyll content in both genotypes (Table 2).

Gamma radiation induces various physiological and biochemical alteration in plants. The irradiation of plants with high dose of gamma rays disturbs the hormone balance, leaf gas-exchange, water exchange and enzyme activity (Kiong *et al*., 2008). These effects include changes in the plant cellular structure and metabolism such as dilation of thylakoid membranes, alteration in photosynthesis, modulation of the antioxidant system, and accumulation of phenolic compounds. Based on transmission electron microscope observations, chloroplasts were extremely sensitive to gamma radiation compared to other cell organelles, particularly thylakoids being heavily swollen (Wi *et al*., 2007). In this study, the chlorophyll content of gamma irradiated wheat displayed a gradual decrease at 200 Gy dose. In addition, it can be observed that the concentration of chlorophyll *a* was relatively higher than chlorophyll *b* in irradiated and non-irradiated plants. Kiong *et al*., (2008) reported that the reduction in chlorophyll *b* is due to a more selective destruction of chlorophyll *b* biosynthesis or degradation of chlorophyll *b* precursors. Furthermore, Kim *et al*., (2004) have evaluated the chlorophyll content on irradiated red peper plants; their results showed that plants exposed at 16 Gy may have some significant increase in their chlorophyll content that can be correlated with

stimulated growth. Modulation in photosynthesis in irradiated plants might partly contribute to increased growth (Kim *et al*., 2004; Wi *et al*., 2007). Data presented in Table 3 illustrated that the correlation between total chlorophyll content and shoot length was significant ($p<0.05$). These results are in agreement with the findings of Wi *et al*., (2007).

Effect of gamma radiation on proline content: Biochemical differentiation based on proline content revealed that seedlings irradiated at 100, 200 and 300 Gy, exhibited proline content of 1.4 mg/g FW, 1.7 mg/g FW and 1.5 mg/g FW, respectively which were not significantly different as compared to those in non- irradiated seedlings (Table 1). However, there was no significant difference among the seedlings irradiated with 100, 200 and 300 Gy. In T-65-58-8 genotype, proline contents were slightly increased after imposing different levels of gamma radiation of seeds as compared with non-irradiated control (Table 2). However, in cv. Roshan, proline contents were higher after irradiation with 100, 200 and 300 Gy as compared to non-irradiated control. A maximum increase in proline contents was observed after 200 Gy dose in Roshan genotype (Table 2).

Gamma radiation was reported to induce oxidative stress with overproduction of reactive oxygen species (ROS) such as superoxide radicals, hydroxyl radicals and hydrogen peroxide, which react rapidly with almost all structural and functional organic molecules, including proteins, lipids and nucleic acids causing disturbance of cellular metabolism (Al-Rumaih & Al-Rumaih, 2008; Ashraf, 2009; Noreen & Ashraf, 2009). To avoid oxidative damage, plants have evolved various protective mechanisms to counteract the effects of reactive oxygen species in cellular compartments (Kiong *et al*., 2008). This defense was brought about by alteration in the pattern of gene expression. This led to modulation of certain metabolic and defensive pathways. One of the protective mechanisms in the synthesis of osmolytes which is essential to plant growth was proline synthesis (Esfandiari *et al*., 2008).

The results of this study revealed that increase in proline content was observed in irradiated plants. There was a convincing evidence which showed that the osmolyte synthesis such as proline involved in protective mechanisms were altered with several environmental stresses, including gamma irradiation (Al-Rumaih & Al-Rumaih, 2008). Proline is a compatible osmolyte and it may interact with enzymes to preserve enzyme structure and activities. Indeed, proline has been shown *In vitro* to reduce enzyme denaturations caused due to heat, NaCl stress, gamma stress, etc.(Kavi Kishor *et al*., 2005; Ashraf & Foolad, 2007). The present increase in proline content was reported to cope with the problem of oxidative reagents (Falahati *et al*., 2007). In this study the proline contents of gamma irradiated seedlings showed a slight increase as the gamma doses increased. However, Falahti *et al*., (2007) contradicted this statement by proposing that the radiation may have promoted the level of antioxidants and consequently there would be no need for extra amount of proline to cope with the same problem of oxidative reagents.

The results of this research showed that different doses of gamma radiation has different effects on biochemical plant characteristics, such as increasing of proline and chlorophyll content, stimulation of germination and seedling growth. It is clear that this technique can be used for production of a mutant with ability for environmental stress tolerance.

Table 3. Correlation coefficient between root and shoot length, root and shoot weight, proline and total chlorophyll content of wheat (*Triticum aestivum* L.) genotypes.

	Parameter		1	2	3	4	5	6	7
1.	Root Length	1	1						
2.	Shoot length	2	0.933**	1					
3.	Root length	3	0.932**	0.840**	1				
4.	Shoot weight	4	0.839**	0.756*	0.899**	1			
5.	Root number per seedling	5	0.669*	0.480	0.575	0.712*	1		
6.	Proline content	6	0.109	-0.037	0.184	0.229	0.430	1	
7.	Total chlorophyll content	7	0.615	0.637*	0.442	0.516	0.573	0.258	1

* $p<0.05$

** $p< 0.01$

References

Abdel-Hady, M.S., E.M. Okasha, S.S.A. Soliman and M. Talaat. 2008. Effect of Gamma radiation and gibberellic acid on germination and alkaloid production in *Atropa belladonna*. *Aust. J. Basic Appl.*, 2(3): 401-405.

Al-Rumaih, M.M. and M.M. Al-Rumaih. 2008. Influence of ionizing radiation on antioxidant enzymes in three species of *Trigonella*. *Am. J. Environ. Sci*., 4(2):151-156.

Ashraf, M. 2009. Biotechnological approach of improving plant salt tolerance using antioxidants as markers. *Biotechnol. Adv*., 27: 84-93.

Ashraf, M. and M.R. Foolad. 2007. Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environ. Exp. Bot*., 59(2): 206-216.

Ashraf, M., A.A. Cheema, M. Rashid and Z. Qamar. 2003. Effect of gamma rays on M1 generation in Basmati rice. *Pak. J. Bot*., 35(5): 791-795.

Barbara, G, S. Maria, W. Anna, G. Slawa and G. Magdalena. 2003. Changes in the ultra structure of chloroplasts and mitochondria and antioxidant enzyme Activity in *Lycopersicon esculentum* Mill. leaves prayed with acid rain. *Plant Sci*., 164: 507-516.

Bates, L.S. 1973. Rapid determination of free proline for water-stress studies. *Plant Soil*, 39: 205-207.

Chaudhuri, K.S. 2002. A simple and reliable method to detect gamma irradiated lentil (*Lens culinaris* Medik.) seeds by germination efficiency and seedling growth test. *Radiat. Phys. Chem*., 64: 131-136.

Esfandiari, E., M.R. Shakiba, S.A. Mahboob, H. Alyari and S. Shahabivand. 2008. The effect of water stress on antioxidant content, protective enzyme activities, proline content and lipid per oxidation in seedling wheat. *Pak. J. Biol. Sci*., 11(15): 1916-1922.

Falahati, A., S.K. Kazemitabar, A. R. Bahrami, M. Lahouti and M.F. Rahimi. 2007. The study of gamma irradiation effects on drought tolerance in rice *(Oryza sativa* L.). *Indian J. Crop Sci*., 2(1): 155-158.

Foyer, C.H., M. Lelandias and K.J. Kunert. 1994. Photo-oxidative stress in plants. *Physiol. Plant*., 92: 696-717.

Irfaq, M. and K. Nawab. 2001. Effects of gamma irradiation on some morphological characteristics of three wheat cultivars. *Int. J. Biol. Sci*., 1(10): 935-937.

Kavi Kishor, P.B., S. Sangam, R.N. Amrutha *et al*. 2005. Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: Its implications in plant growth and a biotic stress tolerance. *Curr. Sci*., 88: 424-438.

Khajeh-Hosseini, M., A.A. Powell and I.J. Bingham. 2003. The interaction between salinity stress and seed vigour during germination of soybean seeds. *Seed Sci. Technol*., 31: 715-725.

Kim, J.H., M.H. Baek, B.Y. Chung, S.G. Wi and J.S. Kim. 2004. Alterations in the photosynthesis pigments and antioxidant machineries of red pepper (*Capsicum annuum* L.) seedlings from gamma-irradiated seeds. *J. Plant Biotechnol*., 47: 314-321.

Kim, J.S., E.K. Lee, M.H. Back, D.H. Kim and Y.B. Lee. 2000. Influence of Low dose *y* radiation on the physiology of germinative seed of vegetable crops. *Korean J. Env. Agric*., 19: 58-61.

Kiong, A., A., Ling Pick, S.H. Grace Lai and A.R. Harun. 2008. Physiological responses of *Orthosiphon stamineus* plantlets to gamma irradiation. *Am-Eurasian J. Sustain. Agric.,* 2(2): 135-149.

Kovacs, E. and A. Keresztes. 2002. Effect of gamma and UV-B/C radiation on plant cell. *Micron* 33: 199-210.

Ling, A.P.K., J.Y. Chia, S. Hussein and A.R. Harun. 2008. Physiological Responses of *Citrus sinensis* to Gamma Irradiation. *World Appl. Sci. J*., 5: 12-19.

Melki, M. and A. Marouani. 2009. Effects of gamma rays irradiation on seed germination and growth of hard wheat. *Environ Chem Lett*., Doi: 10.1007/s10311-009-0222-1.

Melki, M. and D. Salami. 2008. Studies the effects of low dose of gamma rays on the behavior of *Chickpea* under various conditions. *Pak. J. Biol. Sci*., 11(19): 2326-2330.

Mokobia, C.E. and O. Anomohanran. 2005. The effect of gamma irradiation on the germination and growth of certain Nigerian agricultural crops. *J. Radiol. Prot*., 25: 181-188.

Molas, J. 2002. Changes of chloroplast ultra structure and total chlorophyll Concentration in cabbage leaves caused by excess of organic Ni (II) complexes. *Environ. Exp. Bot*., 47: 115-126.

Noreen, Z. and M. Ashraf. 2009. Changes in antioxidant enzymes and some key metabolites in some genetically diverse cultivars of radish (*Raphanus sativus* L.). *Environ. Exp. Bot*., 67: 395-402.

Oldacay, S. and G. Erdem. 2002. Evaluation of chlorophyll contents and peroxidase activities in Helianthus annuls genotypes exposed to radiation and magnetic fields. *Pak. J. Appl. Sci*., 2: 934-937.

Preussa, S.B., A.B. Britta. 2003. A DNA-damage-induced cell cycle checkpoint in *Arabidopsis*. *Genetics*, 164: 323-334.

Quaggiotti, S., A.R. Trentin, F.D. Ecchia and R. Ghisi. 2004. Response of maize (*Zea mays* L.) nitrate reductase to UV-B radiation. *Plant Sci*., 167: 107-116.

Toker, C., B. Uzun, H. Canci, F. Oncu Ceylan. 2005. Effects of gamma irradiation on the shoot length of *Cicer* seeds. *Radiat. Phys. Chem*., 73: 365-367.

Wi, S.G., B.Y. Chung, J.S. Kim and *et al*. 2007. Effects of gamma irradiation on morphological changes and biological responses in plants. *Micron*, 38: 553-564.

(Received for publication 10 January 2010)