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BIOCHEMICAL RESPONSE OF TWO WHEAT CULTIVARS (*TRITICUM AESTIVUM* L.) TO GAMMA RADIATION

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Abstract

This study assessed the biochemical changes in wheat plants exposed to gamma radiation. Seeds of two wheat cultivars Roshan and Bam were irradiated with 100, 200, 300 and 400 Gy and were immediately planted into soil plots in greenhouse. Leaf samples for various measurements were taken from flag leaves on the 7th day after anthesis. The results demonstrated that seeds of cv. Roshan did not germinate above 200 Gy dose. Gamma irradiation increased the malondialdehyde content remarkably in both cultivars. In both cultivars the chlorophyll content increased at 100 Gy. However, the highest amount of proline was obtained at 200 Gy. Cultivars Roshan and Bam at 200 Gy showed the highest (70.98 µg/g Fw) and lowest (38.91 µg/g Fw) amount of total soluble protein, respectively. Increasing gamma doses improved the activity of peroxidase in both cultivars, but the effect was much greater in cv. Bam than that in cv. Roshan. It seems that cv. Roshan was more sensitive to irradiation stress than cv. Bam. Overall, leaf soluble protein, chlorophyll contents, and lipid peroxidation were found to be effective in assessing the response of wheat cultivars to gamma radiation.

Introduction

Genetic variability for several desired traits has high practical implications in different breeding programs aimed at improving yield and quality traits in different crops under both normal and stress conditions (Ashraf *et al*., 1987). However, genetic variability can be successfully induced through mutations (Karimi *et al*., 2008; Hegazi and Hamideidin, 2010, Bilgin *et al*., 2011).

Gamma irradiation is one of common physical mutagens imposing considerable effects on physiological and biochemical processes in plants (Begum & Dasgupta, 2011; Heidarieh *et al*., 2012,). The biological effect of gamma-rays is mediated through their interaction with atoms or molecules in the cell, particularly water, to produce free radicals (Kovacs & Keresztes, 2002). These radicals in turn 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 intensity of radiation (Ashraf *et al*., 2003). The synthesis of protein, chlorophyll, lipid peroxidation, enzyme activity and accumulation of phenolic compounds are affected by irradiation of seeds with gamma rays (Hameed *et al*., 2008). Plant responses to different stresses vary considerably (Ashraf, 2003) and this is true for mutagens including a range of gamma doses which are strongly dependent on the physiological parameters such as the species, variety or cultivar, the developmental stage at the time of irradiation and even among the genotypes of the same species (Kim *et al*., 2009). However, physiological traits such as chlorophyll and protein contents, peroxidase and lipid peroxidation, which have an important role in oxidative stress and are indicators of cellular damage should have been taken as important criteria, in the wheat mutation breeding studies. Thus, in the present study, an attempt had been made to (1) assess the effect of different doses of gamma irradiation on physiological characteristics in wheat; (2) find out the potential role of these biochemical parameters (lipid peroxidation, peroxidase activity, proline, chlorophyll and carbohydrate contents) in determination of appropriate radiation dose for an effectively inducing mutation in wheat; and (3) study differential radio sensitivity of two wheat cultivars Bam and Roshan based on the above-mentioned physiological parameters.

Materials and Methods

Plant materials: Seeds of two wheat cultivars Roshan and Bam were used for gamma irradiation. Moisture content of the seeds was adjusted at 13% before the irradiation treatment. The irradiated seeds were immediately planted in soil in a greenhouse. Samples for various assays and estimation were taken from flag leaves on the 7th day after anthesis. The experiment was carried out in the greenhouse of the Agricultural, Medical and Industrial Research School, Karaj, Iran. during 2011-2012, where the average *PAR* measured at noon ranged from 848 to 1254 µmol/m^2/s, day/night relative humidity 58/74%, and temperature 24/8°C.

Irradiation treatments: Gamma irradiation was conducted using ^{60}Co gamma source at a dose rate of 0.864 kGy/h at Agricultural, Medical and Industrial Research School, Nuclear Science and Technology Research Institute, Karaj, Iran. The dose levels of 0-400 Gy, with increment of 100 Gy, were used in order to estimate appropriate radiation dose for breeding purposes.

Chemical analysis: For different biochemical estimations, the irradiated and non-irradiated plants were frozen in liquid nitrogen. Lyophilized leaf powders (0.2g) were homogenized with 4 mL of ice-cold extraction buffer (100 mM potassium phosphate buffer, pH 7, 0.1 mM EDTA) by a mortar and pestle. The homogenate was filtered through muslin cloth and centrifuged at 16,000×*g* for 15 min. The supernatant fraction was used as crude extract for further analyses.

Determination of chlorophyll content: The chlorophyll content (CHL) was determined as described by Chutipaijit *et al*., (2012). Quickly, leaf (0.1 g) powder was lyophilized in 80% acetone and centrifuged at 10000×*g* for 10 min. Absorbance was recorded at 646 and 663 nm, and chlorophyll contents calculated.

Determination of proline accumulation and MDA content: Malondialdehyde content was measured using a thiobarbituric acid reaction according to the method of Heath and Packer (1968). Proline content was determined following the method of Bates *et al*., (1973).

Enzyme assays and protein determination: Peroxidase (POD, EC 1.11.1.17) activity was determined according to Herzog & Fahimi (1973) with minor modifications. Protein concentrations in the enzyme extract were determined by the method of Bradford (1976) using bovine serum albumin as a standard.

Statistical analysis: The experiment was arranged in completely randomized factorial design. The factors were cultivars (Roshan and Bam) and gamma irradiation (4 levels) with three replications. Tukey's Honestly Significant Difference (Tukeys HSD) test ($p<0.05$) was used to determine the differences in average of all tested parameters among irradiated and non-irradiated plants.

Results and discussion

Effect of gamma irradiation on chlorophyll content: The highest chlorophyll content was recorded after 100 Gy dose in both cultivars, but in cv Roshan the increase of chlorophyll content after 100 Gy was lower than in the other cultivar (Fig. 1). By increasing the radiation dose to 200, 300 and 400 Gy the chlorophyll content of Bam cultivar declined 12.8, 26 and 29%, respectively compared to the control (Fig. 1). Irradiation above 200Gy inhibited the germination of Roshan seeds in soil. However, minimum chlorophyll content was observed at 200 Gy in cv. Roshan (Fig. 1).

The concentration of chlorophyll *a* was higher than chlorophyll *b* in both groups (irradiated and non-irradiated treatments) (Fig. 1). According to the results of this study, it could be concluded that radiation above 100 Gy caused an obvious depression of chlorophyll *a*, *b* and total chlorophyll content in both cultivars (Fig. 1).

Photosynthetic pigments can be damaged by gamma irradiation, with concomitant loss of photosynthetic capacity (Kiong *et al*., 2008). Based on transmission electron microscope observations, chloroplasts were extremely sensitive to gamma radiation compared to other cell organelles, particularly thylakoids being heavily swollen (Borzouei *et al*., 2010). Marwood and Greenberg (1996) reported that the arranged pattern of grana and stroma thylakoids has been lost by gamma rays. The sensitivity response of soybean to gamma radiation was determined by Alikamanoglu *et al.,* (2010), it was found that total chlorophyll content was decreased up to 81.36% at 400 Gy and 80.91% at 500 Gy gamma ray. Furthermore, Borzouei *et al*., (2010) have evaluated the chlorophyll content on irradiated wheat seedling and they reported, total chlorophyll increased 64.5% at 100 Gy, which can be correlated with stimulated growth. It has also been indicated the chlorophyll content of gamma irradiated wheat seedling displayed a gradual decrease at 200 Gy dose (Borzouei *et al*., 2010). These results are in agreement with our findings.

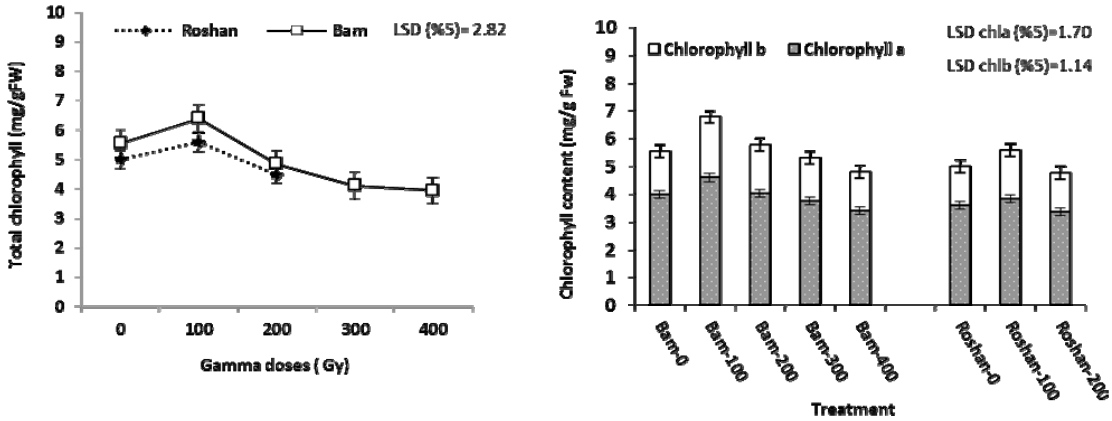

Fig. 1. Effects of gamma doses (100, 200, 300 and 400 Gy) on leaf chlorophyll (CHL) content of wheat cultivars: Roshan (dark bars) and Bam (open bars) plants at 7th days after anthesis stage.

Effect of gamma radiation on malondialdehyde content: Lipid peroxidation expressed as malondialdehyde (MDA) content was significantly increased with increasing irradiation dose for both Roshan and Bam cultivars (Table 1). According to the results, seeds of cv. Roshan were found to be more sensitive to gamma radiation at 300 and 400 Gy because of, the seeds emergence was failed after higher irradiation doses (300 and 400 Gy). Plants of Bam, with irradiated seeds at 300 and 400Gy, which had the MDA content of 5.9 µmol/g Fw and 6.1 µmol/g Fw respectively, showed a further increase of 31.1% and 35.5% in MDA as compared to the non-irradiated seeds but were not remarkably different from each other (Table 1). The maximum increase of MDA content in Roshan and

Bam cultivars was obtained in response to 200 and 400 Gy dose in comparison with the their respective control (Table 1). The essential effect of radiation on cellular membranes is supposed to induce lipid peroxidation by the production of free radicals (Jan *et al.,* 2012). In a study by Hameed *et al.,* (2008) which analyzed the effect of gamma irradiation on some biochemical parameters of chickpea seedlings, it had been reported that the MDA content (that is an index of lipids peroxidation) is increased when the irradiation dose increased from 100 to 1000Gy. In agreement with the results obtained by Stajner *et al.,* (2007), at the highest irradiation dose (140 Gy) lipid peroxidation (LP) of soybean increased by 21.6%, and $HO^{\cdot}$ radical quantity by 79.3% compared to the non-irradiated treatment. Consequently, this study follow a line of previous investigations which means, the increase in gamma doses levels causes the higher concentration of MDA in experimented cultivars.

Table 1. Effects of different gamma treatments on soluble proteins, malondialdehyde (MDA), proline contents and proxidase activity of two wheat cultivars (*Triticum aestivum* L.)

Cultivars	Gamma treatments (Gy)	Soluble proteins(µg/g Fw)	Malondialdehyde (µmol/g Fw)	Proline (µmol/g Fw)	Proxidase activity (µmol guaiacol oxidized/min/g Fw)
Roshan	0	55.57*^{a}	4.25^{d}	5.34^{ab}	9.13^{cd}
	100	67.7^{a}	4.90^{bcd}	4.09^{b}	16.79^{b}
	200	70.98^{a}	6.47^{a}	6.45^{a}	20.85^{ab}
	300	-	-	-	-
	400	-	-	-	-
Bam	0	59.95^{a}	4.53^{cd}	6.15^{a}	10^{c}
	100	53.35^{a}	4.72^{cd}	5.58^{ab}	18.73^{ab}
	200	38.91^{a}	4.47^{cd}	6.89^{a}	20.01^{ab}
	300	56.13^{a}	5.89^{abc}	6.2^{a}	22.5^{ab}
	400	66.63^{a}	6.16^{ab}	6^{ab}	23.01^{a}

*Values followed by different letters in the same column, are significantly different at $P \leq 0.05$.

Effect of gamma radiation on soluble protein content: Data presented in Table 1 showed that gamma treatment did not have significant effect on protein content. A maximum increase in protein contents was observed after 300 and 400 Gy doses in Bam cultivar. Radiation at 200 Gy causes the highest amount of soluble protein, 70.98 ug/g Fw, in cv Roshan and the lowest soluble protein, 38.91 ug/g Fw, in cv. Bam; which were 27.7% higher and 35.1% lower than that of the non-irradiated controls, respectively (Table 1).

Developing of defensive systems is the most important reaction of plant cells to gamma irradiation (Qui *et al.,* 2000; Jan *et al.,* 2012). Al-Rumaih and Al-Rumaih (2008) pointed that increase the amount of soluble protein has been used as a protective mechanism against damages of gamma irradiation. In this study, two cultivars were different based on sensitivity to radiation doses. Nevertheless, chemical changes in the proteins caused by gamma irradiation include fragmentation, cross-linking, aggregation and oxidation by oxygen radicals that are generated in the radiolysis of water (Kiong *et al.,* 2008; Afify *et al.,* 2011).

Data obtained by other authors also showed that gamma treatment with up to 10 KGy, did not induce significant loss in water soluble components such as minerals, nitrogenous constituents, sugars and proteins (Stajner *et al.,* 2007). In cv Roshan, decline in protein oxidation by dose of 200 Gy could be elucidated by the high and significant increase of proline content (Table 1). This observation implicates that proline and soluble sugar content, induced by dose of 200Gy, act as protective agents against protein oxidation.

Effect of gamma radiation on proline content: The maximum increase in proline content was observed at the dose of 200 Gy [16% when compared to the non-irradiated control] (Table 1). Seeds of Bam and Roshan cultivars which irradiated at 100 Gy showed a slight decrement of proline content, 9.3%, and 23.6% as compared to the non-irradiated, respectively (Table 1). The results in Table 1 exhibited that, after seeds irradiation with 200 Gy, proline content of both cultivars was increased.

One of the approaches of plant survival under stress conditions is the accumulation of compatible solutes (proline, citrulline, glycine betaine, and polyols) (Chen *et al.,* 2007). Proline as compatible solutes, contribute to stress toleance by acting as osmuregolators and have many other properties such as hydrophilicity, protection of macromolecules, active oxygen scavengers, the involvement in the system maintaining the cell pH-stat (Kuznetsove and Shevyakova 2007). Morever the hydrophilicity of proline and other compatible solutes helps to replace water molecules around nucleic acid, proteins and membrances during water shortage. Therefore the interaction of proline and non-aqueous tails of protein surface causes to increase the stability of proteins (Irigoyen *et al.,* 1992). With comparing the results of protein and proline content (Table 1), we can state that an increase in proline content of cv. Roshan at

200 Gy has preserved the soluble proteins content; thereby this cultivar might also tolerate to gamma stress at mentioned dose. Additionally increase in proline content of cv. Bam after 200, 300 and 400 Gy doses is a defensive mechanism for proteins protection and other important molecules in other to increasing gamma radiation tolerance.

Effect of gamma irradiation on activity content of peroxidase: Gamma irradiation had a significant effect ($P<0.05$) on peroxidase (POD) activity of two cultivars as compared with non-irradiated control (Table 1). A considerable increase in peroxidase activity was observed by gamma irradiation especially at 300 and 400 Gy. Irradiated seeds of cv Roshan at 100 and 200 Gy recorded an increase of peroxidase activity from 83.9 to 128.4%, over the non-irradiated seeds (Table 1). Conversely, seeds of cv. Bam that exposed to higher dose of irradiation (400Gy) indicated a significant enhancement of peroxidase activity of 23.01 µmol guaiacol oxidized/min/g Fw which was approximately three times the peroxidase activity of the non-irradiated seeds (Table 1). However, the peroxidase activity of Bam seeds which irradiated at 300 and 400Gy were not significantly different from each other (Table 1).

There are some reports showing that the activities of enzymes involved in reactive oxygen species scavenging were altered by several environmental stresses, including gamma irradiation (El-Beltagi *et al.,* 2010; El-Beltagi *et al.*, 2011). It was suggested by Kiong *et al.,* (2008) measurement the changes in peroxidase activity after gamma irradiation is very importance, because peroxidase was known to be essential for a variety of cellular functions such as lignification, cell wall biosynthesis and plasticity, which all may be altered upon exposure to gamma irradiation. Increase of peroxidase activity with a corresponding decline in growth *Triticum aestivum* plants under higher irradiation doses (20, 40, 60, 80Gy) was reported by Chaomei and Yanlin (1993). In another study by Moussa (2009) an increase in the activities of GR, SOD and APOX was observed in Broad beans (*Vicia faba* L.) plants that were exposed to gamma irradiation. In *N. tabacum*, the genes GST, CuZnSOD, POD and CAT are up-regulated and the gene for cytosolic and stromal APX is down-regulated (Jan *et al.,* 2012). In other words, the results of this study about peroxidase activity were in agreement with the other reports.

Gamma radiations can be used to improve occurrence of genetic variability. The effect of climate changes on agriculture dose not forecast and therefore new varieties must be developed and distributed regularly at the national and regional levels for sustainable crop production. The present study confirms that gamma irradiation has different effects on plant biochemical characters, such as increasing of MDA content, chlorophyll content, soluble protein content, and PODs activity. It is obvious that this technique can be used to product a mutant with ability of tolerance against environmental stress and minimizes the adverse effects of increasing or decreasing rainfall and temperatures or other extreme weather conditions.

References

Afify, A.M.R., M.M. Rashed, E.A. Mahmoud and H.S. El-Beltagi. 2011. Effect of gamma radiation on protein profile, protein fraction and solubility's of three oil seeds: Soybean, Peanut and Sesame. *Not. Bot. Horti. Agrobo.,* 39: 90-98.

Alikamanoglu, S., O.Yaycili and A. Sen. 2010. Effect of gamma radiation on growth factors, biochemical parameters, and accumulation of trace elements in soybean plants (*Glycine max* L. Merrill). *Biol. Trace Ele. Res.,* (1-3):283-93.

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: 151-156.

Ashraf, M. 2003. Relationships between leaf gas exchange characteristics and growth of differently adapted populations of Blue panicgrass (*Panicum antidotale* Retz.) under salinity or waterlogging. *Plant Sci.* 165(1): 69-75.

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: 791-795.

Ashraf, M., T. McNeilly and A.D. Bradshaw. 1987. Selection and heritability of tolerance to sodium chloride in four forage species. *Crop Sci*. 27: 232-243.

Bates, L.S., R.P. Waldren and I.D. Teare. 1973. Rapid determination of free proline for water-stress studies. *Plant Soil,* 39: 205-207.

Begum, T. and T. Dasgupta. 2011. Effect of mutagens on character association in sesame (*Sesamum indicum* L.). *Pak. J. Bot*., 43: 243-251.

Bilgin, O., K.Z. Korkut, I. Baser, O. Daglioglu, I. Özturk, T. Kahraman and A.Alpaybalkan. 2011. Genetic variation and inter-relationship of some morpho-physiological traits in durum wheat (*Triticum aestivum*) (L.) Desf. *Pak. J. Bot*., 43: 253-260.

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

Bradford, M. 1976. A rapid and sensitive method for the quantification of microgram quantities in utilizing the principle of protein-dye binding. *Anal. Biochem.,* 72: 284-254.

Chaomei, Z. and M. Yanlin. 1993. Irradiation induced changes in enzymes of wheat during seed germination and seedling growth. *Acta. Agric. Nuc. Sini.,* 7: 93-97.

Chen, Z., A.C. Tracey, Z. Meixue, T. Amanda, P.N. Bodapati and S. Sergey. 2007. Compatible solute accumulation and stress-mitigating effects in barley genotypes contrasting in their salt tolerance. *Environ. Exp. Bot.*, 58: 4245-4255.

Chutipaijit, S., S. Cha-um and K. Sompornpailin. 2012. An evaluation of water defficit tolerance screening in pigmented indica rice genotypes. *Pak. J. Bot*., 44: 65-72.

El-Beltagi, H.S., A.A. Mohamed and M.M. Rashed. 2010. Response of antioxidative enzymes to cadmium stress in leaves and roots of Radish. *Not. Sci. Biol.,* 2: 76-82.

El-Beltagi, H.S., O.K. Ahmed and W. El-Desouky. 2011. Effect of low doses γ-irradiation on oxidative stress and secondary metabolites production of Rosemary (*Rosmarinus officinalis* L.) callus culture. *Rad. Phys. Chem.,* 80: 968-976.

Hameed, A., T. Mahmud shah, B. Manzoor Atta, M. Ahsanul haq and H. Syed. 2008. Gamma radiation effects on seed germination and growth, protein content, peroxides and protease activity, lipid per oxidation in desi and kabuli chickpea. *Pak. J. Bot.,* 40: 1033-1041.

Heath, R.L. and L. Packer. 1968. Photoperoxidation in isolated chloroplast. I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch. Biochem. Biophys.,* 125: 189-198.

Hegazi, A.Z. and N. Hamideldin. 2010 The effect of gamma irradiation on enhancement of growth and seed yield of okra [*Abelmoschus esculentus* (L.) Monech] and associated molecular changes. *J. Hort. Forest.*, 2: 038-051.

Heidarieh, M., A. Borzouei, S. Rajabifar, F. Ziaie and Sh. Shafiei. 2012. Effects of gamma irradiation on antioxidant activity of Ergosan. *Iran. J. Radiat. Res.,* 9: 245-249.

Herzog, V. and H. Fahimi. 1973. Determination of the activity of peroxidase. *Anal. Biochem.,* 55: 554-562.

Irigoyen, J.J., D.W. Emerich and M. Sanchez-diaz. 1992. Water stress induced change in concentration of proline and total soluble sugars in nodulated alfalfa (*Medicago sativa*) plants. *Physiol. Plant.,* 84: 55-60.

Jan, S., T. Parween, T.O. Siddiqi and M. Zafar. 2012. Effect of gamma radiation on morphological, biochemical, and physiological aspects of plants and plant products. *Environ. Rev.,* 20: 17-39.

Karimi, K.M.R., A.K.M.R. Islam, M.M. Hossein, H.M.S. Azad and M.W. Rahman. 2008. Effect of gamma rays on yield and yield attributes of large seeded chickpea. *J. Soil. Nature,* 2: 19-24.

Kim, J.H., M.H. Lee, Y.R. Moon, J.S. Kim, S.G. Wi, T.H. Kim and B.Y. Chung. 2009. Characterization of metabolic disturbances closely linked to the delayed senescence of Arabidopsis leaves after g-irradiation. *Environ. Exp. Bot.,* 67: 363-371.

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

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

Kuznetsove, V.V. and N.I. Shevyakova. 2007 Polyamines and stress tolerance of plant. *Plant Stress,* 1: 50-71.

Marwood, C.A. and B.M. Greenberg. 1996. Effect of supplementary gamma irradiation on chlorophyll synthesis and accumulation of photosystems during chloroplast development in *Spirodela oligorrhiza*. *Photochem. Photobiol.,* 64: 664-670.

Moussa, H. 2009. Effect of gamma radiation on antioxidant enzymes and G6PDH activities in *Vicia faba* plants. *Acta. Agron. Hung.,* 57: 79-86.

Qui, X., F. Wang, X. Wang, G. Zhou and Z. Li. 2000. Effect of combined treatment of 60Co γ- ray and EMS on anti-oxidase activity and ODAP content in *Lathyrus sativus*. *Chin. J. Appl. Ecol.,* 11: 957-958.

Stajner, D., M. Milosevic and B.M. Popovic. 2007. Irradiation Effects on Phenolic content, lipid and protein oxidation and scavenger ability of soybean seeds. *Int. J. Mol. Sci.,* 8: 618-627.

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