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Research article

Biochemical Responses of Sainfoin (*Onobrychis viciifolia* Scop.) Callus Tissue to Low Dose Gamma Radiation

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ABSTRACT

In this study, biochemical changes in callus tissue caused by low dose (50 Gray) gamma radiation were investigated. For this purpose, seedlings were developed *in vitro* from seeds exposed to gamma radiation. The petioles of these seedlings were used as explants for callus formation. Biochemical parameters such as antioxidative enzyme activities (superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR)), malondialdehyde (MDA) and proline content were measured in 30-day callus tissue. As a result of the study, it was determined that the activities of antioxidative enzymes (except for APX), malondialdehyde and proline content were statistically increased in callus tissues. Considering the increase rate in callus tissues, it was determined that the highest increase in biochemical parameters was in SOD activity and proline content. The importance of this study is to show that irradiation of seeds with gamma radiation is sufficient to change the biochemical content of callus tissues obtained from any plant part under *in vitro* conditions. As a result, a plant breeders who uses biotechnological approaches in their studies can easily benefit from the evidence presented in this study.

Key words*:* Gamma radiation, Antioxidant enzymes, Malondialdehyde (MDA), Proline

Araştırma makalesi

Korunga (*Onobrychis viciifolia* Scop.) Kallus Dokusunun Düşük Doz Gama Radyasyonuna Biyokimyasal Yanıtları

ÖZET

Bu çalışmada, düşük doz (50 Gray) gamma radyasyonunun kallus dokusunda ortaya çıkardığı biyokimyasal değişimler incelenmiştir. Bu amaçla, gamma ışınımına maruz bırakılan tohumlardan *in vitro* koşullarda fide geliştirilmiştir. Geliştrilen bu fidelerin yaprak sapları kallus oluşumu için eksplant olarak kullanılmıştır. 30 günlük kallus dokusunda antioksidatif enzim aktiviteleri (süperoksit dismutaz (SOD), katalaz (CAT), askorbat peroksidaz (APX) ve glutatyon redüktaz (GR)), malondialdehit (MDA) ve prolin içeriği gibi biyokimyasal parametlerin ölçümleri yapılmıştır. Çalışma sonucunda, antioksidatif enzimlerin aktivitlerinin (APX hariç), malondialdehit ve prolin içeriğinin kallus dokularında istatistiki açıdan önemli derecede arttığı tespit edilmiştir. Kallus dokularında artış oranı dikkate alındığında biyokimysal parametreler içerisinde en fazla artışın SOD aktivitesinde ve prolin içeriğinde olduğu tespit edilmiştir. Bu çalışmanın önemi, tohumların gama radyasyonu ile ışınlanmasının, *in vitro* şartlarda herhangi bir bitki parçasınından elde edilicek kallus dokularının biyokimyasal içeriğinde değişiklik yaratmaya yeterli olduğunun gösterilmesidir. Sonuç olarak, çalışmalarında biyoteknolojik yaklaşımları kullanan bir bitki ıslahçı bu çalışmada sunulan kanıtlardan kolaylıkla faydalanabilir.

Anahtar Kelimeler*:* Gamma radyasyonu, Antioksidan enzimler, Malondialdehit (MDA), Prolin

Introduction

Sainfoin (Fabaceae) is an important animal feed plant. 160 species of it are known around the world. While sainfoins are spread in a very broad geography from the Baltic Sea to the Mediterranean, Asia Minor and Siberia. They have accumulated and diversified especially in the Anatolia-Iran-Caucasus triangle. In these regions, 32 out of the 53 species in Iran (60.4%), 27 out of the 52 species in Turkey (51.9%) and 21 out of the 39 species in the Caucasus (53.4%) are endemic. In the light of these data, it may be seen that Turkey is one of the important centers of development for this genus (Avcı 2010). Sainfoin has several superior characteristics. It may be grown in arid, gravelly and calcareous soils. It is more resistant to arid conditions in comparison to other feed plants. Despite all these advantages, one of the biggest problems in farming sainfoin is that it does not have enough varieties that have good agricultural characteristics which can adapt to different environmental conditions (Kempf et al. 2016). Therefore, it is important to develop varieties of sainfoin that have good agricultural characteristics.

Different practices are used in variety development studies. One practice is gamma irradiation. The basis of the biological effect of gamma rays is that they interact with atoms or molecules.

They especially interact with water and lead to the formation of free oxygen radicals or reactive oxygen species (ROS), namely oxidative stress. These free radicals destroy plant cells or lead to substantial modifications in cell contents. These radicals also cause biochemical, physiological and molecular differentiations in plants on a cellular level. In order to protect themselves against ROSs, plants have a comprehensive defense mechanism including main antioxidative enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione reductase (GR), and catalase (CAT) and important osmolytes like proline. Several researchers have reported that resistance against oxidative stress may be increased by increasing the quantities and/or activities of elements of the defense system in the cell by low doses of gamma irradiation (Beyaz et al. 2016). Thus, it is possible to develop new mutant plants in *in vitro* conditions against stress factors by using gamma irradiation. However, it is firstly required to determine the biochemical changes on the cellular level that gamma irradiation will create, and therefore, the biochemical mechanism. Tissue culture techniques that are increasingly becoming prevalent in recent years have found a broad area of usage in both plant breeding processes and practices outside breeding. In addition to this, tissue cultures may be used in determining the molecular fundamentals of stress, mechanisms of resistance against stress factors, physiological and biochemical events and changes that emerge under stress conditions (Özcan et al. 2004). As the source of explants, this study used the pedicle parts of seedlings that developed under *in vitro* conditions from seeds that were exposed to different doses of gamma rays, and it was aimed to investigate the biochemical changes (antioxidative defense systems) that occurred in the callus tissues developed from these explants. In the literature, there are studies where not the seed material but the callus tissue consisting of parts (explants) taken from seedlings that have developed from normal seed material under *in vitro* conditions is irradiated and biochemical changes (antioxidative defense systems) are investigated (Vardhan and Shukla 2017, Azeez et al. 20017, Ramakrishna et al. 2018). In difference to previous studies, this study investigated irradiation of seed material and whether this process increases the quantities and/or activities of elements of the antioxidative defense system in the callus tissue that is formed by pieces (explants) taken from seedlings that develop from this material.

Material and Methods

Plant Material and Gamma Irradiation

Seeds from the "Koçaş" ecotype of the sainfoin plant were irradiated with 50 Gy using a Cobalt-60 (Ob-Servo Sanguis Co^{60}, Izotop Company, Hu) gamma cell source with a 491 Gy/Hour irradiation power. The irradiation took place at the Turkish Turkish Energy, Nuclear and Mining Research Institute (TENMAK). A dosimeter was used to calibrate the area before utilizing the gadget. The samples were put on a tray that rotated 360 degrees and were uniformly bombarded by gamma rays coming directly from a window that opened to a height of 30 cm in the apparatus.

Seed surface sterilization, germination of seeds in in vitro conditions, explant isolation and callus formation

The irradiated and peeled sainfoin seeds (obtained from the Republic of Turkey Ministry of Agriculture and Forestry) were kept in a 20 percent commercial bleach solution (ACE-Turkey, 5 percent NaOCl) for 20 minutes for surface sterilization before being rinsed three times with sterile distilled water. The sterilized seeds (both irradiated and non-irradiated) were planted in sterile Magenta boxes in MS (Murashige and Skoog 1962) nutritional mix (Caission, USA) that included 3 percent sucrose (and was solidified by 0.65 percent agar (Caission, USA). At 24°C, all of the cultures were kept under white fluorescent light (27 mol m^2 s^1) for 16 hours of light and 8 hours of darkness. Leaf stalk explants from seedlings that grew 30 days after seed germination were cultivated in conditions with 1 mg/l 6-Benzylaminopurine (BAP) (Sigma) and 4 mg/l 2,4-Dichlorophenoxyacetic Acid (2-4 D) (Garshasbi et al. 2012) plant growth regulators for callus production. Biochemical tests were performed on callus tissues that had formed four weeks after the cultivation procedure began.

Biochemical Analysis

Contents of malondialdehyde (MDA), proline and activities of antioxidative enzymes (CAT, SOD, GR, and APX) were measured on the callus tissues after 4 weeks of cultivation.

Antioxidant Enzyme Analyses

Superoxide dismutase (SOD) activity: The activity of superoxide dismutase (SOD) was evaluated using Çakmak and Marschner (1992) and Çakmak et al. (1995) methods based on the reduction of NBT (nitro blue tetrazolium chloride) (Sigma, Catalogue Number: N6876) by O_2- in the presence of light. In the reaction medium, all of the solutions were added: The enzyme extract (25 to 100 l) was followed by 0.5 ml of 50 mM Na_2CO_3 (Merck: Catalogue Number: 106392) (pH of 10.2), 0.5 ml of 12 mM L-methionine (Sigma, Catalogue Number: M9625), and 0.5 ml and 75 mM p-nitro blue tetrazolium chloride (NBT) (Sigma, Catalogue Number: N6876). The materials were exposed to light for 15 minutes before being measured at 560 nm.

Ascorbate peroxidase (APX) activity: The activity of ascorbate peroxidase (APX) was determined using the method given by Çakmak and Marschner (1992) and Çakmak et al. (1995), which was based on ascorbate oxidation at 290 nm (E = 2.8 mM cm^{-1}). The final volume of the reaction medium was adjusted to 1 ml by adding 0.1 mM of EDTA containing a 50-mM phosphate buffer (pH of 7.6), 0.1 ml and 10 mM of EDTA containing 12 mM of hydrogen peroxide (H_2O_2) (Merck, catalogue number: 108600), 0.1 ml and 0.25 mM of L-ascorbic acid (Sigma, catalogue number: 33034).

Glutathione reductase (GR) activity: Glutathione reductase (GR) activity was determined using the method proposed by Çakmak and Marschner (1992) and Çakmak et al. (1995) based on the oxidation of -Nicotinamide adenine dinucleotide 2′-phosphate reduced tetrasodium salt hydrate (NADPH) (Sigma, catalogue number: N1630) (E = 6.2 mM cm^{-1}) at 340 nm (E). The final

volume of the reaction medium was adjusted to 1 ml by adding 0.1 mM EDTA containing a 50 mM phosphor buffer (pH 7.6), 0.1 ml and 0.5 mM of oxidized glutathione (GSSG) (Sigma, catalogue number: G4376), 0.1 ml and 0.12 mM of NADPH and enzyme extract into the medium, and the NADPH oxidation level was measured spectro

Catalase (CAT) activity: The breakdown rate of H_2O_2 at 240 nm (E = 39.4 mM cm^{-1}) was used to determine catalase activity (Çakmak and Marschner 1992; Çakmak *et al.* 1995). The final volume of the reaction media was adjusted to 1 ml in this enzyme study by adding 0.1 mM of EDTA containing a 50-mM phosphate buffer (pH 7.6), 0.1 ml and 100 mM of H_2O_2 and enzyme extract to the reaction medium.

*Measurement proline and lipid peroxidation (MDA content):*The content of malondialdehyde (MDA) was determined using the method published by Lutts et al. (1996). In a nutshell, 200 mg of fresh leaves were mixed with 5 ml of 0.1 percent trichloroacetic acid (TCA) (Sigma, Catalogue Number: 27242) and centrifuged at 16,246 g for 20 minutes. From 5 ml extracts, 3 ml of the supernatant was collected. Equal volumes of each of the supernatants were treated with 3 ml of 0.1 percent thiobarbituric acid (Merck, Catalogue Number:108180) in 20 percent trichloroacetic acid (weight/volume). Using a spectrophotometer, the samples' A-absorbance was evaluated spectrophotometrically at 532 and 600 nm (UVmini-1240, Shimadzu, Japan).

The proline assay was based on a method developed by Bates et al. (1973), which grinds fresh plant materials with 3 percent sulfosalicylic acid (Sigma, Catalogue Number:390275). The ground samples were placed in tubes containing the ninhydrin reagent (Sigma, catalogue number: 151173), which were then immersed in a water bath at 100°C for 1 hour. 4 mL toluene (Merck, catalogue number: 108325) was added to the samples once they had cooled. At 520 nm, the samples were inspected.

Statistical Analysis

The study was designed with a completely randomized block design with 3 replications. Data were analyzed using Independent-Samples t-test of SPSS 22.

Results and Discussion

The physiological and biochemical processes in plants are significantly affected by gamma irradiation stress (Hameed et al. 2008). In this study, biochemical changes (SOD, CAT, APX and GR activity; MDA and proline contents) were investigated in callus tissues, which were derived from the explants of seedlings grown from non-irradiated and low dose (50Gy) degrees of gamma-irradiated seeds of sainfoin (*O. viciifolia* Scop.) under *in vitro* conditions.

Responses of Antioxidative Enzymes to Gamma Irradiation

The results of this study indicated that the responses of the antioxidative enzyme activities to gamma irradiation were various in the callus tissues (Table 1.). Superoxide dismutase (SOD) catalyzes the dismutation of $O_2^{\cdot -}$ radicals to molecular O_2 and H_2O_2. The enzyme SOD is

considered the first-line of defense because it catalyzes the first reaction in the ROS detoxification process (You and Chan 2015). Therefore, SOD activity upon gamma irradiation stress exposure provides valuable information about the biochemical mechanism of callus tissues. The results of this study showed that the activities of SOD was significantly ($P \leq 0.01$) stimulated (19.98%) by the irradiation low dose treatment (50 Gy) in the callus tissues, when compared to the control group (Table 1.). El-Beltagi et al. (2011) reported that SOD activity was positively correlated with the doses of gamma irradiation in rosemary callus. These results were in line with the points by some scientists such as Foyer (1993) and Aly and El-Beltagi (2010) who provided evidence of enhanced activities of SOD by gamma irradiation treatment in callus tissues. On the contrary, Alikamanoglu et al. (2011) reported that the activities of superoxide dismutase were significantly decreased depending on the irradiation dosages. Catalase (CAT) is another important antioxidant enzyme that detoxifies H_2O_2. A significantly dose-rate-dependent ($P \leq 0.01$) increase (13.98%) was seen for catalase activity (CAT) in the callus tissues (Table 1). The combined action of CAT and SOD converts the toxic superoxide radical (O_2^-) and hydrogen peroxide (H_2O_2) into water and molecular oxygen (O_2), thus averting cellular damage under unfavorable conditions (Chaitanya et al. 2002; El-Beltagi et al. 2011). This study results showed that gamma irradiation promoted SOD activity and CAT activity in the sainfoin callus tissues. These results confirm the previous findings of Kim et al. (2004) and Kim et al. (2015). APX plays an essential role in the control of intracellular ROS levels (Kim et al. 2015). In this study, the results showed that APX activity was inhibited (2.52%) in the callus tissues at dose of 50 Gy (Table 1). APX has a higher affinity for H_2O_2 than CAT (Kim et al. 2015). On the contrary, the results indicated that CAT works more than APX in the callus tissue of sainfoin. The GR activity was positively correlated with the low dose of gamma irradiation (Table 1.). Compared to control groups, the activity of GR significantly ($P \leq 0.05$) increased (24.20%) with gamma irradiation of 50 Gy. Moussa (2008) reported that gamma irradiation at all doses (0–100 Gy) used on seeds of fava beans caused increases in GR and APX activities. These results indicated that exposure to low dose gamma-irradiation significantly increased the activities of the antioxidant enzymes (SOD, CAT, GR, except APX) in the callus tissues of sainfoin. The findings that were obtained in this study also clearly demonstrated the SOD and GR key components of the antioxidant defense systems of the callus tissues.

Table 1. Changes in biochemical parameters of 30-day-old callus tissue that derived from the explants of seedlings grown from seeds that were exposed to 50 Gy dose of gamma irradiation

	APX		CAT		GR		SOD		MDA		Proline	
	(μmol min^{-1} mg^{-1} FW)						(U min^{-1} mg^{-1} FW)		(μmol g^{-1} FW)			
	Cont.	Gamma	Cont.	Gamma	Cont.	Gamma	Cont.	Gamma	Cont.	Gamma	Cont.	Gamma
	221.7	216.1	251.7	286.9	122.7	152.4	352.8	423.3	2.3	3.6	15.0	24.1
t value	3.532*		5.427**		3.086*		3.909*		2.992*		3.316*	

*, ** Significant difference ($p < 0.05$ and $p < 0.01$) compared to control. Cont.: Control

Effects of Gamma Irradiation on MDA and Proline Contents

Proline plays an important role in osmoregulation and osmotolerance. Moreover, it has been demonstrated to protect enzymes from inactivation by stress factors (Chen et al. 2011). In this study, significant differences ($P \leq 0.05$) were found in the proline contents between the gamma treatment groups and the control groups (Table 1). The findings showed that the dose of 50 Gy led to a remarkable increase (60.66%) in proline contents (24.1 μmol g^{-1} FW). These results confirmed the previous findings of Shojaie et al. (2010) who reported that proline contents decreased in irradiated calli of potato; however, they emphasized that the dose of 60 Gy causes increasing proline contents in potato calli. On the other hand, Chandrashekar et al. (2013) observed that proline contents increased with an increase in the dosage of irradiation in *Terminalia arjuna* plants (medicinal plants). Different doses of gamma irradiation have different effects on biochemical plant characteristics, such as increase in total soluble protein and total soluble amino acid proline content (El-Beltagi et al. 2011). We speculated that the dose of 50 Gy caused increases in the proline content in the callus tissues due to the appropriate dose to create a low-dose effect or hormetic effects.

Reactive oxygen species can react with nearly all cell constituents. Such an interaction triggers free radical chain reactions, eventually causing membrane lipid peroxidation (Marcu et al. 2013). In this study, lipid peroxidation was characterized by the malondialdehyde (MDA) contents. Our results revealed that the MDA contents of the callus tissues were increased (56.52%) significantly ($P \leq 0.05$) by 50 Gy of gamma irradiation (Table 1). El-Beltagi et al. (2011) reported that, at a high irradiation dose (20 Gy), the MDA content of rosemary (*Rosmarinus officinalis* L.) callus tissues increased. Kim et al. (2015) reported that MDA levels

increased with the increase in gamma irradiation doses. Hameed et al. (2008) also stated that lipid peroxidation content was significantly higher in the irradiated plants than those in the control plants. The findings of this study exhibit that low dose of gamma radiation causes increase in the MDA contents of callus tissues.

Conclusion

Low-dose (50 Gy) of gamma-irradiation can be useful for the alteration of one or a few biochemical characters in callus tissue. On the basis of the data collected here, the biochemical parameters were regularly increased in the callus tissues with increasing doses of gamma irradiation, except for ascorbate peroxidase (APX). However, based on these finding, it is concluded that, among the antioxidant enzymes, SOD and GR were more active in the callus tissues and it was provoked by the dose of 50 Gy. Accumulation of malondialdehyde (MDA) in the callus tissues showed a linear increase depending on the doses. The dose of 50 Gy led to an increase proline accumulation in the callus tissues. In the light of these findings, 50 Gy and below are recommended doses for future studies to create a positive effect on antioxidant defense systems and other biochemical parameters of callus tissues. However, these findings should be supported by morphological parameters such as callus fresh and dry weight, percentage of callus formation, and increase in the number of shoots formed from callus.

Many researchers generally choose to expose callus tissues to gamma irradiation after they derived callus tissues to create changes in antioxidant defense systems, and therefore, to improve new plant varieties that are resistant against stress factors. However, unlike in other studies, this study demonstrated that there is no need to expose callus tissues to irradiation, and it would be sufficient to irradiate seeds with gamma rays to create positive changes in the biochemical contents of cells. This study also stated the positive and not transient effect of gamma irradiation on the antioxidative defense systems of the callus tissues of *O. viciifolia* Scop. The contribution of this study is that the low dose of gamma rays caused a positive effect on the elements of antioxidative defense systems.

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Conflict of Interest

No known or potential conflict of interest exist for any author.

References

Alikamanoglu S, Yaycili O, Sen A (2011). Effect of gamma radiation on growth factors, biochemical parameters, and accumulation of trace elements in soybean plants (*Glycine max* L. Merrill). - Biol Trace Elem Res. 141:283-293Aly AA and El-Beltagi HES (2010). Influence of ionizing irradiation on the antioxidant enzymes of *Vicia faba* L. -Grasas Aceites 61(3):288-294

Avcı S (2010) Collection of wild sainfoin (*Onobrychis* Sp.) species of Turkey and determination of their morphological characteristics, Ph.D. Thesis, Ankara University

Azeeza H, Ibrahim K, Pop R, Pamfil D, Hârţad M, Bobiș O (2017). Changes induced by gamma ray irradiation on biomass production and secondary metabolites accumulation in Hypericum triquetrifolium Turra callus cultures. -Industrial Crops & Products 108:183–189

Bates LS, Waldren RP, Teare ID (1973). Rapid determination of free proline for water stress studies. -Plant Soil 39: 205-207

Beyaz R, Sancak C, Yildiz Ç, Kuşvuran Ş, Yildiz M (2016). Physiological responses of the M_1 sainfoin (*Onobrychis viciifolia* Scop) plants to gamma radiation. -Appl Radiat Isot. 118:73-79

Cakmak I and Marschner H (1992). Magnesium defficiency and higlight intensity enhance activities of superoxide dismutase, ascorbate peroxidase and glutathione reductase in bean leaves. Plant Physiol. 98:1222-1226

Çakmak I, Atlı M, Kaya R, Evliya H, Marschner H (1995). Association of high light and zinc deficiency in cold-ınduced leaf chlorosis in grapefruit and mandarin trees. J. Plant Physiol. 146: 355-360

Chaitanya K, Sundar D, Masilamani S, Ramachandra RA (2002). Variation in heat stress-induced antioxidant enzyme activities among three mulberry cultivars. Plant Growth Regul. 36:175-180

Chandrashekar AKR, Somashekarappa HM, Souframanien J (2013). Effect of gamma irradiation on germination, growth, and biochemical parameters of Terminalia arjuna Roxb. -Radiation Protection and Environment 36(1): 38-44

El-Beltagi HS, Ahmed OK, El-Desouky W (2011). Effect of low doses γ -irradiation on oxidative stress and secondary metabolites production of rosemary (*Rosmarinus officinalis* L.) callus culture. -Radiat Phys Chem 80: 968-976

Foyer CH (1993). Ascorbic acid. In: Alscher, R.G., Hess, J.L. (Eds.), Antioxidants in higher plants. CRC Press, Florida, USA, pp. 31-58

Garshasbi H, Omidi M, Torabi S, Davodi D (2012). The study of phytohormones and explants on callus induction and regeneration of sainfoin (*Pnobrychis sativa*). Pak. J. Agri. Sci. 49:319-322

Hameed A, Shah TM, Atta BM, Haq MA, Hina S (2008). Gamma ırradiation effects on seed germination and growth, protein content, peroxidase and protease activity, lipid peroxidation ın desi and kabuli chickpea. Pak. J. Bot., 40(3):1033-1041

Kempf K, Mora-Ortiz M, Smith L, Kölliker R, Skøt L (2016). Characterization of novel SSR markers in diverse sainfoin (*Onobrychis viciifolia*) germplasm. -BMC Genetics 17(1):124

Kim DY, Hong MJ, Park CS, Seo YW (2015). The effects of chronic radiation of gamma ray on protein expression and oxidative stress in *Brachypodium distachyon*. - Int. J. Radiat. Biol. 91(5): 407-419

Kim JH, Baek MH, Chung BY, Wi SG, Kim JS (2004). Alterations in the photosynthetic pigments and antioxidant machineries of red pepper (*Capsicum annuum* L.) seedlings from gamma-irradiated seeds. -J Plant Biotechnol. 47:314-321

Lutts S, Kinet JM, Bouharmont J (1996). NaCl-Induced senesence in leaves of rice (*Oryza sativa* L.) cultivars differing in salinity resistance. Ann. Bot. 78: 389-398

Marcu D, Damian G, Cosma C, Cristea V (2013). Gamma radiation effects on seed germination, growth and pigment content, and ESR study of induced free radicals in maize (*Zea mays*). -J Biol Phys 39: 625-634

Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum 15:473-497

Özcan, S., Gürel, E., Babaoğlu, M.: Bitki Biyoteknolojisi II Genetik Mühendisliği ve Uygulamaları (In: Turkhis)S. U. Vakfı Yayınları, Konya. pp.1-456 (2004)

Ramakrishna D, Chaitanya G, Suvarchala V, Shasthree T (2018). Effect of gamma ray irradiation and ethyl methane sulphonate on *in vitro* mutagenesis of Citrullus colocynthis (L.) Schrad. -J Plant Biotechnol 45:55-62

Rejili M, Telahigue D, Lachihe B, Mrabet A, Ferchichi A (2008). Impact of gamma radiation and salinity on growth and K+/Na+ balance in two populations of Medicago sativa (L.) cultivar Gabe`s. -Progress in Natural Science 18:1095-1105

Shojaie B, Ehsanpour AA, Abdi MR (2010). Proline, sodium and potassium concentration changes in gamma rays and NaCl treated potato calli. -Journal of Cell and Molecular Research 2(2):74-80

Vardhan PV and Shukla LI (2017). Gamma irradiation of medicinally important plants and the enhancement of secondary metabolite production. - Int. J. Radiat. Biol. 93(9):967-979

You J, Chan Z (2015). ROS regulation during abiotic stress responses in crop plants. Frontiers in Plant Science 6: 1-15