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Investigation of Gamma-Ray Effect on Physiological and Biochemical Traits of Triticale Plant under Salinity Stress

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ABSTRACT

Salinity stress alters several physiological and biochemical traits, resulting in reduced yields in different plants. Triticale, which is a hybrid of wheat and rye, is one of the most interesting and valuable plants in the late nineteenth century. Unfortunately, in recent years, due to the problem of salinity stress in most agricultural environments in Iran, it is impossible to grow this valuable plant, and its production and cultivation in the country have stopped. The source used in this study was an iodine-131 source with different activities in which triticale seeds have been exposed to gamma iodine-131 radiation at intervals of 1 to 6 days. Samples were irradiated at doses of 0-23-50-63-80-95-110 Gy, and then cultured in the laboratory. Among the irradiated samples, 63 Gy sample was the best sample in terms of germination rate and was selected to apply salinity stress with a range of 0 and 150 mM NaCl. After 10 days of stress application, different physiological and biochemical traits of triticale seedlings were tested. The results of analysis of variance showed that salinity stress had a significant effect on all measured physiological and biochemical traits. In addition, the interaction of salinity and radiation on all traits except for peroxidase was significant. By radiation, enzymatic, and non-enzymatic antioxidant defense systems, increased, while oxidative stress parameters, such as hydrogen peroxidase and malondialdehyde reduced considerably. Consequently, radiation at a dose of 63 Gy improved the biological traits of the plant and created more resistance to salinity stress in the triticale plant.

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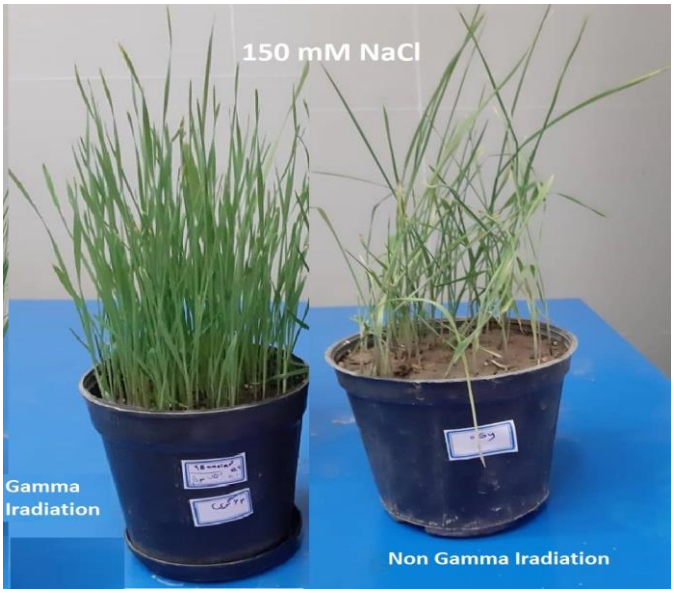
GRAPHICAL ABSTRACT



Introduction

The triticale is a plant produced by humans based A cross between wheat and rye, which is much richer than that wheat, a completely cold-resistant plant [1]. This plant is one of the main food feeds for animals that can be produced in different regions of Iran [2]. Salinity is a major biological stress affecting the plant negatively [3]. Excessive values of the Na^+ ions lead to non-ionic equilibrium and decrease the adsorption of useful ions, such as K^+, Ca^{2+}, and Mn^{2+} ions [4]. It is necessary to identify molecular compounds in the signaling pathways related to salt stress**.** The wide application of gamma rays is influenced by the penetration of plant modification techniques. The effects of gamma radiation on morphological changes and the biological response of plants depend on the dose of radiation [5]. The use of gamma rays with in low doses has a positive effect on various plants. Exposure to gamma radiation with lower doses not only causes growth, but also increases the seed germination index, root length, stem length, and so forth [6, 7] . One of the main problems of Iran is the lack of water and planting plants in salty lands. Owing to the existence of many sources of saline land in Iran, usually, the parameters and genes of plants are sensitive to the salinity of water, land, and it is possible to produce nutritious edible plants with genetic changes and changes in enzyme and its parameters with genetic changes and changes in the parameters of the enzymatic and non-enzymatic antioxidant defense system [8]. Electromagnetic radiation is one of the cases where genes may change [9]. By exposing plant seeds to UV radiation, electrical discharges, plasma, gamma, beta, and neutron radiation [10]. The possibility of genetic changes and the plant resistance to salinity has been investigated [11]. The results of various research indicate that in a certain dose of gamma, neutron, beta, and radiation, it is possible to produce plants in areas with high salinity [12]. The effects of gamma radiation have been investigated by studying the germination, growth, development of plants, and the biochemical characteristics of corn [13]. Plan breeding of wheat and triticale in Argentina is based on the objective of improving an individual crop, concerning resistance to drought stress. The use of gamma radiation holds promise for physiological crop improvement [14]. Much researches has been done on different samples of wheat, corn, barley, etc. with different treatments, but so far no research has been done on the use of gamma-ray radiation to increase resistance to salt stress in triticale.

Material and Methods

In the first phase, triticale seeds were packed in 21 batches of 10 g and were exposed to gamma radiation from iodine 131 at 1-6 days.

Then, the samples of seeds were categorized, and after disinfection for 48 hours, they were placed in 50 ml of Hoagland's solution. All tests and protection related to the radiation of triticale seeds were carried out in the protected storage center of the Nuclear Medicine Department of Shafa Hospital under the supervision of an experienced expert with a license from the radiation protection center of the Atomic Energy Organization of Iran.

At the end of the desired time, different samples irradiated with 0-23-50-63-80-95-110Gy were planted in sterilized plastic pots with soil (50% soil, 10% leaf soil, and 40% sand with some perlite).

This study has attempted to investigate the possibility of genetic changes of triticale in waters with different salinity using gamma radiation with a dose between 0 and 110 Gy. Different sources can be used for gamma radiation [15]. Cobalt 60 and iodine 131 sources can also be used for radiation [16]. In this study, an iodine 131 source has been used, which is a gamma and beta source [17].

The dose of this radiation in the triticale plant is investigated using the MCNPX code. The MCNPX code is a Monte Carlo code that can detect 32 nuclear atoms [18]. The elements in the triticale, which is are extracted from scientific sources [19] and applied as the material card to the MCNPX code. The characteristics of the gamma source and iodine-131 will also be extracted from the scientific sources and will be defined as the source in the code. An input file for the MCNPX code is written to check the dose received. The percentage of elements in the triticale plant that was extracted from reliable sources are presented in Table 1 [20].

The amount of gamma and beta energy of iodine source 131 is extracted and is presented in Table 2 and is entered as a source in the MCNPX code.

Table 1: Elements in the triticale plant

Element	Total	Element	Total
Ca	0.468511	Ni	2.75E-05
K	1.258239	Cr	0.000436
P	0.357776	Pb	1.18E-05
S	0.04986	As	1.18E-05
Mn	0.004457	H	6.473433
Fe	0.031486	O	45.4708
Cu	0.000302	C	44.39941
Zn	0.0006	N	1.484635

Table 2: Gamma and beta radiation of iodine source 131

Gamma rays		Beta rays	
Energy(Mev)	Abundance %	Energy(Mev)	Abundance %
0.284	5	0.25	2.8
0.364	78.4	0.34	9.3
0.637	9	0.61	87.2
0.722	3	0.81	0.7

The pots are then placed in different doses in the germinator to grow under the same conditions for 12 days at approximately 26 °C and 70% humidity.

The studied traits were selected in the first phase by focusing on the general growth rate and germination percentage. The speed of growth and germination of one of the samples was higher than other samples.

In the second phase, the plants treated with the optimized radiation dose (63Gy) and the non-irradiated sample (as normal) were subjected to salinity stress of 0 and 150 mM NaCl and were evaluated in the form of a factorial test in a completely randomized design with two repetitions and the oxidative stress traits: hydrogen peroxidase enzyme – malondialdehyde, enzymatic antioxidant defense system: superoxide dismutase - catalase – peroxidase, and non-enzymatic defense system: total enzymatic defense Free amino acids – protein were evaluated. The obtained data were statistically analyzed by SPSS software and Excel software was used to draw graphs.

Results and Discussion

In the first phase, the sample of 63 Gy was more favorable than the other doses (23, 50, 80, 95, and 110 Gy) according to the indicators of growth rate and germination percentage (Figure 1), and for the continuation of the work and to apply salinity stress, 0 and 150 mM NaCl was selected in the second phase. The control and irradiated samples with a dose of 63 Gy were placed in the germinator with salinity stress of 150 and 0 mM NaCl. Figures 2a,b shows the triticale plant in a dose of 63 Gy and 0 Gy (normal sample) after applying salt stress. By calculating the amount of sodium chloride added to 1 liter of water, we reached the desired salt tension. As results show, the growth and germination rate of the irradiated plant at a dose of 63 Gy at a salinity of 150 mM NaCl is higher than that of other samples at the same salinity.

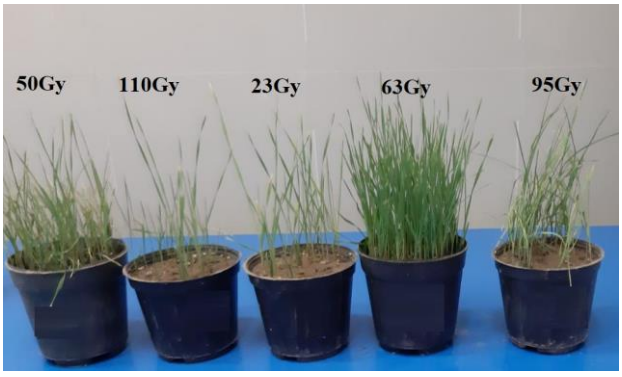


Figure 1: Growth and germination rate of Triticale in various gamma doses

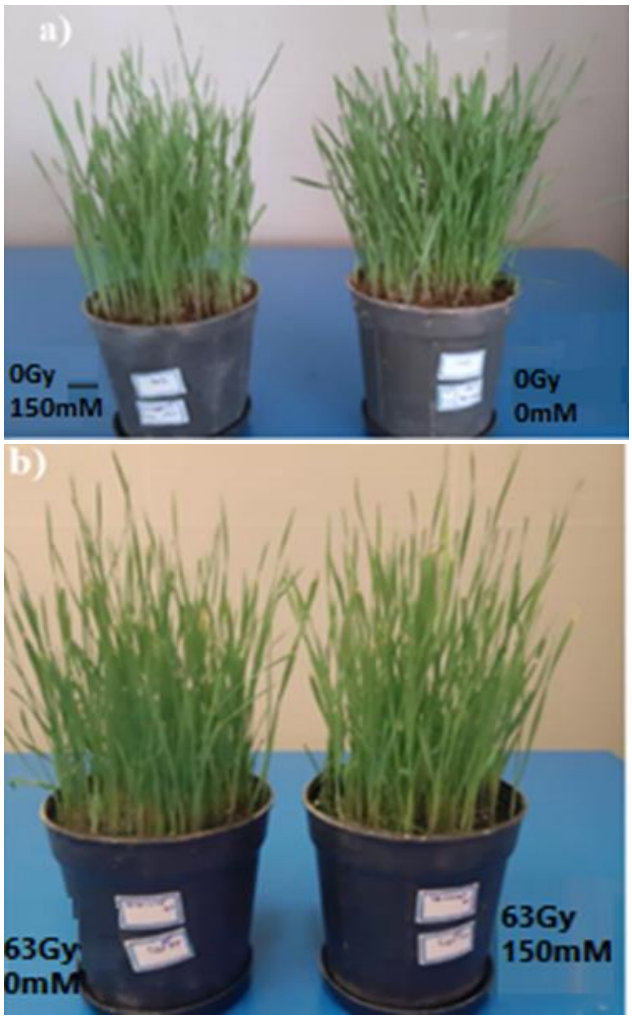


Figure 2: Growth rate of the triticale plant at: (a) 0 Gy and salinity of 0 and 150 mM NaCl and (b) 63Gy and salinity of 0 and 150 mM NaCl

Wilting and yellowing of plant leaves were observed in the control sample. Traits of Oxidative stress and enzymatic and non-enzymatic antioxidant defense systems including hydrogen peroxidase, malondialdehyde, proline, catalase, superoxide dismutase, peroxidase, total sugar, free amino acid, chlorophyll, and protein in samples of 0 and 63 Gy in 0 and 150 mM NaCl with different solutions is measured with a spectrophotometer according to the resulting wavelengths. Figure 3a shows the results of the amount of hydrogen peroxidase. As Figure 3a and the results show, owing to the application of salinity stress of 0 and 150 mM NaCl, 63 Gy samples showed resistance, and the antioxidant defense system of the irradiated sample became more active to the extent of stress resistance.

Increase of salinity and reduction of the amount of hydrogen peroxidation enzyme are a part of the oxidative stress system. The diagram shows better resistance at a salinity of 150 mM NaCl. Figure 3b presents the results of enzyme malondialdehyde, which is part of the oxidative stress system. 63 Gy sample at a salinity of 150 mM NaCl had a relative decrease, indicating an increase in plant resistance. Figure 3c demonstrates the enzyme catalase. Catalase is one of the most important enzymes in protecting cells against oxidative contamination by oxygenated water. This enzyme decomposes oxygenated water into oxygen and water, since oxygenated water or hydrogen peroxide (H_2O_2) is a toxic substance, which is increased by salinity stress, and then catalase is increased by radiation to decompose this toxic substance. Superoxide dismutase is one of the most important types of the antioxidant defense system and is present in almost all cells exposed to hydrogen peroxide production. Figure 3d presents the results of this enzyme in which 63 Gy radiation increases the immune system against salinity tension. Figure 3e shows the results of the peroxidase enzyme. Peroxidase is a large group of enzymes involved in various biological processes and usually cause the breakdown of peroxides. Figure 3f depicts ascorbate peroxidase as one of the enzymes of the antioxidant defense system. In addition, the 63 Gy sample with a salinity stress of 150 mM NaCl is more resistant. Figure 3g presents the results of proline measured in samples 0 and 63 Gy with a salinity stress of 0 and 150 mM NaCl. Proline is a unique compound in the structure of proteins and is known as one of the 20 major amino acids constituting the structure of proteins. It helps to repair damaged tissues and build proteins.

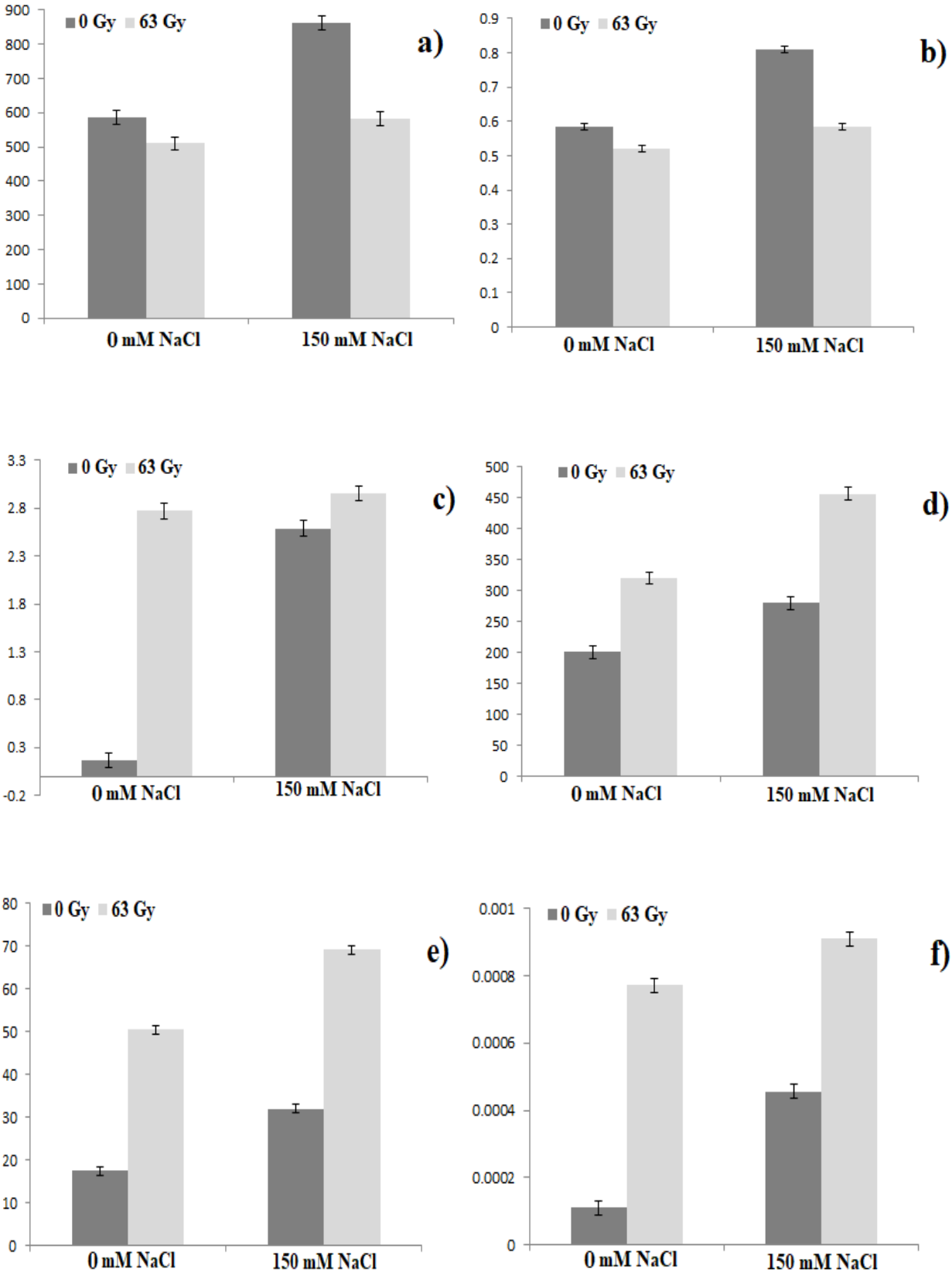
Total sugars, or carbohydrates consisting of carbon, hydrogen, and oxygen atoms, act mostly as energy-saving molecules and are divided into three categories: simple, compound, and complex. Salinity stress reduces energy levels. However, the radiation combined with salinity stress increases total sugar levels as shown in Figure 3h. Figure 3i presents the results of the study of total free amino acids. Amino acids are the building blocks of proteins and control vital functions. Salinity stress reduces amino acid levels. However, 63 Gy radiation, along with salinity increases amino acid levels.

Figure 3j depicts the measurement results of total chlorophyll that show the rate of change of plant pigments in irradiated and non-irradiated samples in the amounts of 0 and 150 mM NaCl. Radiation at a rate of 63 Gy protects plant pigments against salinity stress. Figure 3k shows the amount of total protein. Proteins are one of the largest molecules, like a chain of a three-dimensional polymer coil composed of amino acids. These biological macromolecules play an essential role in structural and enzymatic functions. Radiation has a positive effect on the amount of protein.

Table 3 lists the changes in the traits and enzymes of triticale plants against the effects of salinity, radiation, and the interaction of stress and radiation on the plant. Analysis of variance can be observed in the table as significant and ns (non-significant).

Table 4 deals with the correlation between simple plant traits. For example, in the first column, a high and significant correlation (**) between hydrogen peroxidase and malondialdehyde with a value of 0.98 is specified, revealing differences with other enzymes and traits. The correlation between the enzymatic antioxidant defense systems is clear and significant and is contrary to hydrogen peroxidase and malondialdehyde, demonstrating an increase in the plant resistance to increased hydrogen peroxidase and malondialdehyde.

In Table 5, the studied traits were grouped into two factors that explained 92.50% of the data changes as follows: The first factor explained 70.28% of the total data changes. In this factor, the highlighted traits (due to a factor coefficient greater than 0.5) had a higher correlation than other traits associated with the first factor, which were positive coefficients of the traits. The high coefficients of the mentioned traits indicate that these traits have the highest degree of diversity in this factor, and other traits have less diversity. Therefore, the choice to improve or increase these traits will be effective in this factor.


a)
0 Gy
63 Gy
900
800
700
600
500
400
300
200
100
0
0 mM NaCl
150 mM NaCl
b)
0 Gy
63 Gy
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0 mM NaCl
150 mM NaCl
c)
0 Gy
63 Gy
3.3
2.8
2.3
1.8
1.3
0.8
0.3
-0.2
0 mM NaCl
150 mM NaCl
d)
0 Gy
63 Gy
500
450
400
350
300
250
200
150
100
50
0
0 mM NaCl
150 mM NaCl
e)
0 Gy
63 Gy
80
70
60
50
40
30
20
10
0
0 mM NaCl
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f)
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63 Gy
0.001
0.0008
0.0006
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0.0002
0
0 mM NaCl
150 mM NaCl

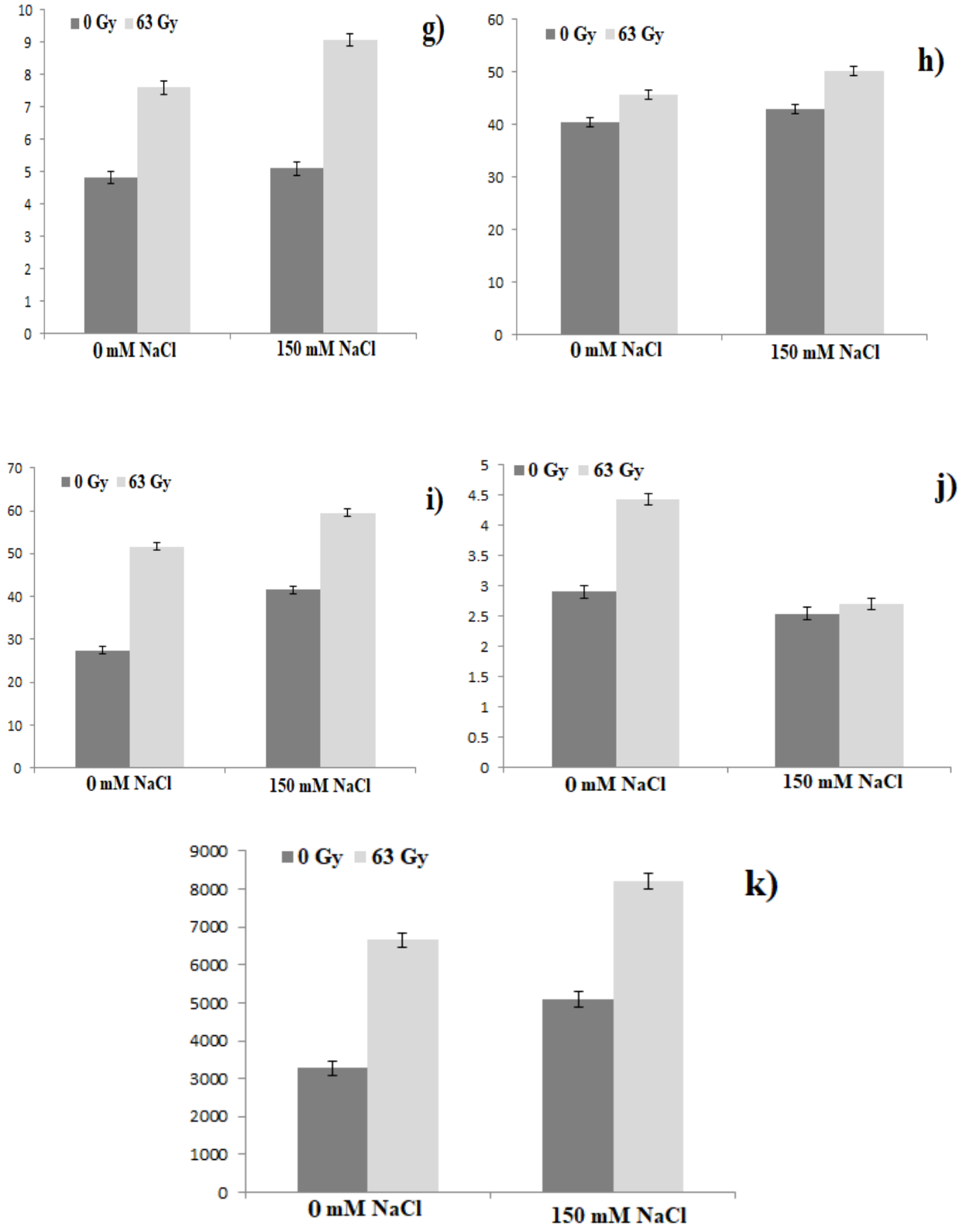

Figure 3: Results of the measurement: (a) hydrogen peroxidase enzyme, (b) malondialdehyde enzyme, (c) catalase, (d) superoxide dismutase enzyme, (e) peroxidase, (f) ascorbate peroxidase, (g) proline, (h) total sugars, (i) total free amino acids, (j) total chlorophyll, and (k) total protein

Table 3: A simple analysis of the variance for traits

		Average of squares					
Sources of changes	Degrees of freedom	Hydrogen peroxidase	Proline	Total sugar	Total free amino acids	Total chlorophyll	Peroxidase
Salinity	1	9.07E4**	2.28**	3.81E1**	3.65E2**	3.30**	8.28E2**
Ray	1	9.56E4**	3.42E1**	1.17E2**	1.33E3**	2.12**	3.71E3**
Salinity × Ray	1	3.17E4**	1.10**	3.16**	3.06E1**	1.41**	1.23E1 ns
Error	4	1.49E1	0.00	4.00E-1	7.60E-1	1.00E-2	1.07E1
%CV	-	6.10E-1	1.05	4.70E-1	1.93	3.47	7.74

ns,: Non-significant at probability levels of 1% and 5%.*

*** Significant at probability levels of 1% and 5%.*

Continuation of **Table 3**

		Average of squares				
Sources of changes	Degrees of freedom	Catalase	Malon De Aldehyde	Superoxide dismutase	Total protein	Ascorbate peroxidase
Salinity	1	5.09**	6.00E-2**	3.48E4**	8.57E6**	2.00E-7**
Ray	1	6.65**	6.00E-2**	6.58E4**	3.17E7**	9.00E-7**
Salinity × Ray	1	3.76**	2.00E-2**	2.49E3**	4.99E4**	0.00**
Error	4	1.00E-2	0.00	1.92E1	2.80E3	0.00
%CV	-	4.56	4.09	1.40	9.10E-1	7.30

*** Significant at probability levels of 1% and 5%.*

Table 4: Simple correlation for traits

	Hydrogen peroxidase	Proline	Total sugar	Total free amino acids	Total chlorophyll	Peroxidase	Catalase	Malon De Aldehyde	Superoxide dismutase	Total protein	Ascorbate peroxidase
Hydrogen peroxidase	1										
Proline	-0.54	1									
Total sugar	-0.31	0.96**	1								
Total free amino acids	-0.23	0.92**	0.96**	1							
Total chlorophyll	0.64*	0.28	0.07	0.23	1						
Peroxidase	-0.34	0.97**	0.98**	0.97**	0.18	1					
Catalase	0.12	0.68*	0.88**	0.9**	0.19	0.8**	1				
Malon De Aldehyde	0.98**	-0.52	-0.29	-0.22	0.65*	-0.32	0.13	1			
Superoxide dismutase	-0.21	0.93**	0.99**	0.94**	-0.03	0.97**	0.78**	-0.2	1		
Total protein	-0.28	0.95**	0.98**	0.99**	0.19	0.98**	0.86**	-0.26	0.97**	1	
Ascorbate peroxidase	-0.28	0.92**	0.94**	0.99**	0.31	0.95**	0.89**	-0.27	0.92**	0.98**	1

ns,: Non-significant at probability levels of 1% and 5%.*

***: Sgnificant at probability levels of 1% and 5%.*

Table 5: Coefficients of common factors, relative and cumulative variances, and the degree of commonality of factors in different traits

Traits	Common factor coefficients		Common variance
	1	2	
Hydrogen peroxidase	-0.38	0.9	0.96
Proline	0.98	-0.15	0.98
Total sugar	0.98	0.11	0.97
Total free amino acids	0.98	0.14	0.99
Total chlorophyll	0.27	-0.72	0.6
Peroxidase	0.99	0.06	0.98
Catalase	0.82	0.4	0.83
Malon De Aldehyde	-0.37	0.91	0.96
Superoxide dismutase	0.95	0.21	0.95
Total protein	0.99	0.11	1
Ascorbate peroxidase	0.98	0.08	0.97
Relative variance	70.28	22.22	
Cumulative variance	70.28	92.5	
Special values	7.73	2.44	

The second factor explained 22.22% of the total data changes. The largest factor coefficients were related to the highlighted traits, and the factor coefficient of all traits except total chlorophyll was positive.

Dendrogram or hierarchical clustering is a clustering method aiming at building a hierarchy of clusters. Agglomerative hierarchical clustering differs from partition-based clustering since it builds a binary merge tree starting from leaves containing data elements to the root that contains the full data set. The graphical representation of that tree that embeds the nodes on the plane is called a dendrogram. To implement a hierarchical clustering algorithm, one has to choose a linkage function (single linkage, average linkage, complete linkage, Ward linkage, etc.) that defines the distance between any two sub-sets (and relies on the base distance between elements). In this diagram, what is important is the height, so that whatever clusters or observations are similar. If there are more together, a lower height will be created and vice versa.

Dendrogram can be drawn based on both distance and similarity levels of the hierarchy displays a classification of data that can be viewed as a tree. Each leaf of the tree represents an initial observation, and the root of the tree is the set of all observers. The results of a hierarchical clustering are usually depicted as a dendrogram. In Figure 4, samples of triticale plant traits were evaluated, and standardized data were entered using the entered method. In this diagram, what is observed is the lowest level of amino acid and ascorbate peroxidase enzymes and the subsequent similarity relationship of total sugar and superoxide dismutase. Figure 4 demonstrates the importance of the last classification and the level of hydrogen peroxidase and malondialdehyde, as well as the effect of these two on other traits.Similar research and results have been conducted in this field regarding the effect of gamma radiation in increasing tolerance against salinity stress in different plants, which are mentioned in a few cases:

Experimental results showed that the safflower response against gamma radiation and salinity varied. Salinity decreased root and shoot length, root and shoot dry weight, leaf number and area, and stomatal conductance. Root dry weight at the dose of 50 Gy and salinity levels of 3 and 6 dsm^{-1} and at 100 Gy at all salinity levels increased. Gamma irradiation doses of 300 and 400 Gy decreased the root and shoot growth characteristics of safflower. According to the results of the experiment, it can be concluded that doses less than 200 Gy in safflower are capable of producing salt- resistance crops [21]. In another study, the effect of gamma-ray

radiation under salinity stress conditions has been done on beans. The effects of gamma rays on vegetative growth and amino acid profile were different in stressed and non-stressed conditions. The results of this study have shown that the application of low-dose gamma in moderate stress conditions has a hormetic effect, not under severe salt stress [22]. Likewise, the combined effect of gamma radiation and salt concentration on proline content and the growth of Thai aromatic rice has been investigated in another study. 2AP acts as the characteristic compound in fragrant-rice cultivars. The 2AP content of gamma irradiated rice-seedlings under 20 mM of NaCl concentration had approximately 2.6 to 3.1 times higher than in the growth condition without salt concentration of gamma irradiated rice-seedlings. Other results indicate that the combination of gamma irradiation technique and salt concentration can be used for improving the 2AP content in rice [23]. In addition, the effect of gamma-ray pretreatment on the biochemical and molecular responses of potatoes growing under salt stress has been investigated. It was concluded that irradiation of potato callus by 20 Gy gamma rays is an effective process for inducing salt resistance. However, this finding needs to be confirmed in field conditions [24]. In a research, it was found that gamma radiation in low doses (50 Gy), possibly by modulating physiological responses, and also stimulating stress signal transmission in Arabidopsis seedlings, reduces salt stress [25].

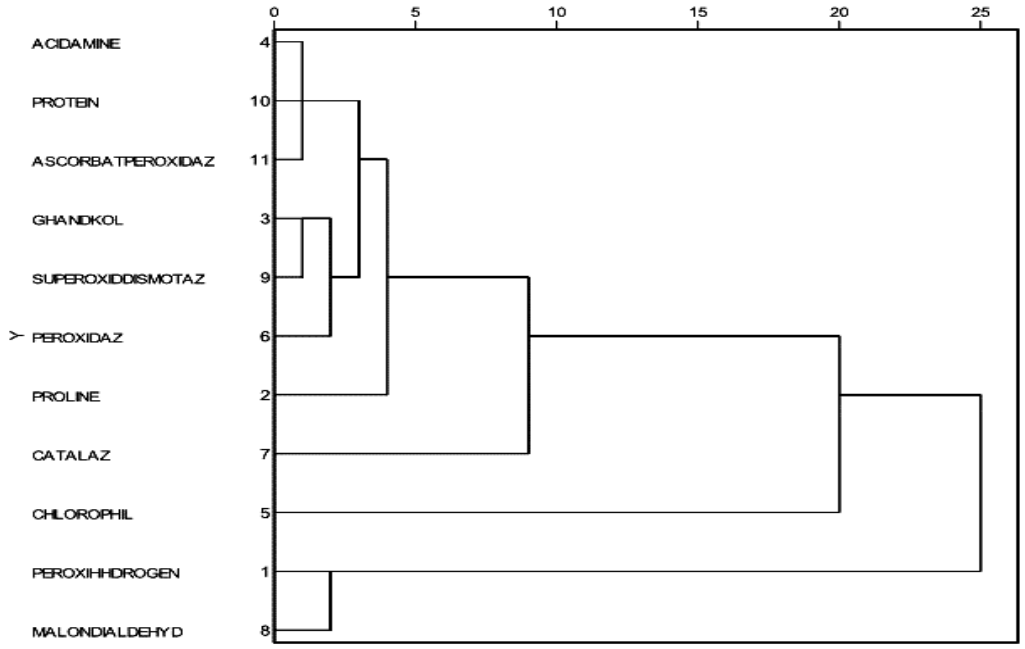

Figure 4: Cluster analysis dendrogram using the Ward method

In another research on the effect of γ-ray seed treatment on corn plant growth and chlorophyll synthesis under salt stress conditions, it was found that radiation before planting seeds with a dose of 50 Gy stimulates plant growth [26].

In this research, gamma radiation in increasing the resistance of triticale plants with a salinity of 0 and 150 mM NaCl has been investigated using practical results. The samples were irradiated at a calculated time interval of 1 to 6 days. Packages of 10 g of triticale seeds were exposed to gamma irradiation of an iodine 131 source. The obtained doses according to Table 3 included doses of 23, 50, 63, 80, 95, and 110 Gy. In addition, the beta radiation dose of this source in triticale samples was calculated which was negligible. Irradiated and non-irradiated samples were planted in plastic pots with a mixture of sand and soil, and their growth rates were compared. The 63 Gy sample had a higher

germination rate and better growth than the other samples.

Then, 63 Gy and normal samples were exposed to salinity stress of 0 and 150 mM NaCl for 10 days, and then transferred to the laboratory. In the laboratory, different physiological and biochemical traits of the plant (oxidative stress parameter: hydrogen peroxidase enzyme-malondialdehyde-enzymatic antioxidant, defense system: superoxide dismutase-catalase-peroxidase, and non-enzymatic defense system: free amino acid-proline-Protein) were measured using different concentrations of the solution and with a spectrophotometer according to different enzymatic wavelengths (Figure 3). The results indicate that salinity stress in plants "in the research in which triticale was studied" causes a change in the oxidative stress system, demonstrating the imbalance of systematic manifestations of "reactive oxygen species" (ROS), and the ability of a biological system to neutralize and inhibit its toxic mediators or to repair the damage. Any disturbance in the natural state of oxidation (oxidation: the reaction of a substance with oxygen and combining with it, and all reactions in which the process loses a substance electron) through the peroxide production (a complex composed of a single oxygen bond). It is an oxygen or ion peroxide. The O – O group is called the peroxide group or peroxy group.

The simplest stable peroxide is hydrogen peroxide. In addition, free radicals (called atoms, molecules, or ions that have molecules or atoms with unpaired electrons in the electron shell. In other words, their electron shell is incomplete and they accumulate in the body, which is dangerous and causes dangerous diseases like cancer). It produces toxic effects and causes damage to all intracellular components and structures, including proteins, lipids, and DNA. Oxidative stress, due to salinity stress, can cause damage to nucleotide bases and breakage in DNA strands. Base damage is often indirectly caused by reactive oxygen species, such as O_2^- (superoxide radical), OH (hydroxyl radical), and H_2O_2 (hydrogen peroxide). Hydrogen peroxide or hydrogen peroxide (H_2O_2) is a common oxidant. Hydrogen peroxide is the simplest peroxide (peroxides are compounds that have a single oxygen-oxygen bond). Decomposition of this substance causes OH radicals that are unavailable for more than a few seconds, during which it oxidizes organic and inorganic materials with its strong oxidizing properties. In this research, the number of changes in hydrogen peroxidase, and malondialdehyde, as well as the amounts of superoxide dismutase enzymes, catalase, peroxidase, ascorbate peroxidase, proline, total sugar, total free amino acids, and total protein were measured. Salinity stress has caused significant changes.

The oxidative stress system, namely hydrogen peroxidase, and malondialdehyde, is increased by salinity stress, indicating cell damage and the formation of free radicals causing premature aging in the plant. In samples irradiated with a dose of 63 Gy, this defense system has increased so much, so that the defense system has been able to reduce the amount of hydrogen peroxidase and malondialdehyde, thereby causing serious damage to the plant. In general, the sample irradiated with salinity stress had higher resistance than control sample, and with increasing the amount of sodium chloride up to 150 mM NaCl, the resistance to salinity stress tolerance increased more in the 63 Gy sample than in the control sample; this was found suitable for planting in saline soils. The innovation and novelty in this study is the research on the triticale plant, which until now has not been studied about gamma-ray radiation on it to increase tolerance to salt stress and planting in salty lands or saline water has not been done, which aims to increase the performance of this sample of the plant against saline stress, its production, and planting in saline lands. Unfortunately, in recent years in Iran, due to the lack of water and salinity of water and land, planting and production of triticale plant has decreased significantly in Iran. We hope that with the passage of time and more research using controlled radiation, it will be possible to increase the agricultural problems and the resistance to stress in the performance of plants against it.

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Authors' Contributions

All authors contributed to data analysis, drafting, and revising of the paper and agreed to be responsible for all the aspects of this work.

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