



## OPEN Molecular, biophysical, and biochemical studies on irradiated *Zea mays* seeds using various sources of gamma rays for dosimetical applications

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Gamma rays are a powerful tool for enhancing crop quality and production. They can cause mutations that improve plant traits and are commonly used in agriculture. The present study aimed to examine the effects of gamma irradiation on maize hybrids' triple white seeds (Giza 321) using different doses (10, 20, and 50 Gy) from different radiation sources <sup>60</sup>Co or <sup>137</sup>Cs. The maize treated with gamma rays from the Co-60 source at 10 Gy exhibited the lowest shoot length percentage of 37.5%, compared to control groups, while root lengths were unaffected at 10 and 50 Gy Cs-137 doses. In addition, the study revealed that gamma irradiation stimulated the excess production of proline, protein, and antioxidant enzymes, which revealed the defense strategy of the plant that tolerates stress. The study also revealed that gamma rays caused a significant reduction in chlorophyll content for all doses, while carotenoid content increased. DNA tail length indicated that minimal damage occurred at 50 Gy of <sup>60</sup>Co and <sup>137</sup>Cs, respectively. Moreover, the analysis of tail DNA% and tail moment showed that the lowest damage was determined for 20 Gy of <sup>60</sup>Co and <sup>137</sup>Cs, respectively. SDS-PAGE analysis showed that the 20 Gy Co treatments had the largest number of bands (15), while the 20 Gy Cs dose had the minimum number of bands (10). Ultimately, the proline content and peroxidase enzymes respond exponentially with the dose, making them potential radiation biomarkers for dosimetric purposes. However, further dosimetric features of these two parameters are necessary to be defined in future work. The present results showed that the treatment of plants with gamma rays enhanced the defense system of the maize at a specific dose, thereby, a large-scale study is recommended for using this radiation to enhance the defense and/or the tolerance of a wide range of crops as well as evaluate its safety, applicability, and reproducibility at field scale.

**Keywords** Maize, gamma-rays, DNA damage, SDS-PAGE, Proline, Antioxidant

Maize (*Zea mays* L.) is the third most significant grain crop of the Poaceae family, behind rice and wheat<sup>1</sup>. It is a cereal grown in various climates, from tropical to temperate. It was domesticated in Mexico around 8,000 years ago from a wild grass species called teosinte<sup>2</sup>. Maize is considered a unique and significant crop in worldwide agriculture due to its widespread uses for human consumption, animal feeding, and as a source for various industries<sup>3</sup>. Over the past decade, new technologies like improved high-yielding seeds were introduced in maize production. However, contrary to the government's expectations, maize production has not experienced exponential growth<sup>1</sup>.

Improving the quality and quantity of economically important crops has been a human priority for many years. Cultivated and wild plants are crucial for providing food, maintaining ecosystem stability, and serving as a valuable resource for medications and other products<sup>4</sup>. Classical plant breeding is the oldest and most

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intensively used technique for improving plant traits in response to environmental changes, pathogen invasions, and increasing production, but it can be labor, reduce gene variants, and time-consuming<sup>5</sup>. To solve problems associated with classical breeding, cost-effective and efficient alternative methods are urgently needed to improve crop productivity. Mutation and molecular breeding offer a reliable and attractive choice for enhancing plant-desirable traits<sup>6</sup>.

Mutation breeding has become a popular and unusual technique for breeders, as it offers the opportunity to acquire new necessary traits that have either not existed naturally or have been lost through evolution<sup>7</sup>. Radiation technology is commonly used to induce mutations and develop new crops. Gamma irradiation (GR) is the most commonly used radiation in mutation breeding because it's relatively easy to use compared to other methods<sup>8</sup>. Moreover, GR enhances the plant's genetic characteristics, making the use of radiation practice more appealing; thus, there is an attractive and probable space for research on radiation-induced mutations, which can enhance the quality of plant species through breeding<sup>4</sup>. Radiation-induced mutations (RIM) were used in many crops, including tomato<sup>9</sup>, wheat<sup>10</sup>, soybean<sup>11</sup>, and maize<sup>12,13</sup>.

One of the primary features of GR is the direct loss of energy and biological macromolecule damage, like DNA, via direct or indirect pathways. This resulting in single and double-strand breaks (DSBs), which can influence functional responses of cells and trigger apoptosis, ultimately leading to growth suppression<sup>14</sup>. Conger<sup>15</sup> showed how oxygen contributes significantly to the start and progression of radiation damage in seeds through interactions with radiation-induced free radicals. During irradiation and post-irradiation storage, environmental factors-particularly temperature and water content have a significant impact on the density of radicals. Furthermore, the oxygen effect can be totally eliminated or, at the other extreme, increased to the maximum level (for a given dose) by adjusting temperature and seed moisture content. Controlling the interactions between seed water content, temperature, and post-irradiation time should be used instead of directly controlling oxygen levels because this is challenging<sup>16</sup>. Furthermore, indirectly through the radiolysis products, resulting in a cascade of reactive molecules<sup>17</sup>.

Exposing seeds to gamma irradiation can cause mutagenic changes that penetrate cells and tissues deeply before causing significant alterations. Radiation-induced DNA modification in plant seeds can create differences in offspring, developing novel varieties with desired qualities<sup>18</sup>. Consequently, there is an increasing interest in studying DNA damage in irradiated plants, and sensitive techniques for detecting DNA damage have been utilized<sup>19</sup>. Comet assay has become the standard method for measuring DNA damage because it is simple, accurate, sensitive, and cheap<sup>20</sup>. The GR has been demonstrated to cause DNA damage following exposure, as directly assessed by the single-cell gel electrophoresis (Comet assay)<sup>21,22</sup>. When particular agronomic features are molecularly identified, it facilitates the screening of DNA polymorphisms among mutants, which improves the genetic breeding process via GR<sup>23</sup>. Molecular markers are extensively utilized to distinguish the genetic variances between the mutant and the original plants by describing the differences at DNA level<sup>24</sup>. SDS-PAGE protein patterns are commonly used to evaluate plant mutations and genetic diversity<sup>25</sup>. Also, GR caused some protein bands to develop or vanish, which changed the patterns of the protein profiles. Also, low doses of gamma rays can stimulate amino acid biosynthesis, thereby altering the protein content that plays crucial roles in plant development<sup>26</sup>.

Gamma rays can impact plant growth and induce variations in the cells' genetic level, biochemical, physiological, and morphogenetic nature. Also, it induces the breakdown of the bonds between chains, cross-linking occurs in both DNA and protein molecules and produces biochemical mutant plants<sup>27</sup>. Gamma radiation had damaging and beneficial effects on maize at all vegetative growth physiological and biochemical criteria, resulting in total phenolics, proline, chlorophyll a, b, and chlorophyll contents. Indeed, the degree of sensitivity or tolerance was dose-dependent<sup>13</sup>. Mohammadi et al.<sup>28</sup> reported that gamma rays at 50, 100, 200, and 300 Gy had a detrimental effect on the growth and the pigments of savory seeds, while increasing the number of nodes, shoot branching, the diameter of the stem, total phenolics, starch, soluble sugar, and flavonoids contents. Also, Kesavan, et al. found a linear response between the seedling injury and O<sub>2</sub> concentration in the range of post-hydration medium 0–80% of dry barley seeds irradiated (350 Gy of <sup>60</sup>Co-rays)<sup>29</sup>.

Radiation doses in various media (such as air, water, or tissue), exposure dose, equivalent dose, ambient dose, or/and their rates can all be measured or evaluated using physical, chemical, biological, or electronic radiation dosimeters. Since no dosimeter can have all the needed attributes, research for new dosimeters is ongoing, and the enhancement of known existing dosimeters with improved features is crucial. The choice of dosimeters depends on the specific requirements and conditions of the measurements<sup>30</sup>. Currently, a significant amount of food used by humans now comes from plants that have been enhanced through radiation-induced mutations. Over 2500 crop varieties, used in modern agriculture, have been developed with high-dose ionizing radiation<sup>31</sup>, including the most important crops, e.g., wheat and rice<sup>32</sup>. However, there are no published works discussing the use of the molecular, biophysical or biochemical indicators as potential radiation biomarkers to aid in the radiation dosimetry applications. FAO/IAEA Mutant Variety Database records several new cultivars each year, many of which are produced using ionizing radiation.

The present work was conducted to (1) evaluate the impact of gamma radiation from different sources (<sup>60</sup>Co or <sup>137</sup>Cs) on maize growth, (2) investigate the various responses of physiological and biochemical properties of the treated maize through investigating DNA damage and genetic variations using the comet assay and molecular markers, also biochemical and morphological parameters to understand the possible challenges and advantages related to gamma mutagenicity and (3) determine the potential use of the studied indicators in the radiation dosimetry applications.

## Materials and methods

### Seed irradiation and plant cultivation experiment

Maize (*Zea mays* L. Var Giza 368 (triple hybrid)) seeds at  $(13 \pm 2)$  % moisture contents were provided from the Agriculture Ministry, Sakha Research Center, Kafr El-Sheikh, Egypt. They were classified into seven groups: three of them were irradiated to different gamma doses from Cs-137 source (GB 150, Atomic energy of Canada limited) with a dose rate of 5.5 Gy/h, the other three groups were irradiated to different doses from Co-60 source (Theratron 780E, Nordion, Inc (Canada)) with a dose rate of 63 Gy/h, and the 7th group was kept unirradiated (control). In order to avoid the results variations which may be affected by the irradiation temperature<sup>33</sup>, all seeds were irradiated at the National Institute of Standards (NIS) at a temperature of  $20 \pm 1$  °C. The doses were calibrated in a unit of air kerma rate using a secondary standard system that is traceable to the SI unit through the Bureau International des Poids et Mesures (BIPM). In order to achieve the charged particle equilibrium (CPE), one layer of seeds with a thickness of  $5.5 \pm 0.4$  mm was irradiated behind 3 mm and 5 mm of Perspex for Cs-137 and Co-60, respectively. The expanded uncertainty of the irradiation doses was about 2%. After five days of the irradiation, a completely randomized pot (30 cm in diameter with 6 kg of clay soil inside of them) experiment was established at the Botanical Garden, Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Nasr City, Cairo, Egypt. After 40 days of the treatment, which represented the vegetative growth stage and occurred between germination and flowering, samples from the different treatments and the control were collected for morphological and biochemical analyses. During the vegetative phase, plants are busy carrying out photosynthesis and accumulating resources that will be needed for flowering and reproduction.

### Quantification of DNA damage using single-cell electrophoresis

A small section of leaves was used to quantify DNA damage via a comet test<sup>34</sup> with some modifications<sup>35</sup>. Briefly, this involved isolating cell nuclei and conducting electrophoresis under alkaline pH, resulting in damaged DNA moving out of the nuclei and creating a COMET-like appearance. The amount of DNA in the COMET tail was then quantified by fluorescence microscopy. Approximately 50 nuclei from three replicates per dose were analyzed to assess DNA damage. OpenComet v1.3.1 computer-assisted image analysis system (comet score software) was used to measure the tail DNA (% tail DNA =  $100 - \% \text{ head DNA}$ ) for quantifying DNA damage<sup>36</sup>.

### SDS-PAGE analysis

SDS-PAGE (Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis) was utilized to assess the variation of total proteins<sup>37</sup>. Leaves samples were homogenized and then dissolved in an equivalent volume of 2% SDS sampling buffer containing 4% SDS, 100 mM Tris-Cl (pH 6.8), 0.2% bromophenol blue, 14 M mercaptoethanol and 20% glycerol; then samples boiled for 5 min in a water bath, followed by centrifugation at 14,000 rpm for 1 min and place on ice to cool. A stacking gel was then prepared, and the polyacrylamide gel was separated. Then, SDS gel electrophoresis was performed in a 1x Tris-glycine buffer at 100 V for 60 to 90 min. After running, the gel was placed in a Coomassie blue staining solution overnight. Finally, the excess stain was removed using a de-staining solution buffer for 3–4 h at room temperature, and the gel was then ready for imaging under white light.

### Estimation of pigments

A technique to quantify the pigments present in plants' leaves was discovered by Vernon and Seely<sup>38</sup>. This method involved chopping the green tissues into tiny pieces and weighing them in 1g portions. The tissue fragments were blended in 100 ml acetone 80% for two minutes to eliminate the plant dyes. The mixture was quantitatively transferred and filtered via a Buchner filter with No. 1 filter paper. To make 100 ml, 80% acetone was added to the filtrate after being transferred to a 100 ml flask. The optical density of the extract at three distinct wavelengths (649, 665, and 470 nm) was measured with a Carl Zeiss spectrometer. These wavelengths are found in the region where carotenoids and chlorophyll "a" and "b" absorb the lightest. The following formulas were used to calculate the content of chlorophylls a, b, and total chlorophyll in plants:

$$\text{Chlorophyll a} = 2.39 OD_{649} - 11.63 OD_{665}$$

$$\text{Chlorophyll b} = 20.11 OD_{649} - 5.18 OD_{665}$$

$$\text{Chlorophyll a + b} = 17.72 OD_{649} + 6.45 OD_{665}$$

However, the carotenoid content (mg/g FW) was determined using the following equation:

$$A = \frac{1000 (OD_{470}) - 1.82 \text{ Chlorophyll a} - 85.02 \text{ Chlorophyll b}}{198}$$

### Antioxidant enzymes

#### Enzyme extraction

The process of enzyme extraction was performed as described by Mukherjee and Choudhuri<sup>39</sup>. In brief, 2 g of plant terminal buds and the first and second new leaves were homogenized with 10 ml (0.1 M) phosphate buffer at 6.8 pH<sup>39</sup>. The resulting mixture was centrifuged at 2 °C for 20 min at 20,000 rpm. The enzymes were extracted from the clear supernatant, which contained the enzymes.

#### Determination of peroxidase (POX)

The POX was determined according to the methodology of Bergmeyer et al.<sup>40</sup>. In this technique, the rate of increase in absorbance as pyrogallol was measured using UV/Vis spectrophotometer in 60 s at 470 nm and

25 °C. The reaction mixture contained 5.8 ml of a 50 mM phosphate buffer at 7.0 pH, 0.2 ml of enzyme extract, and 2 ml of 20 mM H<sub>2</sub>O<sub>2</sub>.

*Determination of polyphenol oxidase (PPO)*

Polyphenol oxidase activity was assessed using Kar and Mishra’s method<sup>41</sup>. The substrate was made of 0.1 M catechol in a pH 5.0 sodium acetate buffer. For 60 min, the reaction was conducted at 30 °C, and measurements were taken using a spectrophotometer at 395 nm (Ultrospec 2000, Pharmacia Biotech, Country). The expression for polyphenol oxidase activity was changed in optical density min<sup>−1</sup>g<sup>−1</sup> fresh mass (FM).

*Determination of proline content*

Fresh leaves (0.5 g) from each treatment were homogenized in 10 ml of aqueous sulfosalicylic acid (3%), in order to ascertain the proline content in the studied plant samples. The filtrate was combined with 2 ml of acid ninhydrin reagent and 2 ml of glacial acetic acid, and the mixture was left at 100 °C for one hour. Following cooling, 4 ml of toluene was added to the reaction mixture in order to extract the proline content. Toluene was used as a blank in a spectrophotometer (VEB Carl Zeiss) to measure the absorbance at 520 nm<sup>42</sup>.

*Determination of protein content*

The amounts of total soluble protein were ascertained using the Bio-Rad protein assay and a UV spectrophotometer (UNICO Vis Model 1200, USA) in accordance with the procedures outlined by Lowry et al.<sup>43</sup>.

**Statistical analysis**

The data of morphological, physiological, and biochemical analyses, in triplicate, were subjected to one-way ANOVA, followed by post hoc Tukey’s test (HSD) at the probability level of 0.05 using Minitab version 19. All results are the means of three independent replicates. For the fitting of dose response curves, the statistical OriginPro 8.5 software program, created by OriginLab (<https://www.originlab.com>) was used.

**Results**

**DNA damage assessment by comet assay**

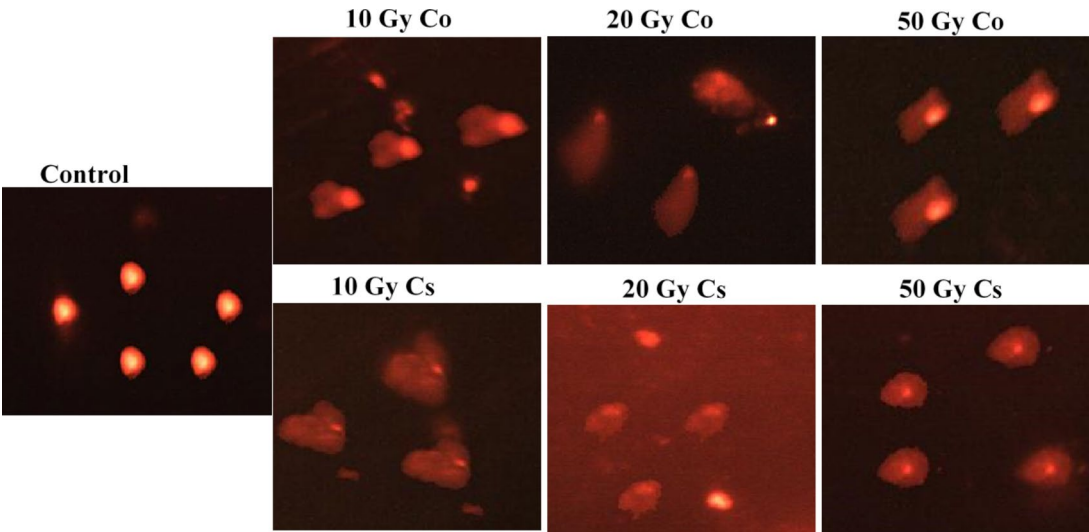
Comet assays use fluorescence microscopy and agarose electrophoresis technique to detect and measure DNA strand breaking at the single-cell level. In an electric field, cells with damaged DNA show enhanced chromosomal DNA migration from the nucleus. The DNA migratory pattern is shaped like a conventional “comet” with a head and a tail. During electrophoresis, the bulk DNA, also known as the nucleoid or “the head,” travels from the cathode to the anode more slowly than the short, broken DNA fragments or “the tail”. At this point, the DNA resembles a comet moving ahead with the tail. In an attempt to understand the genetic variants between the irradiated and non-irradiated maize plants, the assessment of DNA damage (tail length, tail DNA%, and tail moment) in leaves derived from irradiated seeds reveals precise parameters for determining the effect of GR on DNA within the cell. The study showed that DNA damage varies independently in response to different gamma-ray sources <sup>60</sup>Co and <sup>137</sup>Cs and doses (10, 20, and 50 Gy) (see Table 1; Fig. 1). DNA tail length indicated maximum DNA damage at 10 and 20 Gy of <sup>137</sup>Cs and <sup>60</sup>Co, respectively, while minimal damage occurred at 50 Gy of <sup>60</sup>Co and <sup>137</sup>Cs, respectively. Moreover, the analysis of tail DNA% and tail moment at 10, 20, and 50 Gy of <sup>60</sup>Co and <sup>137</sup>Cs demonstrated the highest damage at 10 and 50 Gy of <sup>60</sup>Co and <sup>137</sup>Cs, respectively, and the lowest damage at 20 Gy of <sup>60</sup>Co and <sup>137</sup>Cs, respectively. On the other hand, the assessment of tail moment (TM) showed the maximum value of damage at 10 and 50 Gy of <sup>137</sup>Cs and <sup>60</sup>Co, respectively, compared with the control and the minimal damage at 20 Gy of <sup>60</sup>Co and <sup>137</sup>Cs.

**SDS-protein electrophoresis**

Our SDS-PAGE study revealed distinct protein profiles in irradiated and non-irradiated maize plants, with gamma radiation exposure leading to the emergence or removal of bands and alterations in band densities. The polypeptide profile consisted of 15 bands ranging in size from 22 kDa to 110 kDa, with 4 polymorphic and 10 monomorphic bands (Table 2). The 20 Gy Co treatments had the largest number of bands (15), while the 20 Gy

Treatments	Tail length (μm)	Tail DNA (%)	Tail moment
Control	8.03 ± 0.39b	3.98 ± 0.26d	0.29 ± 0.01c
10 Gy Co	8.64 ± 0.31b	8.47 ± 0.51a	0.64 ± 0.03b
10 Gy Cs	15.35 ± 0.68a	6.58 ± 0.39b	1.05 ± 0.4a
20 Gy Co	10.95 ± 0.54b	5.07 ± 0.42c	0.61 ± 0.04b
20 Gy Cs	13.27 ± 0.50a	6.74 ± 0.47b	0.78 ± 0.01b
50 Gy Co	7.42 ± 0.48c	7.06 ± 0.44a	0.65 ± 0.01b
50 Gy Cs	9.83 ± 0.41b	7.44 ± 0.34a	0.86 ± 0.03a
HSD at 5%	2.36	1.42	0.21

**Table 1.** Percentage of DNA damage (tail length, tail DNA, and tail moment) of cells in response to Co and Cs (10, 20, and 50) gy sources of gamma radiation. Tukey’s Honestly Significant Difference (HSD) at the 5% significance level. HSD The different superscript letters among each treatment mean significant variation at a probability level of 0.05.



**Fig. 1.** Comet assay images showed DNA damage of maize in response to Co and Cs (10, 20, and 50) Gy as sources of gamma irradiation.

Band no.	M.W. (kDa)	Control	10 Gy Co	10 Gy Cs	20 Gy Co	20 Gy Cs	50 Gy Co	50 Gy Cs
Band 1	~ 110	1	0	1	1	0	0	1
Band 2	~ 85	0	0	0	1	0	0	0
Band 3	~ 70	1	1	1	1	1	1	1
Band 4	~ 68	1	1	1	1	1	1	1
Band 5	~ 45	0	0	0	1	0	1	1
Band 6	~ 37	1	1	1	1	1	1	1
Band 7	~ 36	1	1	1	1	1	1	1
Band 8	~ 35	1	1	1	1	1	1	1
Band 9	~ 34	1	1	1	1	1	1	1
Band 10	~ 32	1	1	1	1	1	1	1
Band 11	~ 30	1	1	1	1	1	1	1
Band 12	~ 28	1	1	1	1	1	1	1
Band 13	~ 27	1	0	0	1	0	0	1
Band 14	~ 24	1	1	1	1	0	0	0
Band 15	~ 22	1	1	1	1	1	1	1
Total	15	13	11	12	15	10	11	13

**Table 2.** Effect of different sources of gamma irradiation on protein profile pattern of the tested maize plant.

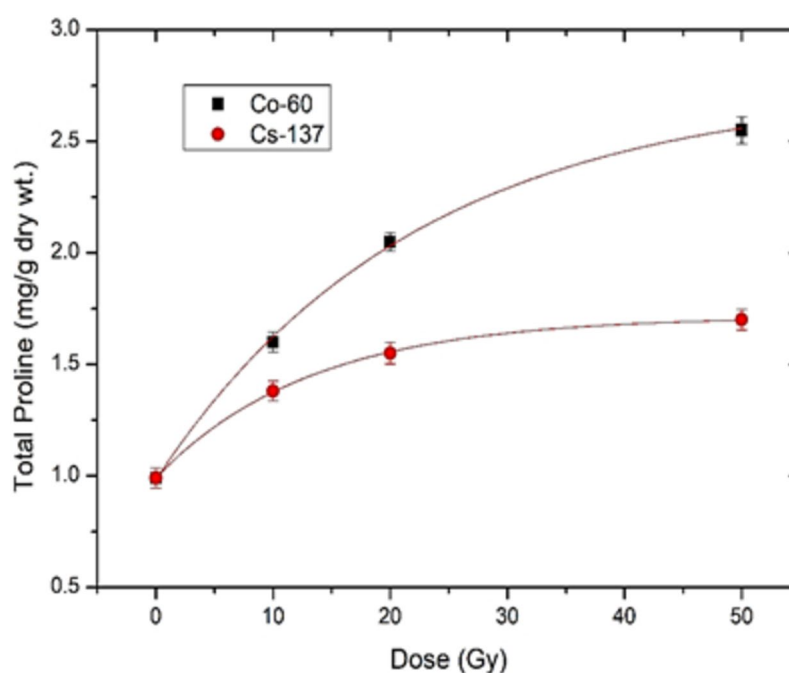
Cs dose had the minimum number of bands (10). The 10 Gy Co and 50 Gy Co treatments had the same number of bands (11), and the 50 Gy Cs dose and control had the same number of bands (13). While control shares band with 110 kDa with 20 Gy Co and 10, 50 Gy Cs. On the other hand, 20, 50 Gy Co, and 50 Gy Cs share the same band 45 kDa, which disappeared from other treatments and the control.

**Morphological traits**

Effects of gamma irradiation at different doses from different sources, <sup>60</sup>Co or <sup>137</sup>Cs, on morphological parameters of maize hybrids triple white (Giza 321) are depicted in Table 3. Plant heights and fresh weight (g/plant) were severely reduced by gamma irradiation dosages; the highest plants were found in the control and treated plants (10, and 50 Gy from the Cs source and 20 Gy from the Co source, respectively), with no discernible differences between them. Gamma rays from the Co source were observed at 10 Gy groups with the lowest shoot length percentage compared to the control and other groups. Compared to the control, the lengths and fresh weight of the shoot were significantly reduced by gamma rays from the Cs source at 20 Gy and gamma rays from the Co source at 10 and 50 Gy. When exposed to gamma rays from the Cs source at 10 Gy, root lengths were significantly promoted.

Treatments	Shoot lengths (cm)	Root lengths (cm)	F.W. of shoot (g)	Chlorophyll a	Chlorophyll b	Chlorophyll a + b	Carotenoids	Total protein mg/g dry weight	Total proline mg/g dry weight	Peroxidase (unit/g. F. wt./hour)	Polyphenol oxidase (unit/g. F. wt./hour)
Control	57.30 ± 3.52 <sup>a</sup>	8.90 ± 1.21 <sup>ab</sup>	8.14 ± 1.43 <sup>a</sup>	23.55 ± 1.21 <sup>a</sup>	19.37 ± 1.21 <sup>a</sup>	42.92 ± 1.25 <sup>a</sup>	0.02 ± 0.01 <sup>d</sup>	6.76 ± 0.05 <sup>d</sup>	0.99 ± 0.03 <sup>d</sup>	15 ± 0.59 <sup>d</sup>	4.80 ± 0.76 <sup>d</sup>
10 Co	35.80 ± 3.43 <sup>c</sup>	6.60 ± 0.51 <sup>b</sup>	2.92 ± 0.43 <sup>c</sup>	19.34 ± 2.02 <sup>b</sup>	12.54 ± 2.21 <sup>bc</sup>	31.87 ± 2.01 <sup>c</sup>	2.11 ± 0.02 <sup>c</sup>	7.66 ± 0.04 <sup>c</sup>	1.60 ± 0.03 <sup>c</sup>	22 ± 0.55 <sup>bc</sup>	8.60 ± 0.59 <sup>c</sup>
20 Co	48.25 ± 3.17 <sup>a</sup>	8.33 ± 0.55 <sup>ab</sup>	6.31 ± 1.06 <sup>a</sup>	22.89 ± 1.04 <sup>a</sup>	14.98 ± 1.32 <sup>b</sup>	37.87 ± 1.20 <sup>b</sup>	1.94 ± 0.12 <sup>c</sup>	8.26 ± 0.04 <sup>b</sup>	2.05 ± 0.02 <sup>a</sup>	28 ± 0.59 <sup>b</sup>	13.20 ± 0.14 <sup>b</sup>
50 Co	41.60 ± 3.90 <sup>bc</sup>	7.60 ± 1.03 <sup>ab</sup>	4.20 ± 0.65 <sup>bc</sup>	16.79 ± 1.56 <sup>bc</sup>	11.54 ± 2.12 <sup>c</sup>	28.33 ± 2.21 <sup>cd</sup>	3.52 ± 0.02 <sup>a</sup>	7.96 ± 0.04 <sup>b</sup>	1.55 ± 0.04 <sup>a</sup>	35 ± 0.38 <sup>a</sup>	16.60 ± 0.12 <sup>a</sup>
10 Cs	46.20 ± 2.35 <sup>a</sup>	12.10 ± 1.63 <sup>a</sup>	5.28 ± 0.47 <sup>abc</sup>	12.79 ± 1.32 <sup>d</sup>	9.80 ± 1.02 <sup>d</sup>	22.59 ± 2.36 <sup>d</sup>	3.44 ± 0.032 <sup>a</sup>	7.39 ± 0.04 <sup>c</sup>	1.38 ± 0.03 <sup>c</sup>	18 ± 0.26 <sup>d</sup>	6.00 ± 1.48 <sup>d</sup>
20 Cs	40.83 ± 1.44 <sup>bc</sup>	8.16 ± 1.08 <sup>ab</sup>	3.53 ± 0.60 <sup>c</sup>	12.69 ± 2.32 <sup>d</sup>	8.80 ± 2.21 <sup>d</sup>	21.49 ± 2.36 <sup>d</sup>	3.47 ± 0.21 <sup>a</sup>	8.76 ± 0.04 <sup>b</sup>	2.55 ± 0.03 <sup>b</sup>	22 ± 0.89 <sup>bc</sup>	8.40 ± 0.48 <sup>c</sup>
50 Cs	46.80 ± 3.84 <sup>a</sup>	9.60 ± 1.08 <sup>ab</sup>	4.94 ± 0.51 <sup>abc</sup>	14.87 ± 2.21 <sup>c</sup>	8.78 ± 0.65 <sup>d</sup>	23.65 ± 1.65 <sup>d</sup>	3.36 ± 0.14 <sup>a</sup>	7.08 ± 0.04 <sup>bc</sup>	1.70 ± 0.03 <sup>b</sup>	25 ± 0.71 <sup>b</sup>	7.20 ± 0.59 <sup>c</sup>
HSD at 5%	12.36	4.01	3.34	3.36	2.01	4.34	0.65	0.45	0.51	4.34	2.65

**Table 3.** Effect of different sources and doses of gamma rays on morphological traits, pigment traits, protein, proline contents, and antioxidant enzymes activity of maize hybrids triple white (Giza 321) plants. Data represents means ± standard error ( $n=3$ ). Different uppercase letters in the same species within a column indicate significant differences ( $P \leq 0.05$ ) by Tukey's test (HSD).



**Fig. 2.** The relation between the total proline content and both Co-60 and Cs-137 doses. Error bars represent the standard deviation of 3 aliquots per dose.

### Pigments contents

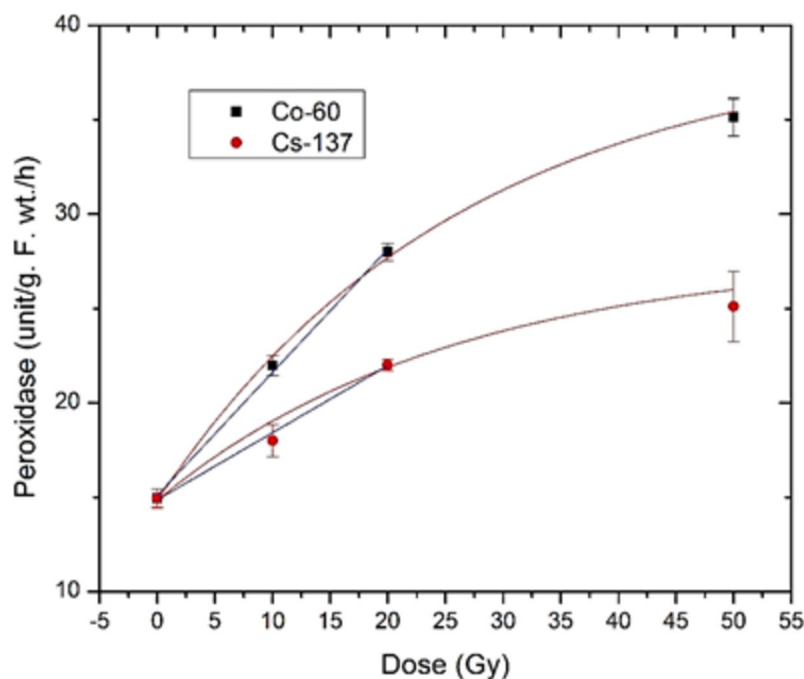
A significant decrease in chlorophyll a, b, and a + b in response to all doses of GR related to control groups is shown in Table 3. In regard to carotenoids, maize leaf content increased significantly when gamma irradiation levels were raised from 10 to 50 Gy. In contrast to non-irradiated plants, this had the lowest carotenoid concentration. Plants exposed to 50 Gy from a Co source had the highest carotenoid content.

### Total protein and proline contents

Data found in Table 3 also indicated that the pretreatment with doses of gamma-ray caused a progressive increment in total protein and proline content compared with that of the corresponding unirradiated control plants. The lowest values of protein and proline contents appeared in the control groups. The highest value of total protein content was observed in 20 Gy irradiated plants from  $^{60}\text{Co}$  and  $^{137}\text{Cs}$  sources, reaching around 22 and 29.4%, respectively, as compared to the control group. On the other hand, the proline concentration increases exponentially as the exposure dose increases in both  $^{60}\text{Co}$  and  $^{137}\text{Cs}$  irradiation. Figure 2 portrays the relation between the proline contents and the dose irradiated by the two used sources, indicating that this parameter can be used in radiation dosimetry in the intermediate dose range (0–50 Gy). The fitting parameters of this relationship are summarized in Table 4. It can also be noticed that the impact of the dose from the  $^{60}\text{Co}$  source on proline content is greater than that of irradiated by the Cs-137 source.

Radiation source	Factor	Type of fitting	Equation	Equation constants	R-value
$^{60}\text{Co}$	Proline	Exponential	$y = Ae^{(R_0 x)} + y_0$	$y_0 = 2.74 \pm 0.088$ $A = -1.76 \pm 0.087$ $R_0 = -0.045 \pm 0.005$	0.9995
	Peroxidase	Linear	$y = a + bx$	$a = 15.1 \pm 0.37$ $b = 0.652 \pm 0.027$	0.999
		Exponential	$y = Ae^{(R_0 x)} + y_0$	$y_0 = 39.1 \pm 2.77$ $A = -24.3 \pm 2.71$ $R_0 = -0.038 \pm 0.007$	0.999
$^{137}\text{Cs}$	Proline	Exponential	$y = Ae^{(R_0 x)} + y_0$	$y_0 = 1.71 \pm 0.009$ $A = -0.72 \pm 0.011$ $R_0 = -0.076 \pm 0.003$	0.9999
	Peroxidase	Linear	$y = a + bx$	$a = 14.87 \pm 0.25$ $b = 0.356 \pm 0.015$	0.999
		Exponential	$y = Ae^{(R_0 x)} + y_0$	$y_0 = 27.9 \pm 5.58$ $A = -13 \pm 5.54$ $R_0 = -0.039 \pm 0.026$	0.994

**Table 4.** Summary of the fitting parameters of the dose-response of the peroxidase enzyme and proline content for different sources.



**Fig. 3.** The dose-response of peroxidase enzyme for Co-60 and Cs-137 irradiation. Linear fitting regression is in blue, and exponential fitting lines are in red.

### Antioxidant enzymes

In our study, the maize plants exposed to  $\gamma$  radiation exhibited notable changes in the activity of peroxidase (POD) and polyphenol oxidase (PPO) (see Table 3). The radiation treatment elevated the activity of the POD and PPO enzymes. The peroxidase enzyme levels behave similarly for both radiation sources  $^{60}\text{Co}$  and  $^{137}\text{Cs}$ . Compared to the control group, peroxidase levels increase exponentially in plants exposed to Co or Cs source as the radiation dose increases, as demonstrated in Table 3. In the case of polyphenol oxidase, activity rises with increasing Co-60 doses, while for Cs-137 radiation, activity increases with doses until 20 Gy, then falls.

It is worth noting that Fig. 3 illustrates the dose response of the peroxidase enzyme for both  $^{60}\text{Co}$  and  $^{137}\text{Cs}$  irradiation from the perspective of radiation dosimetry. It is clear from this figure that the relation between the concentration of the peroxidase enzyme and the dose for both cobalt and cesium is linear until 20 Gy and exponential up to 50 Gy. This indicates that the peroxidase enzyme can be used as a detector in the range of up to 50 Gy. With a minimum R of 0.994, the fitting parameters of these relations in Fig. 3 are listed in Table 4.

## Discussion

Nowadays, nuclear power is used in the process of mutant breeding to increase genetic variety and improve crops. The comet test is a simple and highly sensitive technique for detecting DNA radio-sensitivity which can identify single and double-stranded DNA breaks at the cellular level. Our study compared the values of Co and Cs as sources of gamma radiation at doses of 10, 20, and 50 Gy. It was found that the maximum DNA damage occurred at 10 and 20 Gy of Cs and Co, respectively, while the minimal damage occurred at 50 Gy of Co and Cs, respectively, as presented by DNA tail length. Tailed DNA % showed the highest damage at 10 and 50 Gy of Co and Cs, respectively, and the lowest damage at 20 Gy of both sources. On the other hand, TM showed the maximum value of damage at 10 and 50 Gy of Cs and Co, respectively, and minimal damage at 20 Gy of both sources, compared to the control. Based on these findings, we propose that gamma radiation-induced DNA damage may be a result of direct energy impacting the DNA molecules and/or oxidation of DNA caused by excessive generation of reactive oxygen species (ROS). Strong ionizing agents like gamma radiation produce ROS, which harm DNA and other biological macromolecules. In order to neutralize ROS and stop oxidative damage, antioxidants are essential.

In our investigation, however, there is no evidence to determine whether the DNA damage was caused by the direct or indirect effects of gamma radiation. Ionizing radiation is a familiar factor that causes damage to DNA, and it has been suggested that acute high doses of 10–1000 Gy could be lethal to plants<sup>44</sup>. Gamma rays can interrupt the integrity of genetic material and damage genomic stability by inducing DNA damage. Significantly elevated DNA damage is primarily due to the direct ionization by gamma irradiation or linked to ROS-induced oxidative stress<sup>45,46</sup>. GR can cause significant genomic instability, and unrepaired DNA damage can impair cell functions by disrupting DNA replication and transcription, resulting in reduced growth<sup>47</sup>. On the other hand, numerous DNA repair mechanisms are activated at different cell cycle stages, enabling cells to repair their damaged DNA<sup>48</sup>. The interaction of fission neutrons and <sup>60</sup>Co gamma-radiation causing somatic mutations in maize embryos that are just beginning to germinate was examined by Conger<sup>49</sup>. He found that whereas mutagenic efficacy rose with increasing gamma radiation dose from 0.3 to 6 Gy, it fell with increasing fission neutron dose ranging from 5 to 320 mGy. It was also mentioned that the high frequency of mutations was observed, particularly at low neutron doses.

Plant stress responses often result in alterations in protein content. Therefore, we used SDS-PAGE in this study, to analyze the protein profiles of irradiated and non-irradiated maize plants to understand the genetic differences between the two groups. The SDS-PAGE analysis revealed 15 bands ranging in size from 22 to 110 kDa, with 4 polymorphic and 10 monomorphic bands. The largest number of bands was determined for 20 Gy of Co, while the lowest number of bands was assessed for the treatment of 20 Gy of Cs doses. Gamma irradiation induces changes in the cells' genetic, physiological, and morphological nature, as well as biochemical changes and breakdowns in protein bonds<sup>27</sup>. This indicates that GR has caused alterations in gene expression, resulting in changes in the types of protein found in the SDS protein profiles<sup>11</sup>. From the obtained results the alterations in protein bands may result from the direct effect of GR on stored protein molecules and/or affecting DNA which results in the production of new proteins or the loss of certain proteins due to damaged/lost pieces of DNA.

Seeds exposed to GR undergo mutagenic changes, and penetrating tissues and cells before undergoing drastic changes. GR resulted in alterations in protein patterns by causing the emergence or vanishing of particular protein bands. Additionally, low doses of GR can stimulate the biosynthesis of amino acids, thereby altering the content of proteins that play crucial roles in plant development<sup>26</sup>. Moreover, the presence and/or absence of some protein bands might be connected to environmental stresses that lead to alternation in the expressions of certain genes<sup>50</sup>. Several Proteins are synthesized and stored in plant tissues in response to various stresses, and Protein polymorphisms arise from insertion or deletion in mutated protein bands<sup>51</sup>. New bands usually form as a result of different changes in the structural DNA (such as splits, deletions, and transpositions), leading to alterations in amino acids and, consequently, the shape of the protein<sup>52</sup>. Furthermore, irradiation may stimulate chemical changes in proteins as cross-linking, degradation, disturbance of the organized structures of protein molecules, and assemblage of the polypeptide chain due to oxygen free radicals<sup>53</sup>.

Our result revealed that gamma rays from the <sup>60</sup>Co source at 10 and 50 Gy caused a high decrease in shoot length compared to other groups. The lowest shoot length was observed in exposure of gamma rays from the Co-60 source at 10 Gy groups by 37.5% compared to control groups. These observations in contrary to the findings of Bhosale and More<sup>54</sup>, who observed a progressive reduction in the height of *Withania somnifera* seedlings upon exposure to gamma rays rising from 10 to 40 kR. The exposure of *Brassica napus* L. seeds to gamma-ray doses of 0, 10, 15, 20, 25, and 30 kR portrayed that, the higher radiation doses significantly decreased plant height in comparison to the control<sup>55</sup>. It also reported that barley (*Hordeum vulgare* L.) seeds exposed to gamma rays at levels (0.2 and 0.4) kGy showed a significant drop percentage of germination, however, seeds exposed to 0.1 kGy did not differ from the control<sup>56</sup>.

Since gamma rays ionize the irradiated materials, a significant number of free radicals are created, simulating environmental stressors. Plants employ antioxidant defense systems to avert oxidative damage caused by gamma radiation. Plant germination, growth, and metabolite production can all be affected by radiation. Reduced seed moisture content and decreased meristematic tissue mitotic activity are the likely causes of the plant height reduction caused by high radiation exposures<sup>57</sup>. Additionally, Shala stated that, as a protective response to the higher irradiation doses, germination percentage and vegetative development characteristics were significantly reduced by increasing gamma-ray doses<sup>58</sup>. In our study, irradiated of Co-60 at 20 Gy caused the highest value of lengths and fresh weight of shoot compared to other doses, also exposed to gamma rays from the Cs-137 source at 10 Gy, root lengths were dramatically promoted by about 36% compared to control. The results of Hamideldin and Eliwa indicate that increasing the dosage of gamma rays from 10 to 50 Gy increased significantly the total biomass of mustard plants<sup>59</sup>. This suggests that the largest effect of gamma irradiation doses on plant biomass

was consistent with their findings. Furthermore, Bhat et al.<sup>60</sup> noted that plants of *Linum usitatissimum* L. showed a discernible decrease in dry weight in response to gamma radiation at 15 kR. Similarly, it was found that at 20 kR of gamma rays, fennel vegetative growth metrics were reduced by almost 50%<sup>61</sup>. The drop in plants' fresh and dry weights could be the result of radiation stress-induced moisture content loss, particularly in cases where significant gamma radiation doses are received. The morphological parameters and survival rate of stevia declined along with an increase in the gamma irradiation dose (0, 100, 300, 500, 700, 900, and 1100 Gy). Furthermore, seeds subjected to higher amounts of radiation (> 500 Gy) did not germinate<sup>57</sup>. This is acceptable as there is a correlation between reduced seedling development and cytogenetic and genetic damage caused by gamma rays<sup>62</sup>.

All treatments caused a significant increase in carotenoid contents compared to control groups but caused significant decrease in chlorophyll a, b, and a + b, except gamma rays from the Co source at 20 Gy groups, which caused a non-significant decrease compared to the control group. Gamma rays from the Cs source at either 20–50 Gy groups appeared a high drop in chlorophyll contents. In this concept, it was stated that, as a protective response to the higher irradiation doses, total carotenoids and total phenolic content rose whereas chlorophyll concentration was significantly reduced by increasing gamma-ray doses of *Ocimum basilicum* plant<sup>58</sup>. Additionally, it was reported that at low dosages (5 Gy), photosynthetic pigments were significantly boosted; at high doses, they were significantly reduced in barley leaves<sup>26</sup>. *Cichorium pumilum* Jacq produced the maximum carotenoids content from irradiated chicory plants with 80 Gy, and which carotenoids content increased as a defensive reaction to the rise in gamma doses<sup>63</sup>. The influence of different levels of gamma dose on chlorophyll was assessed in terms of hormonal imbalance, water exchange, enzyme activity, and leaf gas exchange<sup>64</sup>. The current findings contradict those of Hamideldin and Eliwa, who found that gamma irradiation enhanced the amount of photosynthetic pigments in mustard plant leaves<sup>59</sup>. Also, Aly et al.<sup>65</sup> demonstrated that wheat plants irradiated with 100 and 200 Gy of GR enhanced the photosynthetic pigments' content in response to salt stress conditions.

Gamma radiation from different doses and sources led to a significant rise in proline and antioxidant enzymes. However, the <sup>60</sup>Co source had the most significant effect compared to the control and other source groups. It implies that these antioxidant enzymes' altered roles in processes that guard against oxidative damage brought on by gamma radiation may be the cause of the shift in their activities. Additionally, Kiani et al.<sup>66</sup> reported that gamma irradiation doses ranging from 200 to 300 Gy both decreased *Triticum aestivum* L. plant development and increased the expression of antioxidant enzymes. Also, post-irradiation 350 Gy of cobalt source-O<sub>2</sub>-saturated hydration resulted in maximum harm to 8-day-old barley seedlings, as well as increased peroxidase activity and a corresponding decrease in total peroxides, as demonstrated by Singh and Kesavan<sup>67</sup>. Significant radioprotection against O<sub>2</sub>-dependent damage following irradiation was provided by both coffee and t-butyl alcohol. In the same trend, it was shown that total antioxidant enzymes and total amino acids increased in response to different doses (5, 10, and 20 Gy) of gamma radiation on barley plants<sup>26</sup>. For antioxidant enzymes, all concentrations are incremented with the dose, except polyphenol oxidase the highest level was observed at 20 Gy from Cs-137 sources. The control group has the lowest value compared to other doses of gamma-ray groups. It is worth mentioning that all enzyme concentrations at each dose from Co-60 sources are greater than that from Cs-137, revealing that <sup>60</sup>Co doses have a higher impact than <sup>137</sup>Cs on the antioxidants of maize. This may be attributed to that the energy of Co-60 (1.25 MeV) is higher than Cs-137 (0.662 MeV), leading to higher oxidative damage in the plants. Therefore, and as a defensive action, higher concentrations of antioxidants and proline are produced to cancel the damage.

In terms of radiation dosimetry applications, certain parameters respond in a trendy way, such as proline content and peroxidase enzyme concentration. Our results for the proline contents trend agree with previous reports: fenugreek for doses up to 200 Gy<sup>68</sup> and Iraqi dates<sup>69</sup>. In this context, proline is nominated as an electron spin resonance (ESR) dosimeter by Karakirova for high-dose measurements (1–10 kGy)<sup>70</sup>. As demonstrated above in the paper, both the peroxidase enzyme and proline can quantitatively and precisely measure the dose. However, there is currently no documentation, to our best knowledge, discussing their utilization as biomarkers for ionizing radiation. The dose-response of these parameters shows good fitting lines, making them promising candidates for retrospective detection of ionizing radiation; nevertheless, further investigations are necessary to explore other characteristics.

In conclusion, the study showed that different doses of gamma irradiation have independent effects on the rate of DNA damage, including the length of the tail, the percentage of tail DNA, and the tail moment. Additionally, the irradiation caused some protein bands to appear or disappear. Doses of 10–50 Gy inhibited plant growth, as evidenced by reduced fresh and dry weight and plant length, but also increased the concentration of antioxidant enzymes. Different sources of gamma rays increased protein, proline, and antioxidant enzyme contents. Generally, the doses from <sup>137</sup>Cs have less impact on the studied markers than those from <sup>60</sup>Co, attributed to the low energy of the Cs-137 gamma ray. Amongst all measured parameters, the concentration of proline or peroxidase, which has a promising dose-response behavior, can aid in the detection of radiation in the examined dose range. Further dosimetric features of these two parameters are necessary to be defined in future work to complete the research of their main attributes for dosimetric applications.

## Data availability

Data sets generated during the current study are available from the corresponding author on reasonable request.

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## Author contributions

M.S.A. and M.A.A. wrote the main manuscript, T.C.S. made some statistical analysis, E.A. and M.A.A. prepared figures and tables, and E.A. and A.M.A. rewrite the initial draft and receiving funding. All authors reviewed the manuscript.

## Declarations

## Competing interests

The authors declare no competing interests.

## Additional information

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