TÜBİTAK

Turkish Journal of Agriculture and Forestry

Volume 50 | Number 1 Article 6

2-26-2026

Effects of gamma irradiation on antioxidant enzyme activities in black carrot callus under drought stress

DAMLA TURAN BÜYÜKDİNÇ

DİLRUBA TABAY SÜMBÜL

SHEIKH MANSOOR

Follow this and additional works at: https://journals.tubitak.gov.tr/agriculture

 Part of the Agriculture Commons, and the Forest Sciences Commons

Recommended Citation

TURAN BÜYÜKDİNÇ, D, TABAY SÜMBÜL, D, & MANSOOR, S (2026). Effects of gamma irradiation on antioxidant enzyme activities in black carrot callus under drought stress. *Turkish Journal of Agriculture and Forestry 50* (1): 66-79. https://doi.org/10.55730/1300-011X.3333



This work is licensed under a Creative Commons Attribution 4.0 International License.
This Research Article is brought to you for free and open access by TÜBİTAK Academic Journals. It has been accepted for inclusion in Turkish Journal of Agriculture and Forestry by an authorized editor of TÜBİTAK Academic Journals. For more information, please contact academic.publications@tubitak.gov.tr

TÜBİTAK

Turkish Journal of Agriculture and Forestry

http://journals.tubitak.gov.tr/agriculture/

Research Article

Turk J Agric For
(2026) 50: 66-79
doi:10.55730/1300-011X.3333
© Author

Effects of gamma irradiation on antioxidant enzyme activities in black carrot callus under drought stress

Damla TURAN BÜYÜKDİNÇ[1,*], Dilruba TABAY SÜMBÜL[2], Sheikh MANSOOR[1]
[1]Department of Horticulture, Faculty of Agriculture, Recep Tayyip Erdoğan University, Rize, Turkiye
[2]Central Research and Application Laboratories, Ağrı İbrahim Çeçen University, Ağrı, Turkiye

Received: 04.12.2025 • **Accepted/Published Online:** 05.01.2026 • **Final Version:** 26.02.2026

Abstract: *Daucus carota* L. (carrot) is a commercially significant root vegetable widely cultivated in temperate regions for its edible taproot and valued for its high nutritional content. This study aimed to investigate the effects of drought stress on callus tissues of the Hatay black carrot (*Daucus carota* L. ssp. *sativus* var. *atrorubens* Alef.) genotype and to determine the impact of different gamma radiation doses on antioxidant enzyme activities. Black carrot callus tissues were exposed to gamma irradiation at doses of 0, 7, 8, and 9 Gy to evaluate their responses to drought stress. Antioxidant enzyme activities, including superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX), as well as biochemical parameters such as hydrogen peroxide (H_2O_2) levels, lipid peroxidation measured as malondialdehyde (MDA) content, and soluble protein concentrations, were quantified. Callus formation was completely inhibited at a polyethylene glycol (PEG) concentration of 15%, whereas a gradual decrease in callus production was observed at 5% and 10% PEG. Enzyme activity analyses revealed significant increases in CAT, SOD, and APX activities in gamma-irradiated carrot callus tissues exposed to drought stress compared with control tissues. Furthermore, drought stress resulted in elevated H_2O_2 and soluble protein levels, accompanied by a marked increase in lipid peroxidation, as indicated by MDA content. Multivariate analysis revealed that key oxidative stress markers and antioxidant enzymes significantly influence plant stress responses, indicating the presence of complex regulatory networks. Antioxidant systems play a central role in mitigating stress, with distinct pathways independently regulating oxidative damage markers. The results suggest that differences between gamma-irradiated callus lines and control callus tissues under drought stress conditions may provide valuable insights for future *in vitro* selection and stress-response studies in carrot.

Key words: Carrot, callus, mutation, drought, antioxidant enzyme activity, multivariate analysis

1. Introduction

Drought, an increasingly severe natural hazard exacerbated by rising global temperatures, affects a considerable proportion of the human population, particularly in semiarid and arid regions. Over the past four decades, the land area affected by drought has more than doubled, making drought the most impactful natural disaster globally during this period. Climate change has further amplified drought severity, characterized by increased frequency, intensity, and persistence[1]. Understanding the impact of drought is particularly important for crops such as carrot, which are sensitive to water deficit conditions.

Daucus carota L. (carrot) is a significant vegetable crop cultivated globally in cool climates because of its edible taproot (Bashir et al., 2021). It is a hydrophilic species and is renowned for its high nutritional value (Bonasia et al., 2021). High concentrations of anthocyanins, carotenoids, and phenolics render carrots nutritionally valuable, contributing to substantial consumer demand. However, research on the impact of climate change on carrot production remains limited (Blando et al., 2021; Kowalczyk and Kuboń, 2022). Carrots exhibit diverse pigmentation due to various compounds: beta-carotene imparts an orange color, anthocyanins produce black pigmentation, lycopene generates red coloration, xanthophylls result in yellow coloration, and carrots lacking pigments appear white (Buishand and Gabelman, 1980; Koca Bozalan and Karadeniz, 2011; Zhang et al., 2021). These biochemical characteristics influence the plant's response to environmental stresses such as drought, which can affect both yield and quality.

Drought is a primary abiotic stress factor that significantly impairs plant growth (Duan et al., 2021; Zhu et al., 2021). Carrots are particularly susceptible

[1] FAO (2022). Food and Agriculture Data [online]. Website http://www.fao.org/faostat/en/#data/QC [accessed 01 September 2024].

* Correspondence: damla.turan@erdogan.edu.tr

Authors retain the copyright of their works. Upon acceptance, authors grant the journal a non-exclusive right of first publication and permission to distribute the article in all forms and media under the Creative Commons Attribution 4.0 International (CC BY 4.0) licence.

to abiotic stress, and drought conditions severely affect their production and quality (Razzaq et al., 2017; Junaid et al., 2023). Insufficient soil moisture during taproot development negatively influences carrot yield (Öztürk Gökçe et al., 2022). Furthermore, drought stress alters carrot quality and chemical composition, although dry matter concentrations may increase simultaneously under water deficit conditions (Seleiman et al., 2021). Abiotic stress conditions, such as drought, induce notable changes in the concentrations of metabolites, including proline, glycine betaine, phenolic compounds, and carotenoids (Razzaq et al., 2017). Specifically, drought-sensitive carrot genotypes show a decrease in carotenoid levels compared with drought-tolerant genotypes (Zhang et al., 2021). Cultivars with relatively high dry matter contents tend to exhibit greater drought tolerance (Abid et al., 2018). Severe drought inhibits photosynthesis by disrupting the photosynthetic apparatus, thereby negatively affecting plant chlorophyll content (Wang et al., 2018). Chlorophyll degradation is caused primarily by reactive oxygen species (ROS), which interfere with chloroplast functionality (Qi et al., 2023). These physiological and biochemical changes highlight the importance of studying enzymatic responses under drought conditions.

Drought stress influences enzymes involved in protein metabolism. Protease activity, which is responsible for protein degradation, may increase under drought conditions, leading to reduced protein content. Generally, drought stress negatively affects enzyme activity by damaging plant cells and impairing cellular functions, including enzyme synthesis (Kusvuran et al., 2016; Kuşvuran, 2021; Zia et al., 2021). However, certain enzymes that synthesize compatible solutes (molecules that increase drought resistance) may exhibit increased activity during drought. Additionally, enzymes involved in antioxidant defense mechanisms may become more active in protecting plants from oxidative stress induced by prolonged drought exposure (Zia et al., 2021). Understanding these cellular-level responses requires controlled experimental systems, such as tissue culture.

Tissue culture techniques provide controlled in vitro conditions, facilitating the evaluation of stress tolerance. These cultures yield a homogeneous population of synchronously developing plant cells, which differ from whole plants in their regulatory mechanisms (Ammirato, 1983; Fehér, 2019). In vitro tissue culture is a crucial method for examining physiological and biochemical responses at the cellular level under controlled conditions (Lushchak, 2011; Rai et al., 2011; Efferth, 2019). Culturing explants on media containing selective biotic or abiotic agents to generate new variability is termed the in vitro selection technique (Rai et al., 2011). This method assesses the survival, regeneration, and growth of desired genotypes (somatic variants). Although some researchers emphasize the heritability of somaclonal variation via sexual reproduction, this outcome is not always achievable (Rai et al., 2011). In vitro techniques enable precise stress control and detailed analysis of cellular responses, independent of whole-plant regulatory processes (Lutts et al., 2004). Such controlled systems are particularly useful for studying callus cultures under stress conditions.

Callus culture is among the most widely employed tissue culture techniques because of the ease of manipulating and cultivating callus tissues. Enzymatic activities, which are essential for plant growth and development, change significantly in response to environmental stresses such as drought. Specifically, drought stress substantially alters enzymatic activities in carrot callus tissues, thereby affecting various metabolic and cellular processes (Rai et al., 2011). Polyethylene glycol (PEG) is commonly utilized as an osmotic agent in vitro to simulate drought conditions, facilitating the selection of drought-tolerant mutants (Santos-Diaz and Ochoa-Alejo, 1994; Dami and Hughes, 1997; Afa et al., 2012; Suprasanna et al., 2014; Saepudin et al., 2017; Kacem et al., 2017; Zhang et al., 2021; Nijabat et al., 2023). Notably, previous research has employed PEG-induced drought conditions to select drought-tolerant carrot genotypes in vitro (Fallon and Phillips, 1989). Combining PEG-induced stress with mutagenesis may enhance the efficiency of developing drought-tolerant genotypes.

The use of gamma radiation as a physical mutagen offers several notable advantages, including ease of use, high permanence, accessibility to target cells, and an absence of toxic effects or damage (Schum, 2003). In vitro methods permit the uniform treatment of cells with physical and chemical mutagens, followed by cultivation in homogeneous media (Maliga, 1984; Ahloowalia, 1995). In vitro cultures offer substantial benefits for identifying somaclonal variation in plant breeding studies. In vitro mutagenesis experiments can be performed on large populations within limited space and at any time of year, thereby increasing the probability of obtaining potent mutants. A primary benefit of this approach is that chimeric structures in M1V1 are circumvented. Tissue culture techniques facilitate the management of large populations for mutagenesis treatment, selection, and cloning of specific mutants. Furthermore, this method allows expeditious subculture propagation cycles, which facilitate the separation of mutated sectors from nonmutated sectors (Ahloowalia and Maluszynski, 2001). These advantages make gamma irradiation combined with in vitro selection a powerful tool for crop improvement under abiotic stress.

A narrow genetic base poses a significant obstacle to enhancing crop productivity and ensuring food security

(Spencer-Lopes et al., 2018; Khursheed et al., 2019). Consequently, breeding programs aimed at developing cultivars adapted to climate change must prioritize the expansion of genetic diversity (Piri et al., 2011; Raina et al., 2016; Kökpınar et al., 2021). Mutation induction followed by in vitro or in vivo selection has been widely employed to develop genotypes resistant to drought, salinity stress, aluminum toxicity, pests, and diseases in numerous crop species (Ali and Suryakant, 2024). For example, soybean mutants generated through gamma irradiation (400 rad) were able to tolerate PEG concentrations of up to 20% *in vitro* (Husni et al., 2006). Similarly, mutagenesis combined with PEG-based selection has successfully produced drought-tolerant carrot genotypes (Stintzing et al., 2002). Mutagenic treatments may be applied to seeds or callus tissues, with callus tissues being especially sensitive due to their meristematic properties. Random mutations yield highly diverse genotypes, which can be selectively screened via targeted *in vitro* techniques.

This study aims to utilize tissue culture techniques for cellular-level selection of drought-tolerant genotypes in black carrots, thereby accelerating the identification of beneficial mutations that are rare under natural conditions. Black carrot cultivation has recently shown a decline in yield and quality due to drought stress. The constraints associated with traditional, extensive field-based breeding programs can thus be overcome under controlled laboratory conditions. This approach enables the rapid and precise determination of drought tolerance in callus tissues at the enzymatic level. Consequently, this study proposes a protocol to obtain preliminary data at the callus stage, thereby facilitating the selection of genotypes suitable for cultivation under drought conditions. This provides a strong foundation for subsequent breeding programs aimed at improving carrot resilience under water-limited environments.

2. Materials and methods

The research was conducted at the Tissue Culture Laboratory of the Faculty of Agriculture, Recep Tayyip Erdoğan University, Rize–Pazar, Türkiye, and at the Central Research and Application Laboratories of Ağrı İbrahim Çeçen University, Ağrı, Türkiye.

2.1. Plant material and surface sterilization procedures

Hatay black carrot (*Daucus carota* L. ssp. *sativus* var. *atrorubens* Alef.) seeds were used as plant material to obtain *in vitro* plantlets. The seeds were first rinsed five to six times with tap water, followed by immersion in 70% ethanol for 1 min. Surface sterilization was performed by treating the seeds with a 2% (v/v) liquid detergent solution supplemented with 2–4 drops of Tween-80 for 20 min. The seeds were subsequently rinsed thoroughly three times with sterile distilled water.

2.2. Preirradiation experiments

Carrot seeds were cultured in Petri dishes containing 25 mL of Murashige and Skoog (MS) basal medium (M022; Duchefa Biochemie B.V., Haarlem, Netherlands) (Murashige and Skoog, 1962) and solidified with 7 g L^{-1} plant agar (Duchefa Biochemie B.V.). The pH of the medium was adjusted to 5.8 with NaOH before autoclaving. Each Petri dish contained 10 seeds. Cultures were incubated under cool-white fluorescent illumination at 35 µmol m^{-2} s^{-1} with a 16-h photoperiod, at 24 ± 1 °C, a relative humidity of 50–60%, and a light intensity of 1000 lux.

For callus induction, leaf explants obtained from 3-week-old carrot seedlings were cultured in MS medium supplemented with 0.5 mg L^{-1} kinetin and 1 mg L^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D) under dark conditions. Initiated calli were proliferated on the same medium through three successive subcultures, each lasting 2 weeks, resulting in friable white calli that were used as explants for gamma irradiation.

Black carrot callus tissues (50 calli per dose) were irradiated with gamma rays at doses of 0, 5, 10, 20, 30, 40, 50, and 60 Gy using a cobalt-60 (^{60}Co) gamma-ray source at a dose rate of 360 Gy h^{-1} at the Turkish Energy, Nuclear, and Mineral Research Agency (TENMAK), Ankara, Türkiye. On the basis of plant regeneration rates from callus tissues, regression analysis revealed an optimal irradiation dose of 8.36 Gy (Turan Buyukdinc et al., 2019). The effective mutation dose (EMD_{50}) was determined as ±10% of the optimal dose, corresponding to the lower (7 Gy) and upper (9 Gy) limits, and was employed in the present study. The applied gamma irradiation doses (0, 7, 8, and 9 Gy) were selected based on the estimated EMD_{50} value, representing a biologically effective but nonlethal dose range. This focused range allowed for the evaluation of subtle stress-responsive physiological and biochemical responses without causing irreversible tissue damage.

Immediately following irradiation, the callus tissues were transferred to fresh, hormone-free MS medium to avoid potentially harmful substances generated in the irradiated medium. Plant regeneration from gamma-irradiated calli was subsequently promoted via this medium.

2.3. In vitro selection of drought stress-tolerant carrot genotypes

Plants regenerated from gamma-irradiated calli, following two subcultures, were cultivated for 6 weeks. During this period, leaf explants measuring approximately 0.5 cm were harvested from individual plants. Embryogenic calli derived from these gamma-irradiated carrot explants were subjected to in vitro selection on Murashige and Skoog (MS) medium supplemented with polyethylene glycol (PEG) 6000, which was employed as a drought-stress inducer. To

assess callus formation under drought conditions, tissues were cultured on MS medium supplemented with varying PEG concentrations (0%, 5%, 10%, and 15%).

The stress-induction medium consisted of MS basal medium with vitamins (M022; Duchefa Biochemie B.V.) containing 4.4 mg L^{-1} nutrients and solidified with 7 g L^{-1} plant agar (Duchefa Biochemie B.V.). The medium was supplemented with 30 mg L^{-1} sucrose, 1 mg L^{-1} 2,4-D, and 0.5 mg L^{-1} kinetin, and the pH was adjusted to 5.8 with NaOH before autoclaving. Gamma-irradiated carrot callus tissues were subcultured twice and maintained under drought-stress conditions in vitro for 6 weeks.

2.4. Determination of soluble protein content

The tissue samples (0.5 g) were cryogenically preserved in liquid nitrogen and pulverized into a fine powder. The powder was subsequently homogenized thoroughly in 0.1 M phosphate buffer (pH 6.75) at a ratio of 1:4 (w/v). The homogenate was centrifuged at 15,000 × *g* for 15 min, and the resulting clear supernatant was used for protein quantification. The protein content was determined spectrophotometrically at 562 nm according to Smith et al. (1985).

2.5. Determination of lipid peroxidation and hydrogen peroxide contents

To evaluate lipid peroxidation levels, tissue samples (0.2 g) were homogenized in liquid nitrogen and suspended in 0.1% trichloroacetic acid (TCA). The homogenate was then centrifuged, and the resulting supernatant was mixed with a freshly prepared TCA–TBA–HCl solution (1:1:1, v/v/v). This mixture was incubated at 95 °C for 1 h and cooled to room temperature. The malondialdehyde (MDA) concentration was quantified spectrophotometrically by measuring absorbance at 532 nm and corrected for nonspecific turbidity by subtracting absorbance at 600 nm (Ohkawa et al., 1979).

The content of hydrogen peroxide (H_2O_2) was determined following the method described by Velikova et al. (2000). Tissue samples (0.4 g) were homogenized in cold 0.1% TCA. The resulting supernatant was mixed with 10 mM potassium dihydrogen phosphate (KH_2PO_4) (pH 7.0) and potassium iodide. Absorbance was measured at 390 nm using a UV spectrophotometer, and the H_2O_2 concentration was calculated on the basis of a standard calibration curve and expressed as μmol g^{-1} fresh weight (FW).

2.6. Determination of antioxidant enzyme activities

Following a 6-week treatment period (two subcultures), changes in soluble protein content and antioxidant enzyme activities—superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX)—as well as hydrogen peroxide (H_2O_2) levels were measured in callus tissues exposed to drought conditions. Additionally, alterations in lipid peroxidation, as indicated by malondialdehyde (MDA) accumulation, were assessed. All enzyme activities were quantified using spectrophotometric methods.

For enzyme extraction, 0.5 g of tissue was rapidly frozen in liquid nitrogen and then homogenized in 5 mL of extraction buffer composed of 0.1 M KH_2PO_4 (pH 6.75), 1% polyvinylpyrrolidone, and 1 mM EDTA. The homogenized samples were centrifuged at 15,000 × *g* for 15 min at 4 °C, and the resulting supernatants were collected and used as enzyme extracts.

Catalase (CAT) activity was spectrophotometrically assessed at 240 nm by monitoring the decomposition of hydrogen peroxide (H_2O_2) into water and oxygen (Gong et al., 2001). Superoxide dismutase (SOD) activity was evaluated on the basis of the enzyme's capacity to inhibit the photoreduction of nitro blue tetrazolium by superoxide radicals, thereby preventing the formation of blue-colored formazan (Agarwal and Pandey, 2004; Yordanova et al., 2004). Ascorbate peroxidase (APX) activity was determined by recording the decrease in absorbance at 290 nm due to ascorbate oxidation, calculated using an extinction coefficient of 2.8 mM^{-1} cm^{-1}, following the method described by Nakano and Asada (1981).

2.7. Experimental design and statistical analysis

Data analysis was conducted using IBM SPSS Statistics version 20.0 (IBM Corp., Armonk, NY, USA). The experiment was arranged using three independent biological replicates, each consisting of 10 callus explants per treatment. For each biological replicate, measurements were performed as technical replicates and were averaged prior to statistical analysis. Experimental outcomes were evaluated by one-way analysis of variance (ANOVA). Significant differences among treatment means were determined using Duncan's multiple range test at $p \leq 0.05$. Correlation analysis was performed using the metan package (v1.18.0; Olivoto and Lúcio, 2020) in R software[2].

3. Results

3.1. Callus initiation

Callus induction from explants generally decreased with increasing gamma-ray exposure dose and PEG concentration. The reduction in medium water potential significantly affected in vitro callus initiation in explants exposed to different gamma-ray doses (0, 7, 8, and 9 Gy). Specifically, no callus development was observed in hypocotyl explants to 9 Gy of gamma radiation when cultured on medium supplemented with 10% or 15% PEG. At a PEG concentration of 15%, callus formation was completely inhibited, preventing sufficient tissue

[2] R Core Team (2024). R: A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing [online]. Website https://www.R-project.org [accessed 10 October 2025].

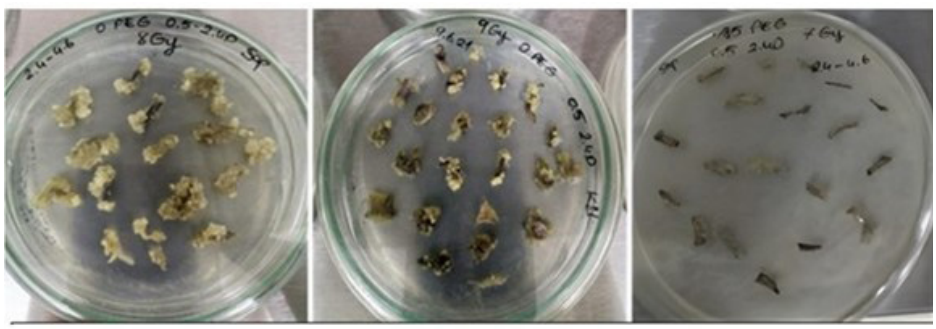
development for biochemical analyses. Consequently, this treatment was not included in further physiological and biochemical evaluations. These results demonstrate that callus initiation was influenced by both gamma-ray dose and PEG-induced drought stress conditions (Figure 1). The amount of callus decreased, and callus tissues progressively darkened with increasing gamma radiation dose and PEG concentration (Table).

3.2. Effects of in vitro drought stress on soluble protein content

The soluble protein content was determined in callus tissues cultivated for 6 weeks on nutrient medium supplemented with varying PEG concentrations, which were employed as an osmotic agent to induce drought stress. The mean values were calculated from three replicates, and statistically significant differences between treatments were identified using Duncan's multiple comparison test ($p \leq 0.05$). Under drought stress conditions, the soluble protein content in callus tissues increased significantly compared with the control, ranging from 0.27 to 2.75 mg g^{-1} FW. The protein content of callus tissues exposed to 5% PEG (P5) was approximately threefold higher than that of the control. At a PEG concentration of 10% (P10), protein levels increased to approximately 4.5-fold higher than those of the control. Conversely, the protein content of callus tissues cultured with 15% PEG was lower than that of the control (Figure 2).

3.3. Effects of in vitro drought stress on MDA and hydrogen peroxide contents

Compared with the control, lipid peroxidation increased significantly under drought stress conditions, ranging from 0.65 to 6.84 mg g^{-1} FW. The highest malondialdehyde (MDA) content was recorded in callus tissues exposed to 8 Gy gamma radiation and treated with 5% PEG. Specifically, the MDA content in callus tissues subjected to 5% PEG was approximately sevenfold greater than that of the control. This increase in MDA levels appears to be associated with the effective mutation dose of 8 Gy gamma radiation (Figure 3a). H_2O_2 levels also increased significantly under drought stress compared with the control, with concentrations ranging from 0.1 to 47.5 mg g^{-1} FW. The highest H_2O_2 concentrations were recorded in callus tissues subjected to 8 Gy gamma radiation combined with 10% PEG (44 mg g^{-1} FW) and 9 Gy gamma radiation combined with 5% PEG (47.5 mg g^{-1} FW). These two treatments showed statistically similar responses to drought stress and were categorized within the same statistical group. Compared with the control



(a) (b) (c)

Figure 1. (a) Effect of PEG on protein content in carrot calli. (b) Effect of PEG on MDA and H_2O_2 levels in carrot calli. (c) Effect of PEG on H_2O_2 levels in carrot calli. (1) Control (C); (2) C + P5; (3) C + P10; (4) 7 Gy + P0; (5) 7 Gy + P5; (6) 7 Gy + P10; (7) 8 Gy + P0; (8) 8 Gy + P5; (9) 8 Gy + P10; (10) 9 Gy + P0; (11) 9 Gy + P5.

Table. Callus formation at different gamma irradiation doses and PEG concentrations.

Irradiation dose (Gy)	Callus amounts as affected by PEG concentration (%)		
	%0	5%	10%
0	4.61 a	4.16 a	3.57 a
7	3.80 b	3.60 a	3.20 a
8	3.40 bc	3.23 b	2.36 b
9	3.20 c	2.33 c	-

Data represent mean values obtained from three independent experiments, each comprising three replicates. Means within the same column followed by the same letter were not significantly different at $p \leq 0.05$.

callus tissues, the highest H_2O_2 concentration represented an approximately 475-fold increase (Figure 3b).

3.4. Effects of in vitro drought stress on antioxidant enzyme activities

These observations indicated that black carrot calli regenerated under PEG-induced drought stress exhibited variable enzyme activities depending on the applied gamma radiation dose and PEG concentration. Specifically, the enzymes SOD, CAT, and APX exhibited similar response patterns. Compared with the control group, the highest enzyme activities were recorded in callus tissues treated with 5% PEG, measuring 223 U mg^{-1} protein for SOD, 22.456 U mg^{-1} protein for CAT, and 1672.743 U mg^{-1} protein for APX (Figures 4a–4c).

Data analysis revealed significant effects of gamma irradiation dose and drought stress on antioxidant enzyme activity. Compared with control tissues, superoxide dismutase (SOD) activity increased markedly with increasing drought stress, reaching its peak (223.82 U mg^{-1} protein) in callus tissues treated with 5% PEG. Similarly, ascorbate peroxidase (APX) activity was highest in calli treated with 5% PEG. Under increased drought stress, APX activity reached its maximum in association with hydrogen peroxide (H_2O_2) accumulation. Compared with the control group, drought conditions led to significant increases in enzyme activities, with CAT, SOD, and APX activities increasing by approximately 964%, 944%, and 943%, respectively.

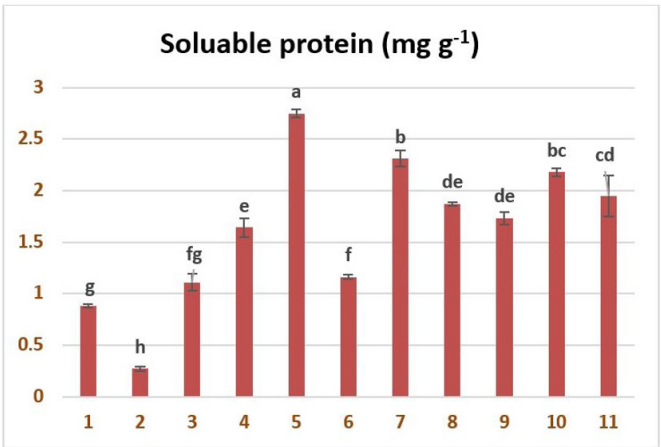


Figure 2. Effect of PEG on protein content in carrot calli (1) Control (C); (2) C + P5; (3) C + P10; (4) 7 Gy + P0; (5) 7 Gy + P5; (6) 7 Gy + P10; (7) 8 Gy + P0; (8) 8 Gy + P5; (9) 8 Gy + P10; (10) 9 Gy + P0; (11) 9 Gy + P5.

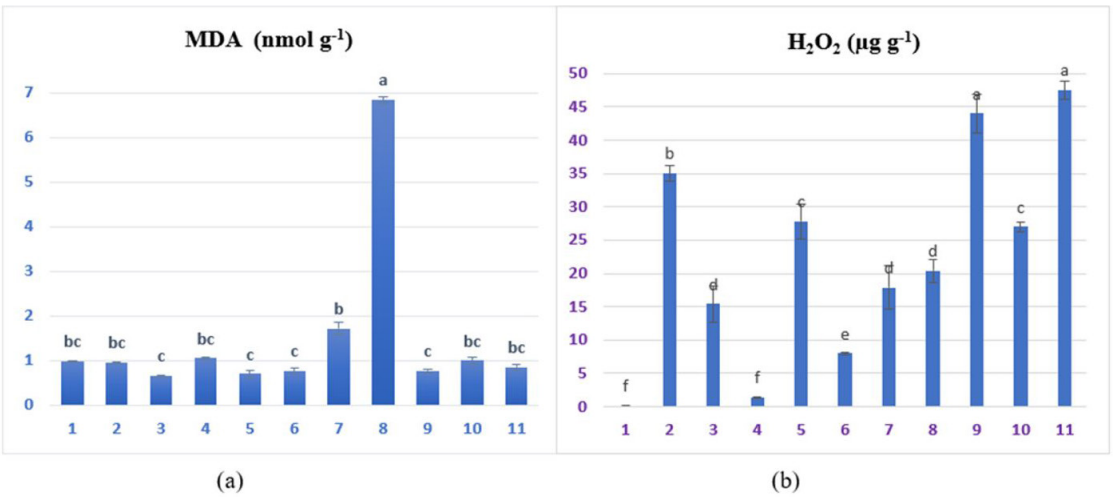


Figure 3. (a) Effect of PEG on MDA levels in carrot calli; (b) effect of PEG on H_2O_2 levels. (1) Control (C); (2) C + P5; (3) C + P10; (4) 7 Gy + P0; (5) 7 Gy + P5; (6) 7 Gy + P10; (7) 8 Gy + P0; (8) 8 Gy + P5; (9) 8 Gy + P10; (10) 9 Gy + P0; (11) 9 Gy + P5.

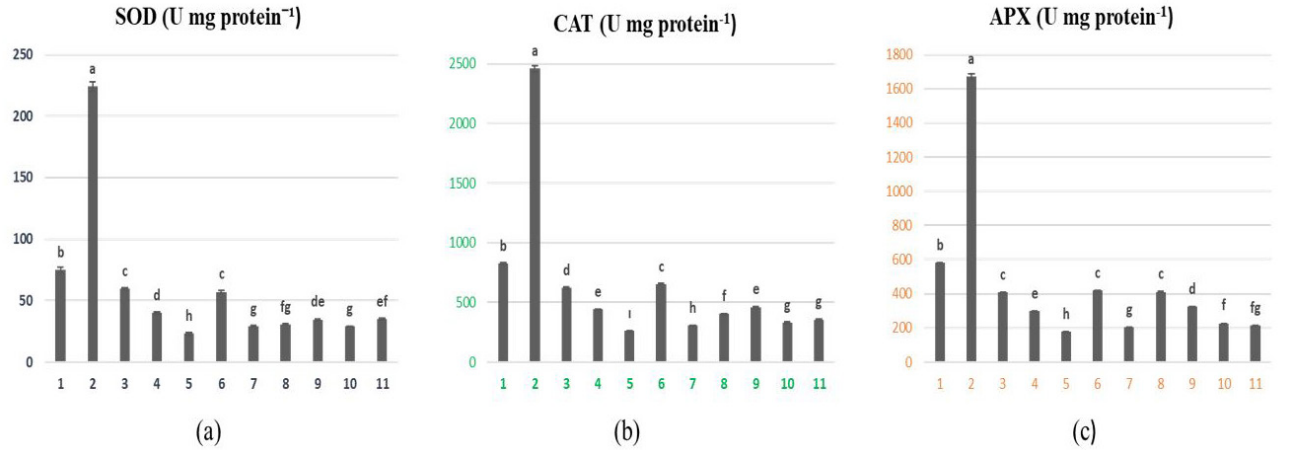


Figure 4. Effect of PEG on SOD, CAT, and APX activities in carrot calli. (1) Control (C); (2) C + P5; (3) C + P10; (4) 7 Gy + P0; (5) 7 Gy + P0; (6) 7 Gy + P10; (7) 8 Gy + P0; (8) 8 Gy + P5; (9) 8 Gy + P10; (10) 9 Gy + P0; (11) 9 Gy + P5.

3.5. Correlation analysis

To examine the relative contributions of individual variables to the second principal component (Dim-2), a contribution analysis was performed (Figure 5a). The bar graph highlights the percentage contribution of several variables to the dimension labelled "Dim-2." Peroxidase (POX) showed the highest contribution, slightly exceeding 40%, and thus represented the dominant variable associated with Dim-2. H_2O_2 contributed approximately 25%, followed by MDA at around 15%, while protein showed a contribution close to 10%. In contrast, SOD, CAT, and APX displayed minimal contributions, each contributing less than 5%. Overall, the analysis indicates that POX, H_2O_2, and MDA were the variables most strongly associated with Dim-2 (Figure 5a). The scree plot presents the proportion of explained variance for each dimension. The first principal component accounted for 53.3% of the total variance, the second explained 23.3%, and the third accounted for 13.9%. Subsequent components contributed less, with the fourth explaining 7% and the fifth 2.4%, while the sixth, seventh, and eighth components contributed negligibly (Figure 5b).

The principal component analysis (PCA) biplot displayed the distribution of variables and treatments based on the first two principal components, which together explained 76.6% of the total variance. POX and H_2O_2 were positioned close to each other, indicating a strong association between these variables. Several treatments (8 Gy + 5% PEG, 7 Gy + 5% PEG, 8 Gy + 0% PEG, and 9 Gy + 0% PEG) clustered on the negative side of Dim-2, suggesting that these treatments shared similar response patterns within the multivariate space. In contrast, APX, CAT, and SOD were located on the positive side of Dim-2, reflecting a distinct association pattern relative to the other variables and treatments (Figure 6a). The PCA further illustrated relationships among the variables based on the first two principal components. POX and H_2O_2 were positioned on opposite sides of the origin, indicating a negative correlation between these variables. MDA was located near POX, suggesting a positive association between these variables, whereas protein was positioned on the opposite side, reflecting a different relationship pattern. APX, SOD, and CAT clustered together on the positive side of the first principal component (Dim-1), indicating that they contributed similarly to the observed variance. The color gradient reflected variable contribution values, with higher contributions represented by warmer colors (Figure 6b).

The correlation heatmap showed significant relationships among oxidative stress markers and antioxidant enzymes. APX, SOD, and CAT exhibited very strong positive correlations ($r \approx 0.99–1.00$; $p < 0.01$), indicating a high degree of covariation among these enzymes. These enzymes were strongly negatively correlated with protein content ($r \approx -0.80$ to -0.81; $p < 0.01$), demonstrating an inverse association between protein levels and antioxidant enzyme activity. In contrast, MDA, POX, and H_2O_2 displayed mostly nonsignificant correlations with the other variables, suggesting that their variation was largely independent within the context of this dataset. Collectively, these patterns reflected coordinated associations among antioxidant enzymes and inverse relationships with protein levels, whereas oxidative stress markers exhibited distinct correlation profiles.

The contribution analysis further showed that SOD, CAT, and APX were the variables most strongly associated with Dim-1, each accounting for more than 20% of the total variance. Protein exhibited a moderate contribution, whereas MDA, H_2O_2, and POX contributed minimally to this dimension (Figure 5c).

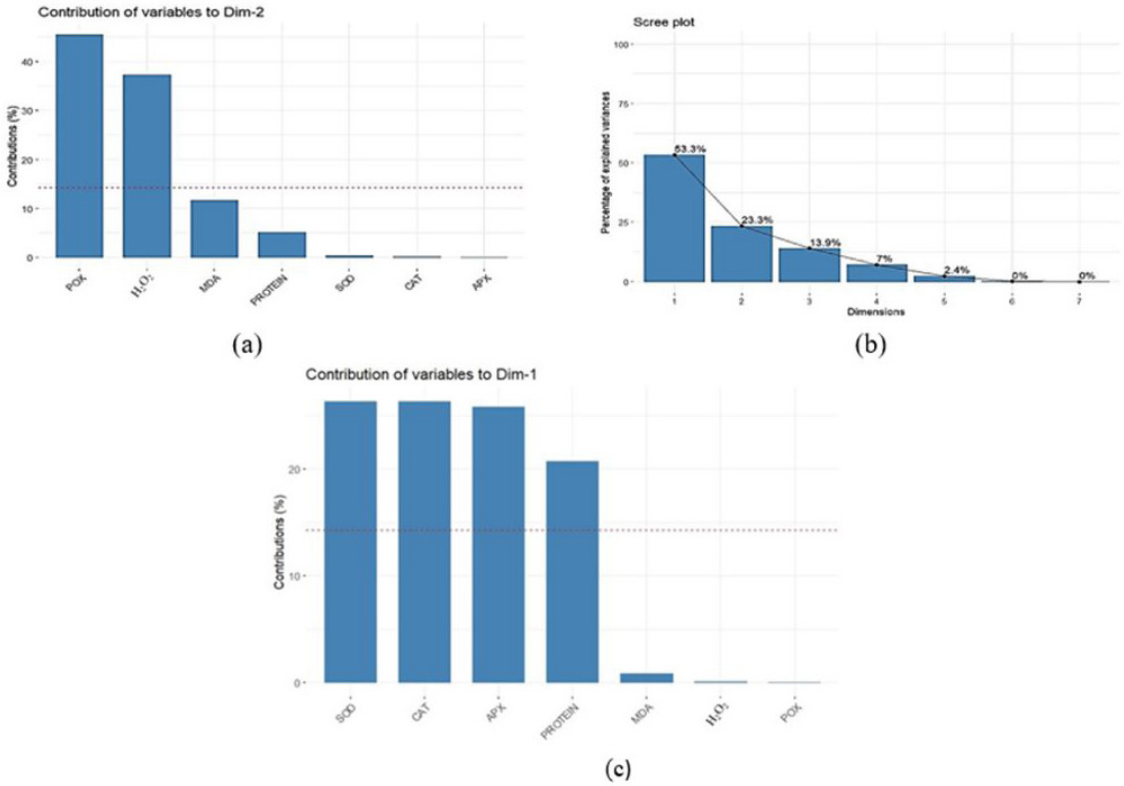


Figure 5. (a) Contribution of variables to Dim-2, highlighting the major contributions of POX, H_2O_2, and MDA. (b) Scree plot showing the proportion of variance explained by each dimension. (c) Contribution of variables to Dim-1, with SOD, CAT, and APX showing the highest contributions, protein showing a moderate contribution, and MDA, H_2O_2, and POX contributing minimally.

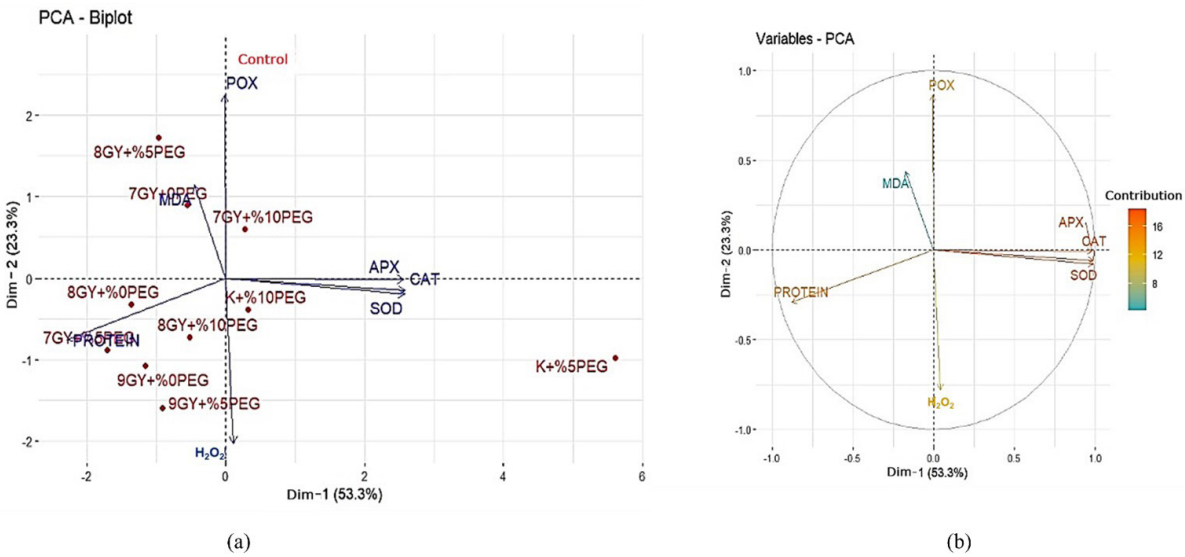


Figure 6. (a) PCA biplot illustrates the distribution of treatments and variables along Dim-1 (53.3%) and Dim-2 (23.3%), highlighting clustering patterns and variable relationships. (b) Correlation plot showing relationships among variables, with position and color gradients indicating relative contribution levels.

4. Discussion

4.1. Effects of drought stress on soluble protein content

Drought and other abiotic stresses induce protective biochemical responses in plants, primarily through the enhanced synthesis of stress-responsive proteins that support physiological adjustments, such as osmotic regulation and the maintenance of cellular functions under limited water availability (Xie et al., 2005). In the present study, PEG-induced drought stress caused a marked increase in soluble protein levels in black carrot callus tissues, indicating the activation of cellular defense pathways. This response likely reflected the induction of stress-associated proteins, including antioxidant enzymes, lipid transfer proteins, and dehydrins, which contribute to cellular stability and metabolic adjustment under drought conditions.

The observed increase in protein accumulation is consistent with previous reports showing that drought-responsive transcription factors activate stress-related genes by binding to cis-regulatory promoter regions, thereby enhancing dehydration tolerance (Samota et al., 2017; Saha et al., 2022). Similar regulatory mechanisms have been documented in several plant species, including rice and soybean, suggesting a conserved molecular response to drought stress (Alam et al., 2010; Samota et al., 2017). The induction of comparable protein responses in PEG-treated black carrot calli supported the involvement of shared stress-adaptive pathways.

Furthermore, drought tolerance has been closely associated with the accumulation of late embryogenesis abundant (LEA) proteins, which stabilize cellular structures, prevent protein denaturation, and maintain membrane integrity during dehydration and rehydration cycles (Kosová et al., 2014; Chen et al., 2021). Increased synthesis of drought-induced proteins has also been reported in maize under water deficit conditions, further corroborating the findings of the present study (Riccardi et al., 1998; Ti-da et al., 2006).

In conclusion, the elevated soluble protein levels observed in drought-stressed callus tissues were in agreement with earlier studies and underscored the pivotal role of stress-responsive proteins in enhancing drought tolerance at the cellular level.

4.2. Effects of drought stress on lipid peroxidation and hydrogen peroxide contents

Drought stress significantly increased oxidative damage in carrot callus tissues, as evidenced by elevated malondialdehyde (MDA) levels. The highest MDA levels were observed under the combined 8 Gy gamma irradiation and 5% PEG-induced drought stress, indicating intensified oxidative damage. This enhanced lipid peroxidation likely resulted from increased accumulation of reactive oxygen species (ROS), which accelerates membrane lipid oxidation under drought stress conditions (Cheng et al., 2018). Elevated malondialdehyde (MDA) levels are a well-established indicator of membrane damage and reflect the severity of oxidative stress imposed on plant tissues (Cheng et al., 2018).

Variations in MDA levels among treatments may be attributed to differences in antioxidant capacity and susceptibility to lipid peroxidation between undifferentiated callus tissues and differentiated plant organs, such as leaves and roots (Taiz, 2006). Due to their homogeneous and undifferentiated nature, callus tissues may also exhibit distinct hormonal and metabolic responses to drought stress and gamma irradiation, leading to the activation of specific signaling pathways regulating oxidative stress responses (Taiz, 2006; Cheng et al., 2018).

Consistent with previous reports, drought stress has been widely associated with increased oxidative stress markers, including MDA, across diverse plant species (Zhong et al., 2021; Zhao et al., 2022). Recent studies have further confirmed that drought-induced ROS production intensifies lipid peroxidation and cellular damage in plant tissues (Ghani et al., 2022a; Ghani et al., 2022b; Qi et al., 2023). Accordingly, the elevated MDA levels observed in this study supported existing evidence linking drought stress with oxidative membrane damage.

In conclusion, the increased MDA concentrations detected under combined drought and gamma irradiation stress highlighted membrane lipid peroxidation as a common physiological consequence of oxidative stress in plant tissues.

4.3. Effects of drought stress on antioxidant enzyme activities

The increased antioxidant enzyme activities observed in gamma-irradiated carrot callus tissues under drought stress were closely aligned with findings from previous studies across various plant species. Enzymes such as catalase (CAT), ascorbate peroxidase (APX), and superoxide dismutase (SOD) are critical components of the plant antioxidant defense system and actively detoxify reactive oxygen species (ROS) generated under stressful conditions. The substantial increase in enzyme activities observed in this study indicated that gamma-irradiated carrot calli strongly activated their antioxidant defense pathways under drought stress, thereby supporting enhanced cellular protection and stress adaptation.

Previous studies have consistently shown that drought stress significantly enhances the activities of key antioxidant enzymes, including catalase (CAT), ascorbate peroxidase (APX), and superoxide dismutase (SOD), supporting the findings of the present study. Increased CAT activity under drought conditions has been closely associated with the detoxification of hydrogen peroxide, thereby preventing oxidative damage to cellular proteins

and membranes (Jung, 2004). Similarly, elevated APX and SOD activities have been linked to improved tolerance against drought-induced oxidative stress in a wide range of plant species, including banana, rice, melon, and pumpkin (Chai et al., 2005; Shehab et al., 2010; Kuşvuran et al., 2010; Kıran et al., 2013; Yasar et al., 2014).

Stress conditions are also known to trigger coordinated genetic responses, leading to the activation of genes associated with somatic embryogenesis. These molecular changes are frequently accompanied by fluctuations in antioxidant enzyme activities, particularly CAT and SOD, during the transition from somatic to embryogenic cells, as reported in *Arabidopsis thaliana* and *Crocus sativus* (Ikeda-Iwai et al., 2003; Blazquez et al., 2009). Such findings suggest a close link between oxidative stress regulation and cellular reprogramming under in vitro stress conditions.

Consistent with the present results, increased APX and SOD activities have been shown to enhance protection against drought-induced oxidative damage in banana and rice calli (Chai et al., 2005; Shehab et al., 2010). Differential antioxidant enzyme responses under abiotic stress have also been reported in *Salicornia* species and cactus tissues, emphasizing the critical roles of CAT and APX in stress adaptation (Torabi and Niknam, 2011; Balen et al., 2013). Moreover, comparative studies indicate that elevated antioxidant enzyme activities are reliable indicators of drought tolerance across genotypes, irrespective of sensitivity, as demonstrated in black pepper, melon, and pumpkin genotypes (Kuşvuran et al., 2010; Vijayakumari and Puthur, 2014; Yasar et al., 2014).

Although most studies report enhanced antioxidant enzyme activities under drought stress, some variability exists. For instance, Smirnoff and Colombe (2000) observed no significant changes in APX activity under drought conditions, suggesting that enzyme responses may vary depending on species, developmental stage, stress severity, and experimental design. Such variability highlights the complexity of antioxidant regulation under stress.

In agreement with the present findings, Razavizadeh and Komatsu (2018) reported increased CAT and SOD activities in *Carum copticum* callus tissues and seedlings exposed to mannitol-induced drought stress, emphasizing the pivotal role of CAT in the antioxidant defense of undifferentiated tissues. Collectively, these findings strongly supported the observed enhancement of CAT, APX, and SOD activities in mutant carrot callus tissues under drought stress, underscoring their central role in stress adaptation and cellular survival.

Multivariate analysis further supported these conclusions by revealing complex interactions among oxidative stress markers and antioxidant defenses. Principal component analysis (PCA) showed that the first two principal components accounted for more than 76% of the total variance. POX, H_2O_2, and MDA were the major contributors to the second principal component, highlighting their central roles in oxidative stress and lipid peroxidation processes. The PCA biplot revealed a negative correlation between POX and H_2O_2, suggesting distinct regulatory mechanisms governing these markers. Treatments clustered on the negative side of the second principal component exhibited similar response patterns, potentially reflecting adaptive antioxidant responses.

Correlation heatmap analysis further demonstrated strong positive correlations among antioxidant enzymes (CAT, APX, and SOD), indicating coordinated regulation under drought stress. In contrast, their inverse relationship with soluble protein content suggested a shift in metabolic resource allocation toward antioxidant defense. The weak correlations between oxidative damage markers and antioxidant enzymes implied the involvement of partially independent regulatory pathways. Overall, these results highlighted the complexity of antioxidant regulation and emphasized the crucial role of coordinated enzymatic defense systems in mitigating drought-induced oxidative stress.

Collectively, these findings indicated that gamma irradiation may be a useful tool for inducing stress-responsive variation under controlled in vitro conditions. The strong contribution of antioxidant enzymes to overall variance confirmed their central role in mitigating stress, whereas the independent regulation of oxidative damage markers suggested that multiple pathways are involved. Through this integrated approach, the understanding of plant stress physiology was enhanced, and future strategies to improve stress tolerance may be informed by targeting key antioxidant systems.

5. Conclusion

In the present study, rapid in vitro development and multiplication of gamma-irradiated black carrot genotypes under drought stress were demonstrated. A preliminary framework for future molecular and physiological investigations of drought stress responses was provided by this study. Specifically, the identification of mutant genes in these drought-resistant genotypes may be used to significantly advance molecular characterization studies, including next-generation sequencing and CRISPR-Cas9-based gene-editing applications. The variation generated through the methodology used in this study was shown to have strong potential to contribute to future efforts aimed at improving drought stress tolerance. Furthermore, it was indicated by these findings that gamma irradiation may be a useful tool for inducing stress-responsive variation under controlled *in vitro* conditions, thereby highlighting the essential role of these mechanisms in supporting plant adaptation and survival under drought-stress conditions.

Acknowledgements
The authors would like to thank the Turkish Atomic Energy Authority (TENMAK) and Dr K. Yaprak Kantoğlu for their valuable support during plant tissue irradiation.

Author contributions
Conceptualization: Damla Turan Büyükdinç, Dilruba Tabay Sümbül. Data curation: Damla Turan Büyükdinç, Dilruba Tabay Sümbül, Sheikh Mansoor. Formal analysis: Damla Turan Büyükdinç, Dilruba Tabay Sumbul; Sheikh Mansoor. Investigation: Damla Turan Büyükdinç, Dilruba Tabay Sümbül, Sheikh Mansoor. Methodology: Damla Turan Büyükdinç, Dilruba Tabay Sümbül, Sheikh Mansoor. Project administration: Damla Turan Büyükdinç. Resources: Damla Turan Büyükdinç, Dilruba Tabay Sümbül. Software: Dilruba Tabay Sümbül, Sheikh Mansoor. Supervision: Damla Turan Büyükdinç. Validation: Damla Turan Büyükdinç, Dilruba Tabay Sümbül, Sheikh Mansoor. Visualization: Damla Turan Büyükdinç, Dilruba Tabay Sümbül, Sheikh Mansoor. Writing – original draft: Damla Turan Büyükdinç. Writing – review and editing: Damla Turan Büyükdinç, Sheikh Mansoor. Funding acquisition: Damla Turan Büyükdinç.

References

Abid M, Ali S, Qi LK, Zahoor R, Tian Z et al. (2018). Physiological and biochemical changes during drought and recovery periods at tillering and jointing stages in wheat (*Triticum aestivum* L.). Scientific Reports 8 (1): 4615. https://doi.org/10.1038/s41598-018-21441-7

Afa LD, Purwoko BS, Junaedi A, Haridjaja O, Dewi IS (2012). Pendugaan toleransi padi hibrida terhadap kekeringan dengan polyetilen glikol (PEG) 6000. Jurnal Agrivigor 11 (2): 292-299.

Agarwal S, Pandey V (2004). Antioxidant enzyme responses to NaCl stress in *Cassia angustifolia*. Biologia Plantarum 48: 555-560. https://doi.org/10.1023/B:BIOP.0000047152.07878.e7

Ahloowalia B (1995). *In vitro* mutagenesis for the improvement of vegetatively propagated plants. In: Induced Mutations and Molecular Techniques for Crop Improvement, Proceedings of IAEA/FAO Symposium; Vienna, Austria. pp. 531-541.

Ahloowalia BS, Maluszynski M (2001). Induced mutations - a new paradigm in plant breeding. Euphytica 118: 167-173. https://doi.org/10.1023/A:1004162323428

Alam I, Lee D-G, Kim K-H, Park C-H, Sharmin SA et al. (2010). Proteome analysis of soybean roots under waterlogging stress at an early vegetative stage. Journal of Biosciences 35: 49-62. https://doi.org/10.1007/s12038-010-0007-5

Ali S, Suryakant TN (2024). Mutation breeding and its importance in modern plant breeding: a review. Journal of Experimental Agriculture International 46 (7): 264-275. Ammirato PV (1983). The regulation of somatic embryo development in plant cell cultures: suspension culture techniques and hormone requirements. Bio/Technology 1 (1): 68-73. https://doi.org/10.1038/nbt0383-68

Balen B, Tkalec M, Rogić T, Šimac M, Štefanić PP et al. (2013). Effects of iso-osmotic NaCl and mannitol on growth, proline content, and antioxidant defense in *Mammillaria gracilis* Pfeiff. *in vitro*-grown cultures. In Vitro Cellular & Developmental Biology – Plant 49: 421-432. https://doi.org/10.1007/s11627-013-9523-y

Bashir R, Riaz HN, Anwar S, Parveen N, Khalilzadeh R et al. (2021). Morpho-physiological changes in carrots by foliar γ-aminobutyric acid under drought stress. Brazilian Journal of Botany 44: 57-68. https://doi.org/10.1007/s40415-020-00676-7

Blando F, Marchello S, Maiorano G, Durante M, Signore A et al. (2021). Bioactive compounds and antioxidant capacity in anthocyanin-rich carrots: a comparison between the black carrot and the Apulian landrace "Polignano" carrot. Plants 10: 564. https://doi.org/10.3390/plants10030564

Blazquez S, Olmos E, Hernández JA, Fernández-García N, Fernández JA et al. (2009). Somatic embryogenesis in saffron (*Crocus sativus* L.). Histological differentiation and implication of some components of the antioxidant enzymatic system. Plant Cell, Tissue and Organ Culture 97: 49-57. https://doi.org/10.1007/s11240-009-9497-y

Bonasia A, Conversa G, Lazzizera C, Gambacorta G, Elia A (2021). Morpho-biometrical nutritional and phytochemical characterization of carrot landraces from Puglia region (Southern Italy). Sustainability 13 (7): 3940. https://doi.org/10.3390/su13073940

Buishand JG, Gabelman WH (1980). Studies on the inheritance of root color and carotenoid content in red× yellow and red× white crosses of carrot, *Daucus carota* L. Euphytica 29: 241-260. https://doi.org/10.1007/BF00025121

Chai TT, Fadzillah NM, Kusnan M, Mahmood M (2005). Water stress-induced oxidative damage and antioxidant responses in micropropagated banana plantlets. Biologia Plantarum 49: 153-156. https://doi.org/10.1007/s00000-005-3156-9

Chen J, Li N, Wang X, Meng X, Cui X et al (2021). Late embryogenesis abundant gene family in *Salvia miltiorrhiza*: identification, expression analysis, and response to drought stress. Plant Signaling & Behavior 16 (5): 1891769. https://doi.org/10.1080/15592324.2021.1891769

Cheng L, Han M, Yang L-M, Li Y, Sun Z et al. (2018). Changes in the physiological characteristics and baicalin biosynthesis metabolism of *Scutellaria baicalensis* Georgi under drought stress. Industrial Crops and Products 122: 473-482. https://doi.org/10.1016/j.indcrop.2018.06.030

Dami I, Hughes HG (1997). Effects of PEG-induced water stress on *in vitro* hardening of 'Valiant' grape. Plant Cell, Tissue and Organ Culture 47: 97-101. https://doi.org/10.1007/BF02318944

Duan R, Huang G, Zhou X, Li Y, Tian C (2021). Ensemble drought exposure projection for multifactorial interactive effects of climate change and population dynamics: application to the Pearl River Basin. Earth's Future 9 (8): e2021EF002215. https://doi.org/10.1029/2021EF002215

Efferth T (2019). Biotechnology applications of plant callus cultures. Engineering 5 (1): 50-59. https://doi.org/10.1016/j.eng.2018.11.006

Fallon KM, Phillips R (1989). Responses to water stress in adapted and unadapted carrot cell suspension cultures. Journal of Experimental Botany 40 (6): 681-687. https://doi.org/10.1093/jxb/40.6.681

Fehér A (2019). Callus, dedifferentiation, totipotency, somatic embryogenesis: What do these terms mean in the era of molecular plant biology? Frontiers in Plant Science 10: 536. https://doi.org/10.3389/fpls.2019.00536

Ghani MI, Ali A, Atif MJ, Ali M, Amin B et al. (2022a). Arbuscular mycorrhizal fungi and dry raw garlic stalk amendment alleviate continuous monocropping growth and photosynthetic declines in eggplant by bolstering its antioxidant system and accumulation of osmolytes and secondary metabolites. Frontiers in Plant Science 13: 849521. https://doi.org/10.3389/fpls.2022.849521

Ghani MI, Saleem S, Rather SA, Rehmani MS, Alamri S et al. (2022b). Foliar application of zinc oxide nanoparticles: an effective strategy to mitigate drought stress in cucumber seedling by modulating antioxidant defense system and osmolytes accumulation. Chemosphere 289: 133202. https://doi.org/10.1016/j.chemosphere.2021.133202

Gong YP, Toivonen PMA, Lau OL, Wiersma PA (2001). Antioxidant system level in 'Braeburn' apple is related to its browning disorder. Botanical Bulletin of Academia Sinica 42 (4): 259-264.

Husni A, Kosmiatin M, Mariska I (2006). Peningkatan toleransi kedelai Sindoro terhadap kekeringan melalui seleksi in vitro. Indonesian Journal of Agronomy 34 (1).

Ikeda-Iwai M, Umehara M, Satoh S, Kamada H (2003). Stress-induced somatic embryogenesis in vegetative tissues of *Arabidopsis thaliana*. The Plant Journal 34: 107-114. https://doi.org/10.1046/j.1365-313X.2003.01702.x

Junaid MD, Öztürk Gökçe ZN, Gökçe AF (2023). Investigation of drought induced biochemical and gene expression changes in carrot cultivars. Molecular Biology Reports 50: 349-359. https://doi.org/10.1007/s11033-022-08050-4

Jung S (2004). Variation in antioxidant metabolism of young and mature leaves of *Arabidopsis thaliana* subjected to drought. Plant Science 166 (2): 459-466. https://doi.org/10.1016/j.plantsci.2003.10.012

Kacem NS, Delporte F, Muhovski Y, Djekoun A, Watillon B (2017). *In vitro* screening of durum wheat against water-stress mediated through polyethylene glycol. Journal of Genetic Engineering and Biotechnology 15 (1): 239-247. https://doi.org.10.1016/j.jgeb.2017.04.004

Khursheed S, Raina A, Parveen K, Khan S (2019). Induced phenotypic diversity in the mutagenized populations of faba bean using physical and chemical mutagenesis. Journal of the Saudi Society of Agricultural Sciences 18 (2): 113-119. https://doi.org/10.1016/j.jssas.2017.03.001

Kıran S, Kuşvuran S, Ellialtıoğlu S, Özkay F (2013). Studies on some morphological and biochemical changes in the melon genotypes exposed to drought stress. Soil and Water Journal 2 (2):1347-1354.

Koca Bozalan N, Karadeniz F (2011). Carotenoid profile, total phenolic content, and antioxidant activity of carrots. International Journal of Food Properties 14 (5): 1060-1068. https://doi.org/10.1080/10942910903580918

Kosová K, Vítámvás P, Prášil IT (2014). Wheat and barley dehydrins under cold, drought, and salinity – what can LEA-II proteins tell us about plant stress response? Frontiers in Plant Science 5: 343. https://doi.org/10.3389/fpls.2014.00343

Kowalczyk Z, Kuboń M (2022). Assessing the impact of water use in conventional and organic carrot production in Poland. Scientific Reports 12: 1-11. https://doi.org/10.1038/s41598-022-07531-7

Kökpınar Ş, Kantoğlu KY, Ellialtıoğlu ŞŞ (2021). Bitkilerde mutagen uygulamaları ile genetik çeşitliliğin artırılması ve sebze ıslahında kullanımı. In: Cengizler I, Duman S (editors). Ziraat, Orman ve Su Ürünlerinde Araştırma ve Değerlendirmeler – I. Ankara, Türkiye: Gece Publishing, pp. 341-362.

Kusvuran S, Kiran S, Ellialtıoglu SS (2016). Antioxidant enzyme activities and abiotic stress tolerance relationship in vegetable crops. In: Shanker AK, Shanker C (editors). Abiotic and Biotic Stress in Plants - Recent Advances and Future Perspectives. London, UK: Intech Open. https://doi.org/10.5772/62235

Kuşvuran Ş (2021). Bitkilerde oksidatif stres ve antioksidan enzimler. In: Ellialtıoğlu ŞŞ, Daşgahn HY, Kuşvuran Ş (editors). Sebzelerde Stres Toleransı ve Islah Stratejileri. Ankara, Türkiye: Gece Kitaplığı, pp. 361-405 (in Turkish).

Kuşvuran S, Daşgan HY, Abak K (2010). Effects of drought stress on antioxidant enzymes activities in melon. In: VIII National Vegetable Symposium; Van, Türkiye. pp. 309-312.

Lushchak VI (2011). Adaptive response to oxidative stress: bacteria, fungi, plants and animals. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology 153 (2):175-190. https://doi.org/10.1016/j.cbpc.2010.10.004

Lutts S, Almansouri M, Kinet J-M (2004). Salinity and water stress have contrasting effects on the relationship between growth and cell viability during and after stress exposure in durum wheat callus. Plant Science 167 (1): 9-18. https://doi.org/10.1016/j.plantsci.2004.02.014

Maliga P (1984). Isolation and characterization of mutants in plant cell cultures. Annual Review of Plant Physiology 35: 519-552. https://doi.org/10.1146/annurev.pp.35.060184.002511

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

Nakano Y, Asada K (1981). Hydrogen-peroxide is scavenged by ascorbate-specific peroxidase in spinach-chloroplasts. Plant Cell Physiology 22 (5): 867-880. https://doi.org/10.1093/oxfordjournals.pcp.a076232

Nijabat A, Manzoor S, Faiz S, Naveed NH, Bolton A et al. (2023). Variation in seed germination and amylase activity of diverse carrot (*Daucus carota* (L.)) germplasm under simulated drought stress. HortScience 58 (2): 205-214. https://doi.org/10.21273/HORTSCI16806-22

Ohkawa H, Ohishi N, Yagi K (1979). Assay for lipid peroxides in animal-tissues by thiobarbituric acid reaction. Analytical Biochemistry 95 (2): 351-358. https://doi.org/10.1016/0003-2697(79)90738-3

Olivoto T, Lúcio ADC (2020). metan: an R package for multi-environment trial analysis. Methods in Ecology and Evolution 11 (6): 783-789. https://doi.org/10.1111/2041-210X.13384

Öztürk Gökçe ZN, Gökçe AF, Junaid MD, Chaudhry UK (2022). Comparative transcriptomics of drought stress response of taproot meristem region of contrasting purple carrot breeding lines supported by physio-biochemical parameters. Functional & Integrative Genomics 22 (4): 697-710. https://doi.org/10.1007/s10142-022-00868-2

Piri I, Babayan M, Tavassoli A, Javaheri M (2011). The use of gamma irradiation in agriculture. African Journal of Microbiology Research 5 (32): 5806-5811.

Qi Y, Ma L, Ghani MI, Peng Q, Fan R et al. (2023). Effects of drought stress induced by hypertonic polyethylene glycol (PEG-6000) on *Passiflora edulis* Sims physiological properties. Plants 12 (12): 2296. https://doi.org/10.3390/plants12122296

Rai MK, Shekhawat NS, Harish, Gupta AK, Phulwaria M et al. (2011). The role of abscisic acid in plant tissue culture: a review of recent progress. Plant Cell, Tissue and Organ Culture 106: 179-190. https://doi.org/10.1007/s11240-011-9923-9

Raina A, Laskar RA, Khursheed S, Amin R, Tantray YR et al. (2016). Role of mutation breeding in crop improvement-past, present and future. Asian Research Journal of Agriculture 2 (2): 1-13. https://doi.org/10.9734/ARJA/2016/29334

Razavizadeh R, Komatsu S (2018). Changes in essential oil and physiological parameters of callus and seedlings of *Carum copticum* L. under in vitro drought stress. Journal of Food Measurement and Characterization 12: 1581-1592. https://doi.org/10.1007/s11694-018-9773-9

Razzaq M, Akram NA, Ashraf M, Naz H, Al-Qurainy F (2017). Interactive effect of drought and nitrogen on growth, some key physiological attributes and oxidative defense system in carrot (*Daucus carota* L.) plants. Scientia Horticulturae 225: 373-379. https://doi.org/10.1016/j.scienta.2017.06.055

Riccardi F, Gazeau P, de Vienne D, Zivy M (1998). Protein changes in response to progressive water deficit in maize, quantitative variation and polypeptide identification. Plant Physiology 117: 1253-1263. https://doi.org/10.1104/pp.117.4.1253

Saepudin A, Khumaida N, Sopandie D, Ardie SW (2017). *In vitro* selection of four soybean genotypes using PEG for drought tolerance. Journal of Agronomy Indonesia 45 (1): 14-22. https://doi.org/10.24831/jai.v45i1.13749

Saha D, Choyal P, Mishra UN, Dey P, Bose B et al. (2022). Drought stress responses and inducing tolerance by seed priming approach in plants. Plant Stress 4: 100066. https://doi.org/10.1016/j.stress.2022.100066

Samota MK, Sasi M, Awana M, Yadav OP, Amitha Mithra SV (2017). Elicitor-induced biochemical and molecular manifestations to improve drought tolerance in rice (*Oryza sativa* L.) through seed-priming. Frontier Plant Science 8: 934. https://doi.org/10.3389/fpls.2017.00934

Santos-Díaz MdS, Ochoa-Alejo N (1994). PEG-tolerant cell clones of chili pepper: growth, osmotic potentials and solute accumulation. Plant Cell Tissue Organ Culture 37: 1-8. https://doi.org/10.1007/BF00048110

Schum A (2003). Mutation breeding in ornamentals: an efficient breeding method. Acta Horticulturae 612: 47-60. https://doi.org/10.17660/ActaHortic.2003.612.6

Seleiman MF, Al-Suhaibani N, Ali N, Akmal M, Alotaibi M et al. (2021). Drought stress impacts plants and different approaches to alleviate its adverse effects. Plants 10 (2): 259. https://doi.org/10.3390/plants10020259

Shehab GG, Ahmed OK, El-Beltagi HS (2010). Effects of various chemical agents for alleviation of drought stress in rice plants (*Oryza sativa* L.). Notulae Botanicae Horti Agrobotanici Cluj-Napoca 38 (1): 139-148. https://doi.org/10.15835/nbha3813627

Smirnoff N, Colombé SV (2000). Drought influences the activity of enzymes of the chloroplast hydrogen peroxide scavenging system. Journal of Experimental Botany 39 (8): 1097-1107. https://doi.org/10.1093/jxb/39.8.1097

Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH et al. (1985). Measurement of protein using bicinchoninic acid. Analytical Biochemistry 150 (1): 76-85. https://doi.org/10.1016/0003-2697(85)90442-7

Spencer-Lopes MM, Forster BP, Jankuloski L (2018). Manual on Mutation Breeding. 3rd ed. Vienna, Austria: FAO/IAEA.

Stintzing FC, Stintzing AS, Carle R, Frei B, Wrolstad RE (2002). Color and antioxidant properties of cyanidin-based anthocyanin pigments. Journal of Agricultural and Food Chemistry 50 (21): 6172-6181. https://doi.org/10.1021/jf0204811

Suprasanna P, Mirajkar SJ, Patade YV, Jain SM (2014). Induced mutagenesis for improving plant abiotic stress tolerance. In: Tomlekova NB, Kozgar MI, Wani MR (editors). Mutagenesis: Exploring Genetic Diversity of Crops. Wageningen, Netherlands: Wageningen Academic Publishers. https://doi.org/10.3920/9789086867967_019

Taiz LE (2006). Plant Physiology. Berlin Heidelberg, Germany: Spektrum Akademischer Verlag.

Ti-da GE, Fang-gong SUI, Li-ping BA, Yin-yan LU, Guang-sheng ZHOU (2006). Effects of water stress on the protective enzymes and lipid peroxidation in roots and leaves of summer maize. Agricultural Sciences in China 5 (4): 291-298. https://doi.org/10.1016/S1671-2927(06)60052-7

Torabi S, Niknam V (2011). Effects of iso-osmotic concentrations of NaCl and mannitol on some metabolic activity in calluses of two *Salicornia* species. In Vitro Cellular & Developmental Biology – Plant 47: 734-742. https://doi.org/10.1007/s11627-011-9371-6

Turan Buyukdinc D, Kantoglu KY, Karatas A, Ipek A, Ellialtıoglu SS (2019). Determination of effective mutagen dose for carrot (*Daucus carota* ssp sativus var atrorubens Alef and *D. carota*) callus culture. International Journal of Science and Technology Research 5 (3): 15-23. https://doi.org/10.7176/JSTR/5-3-02

Velikova V, Yordanov I, Edreva A (2000). Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective role of exogenous polyamines. Plant Science 151 (1): 59-66.

Vijayakumari K, Puthur JT (2014). Drought stress responses in tolerant and sensitive varieties of black pepper (*Piper nigrum* Linn.). Journal of Plantation Crops 42 (1): 78-85.

Wang W, Wang C, Pan D, Zhang Y, Luo B et al. (2018). Effects of drought stress on photosynthesis and chlorophyll fluorescence images of soybean (*Glycine max*) seedlings. International Journal of Agricultural and Biological Engineering 11 (2): 196-201. https://doi.org/10.25165/j.ijabe.20181102.3390

Xie SX, Zhang QM, Xiong XY, Carol L et al (2005). Effect of water-deficit stress on plant gene expression. Journal of Hunan Agricultural University (Natural Science) 31 (5): 574-579.

Yasar F, Uzal O, Kose S, Yasar O, Ellialtioglu SS (2014). Enzyme activities of certain pumpkin (*Cucurbita* spp.) species under drought stress. Fresenius Environmental Bulletin 23 (4): 1093-1099.

Yordanova RY, Christov KN, Popova LP (2004). Antioxidative enzymes in barley plants subjected to soil flooding. Environmental and Experimental Botany 51 (2): 93-101. https://doi.org/10.1016/S0098-8472(03)00063-7

Zhang R-R, Wang Y-H, Li T, Tan G-F, Tao J-P et al. (2021). Effects of simulated drought stress on carotenoid contents and expression of related genes in carrot taproots. Protoplasma 258: 379-390. https://doi.org/10.1007/s00709-020-01570-5

Zhao T, Pan X, Ou Z, Li Q, Zhang W (2022). Comprehensive evaluation of waterlogging tolerance of eleven *Canna cultivars* at flowering stage. Scientia Horticulturae 296: 110890. https://doi.org/10.1016/j.scienta.2022.110890

Zhong L, Liao PR, Liu CZ, Qian JP, He WC (2021). Effects of drought stress on physiological, biochemical, and chemical components of *Cinnamomum cassia* seedlings. China Journal of Chinese Materia Medica 46 (9): 2158-2166 (in Chinese with an abstract in English). https://doi.org/10.19540/j.cnki.cjcmm.20210224.101

Zhu Y, Liu Y, Wang W, Singh VP, Ren L (2021). A global perspective on the probability of propagation of drought: from meteorological to soil moisture. Journal of Hydrology 603 (Part A): 126907. https://doi.org/10.1016/j.jhydrol.2021.126907

Zia R, Nawaz MS, Siddique MJ, Hakim S, Imran A (2021). Plant survival under drought stress: implications, adaptive responses, and integrated rhizosphere management strategy for stress mitigation. Microbiological Research 242: 126626. https://doi.org/10.1016/j.micres.2020.126626