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Improvement of salt stress tolerance in sugar beet: role of gamma irradiation and cultivar-specific responses

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Abstract: This study investigates the effects of salt stress and gamma irradiation on growth, biochemical, and physiological responses in three sugar beet (*Beta vulgaris* L.) cultivars. Control plants were irrigated with fresh water (EC = 1.1 dS/m), whereas salt stress was imposed with an irrigation of 9 dS/m. Seeds were irradiated with gamma rays (0, 50, 100, 200, 400 Gy) before sowing. Exposure to salt stress reduced root yield (RY), sugar yield (SY), chlorophyll content, and antioxidant enzyme activities (catalase (CAT) and superoxide dismutase (SOD)). In contrast, oxidative damage increased, as indicated by elevated malondialdehyde (MDA) concentrations. Interestingly, salt stress enhanced sugar content, with the Eudoro cultivar showing the greatest resilience, maintaining higher RY and SY and lower MDA compared to the other cultivars. Gamma irradiation at moderate doses (50–200 Gy) alleviated the effects of salt stress, with the strongest improvements in SY observed at 100 and 200 Gy. These treatments enhanced RY, chlorophyll content, and antioxidant activities, while also improving photosynthetic efficiency (F_v/F_m) and cellular integrity. Higher doses (> 200 Gy) reduced sugar content, indicating dose-specific effects. Eudoro exhibited superior salt tolerance, maintaining higher root and sugar yields (RY, SY) and reduced oxidative damage (lower MDA) under salt stress. These findings demonstrate that gamma irradiation at optimal doses enhances salt tolerance in sugar beet, offering cultivar-specific benefits for breeding programmes in saline environments.

Keywords: salinity; reactive oxygen species; stimulation; Rubisco; oxidative stress

Sugar beet (*Beta vulgaris* L.), a glycophytic C3 species of the Amaranthaceae family, contributes nearly 30% of global sugar production and is one of the two main sugar-yielding crops worldwide, ranking second to sugarcane (Nap et al. 2025). In 2020, global production reached 252.969 million tonnes across 4.439 million hectares, with Iran cultivating 93 658 hectares, yielding 6 229 795 tonnes at 66.5 t/ha (FAOSTAT 2021). Globally, salinity affects approximately 1.128 million hectares, with Iran's saline lands exceeding 33 million hectares, resulting in over 50%

crop losses (Wang et al. 2019). Salt stress decreases relative water content (RWC), limiting cell expansion, while raising sugar content for osmotic adjustment and oxidative stress markers like malondialdehyde (MDA). Antioxidant enzymes, such as catalase (CAT) and superoxide dismutase (SOD), mitigate damage by neutralising reactive oxygen species (Zhang et al. 2023). Contrary to misconceptions, certain levels of gamma radiation can positively influence physiological functions, including enhancing photosynthesis, cell reproduction, germination, growth

Table 1. Soil physicochemical properties

Depth (cm)	pH	EC (dS/m)	OC (%)	Na	P	K	NH ₄	NO ₃	Soil texture
				(mg/kg)					
0–60	7.8	1.2	0.9	120	12	220	7	15	loam

Physicochemical properties of soil (0–60 cm depth) before planting. Parameters include electrical conductivity (EC), pH, sodium (Na), phosphorus (P, determined by the Egner method), organic carbon (OC), potassium (K, determined by flame photometry), ammonium (NH₄), nitrate (NO₃), and texture. Values are means of composite samples

rates, stress tolerance, and yield (Brahmi et al. 2014). Gamma irradiation may enhance salt tolerance by stimulating antioxidant activity and osmotic regulation. However, its effects on root yield (RY), sugar yield (SY), sugar content (SC), chlorophyll content, quantum yield (F_v/F_m), and enzyme activities remain underexplored across cultivars (Lu et al. 2024). In sugar beet, it may enhance salt tolerance by stimulating antioxidant activity and osmotic regulation, as observed in other crops such as wheat and rice (Riviello-Flores et al. 2022).

This study investigates whether gamma irradiation at 50–400 Gy improves salt tolerance in sugar beet under 9 dS/m, with cultivar-specific responses. We hypothesise that irradiation will enhance root yield, sugar yield, sugar content, chlorophyll content, maximum quantum yield of PSII, and antioxidant enzyme activities in three cultivars – Eudoro, Antek, and Sharif – recommended for autumn planting in Iran’s warm regions. This research evaluates agronomic, physiological, and biochemical traits to provide insights for breeding programmes in saline environments.

MATERIAL AND METHODS

Experimental site and design. Field experiments were conducted at the same field site in Jafarieh, Qom, Iran (34.84°N, 50.48°E, 983 m a.s.l.) during the years 2018–2019 and 2019–2020. Soil EC was measured before planting each year and remained stable, con-

firmed that saline irrigation did not progressively increase soil salinity between years. Soil samples collected from 0 to 60 cm depth, before planting, had an electrical conductivity (EC) of 1.2 dS/m, indicating non-saline conditions. The physicochemical properties are presented in Table 1, and the soil was classified as loam. Meteorological data were recorded for both seasons (Table 2). A factorial split-plot design within a randomised complete block design (RCBD) with three replications was used, with each treatment combination within the split-plot arrangement replicated three times. Each plot (12 m²; 6 × 2 m) had five 6-m rows, spaced 50 cm apart with 20 cm between plants (100 000 plants/ha). The total experimental area was approximately 1 080 m² (90 plots), with a 50 cm spacing between plots and 5 m between irrigation treatments. Planting occurred on 26 and 30 October, with harvests on 9 and 4 July for the 2018–2019 and 2019–2020 seasons, respectively.

Treatments. Main plots had two irrigation levels: normal (EC = 1.1 dS/m, Saveh Dam) and saline (EC = 9 dS/m, local wells), with eight irrigations per season and salinity measured using an electrical conductivity meter. Irrigation scheduling was equal for both treatments (8 irrigations/season, 6 000 m³/ha/year total). The salinity of irrigation water was monitored and kept constant throughout the seasons. Subplots included three cultivars (Eudoro, Antek, Sharif), recommended and supplied by the Sugar Beet Seed Institute (Karaj, Iran) for autumn planting in warm regions due to their agronomic traits (Table 3), and

Table 2. Meteorological data for growing seasons

Season	Mean min temp	Mean max temp	Mean temp	Total rainfall	Mean relative
		(°C)		(mm)	humidity (%)
2018–2019	10.2	24.5	17.3	158.4	61.5
2019–2020	9.8	24.2	17.0	152.9	60.9

Meteorological data for sugar beet growing seasons (October–July), including mean minimum temperature, mean maximum temperature, mean temperature, total rainfall, and mean relative humidity. Data averaged for 2018–2019 and 2019–2020

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Table 3. Agronomic characteristics of sugar beet cultivars

Cultivar	Germ type	Ploidy	Type	Planting time	RY (t/ha)	SC (%)	WSY (t/ha)	Resistance type
Sharif	monogerm	triploid hybrid	normal sugar	autumn–spring	medium	high	medium-high	bolting resistance
Antek	monogerm	diploid hybrid	sugar	autumn–spring	medium	high	high	Rhizomania and bolting resistance
Eudoro	monogerm	diploid hybrid	sugar	autumn–spring	medium	high	high	Rhizomania and bolting resistance

Agronomic traits of sugar beet (*Beta vulgaris* L.) cultivars (Eudoro, Antek, Sharif) recommended by the Sugar Beet Seed Institute, Iran. Includes qualitative assessments of root yield (RY), sugar content (SC), and white sugar yield (WSY). Qualitative categories (e.g., medium, medium high) follow the classification system of the Sugar Beet Seed Research and Breeding Institute of Iran, based on multi-year cultivar evaluations

five gamma irradiation levels (0, 50, 100, 200, 400 Gy). Seeds, sourced from the Sugar Beet Seed Institute (Karaj, Iran), were irradiated using a Cobalt-60 source (activity 4120 Curie, dose rate 0.93 Gy/s) at the Karaj Nuclear Agriculture Research Centre, with exposure times of 54, 108, 215, and 430 s for 50, 100, 200, and 400 Gy, respectively.

Measurements. Biochemical and physiological traits were measured at mid-growth (BBCH 39–41) on the 6th fully expanded leaf, sampled in the morning, 3 days after irrigation. Three leaves per replicate were measured. Root yield was determined from the entire plot at harvest; sugar yield was calculated from subsamples using polarimetry. The polarimetry method was used to measure the sugar content using the MCP Sucromat device (Anton Paar, Graz, Austria), which operates based on the degree of rotation of polarised light. The percentage of sugar was measured in grams of sugar per hundred grams of sugar beet (%) using the Saccharimeter device (Anton Paar, Graz, Austria). Sugar content was quantified using polarimetry, and sodium concentration was measured in root tissues at harvest, dried at 70 °C, digested, and analysed by flame photometry (Clover et al. 1998). Sugar yield was calculated as $SY = RY \times SC$. White sugar yield (WSY) was determined as $WSY = RY \times WSC$ (Cooke and Scott 1993). The percentage of white sugar content (WSC) was calculated according to Reinefeld et al. (1974):

$$WSC = SC - (MS + 0.6)$$

where: MS – molasses sugar content (%). MS was estimated using the following formula: $MS = 0.0343 (K^+ + Na^+) + 0.094 (\alpha - \text{amino} - N) - 0.31$ (Cooke and Scott 1993).

Extraction coefficient of sugar (ECS) was calculated as $ECS = (WSY/SY) \times 100$. Sodium concentration was

measured in root tissues sampled at harvest, dried at 70 °C, digested, and analysed by flame photometry and was expressed as mmol/kg dry weight (DW).

Chlorophyll content was measured by homogenising 0.5 g of fresh leaves with 10 mL of acetone, filtering, and measuring absorbance at 663.2 and 646.8 nm (Lichtenthaler 1987). Chlorophyll *a*, *b* and total chlorophyll contents were determined spectrophotometrically according to Arnon (1949) and expressed as mg/g fresh weight (FW). Quantum yield was measured using a PAM (pulse-amplitude modulated) fluorometer (model FI-OS5 of Hansatech company, King's Lynn, UK) after dark-adapting leaves (Klughammer and Schreiber 2008). Relative water content (RWC) was calculated as:

$$RWC = [((\text{fresh weight} - \text{dry weight}) / \text{turgor weight} - \text{dry weight})] \times 100 \text{ (Turner 1981)}.$$

Rubisco activity was measured *via* NADH oxidation at 340 nm (Sharkey et al. 1991). Leaf area was estimated from leaf length and maximum width using the formula: $\text{area} = \text{length} \times \text{width} \times 0.75$, and Rubisco activity was expressed as $\mu\text{mol CO}_2/\text{m}^2$ leaf area/s. Malondialdehyde content was quantified using thiobarbituric acid, expressed as nmol/mg protein (Ohkawa et al. 1979). Nitrate reductase (NR) activity was measured at 540 nm, expressed as $\mu\text{mol NO}_2^-/\text{g fresh weight/h}$ (Cataldo et al. 2008). Catalase activity was measured as H_2O_2 decomposition at 240 nm, expressed as $\mu\text{mol H}_2\text{O}_2/\text{min/mg protein}$ (Aebi 1984). Superoxide dismutase activity was assessed by measuring the inhibition of nitroblue tetrazolium at 560 nm, expressed as units/mg protein (Beauchamp and Fridovich 1971).

Statistical analysis. Data normality and variance homogeneity were verified using Anderson-Darling

and Bartlett's tests. Combined analysis of variance (ANOVA) was performed using the GLM procedure in SAS v9.1.3 (SAS 9.1.3, SAS Institute, Cary, USA) to assess the significance of main effects (salinity, cultivar, gamma irradiation) and their interactions, with means compared *via* Duncan's test ($P < 0.05$). Standard errors (SE) were derived from the residual mean square of the ANOVA for each parameter. Trait correlations were calculated using Pearson's correlation analysis.

RESULTS AND DISCUSSION

Results represent the mean of two experimental years (2018–2019 and 2019–2020), each with three replicates per treatment. Since year $\times$ treatment interactions were tested and found to be nonsignificant ($P > 0.05$), the data were averaged across the two years to improve the clarity of presentation. Standard errors were calculated across $2 \times 3 = 6$ replicates.

Root and sugar yield. Salt stress significantly reduced root yield across all sugar beet cultivars ($P < 0.01$), with reductions of 30.6, 21.3, and 32.4% in Eudoro, Antek, and Sharif, respectively. Eudoro exhibited the highest RY under normal irrigation (56.67 t/ha) and salt stress (39.30 t/ha, $P < 0.05$ compared to Sharif). In comparison, Sharif showed the lowest (46.56 t/ha and 31.44 t/ha, respectively) (Figure 1A). Gamma irradiation at 50 Gy significantly enhanced RY in Eudoro and Antek under salt stress (46.71 and 39.78 t/ha, respectively, $P < 0.05$). In contrast, Sharif showed maximum RY under normal irrigation (51.29 t/ha), but declined slightly under salt stress. Figure 2A represents average cultivar responses, not individual cultivar data. Sugar yield followed a similar trend, decreasing by 22.8, 14.8, and 20.22% in Eudoro, Antek, and Sharif under salt stress ($P < 0.01$), with Eudoro and Sharif showing the highest and lowest SY, respectively (Figure 1C). Irradiation at 100 and 200 Gy resulted in the highest SY (9.69 and 9.70 t/ha, $P < 0.05$ compared to controls) (Table 4). These reductions in RY and SY, most pronounced in Sharif, are attributed to osmotic stress and ion toxicity, which limit water availability and photosynthesis (Khayamim et al. 2014). Moderate irradiation doses (50–100 Gy) improved RY and SY in Eudoro and Antek, likely by stimulating root growth and sugar accumulation (Li et al. 2020).

Sugar content and extraction efficiency. Saline irrigation significantly increased sugar content by 23.6, 23.7, and 19.32% in Eudoro, Antek, and Sharif,

respectively, compared to normal irrigation ($P < 0.01$). Eudoro exhibited the highest SC (15.45% and 19.10% under normal and saline conditions, $P < 0.05$ compared to Sharif), while Sharif had the lowest (14.49% and 17.29%) (Figure 1B). This apparent increase may partly reflect osmotic regulation, where sugar beet accumulates soluble sugars to maintain turgor and protect against dehydration (Geissler et al. 2009). However, higher SC under salt stress could also result from a concentration effect due to reduced water content in roots, as reported in water stress studies (Walsh et al. 2023). However, irradiation at 200 Gy reduced SC to 16.07% compared to 17.31% in controls and 400 Gy-treated plants ($P < 0.05$) (Table 4), suggesting a dose-dependent trade-off, possibly due to metabolic resources redirecting toward stress defence pathways (Lu et al. 2024). The extraction coefficient of sugar (ECS) decreased by 5, 3.2, and 16.6% in Eudoro, Antek, and Sharif, respectively, under salt stress ($P < 0.01$), with Eudoro and Antek outperforming Sharif. Irradiation at 100 and 200 Gy resulted in lower ECS (72.17% and 72.12%, $P < 0.05$) compared to controls (77.12%), 50 Gy (75.87%), and 400 Gy (76.99%) (Table 4). White sugar yield decreased by 26.7, 17.5, and 31.5% in Eudoro, Antek, and Sharif under salt stress ($P < 0.01$), with Antek showing the smallest reduction, indicating better tolerance (Figure 1D, Khayamim et al. (2014)).

Chlorophyll content and photosynthetic efficiency. Salt stress reduced chlorophyll content by 32.67% (281.67 mg/m² vs. 418.02 mg/m² under normal irrigation, $P < 0.01$). Gamma irradiation at 100 and 200 Gy significantly increased chlorophyll content by 17.29% under normal irrigation and 19.14% under salt stress ($P < 0.05$) (Figure 2B), suggesting enhanced photosynthetic activity (Borzouei et al. 2013). All cultivars exhibited 15–16% higher chlorophyll levels at these doses compared to the controls ($P < 0.05$) (Figure 3A). This reduction under salt stress impairs photosynthetic capacity due to oxidative damage and disruption of the chloroplast membrane (Mohamed et al. 2021). Eudoro and Antek exhibited better chlorophyll content, supporting higher photosynthetic efficiency. Their superior content may be related to stronger antioxidant defence systems and more stable chloroplast membranes, which limit pigment degradation and preserve the function of PSII (Fugate et al. 2024). Consequently, quantum yield was highest in Eudoro under normal irrigation but decreased under salt stress across all cultivars ($P < 0.01$; Table 4), reflecting reduced energy transfer and Rubisco re-

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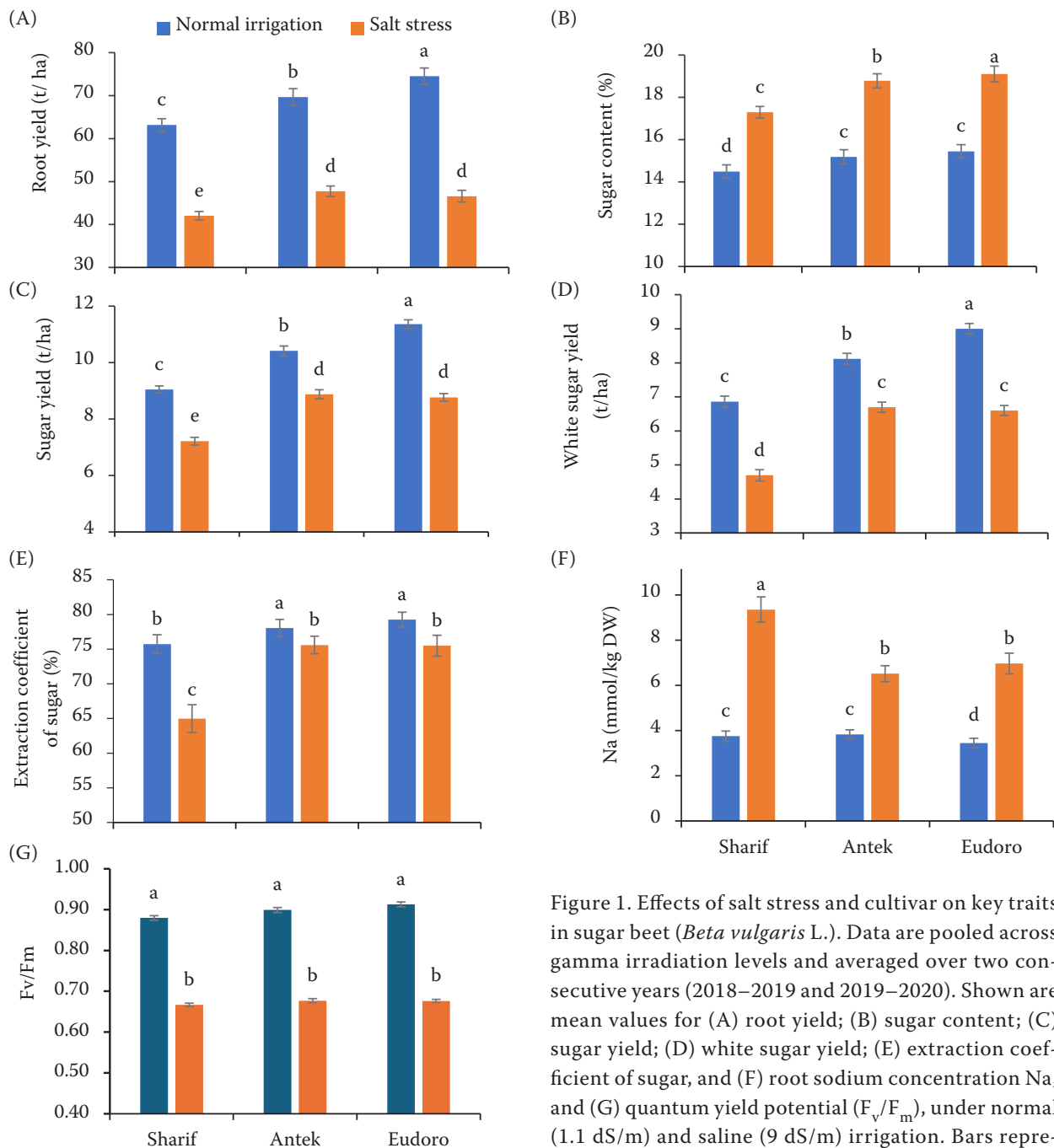


Figure 1. Effects of salt stress and cultivar on key traits in sugar beet (*Beta vulgaris* L.). Data are pooled across gamma irradiation levels and averaged over two consecutive years (2018–2019 and 2019–2020). Shown are mean values for (A) root yield; (B) sugar content; (C) sugar yield; (D) white sugar yield; (E) extraction coefficient of sugar, and (F) root sodium concentration Na, and (G) quantum yield potential (F_v/F_m), under normal (1.1 dS/m) and saline (9 dS/m) irrigation. Bars represent means $\pm$ standard error (SE) of three replicates. Means with different letters are significantly different (Duncan's test, $P < 0.05$). DW – dry weight

generation (Ibrahim et al. 2014). Notably, gamma irradiation at 100 and 200 Gy improved F_v/F_m (0.797 and 0.795, $P < 0.05$), suggesting enhanced efficiency of light energy conversion in Eudoro and Antek, likely due to improved photoprotective mechanisms (Shaebani Monazam et al. 2023, Lu et al. 2024).

Antioxidant enzyme activities and oxidative stress. Salt stress significantly increased catalase

activity by 85% ($2.80 \mu\text{mol H}_2\text{O}_2/\text{min}/\text{mg}$ protein vs. $1.51 \mu\text{mol H}_2\text{O}_2/\text{min}/\text{mg}$ protein, $P < 0.01$) and superoxide dismutase activity by 37.5% ($7.74 \text{ units}/\text{min}/\text{mg}$ protein vs. $5.63 \text{ units}/\text{min}/\text{mg}$ protein, $P < 0.01$) (Table 5). Eudoro and Antek showed increased CAT activity at 50, 100, and 200 Gy ($P < 0.05$), while Sharif's CAT decreased (Figure 3B). SOD activity correlated positively with CAT, Rubisco,

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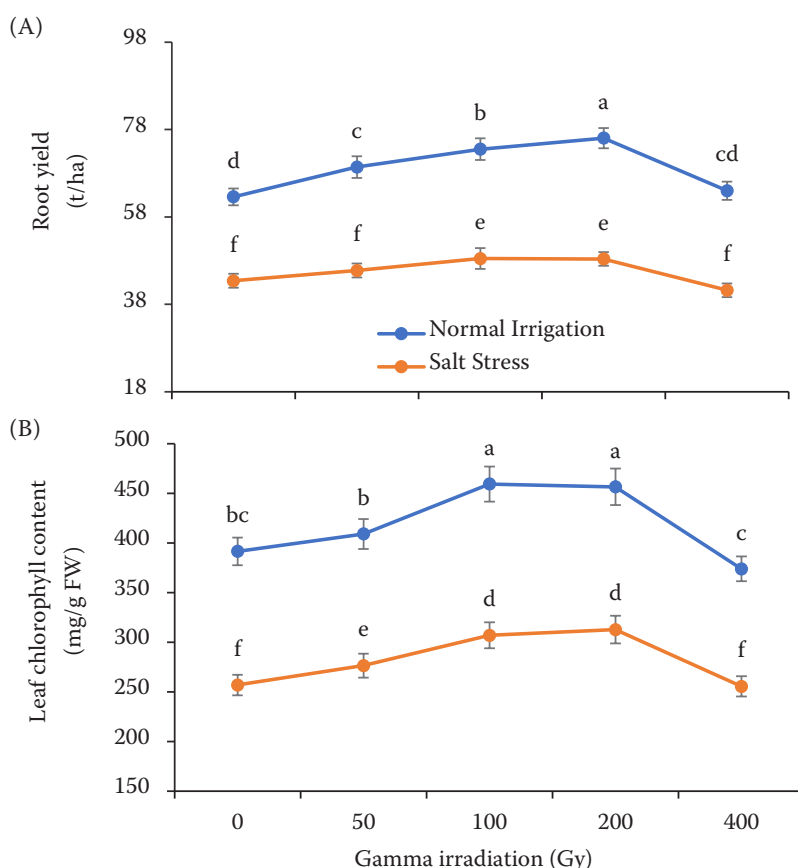


Figure 2. Effects of gamma irradiation and salt stress on yield and chlorophyll. Mean (A) root yield and (B) leaf chlorophyll content in sugar beet (*Beta vulgaris* L.) under normal (1.1 dS/m) and saline (9 dS/m) irrigation across gamma irradiation levels (0, 50, 100, 200, 400 Gy), pooled across cultivars and two years (2018–2019, 2019–2020). Bars represent means $\pm$ standard error (SE) of three replicates. Means with different letters are significantly different (Duncan's test, $P < 0.05$). FW – fresh weight

and F_v/F_m under normal irrigation, and negatively with sodium (Na) and malondialdehyde (Table 6). Under salt stress, SOD correlated positively with CAT, Rubisco, F_v/F_m , and RY, and negatively with Na and MDA ($P < 0.05$) (Table 7). These enzymes mitigate reactive oxygen species (ROS) damage (Wang et al. 2020) by neutralising harmful radicals, such as superoxide and hydrogen peroxide, thereby protecting cellular components from oxidative stress (Sachdev et al. 2021). Irradiation likely enhances CAT and SOD activity by inducing oxidative sig-

nalling pathways and upregulating the expression of antioxidant enzyme-related genes, potentially *via* transcription factors such as WRKY or MYB (Sharma et al. 2020). Irradiation at 50–200 Gy enhanced CAT and SOD in Eudoro and Antek, reducing MDA ($P < 0.05$) (Figure 3C), a marker of lipid peroxidation (Borzouei et al. 2013). Salt stress increased MDA by 12.5% (76.19 nmol/mg protein *vs.* 67.7 nmol/mg protein, $P < 0.01$) (Table 5), but irradiation at 50–200 Gy reduced MDA in Eudoro and Antek. Root Na concentration increased under

Table 4. Effects of gamma irradiation on sugar content and yield

Gamma irradiation (Gy)	SC (%)	SY (t/ha)	ECS (%)	F_v/F_m
Control	17.31 $\pm$ 0.35 ^a	8.95 $\pm$ 0.19 ^c	77.12 $\pm$ 1.11 ^a	0.770 $\pm$ 0.018 ^c
50	16.64 $\pm$ 0.33 ^b	9.30 $\pm$ 0.20 ^b	75.87 $\pm$ 1.05 ^a	0.785 $\pm$ 0.017 ^{ab}
100	16.42 $\pm$ 0.36 ^{bc}	9.69 $\pm$ 0.22 ^a	72.17 $\pm$ 1.23 ^b	0.797 $\pm$ 0.019 ^a
200	16.07 $\pm$ 0.35 ^c	9.70 $\pm$ 0.23 ^a	72.12 $\pm$ 1.19 ^b	0.795 $\pm$ 0.020 ^a
400	17.14 $\pm$ 0.40 ^a	8.77 $\pm$ 0.21 ^c	76.99 $\pm$ 1.15 ^a	0.779 $\pm$ 0.019 ^{bc}

Mean sugar content (SC), sugar yield (SY), extraction coefficient of sugar (ECS), and quantum yield potential (F_v/F_m) across gamma irradiation levels (0, 50, 100, 200, 400 Gy), pooled across irrigation conditions and cultivars (Eudoro, Antek, Sharif) across two years (2018–2019, 2019–2020). Values are means $\pm$ standard error (SE) of three replicates. Means with the same letter are not significantly different (Duncan's test, $P < 0.05$)

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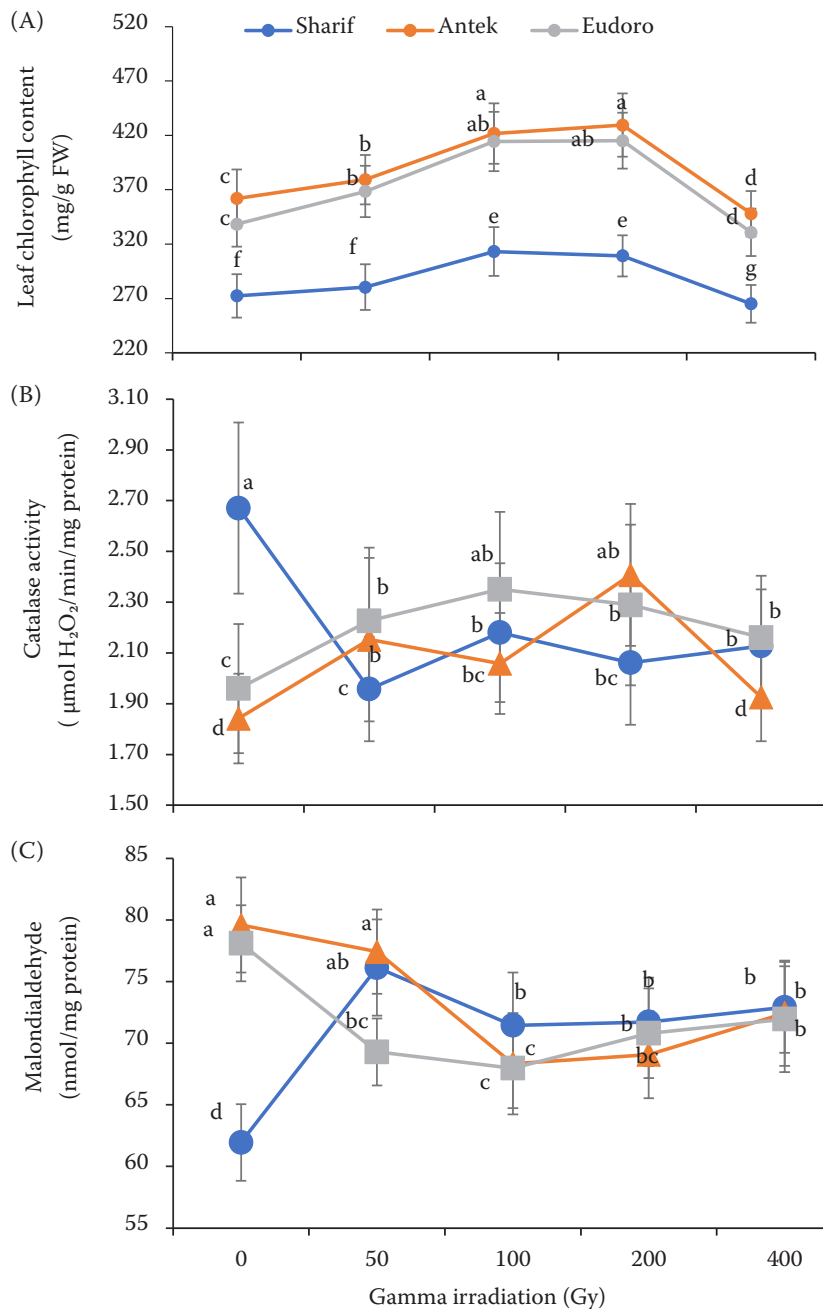


Figure 3. Effects of gamma irradiation and cultivar on physiological traits. Mean (A) leaf chlorophyll content (ChlC); (B) catalase activity (CAT), and (C) malondialdehyde content (MDA) in sugar beet (*Beta vulgaris* L.) cultivars (Eudoro, Antek, Sharif) across gamma irradiation levels (0, 50, 100, 200, 400 Gy), pooled across irrigation conditions and two years (2018–2019, 2019–2020). Bars represent means $\pm$ standard error of three replicates. Means with different letters are significantly different (Duncan's test, $P < 0.05$). FW – fresh weight

salt stress (2-, 1.7-, and 2.5-fold in Eudoro, Antek, and Sharif, respectively, $P < 0.01$; Figure 1F), with Sharif's higher Na accumulation correlating with lower tolerance (Geissler et al. 2009).

Table 5. Effects of irrigation on antioxidant enzymes and oxidative stress

Irrigation type	CAT	SOD	MDA
	($\mu\text{mol H}_2\text{O}_2/\text{min/mg protein}$)	(units/min/mg protein)	(nmol/mg protein)
Normal (1.1 dS/m)	1.51 ± 0.10^b	5.63 ± 0.15^b	67.70 ± 2.00^b
Saline (9 dS/m)	2.80 ± 0.12^a	7.74 ± 0.18^a	76.19 ± 2.20^a

Mean catalase activity (CAT), superoxide dismutase activity (SOD), and malondialdehyde content (MDA) under normal (1.1 dS/m) and saline (9 dS/m) irrigation, pooled across cultivars (Eudoro, Antek, Sharif) and gamma irradiation levels across two years (2018–2019, 2019–2020). Values are means $\pm$ standard error of three replicates. Means with the same letter are not significantly different (Duncan's test, $P < 0.05$)

Table 6. Trait correlations under normal irrigation

Trait	RY	SC	SY	WSY	Na	ECS	Rubisco	MDA	CAT	SOD	NR	ChlC	F _v /F _m
RY	1												
SC	-0.617**	1											
SY	0.666**	0.167	1										
WSY	0.216*	0.592**	0.838**	1									
Na	0.348**	-0.361**	0.102	-0.243*	1								
ECS	-0.562**	0.841**	0.089	0.614**	-0.597**	1							
Rubisco	0.678**	-0.618**	0.261*	-0.106	0.152	-0.553**	1						
MDA	0.011	0.104	0.119	0.149	0.291**	0.092	-0.250*	1					
CAT	-0.409**	0.363**	-0.169	0.037	-0.393**	0.327**	-0.161	-0.605**	1				
SOD	-0.023	-0.127	-0.153	-0.176	-0.358**	-0.088	0.235*	-0.757**	0.603**	1			
NR	0.630**	-0.326**	0.482**	0.239*	0.061	-0.273**	0.417**	-0.030	-0.199	0.039	1		
ChlC	0.773**	-0.388**	0.590**	0.248*	0.193	-0.381**	0.610**	-0.051	-0.224*	-0.002	0.531**	1	
F _v /F _m	0.463**	-0.109	0.482**	0.329**	-0.107	-0.078	0.295**	-0.298**	0.248*	0.245*	0.313**	0.454**	1

Correlation coefficients of traits were calculated using Pearson's correlation method under normal irrigation (1.1 dS/m), including root yield (RY, t/ha), sugar content (SC, %), sugar yield (SY, t/ha), white sugar yield (WSY, t/ha), sodium (Na, mEq/100 g), extraction coefficient of sugar (ECS, %), Rubisco activity ($\mu\text{mol CO}_2/\text{m}^2$ leaf area/s), malondialdehyde (MDA, nmol/mg protein), catalase (CAT, $\mu\text{mol H}_2\text{O}_2/\text{min/mg protein}$), superoxide dismutase (SOD, units/min/mg protein), nitrate reductase (NR, $\mu\text{mol NO}_2^-/\text{g fresh weight/h}$), leaf chlorophyll content (ChlC, mg/m²), and quantum yield potential (F_v/F_m). Data are pooled across two years (2018–2019 and 2019–2020). ** $P < 0.01$; * $P < 0.05$; ns – not significant

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Table 7. Trait correlations under saline irrigation

Trait	RY	SC	SY	WSY	Na	ECS	Rubisco	MDA	CAT	SOD	NR	ChlC	F _v /F _m
RY	1												
SC	-0.516**	1											
SY	0.737**	0.191	1										
WSY	0.259*	0.613**	0.785**	1									
Na	-0.006	-0.429**	-0.340**	-0.731**	1								
ECS	-0.285**	0.762**	0.271**	0.803**	-0.815**	1							
Rubisco	0.673**	-0.461**	0.400**	0.052	-0.013	-0.299**	1						
MDA	-0.308**	0.223*	-0.170	-0.126	0.273**	-0.031	-0.414**	1					
CAT	-0.062	0.154	0.056	0.195	-0.226*	0.221*	-0.007	-0.580**	1				
SOD	0.258*	-0.149	0.168	0.145	-0.312**	0.056	0.379**	-0.820**	0.671**	1			
NR	0.431**	-0.059	0.458**	0.296**	-0.079	0.029	0.412**	-0.029	-0.146	-0.008	1		
ChlC	0.757**	-0.160	0.728**	0.468**	-0.226*	0.038	0.663**	-0.371**	0.030	0.322**	0.554**	1	
F _v /F _m	0.346**	-0.024	0.373**	0.303**	-0.191	0.100	0.214*	-0.247*	0.364**	0.262*	0.115	0.248*	1

Correlation coefficients of traits were calculated using Pearson's correlation method under saline irrigation (9 dS/m), including root yield (RY, t/ha), sugar content (SC, %), sugar yield (SY, t/ha), white sugar yield (WSY, t/ha), sodium (Na, mEq/100 g), extraction coefficient of sugar (ECS, %), Rubisco activity (μmol CO₂/m² leaf area/s), malondialdehyde (MDA, nmol/mg protein), catalase (CAT, μmol H₂O₂/min/mg protein), superoxide dismutase (SOD, units/min/mg protein), nitrate reductase (NR, μmol NO₂⁻/g fresh weight/h), leaf chlorophyll content (ChlC, mg/m²), and quantum yield potential (F_v/F_m). Data are pooled across two years (2018–2019 and 2019–2020).

**P < 0.01; *P < 0.05; ns – not significant

Nitrogen metabolism. Rubisco activity ($\mu\text{mol CO}_2/\text{m}^2$ leaf area/s) decreased under salt stress ($P < 0.01$), with Eudoro and Antek showing higher activity than Sharif. Irradiation at 50, 100, and 200 Gy increased Rubisco activity in Eudoro and Antek ($P < 0.05$), with Sharif peaking at 50 Gy under salt stress (Figure 4A). Nitrate reductase (NR) activity ($\mu\text{mol NO}_2^-/\text{g}$ fresh weight/h) also decreased under salt stress ($P < 0.01$), but Eudoro exhibited the highest NR activity. Irradiation at 50, 100, and 200 Gy enhanced NR in Eudoro under both conditions ($P < 0.05$), with Eudoro and Antek outperforming Sharif (Figure 4B). Reduced NR activity under salt stress likely results from impaired nitrate absorption, affecting protein metabolism (Wang et al. 2019).

Integrated findings. This study demonstrated that moderate gamma irradiation (50–200 Gy) signifi-

cantly enhanced sugar beet performance under salt stress (9 dS/m) by improving root yield, sugar yield, sugar content, chlorophyll content, photosynthetic efficiency, and antioxidant enzyme activities. These improvements were most pronounced in Eudoro, followed by Antek, whereas Sharif showed limited gains. Significantly, the superior performance of Eudoro was associated with higher RY and SY, greater chlorophyll content, and reduced oxidative damage (lower MDA), highlighting the role of enhanced antioxidant defences and osmotic adjustment in conferring stress resilience rather than Na^+ exclusion (Nawaz et al. 2022).

These findings directly address the research question of whether gamma irradiation can improve salt tolerance in sugar beet cultivars. The cultivar-specific responses suggest that genetic background strongly

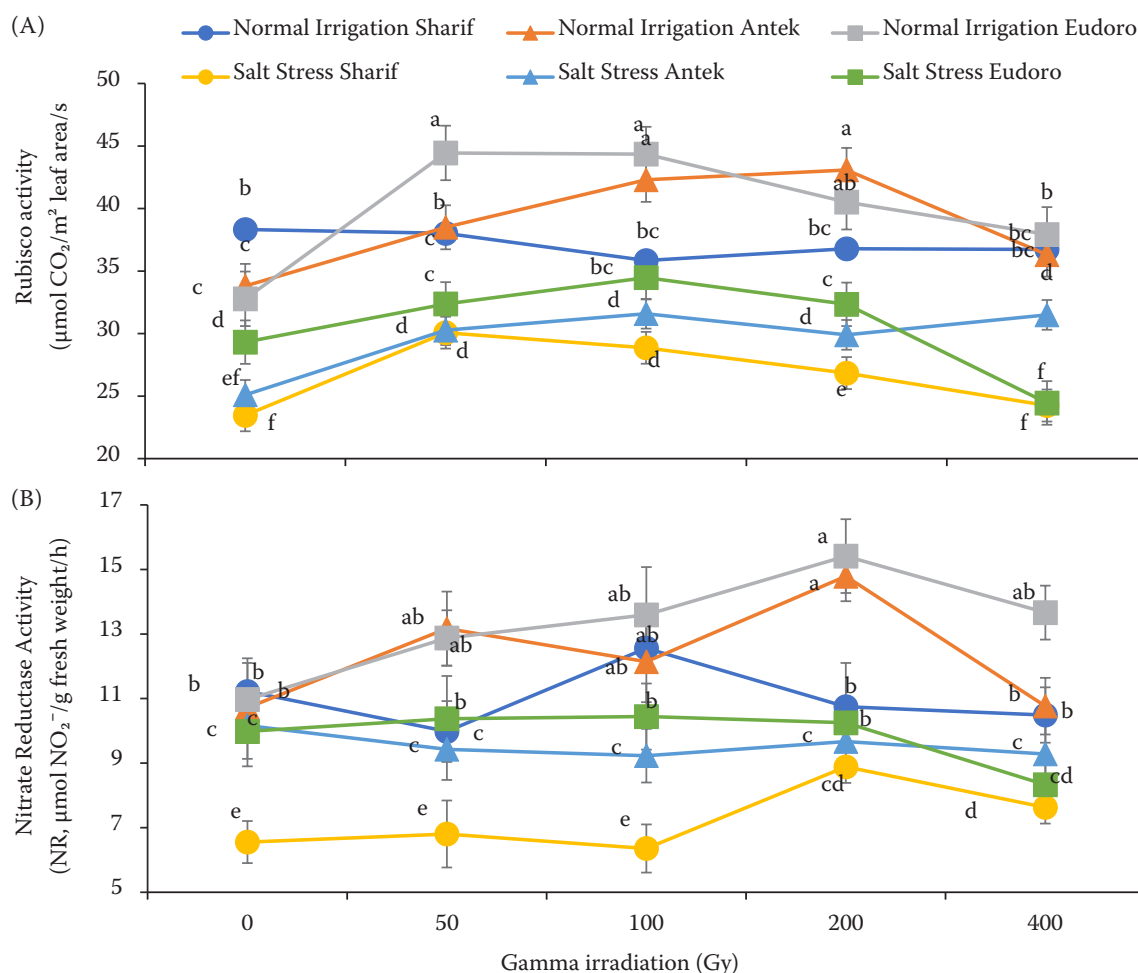


Figure 4. Effects of gamma irradiation and cultivar on enzymatic activities. Mean (A) Rubisco activity (Rubisco) and (B) nitrate reductase activity (NR) in sugar beet (*Beta vulgaris* L.) cultivars (Eudoro, Antek, Sharif) across gamma irradiation levels (0, 50, 100, 200, 400 Gy), pooled across irrigation conditions and two years (2018–2019, 2019–2020). Bars represent means $\pm$ standard error of three replicates. Means with different letters are significantly different (Duncan's test, $P < 0.05$)

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influences irradiation efficacy, with Eudoro emerging as a promising genotype for breeding or agronomic strategies aimed at saline environments. However, higher irradiation doses (e.g., 400 Gy) negatively affected sugar yield (8.77 t/ha; Table 4), emphasising the importance of optimising dosage to maximise benefits without yield penalties (Lu et al. 2024). These results are consistent with radiation-induced hormesis, where low doses (50–100 Gy) stimulate protective mechanisms, including antioxidant activity and chlorophyll stability, whereas higher doses (≥ 400 Gy) become inhibitory and reduce yield.

Broader implications include the potential integration of gamma irradiation into pre-breeding programs to generate stress-resilient sugar beet lines, thereby enhancing the sustainability of sugar production in saline-affected regions. Nonetheless, further research is warranted to clarify the underlying molecular mechanisms, particularly the roles of antioxidant pathways, osmotic regulation, and hormonal signaling. Long-term field trials across diverse environments, combined with omics-based analyses, would provide deeper insights and validate the practical utility of this approach.

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