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Physiological Changes in Different Sugar Beet (*Beta vulgaris* L.) Genotypes Grown under Saline Soil Conditions

Pariya Nouri[1], Sodabeh Jahanbakhsh[*1], Salim Farzaneh[1], Yeganeh Shafiei[3], Bahman Fazeli-Nasab[2]

[1]*Department of Plant Production and Genetics, Faculty of Agriculture and Natural Resources, University of Mohaghegh Ardabili, Ardabil, Iran*
[2]*Department of Agronomy and Plant Breeding, Agriculture Institute, Research Institute of Zabol, Zabol, Iran*
[3]*Pharmaceutical Sciences Research Center, Health Institute, Kermanshah University of Medical Sciences, Kermanshah, Iran*

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ABSTRACT

Salinity is a major abiotic constraint that adversely affects crop growth and physiological performance. Therefore, identifying sugar beet (*Beta vulgaris* L.) genotypes with favorable physiological responses to salinity is important for cultivation and selection under salt-affected conditions. In 2020, a factorial experiment based on a randomized complete block design with three replications was conducted at the University of Mohaghegh Ardabili, Iran, to evaluate the physiological responses of nine sugar beet genotypes under four salinity levels (0, 4, 8, and 12 dS m^{-1}). Salinity significantly affected leaf area, electrolyte leakage, relative water content, soluble protein, chlorophyll pigments, proline, soluble sugars, and the activities of catalase, peroxidase, and polyphenol oxidase. Across the evaluated genotype × salinity combinations, leaf area ranged from 50.00 to 76.33 cm^2, electrolyte leakage from 294.30 to 461.30, proline from 3.68 to 6.86 µg g^{-1} FW, and chlorophyll a from 2.471 to 3.289 mg g^{-1} FW. Increasing salinity from 0 to 12 dS m^{-1} reduced mean relative water content from 67.85% to 53.73% and increased mean soluble sugar content from 50.77 to 62.81 mg g^{-1} FW. Under non-saline conditions, genotypes 15 and 4 showed high chlorophyll a, b, and total chlorophyll contents, whereas genotypes 6, 9, 17, and 19 exhibited comparatively high antioxidant enzyme activities at 12 dS m^{-1}. Overall, the results demonstrate substantial genotype-dependent variation in physiological and biochemical responses to salinity and identify genotypes with potentially useful traits for selection under saline conditions.

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1. Introduction

Sugar beet (*Beta vulgaris* L.) is an herbaceous, dicotyledonous C3 crop belonging to the Amaranthaceae family and is one of the world's major sugar crops. Together with sugarcane, it is a primary source of sucrose for the global sugar industry, with sugar beet contributing substantially to global sugar production (European Commission, 2026). The crop is particularly important in temperate production systems because of its high root sucrose concentration and relatively short production cycle compared with sugarcane (Tayyab *et al.*, 2023). In addition to crystalline sugar, sugar beet processing generates valuable co-products, particularly pulp and molasses, which are used in animal feed and are increasingly explored as feedstocks for biofuels, biochemicals, and other value-added products within circular-bioeconomy systems (Usmani *et al.*, 2022). The economic importance of sugar beet, together with its comparatively high salt tolerance, has stimulated considerable interest in improving its productivity and adaptation to increasingly saline agricultural environments (Wang *et al.*, 2024; El-Mageed *et al.*, 2022). As a cash crop, sugar beet requires meticulous agronomic practices and breeding to enhance its adaptation to biotic and abiotic stresses. Traits of particular interest for improvement include its ability to acclimate to abiotic stresses like drought and salinity (Abu-Ellail *et al.*, 2020). Sugar beet is a temperate crop primarily cultivated in Europe, North America, and the

* Corresponding author.
E-mail address: jahanbakhsh@uma.ac.ir

temperate regions of Asia, with France, Germany, Russia, the USA, and Ukraine being the leading producers (Mohammadzadeh *et al*., 2023). Its rapid growth cycle (5-6 months) allows for harvest after five months, enabling farmers to cultivate a second crop on the same land (Alavilli *et al*., 2023). Integrating sugar beet into existing cropping patterns can foster a sustainable production system, thereby increasing cropping intensity and overall sugar crop output (Alharbi *et al*., 2022).

Globally, nearly 7% of irrigable arable terrains suffer from salinization, resulting in the annual forfeiture of approximately 1.5 million hectares of productive agricultural land (AlKahtani *et al*., 2021) and consequent suboptimal crop viability. *Beta vulgaris* (sugar beet) exhibits inherent halotolerance up to an electrical conductivity threshold of 7.0 dS m^{-1} in the rhizosphere without substantial diminution of output; nevertheless, each incremental unit of EC beyond this level precipitates a 5.9% productivity decrement (Aycan *et al*., 2023). Saline conditions impede vegetative development and persistence by instigating both osmotic desiccation and hydric deficit, thereby generating ionic disequilibrium stemming from the accretion of sodium (Na^+) and chloride (Cl^-) moieties, ultimately eliciting ion-specific cytotoxic effects (Bouras *et al*., 2021). Upon surpassing physiological concentrations, Na^+ becomes highly deleterious to plant ontogeny through perturbation of cellular ionic equilibrium and induction of cytosolic K^+ exudation (Dadkhah and Rassam, 2017). Elevated osmotic tension, concurrent with augmented Na^+ and Cl^- intrusion into root tissues, compromises the assimilation of vital nutrients, notably potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), and nitrate (NO_3^-), further aggravating ionic dysregulation within plant cells (El-Kady *et al*., 2021). Furthermore, ionic toxicity accelerates premature foliar senescence, curtailing the provision of photoassimilates required for vegetative expansion and reducing harvestable yield even under modest saline conditions (El-Mageed *et al*., 2022).

During initial developmental phases, sugar beet specimens encounter hydric deficiency resulting from elevated solute potential in the surrounding matrix (Geng *et al*., 2020). This triggers an array of physiological and biochemical alterations, encompassing suppressed growth and maturation, attenuated photosynthetic activity, impaired respiration and proteosynthesis, and disrupted nucleic acid turnover (Gholipor *et al*., 2022). To endure saline incursion, plants deploy sophisticated adaptive strategies spanning developmental, morphogenic, physiological, and biochemical domains (He *et al*., 2023). For example, numerous genetic determinants implicated in transmembrane transport, signal transduction cascades, redox transformations, and allied pathways have been implicated in salinity response mechanisms (Hossain *et al*., 2021).

Notwithstanding these intrinsic adaptive capabilities, an urgent imperative exists to identify and advocate for highly salt-resilient sugar beet genotypes suitable for sodic environments. Accurate varietal selection necessitates a thorough elucidation of the mechanistic underpinnings conferring saline tolerance in this species. Under ionic duress, sugar beet genotypes have evolved intricate physiological strategies to acclimate to the osmotic and ionic challenges imposed by heightened salinity. Nevertheless, not all genotypic accessions demonstrate equivalent performance across diverse environmental matrices and pedological conditions. Consequently, the current investigation was undertaken to evaluate the growth dynamics and yield attributes of a range of sugar beet genotypes subjected to varying degrees of saline exposure.

2. Materials and methods

The investigation was carried out during the 2020 cultivation cycle at the experimental station of Mohaghegh Ardabili University, situated in Iran (geographic coordinates 45°10′ E, 37°44′ N, at an elevation of 1338 m above mean sea level). This xeric region is distinguished by frigid winters and scant rainfall. Climatic records during the sugar beet growing season indicated mean minimum temperatures of 9.2–10.9°C and mean maximum temperatures of 22.3–23.8°C. Mean relative humidity and wind speed ranged from 41.0 to 41.6% and 1.8 to 2.0 m s^{-1}, respectively. The climatic conditions recorded during the growing season are summarized in Table 1.

2.1. Treatments and experimental design

The experiment was conducted as a factorial experiment based on a randomized complete block design with three replications. Nine sugar beet genotypes (Table 2) were evaluated under four soil

salinity levels: a control and applied salinity levels of 4, 8, and 12 dS m^{-1}. The control treatment was irrigated with well water, whereas the saline treatments were prepared by adding salt to the irrigation water to obtain the target electrical conductivity levels of 4, 8, and 12 dS m^{-1}. The salinity treatments were initiated after plant establishment at the designated growth stage and were subsequently maintained throughout the treatment period. The electrical conductivity of the irrigation water was monitored to ensure that the target salinity levels were maintained. Adequate drainage was provided to allow excess irrigation water to drain from the soil profile and minimize excessive salt accumulation during the treatment period. Each experimental plot measured 18.0 m^2 (3 m × 6 m) and consisted of five ridges, 0.6 m apart and 6.0 m long, with an interplant distance of approximately 0.20 m within each ridge.

Table 1. Climatic conditions during the 2020 sugar beet growing season at the experimental station of Mohaghegh Ardabili University

Climatic variable	Value during the growing season
Mean minimum temperature (°C)	9.2–10.9
Mean maximum temperature (°C)	22.3–23.8
Mean relative humidity (%)	41.0–41.6
Mean wind speed (m s^{-1})	1.8–2.0
Precipitation	0.51*

*The precipitation unit should be verified against the original meteorological record before publication

Table 2. The list of sugar beet genotypes

Genetic material	Cross combination	Code
SHR.1-P12	4=419*SB36=419*KWS=006	33-----41
	5=231*261	31-----42
F-8662=31192	6=SB37*28874=31459	21-----60
	9=419*SB36=419*KWS=006	22-----57
FC709-2/24	12=SB37*28874=31459	38-----48
	15=7112*436	39-----47
S_1-88239=31187	17=SB37*28874=31459	30-----51
	19=7112*SB36=7112*KWS	27-----55
	20=7112*436	29-----54

2.2. Crop management practices

Propagation materials comprising both multigerm and monogerm genotypes of *Beta vulgaris* L., sourced from the Germplasm Repository of the Crop Research Institute under Iran's Agricultural Research Organization, were selected based on their use as commercial sugar beet materials in Iranian agroecosystems. Manual sowing was performed on October 20 and 25, 2020. Plant density was regulated at the octofoliate stage to maintain one plant per sowing mound. Before sowing, soil samples were collected from the experimental field and analyzed for their main physicochemical characteristics, including texture, pH, electrical conductivity, organic matter, total nitrogen, available phosphorus, and available potassium. The results of the initial soil analysis are presented in Table 3. Based on the soil analysis and recommended agronomic practices for sugar beet cultivation, 90 kg ha^{-1} phosphorus and 290 kg ha^{-1} nitrogen were applied to the experimental field. Throughout the growing period, four episodes of surface irrigation were administered. Other agronomic practices, including phytosanitary measures, pest management, and nutrient supplementation, were performed according to local sugar beet cultivation practices. Physiological and biochemical measurements were conducted during the anthesis stage using samples collected from the flag leaves. Plants were harvested on April 28 at the end of the growing period.

Table 3. Initial physicochemical characteristics of the experimental soil before sowing

Soil characteristic	Value
Soil texture	Clay loam
pH	7.8
Electrical conductivity (dS m^{-1})	1.2
Organic matter (%)	1.1
Total nitrogen (%)	0.08
Available phosphorus (mg kg^{-1})	9.6
Available potassium (mg kg^{-1})	285

2.3. Measurements

2.3.1. Leaf area index

Leaf area index (LAI) was measured at the octofoliate developmental stage, preferably during early morning or late afternoon, using a LI-COR LAI-2000 Plant Canopy Analyzer (Lincoln, NE, USA). Measurements were performed according to the manufacturer's recommendations for row crops. For each plot, measurements were obtained along the planting rows and consisted of three measurement sequences. Each sequence included one above-canopy reading and four below-canopy readings distributed across two adjacent rows. The measurements were used to estimate the leaf area index of the crop canopy.

2.3.2. Relative water content (RWC)

The relative water content (RWC) of the leaves was determined according to the method described by

Holaday et al. (1992) (Equation 1). Leaf samples were collected at the specified sampling stage following exposure to the salinity treatments. For each sample, the initial fresh mass (FM) was recorded, after which the leaf samples were immersed in deionized water for 16–18 h to achieve full turgidity. The samples were then gently blotted with absorbent material to remove surface water, and the turgid mass (TM) was recorded. Subsequently, the samples were dried in a forced-air oven at 70°C for 24 h to obtain a constant dry mass (DM) Holaday et al. (1992). RWC was calculated using the following equation (Equation 1):

(1) $RWC = [(FW–DW)/(TW–DW)] \times 100$

2.3.3. *Chlorophyll contents*

Fresh leaf samples (0.5 g) were collected at the specified sampling stage following exposure to the salinity treatments. The samples were immediately frozen in liquid nitrogen, ground, and homogenized with 10 mL of 80% aqueous acetone. The homogenate was centrifuged at 4,000 rpm for 15 min, and the resulting supernatant was used for spectrophotometric determination of chlorophyll a, chlorophyll b, and total carotenoid contents according to the method described by Arnon (1967).

2.3.4. Electrolyte leakage (EL)

Electrolyte leakage was determined using freshly excised leaf samples collected at the anthesis stage, when the physiological and biochemical traits of the sugar beet genotypes were evaluated. Non-frozen leaf samples were rinsed three times with deionized water to remove surface-adhered electrolytes. Uniform leaf discs (0.1 g) were immersed in deionized water at room temperature for 60 min, after which the electrical conductivity of the bathing solution (EC_1) was measured using a conductivity meter. The same samples were subsequently boiled at 100°C for 10 min, cooled to room temperature, and the electrical conductivity of the solution (EC_2) was recorded. Electrolyte leakage was calculated as $EC_1/EC_2 \times 100$, according to Dionisio-Sese and Tobita (1998).

2.3.5. Proline content

Leaf proline content was determined at the anthesis stage, when the physiological and biochemical traits of the sugar beet genotypes were evaluated. Fresh leaf tissue (0.5 g) was homogenized in 10 mL of 3% sulfosalicylic acid and centrifuged at 4,000 rpm for 15 min. The resulting supernatant was used for proline determination using the ninhydrin-based colorimetric method described by Bates et al. (1973). The reaction mixture consisted of the sample extract, glacial acetic acid, and ninhydrin reagent at a 1:1:1 ratio and was incubated at 100 °C for 60 min. The reaction was terminated by rapid cooling in an ice bath. Toluene (4 mL) was then added to extract the chromophore into the organic phase, and the absorbance of the resulting solution was measured at 515 nm using a spectrophotometer. Proline concentration was calculated against a standard curve and expressed as µg g^{-1} fresh leaf weight.

2.3.6. Total soluble sugars

Total soluble sugar content was determined using the phenol–sulfuric acid method described by Irigoyen et al. (1992). Leaf samples were collected at the anthesis stage, when the physiological and biochemical traits of the sugar beet genotypes were evaluated. Fresh leaf tissue (0.5 g) was homogenized in ethanol and filtered to obtain the extract. An aliquot of the extract was then reacted with 5% phenol and concentrated sulfuric acid (98%). The reaction mixture was allowed to stand for 60 min, and absorbance was measured at 625 nm using a spectrophotometer. Total soluble sugar content was calculated using an appropriate standard curve and expressed as mg g^{-1} fresh leaf weight.

2.3.7. Total protein

Total protein content was determined in leaf samples collected at the anthesis stage, when the physiological and biochemical traits of the sugar beet genotypes were evaluated. The Bradford colorimetric assay (Bradford, 1976) was used to determine the concentration of soluble total protein in the prepared leaf extracts. Briefly, a 30 µL aliquot of each sample, previously diluted at a 1:3 ratio with phosphate-buffered saline (PBS), was mixed with 1.5 mL of Bradford reagent (PanReac, AppliChem, ITW Reagents). After incubation for 5 min at room temperature, absorbance was measured at 595 nm using a spectrophotometer. Protein concentration was calculated from a calibration curve prepared using bovine serum albumin (BSA) standards at concentrations ranging from 0.0125 to 1.0 mg mL^{-1}.

2.3.8. Enzyme extractions and assays

Leaf samples were collected at the anthesis stage, when the physiological and biochemical traits of the sugar beet genotypes were evaluated. Freshly collected leaf tissue (0.25 g) was immediately frozen in liquid nitrogen, ground to a fine powder, and homogenized in a pre-chilled mortar and pestle. The extraction medium (4 mL) consisted of 0.05 M disodium hydrogen phosphate/sodium dihydrogen phosphate buffer (pH 7.0), supplemented with 0.2 mM ethylenediaminetetraacetic acid (EDTA) and 1% (w/v) polyvinylpyrrolidone (PVP). The homogenates were centrifuged at 15,000 × g for 20 min at 4°C. The resulting supernatants were carefully collected and used for subsequent determination of enzyme activities.

2.3.9. Catalase activity (CAT)

Catalase enzymatic activity was assessed by quantifying the kinetics of hydrogen peroxide depletion following the protocol established by Chance and Maehly (1955). The reaction cocktail consisted of 2.5 mL of 50 mM phosphate buffer (pH 7.4), 0.3 mL of 1% aqueous hydrogen peroxide, and 60 µL of diluted enzymatic extract. The crude enzyme preparation was appropriately diluted to ensure that all measurements fell within the linear dynamic range of the assay. The progressive reduction in hydrogen peroxide concentration was tracked by recording the diminution in optical absorbance at a wavelength of 240 nm.

2.3.10. Polyphenol oxidase (PPO) activity

The catalytic activity of polyphenol oxidase (PPO) was evaluated employing a modified iteration of the protocol originally described by Kar and Mishra (1976). The assay admixture comprised 50 mM phosphate buffer (adjusted to pH 7.0), 0.02 M pyrogallol as substrate, and 50 µL of the enzymatic extract. The generation of purpurogallin, the chromogenic reaction product, was monitored by recording absorbance values at 420 nm at intervals of 5 seconds over a total duration of 60 seconds.

2.3.11. Ascorbate peroxidase activity (APX)

Ascorbate peroxidase (APX) enzymatic activity was quantified utilizing a slightly adjusted version of the procedure originally reported by Kar and Mishra (1976). The reaction cocktail consisted of 50 mM potassium phosphate buffer (pH 7.0), 0.1 mM ethylenediaminetetraacetic acid (EDTA), 0.5 mM ascorbate, 1.54 mM hydrogen peroxide, and 50 µL of crude enzyme extract. The oxidative conversion of ascorbate was tracked by recording the progressive reduction in optical density at 240 nm.

2.4. Data analysis

The quantitative datasets obtained from this investigation were subjected to statistical scrutiny employing SAS software, version 9.1. Comparative evaluations of treatment means were conducted using Fisher's least significant difference (LSD) test with protection at a significance threshold of $p<0.05$. Graphical representations of the derived results were constructed utilizing Microsoft Excel.

3. Results and discussion

Table 4 analysis of variance showed a significant interaction effect of salinity stress and sugar beet genotype on leaf area index, chlorophyll a, chlorophyll b, total chlorophyll content, ion leakage, proline, soluble protein, and the activity of antioxidant enzymes (catalase, peroxidase, and polyphenol oxidase). Salinity stress and sugar beet genotype also had a significant simple effect on relative moisture content. Soluble sugar, however, was only significantly affected by salinity stress.

Table 4. Analysis of variance of the effect of salinity stress on some physiological traits of different sugar beet genotypes

SoV	df	Leaf area	Electrolyte leakage	Relative water content	Soluble protein content	Proline	Soluble sugars	Chlorophyll a	Chlorophyll b	Total chlorophyll	CAT	PAX	PPO
Block	2	2644.08**	110127	318.97	295.85	5.4	1274.8	0.55	0.74	1.82	118.32	1208.03	1433.92
Salinity stress (SSR)	3	1996.41**	53482.87**	922.42**	752.78**	22.04**	761.4**	1.25**	0.45**	3.2**	697.37**	961.16**	858.32**
Cultivars (CUS)	8	45.81^{ns}	2758.60**	46.38*	52.91**	0.99**	28.05^{ns}	0.17**	0.04**	0.39**	27.08**	74.7**	46.36**
SSR × CUS	24	52.45**	2171.97**	13.95^{ns}	18.15**	0.49*	4.1^{ns}	0.04**	0.13**	0.09**	21.47**	13.97**	30.20**
Error	70	22.36	649.41	18.57	6.36	0.29	13.99	0.02	0.004	0.36	5.69	4.46	6.99
CV (%)	-	7.57	6.61	7.05	5.84	10.69	6.8	4.92	6.04	4.75	5.22	4.02	4.64

**, * and ns, significant at 1% and 5% levels of probability, non-significant, respectively.

3.1. Leaf area

Mean comparison results indicated that increased salt stress led to a greater reduction in leaf area compared to the control group (Table 5; Fig. 3). Specifically, the highest sugar beet leaf area (76.33 cm^2) was observed in cultivar No. 15 without salt application, a value not statistically different from cultivars No. 6 and 20 (Table 5). Conversely, the lowest (50 cm^2) was recorded for cultivar No. 20 under salinity conditions of 12 dS m^{-1} (Table 5).

Salt stress likely reduces leaf area and photosynthesis by inhibiting cell growth due to elemental toxicity, decreased water availability, and reduced nutrient uptake (Aycan *et al*., 2023). While plants employ various mechanisms, such as increased root and aerial part biomass, to absorb soil water under stress, these may be insufficient to compensate for the lack of available moisture (Hozayn *et al*., 2020). Reduced water absorption from the soil subsequently lowers the relative water content of leaves, leading to decreased leaf area, photosynthesis, and ultimately, yield (Khayamim, 2022). Numerous studies corroborate the reduction of relative leaf water in stressed plants (Khozaei *et al*., 2021). Furthermore, high environmental salt concentrations appear to limit cell division and decrease cell density by reducing water availability, thereby diminishing leaf surface area (Kumar *et al*., 2023).

Table 5. Comparison of the mean effect of salinity stress on the physiological traits of some different sugar beet genotypes.

Salinity level	Genotype	Leaf area (cm^2)	Electrolyte leakage	Soluble protein content (%)	Proline (µg g^{-1} fw)	Chlorophyll a	Chlorophyll b	Chlorophyll a+b	CAT	PAX	PPO
						(mg g^{-1} fw)			(µg protein^{-1} min^{-1})		
	4	68.66^{a-f}	342.60^{l-o}	51.24^{ab}	3.68^{m}	3.289^{a}	1.387^{a}	4.676^{a}	40.91^{i-m}	42.87^{q}	49.68^{p-s}
	5	71.33^{a-e}	357.60^{k-n}	48.06^{a-d}	3.76^{lm}	3.267^{ab}	1.378^{a}	4.654^{a}	40.04^{j-m}	46.59^{m-p}	49.14^{rs}
	6	75.33^{a}	333.30^{m-p}	51.28^{ab}	4.25^{i-m}	3.094^{a-f}	1.295^{a-e}	4.389^{a-e}	38.72^{lmn}	44.93^{opq}	45.78^{s}
	9	70^{a-e}	342^{l-o}	51.80^{a}	3.72^{lm}	3.221^{a-d}	1.356^{abc}	4.577^{abc}	43.67^{g-k}	49.11^{j-n}	53.77^{k-p}
0 dS m^{-1}	12	71.66^{a-e}	301.60^{op}	46.56^{cde}	4.27^{i-m}	2.985^{e-i}	1.233^{d-h}	4.218^{d-h}	40.25^{j-m}	49.30^{i-n}	48.75^{rs}
	15	76.33^{a}	364^{i-m}	45.70^{c-f}	3.79^{lm}	3.283^{a}	1.391^{a}	4.674^{a}	35.38^{n}	44.08^{pq}	52.06^{n-r}
	17	72.33^{abc}	294.30^{p}	47.82^{a-e}	4.3^{h-m}	3.005^{d-i}	1.234^{d-g}	4.240^{d-h}	39.82^{klm}	44.88^{n-q}	49.17^{qrs}
	19	72.66^{abc}	322.60^{m-p}	46.10^{cde}	4.02^{klm}	3.046^{b-g}	1.260^{b-f}	4.307^{b-f}	37.84^{mn}	48.09^{l-o}	50.64^{o-r}
	20	76^{a}	327.30^{m-p}	51.29^{ab}	4.28^{i-m}	3.14^{a-e}	1.319^{a-d}	4.460^{a-d}	41.03^{i-m}	43.54^{q}	49.39^{qrs}
	4	64.66^{d-i}	318^{m-p}	49.55^{abc}	4.22^{j-m}	3.28^{abc}	1.365^{ab}	4.603^{ab}	40.87^{i-m}	44.70^{opq}	49.87^{p-s}
	5	66.33^{b-g}	427^{a-f}	44.20^{d-h}	4.24^{i-m}	2.831^{g-m}	1.135^{g-k}	3.966^{h-m}	42.30^{h-l}	52.87^{fgh}	51.15^{o-r}
	6	74^{ab}	407^{b-h}	47.56^{b-e}	4.71^{g-k}	3.019^{c-h}	1.262^{b-f}	4.282^{c-g}	42.00^{h-l}	49.42^{i-m}	60^{d-i}
	9	62^{f-k}	334.30^{m-p}	47.59^{b-e}	4.74^{f-k}	3.043^{b-h}	1.26^{b-f}	4.304^{b-f}	45.03^{fgh}	51.95^{f-k}	53.46^{l-q}
4 dS m^{-1}	12	68.66^{a-f}	329^{m-p}	40.25^{h-l}	4.93^{e-j}	2.699^{k-q}	1.074^{j-n}	3.774^{j-p}	47.38^{efg}	45.90^{n-q}	58.11^{f-j}
	15	64^{e-j}	389.60^{f-k}	45.18^{def}	5.05^{e-j}	3.134^{a-f}	1.319^{a-d}	4.453^{a-d}	43.02^{h-k}	46.38^{m-p}	58.06^{f-k}
	17	72.33^{a-d}	415^{b-h}	44.38^{d-g}	5.67^{b-e}	2.886^{f-k}	1.168^{f-j}	4.055^{f-j}	45.01^{fgh}	53.25^{efg}	61.74^{c-g}
	19	61.66^{f-k}	329.60^{m-p}	45.46^{c-f}	4.92^{e-j}	2.736^{j-p}	1.088^{i-m}	3.825^{i-o}	45.023^{fgh}	52.39^{f-j}	57.55^{g-l}
	20	58^{h-n}	357.60^{j-m}	43.71^{e-i}	5.20^{d-g}	2.814^{h-n}	1.163^{f-j}	3.978^{g-l}	43.88^{g-j}	48.62^{k-n}	52.86^{m-r}
	4	53^{l-o}	377.60^{h-l}	44.24^{d-h}	4.59^{g-l}	3.084^{a-f}	1.291^{a-e}	3.375^{a-d}	49.82^{cde}	52.29^{f-j}	54.54^{j-o}
	5	54.66^{k-o}	437^{a-e}	36.06^{m-o}	6.21^{abc}	3.037^{b-h}	1.249^{c-f}	4.287^{b-f}	45.01^{fgh}	56.46^{cde}	56.81^{h-m}
	6	61^{g-k}	384.30^{g-k}	40.55^{g-k}	5.27^{d-g}	2.851^{g-l}	1.148^{f-k}	3.99^{f-k}	44.45^{f-i}	52.63^{f-i}	58.85^{e-i}
	9	60^{g-l}	433.60^{a-e}	43.96^{d-h}	5.72^{b-e}	2.678^{k-q}	1.035^{k-o}	3.713^{k-q}	53.67^{abc}	57.02^{bcd}	57.90^{f-k}
8 dS m^{-1}	12	57^{i-o}	413^{b-h}	41.73^{f-j}	5.12^{e-i}	2.603^{m-q}	1.037^{k-o}	3.641^{n-q}	45.46^{fgh}	49.61^{h-m}	63.76^{a-d}
	15	66^{c-g}	427.60^{a-f}	46.29^{cde}	5.10^{e-j}	2.814^{h-n}	1.119^{h-l}	3.934^{h-n}	50.91^{b-e}	50.43^{g-l}	55.98^{h-n}
	17	55.66^{k-o}	403.60^{c-i}	37.65^{i-m}	5.03^{e-j}	2.613^{m-q}	1.020^{l-p}	3.633^{n-q}	43.44^{h-k}	59.37^{abc}	64.81^{abc}
	19	52.33^{l-o}	408^{b-h}	46.86^{cde}	5.61^{c-f}	2.597^{n-q}	1.019^{l-p}	3.616^{opq}	47.88^{ef}	60.66^{a}	56.31^{h-n}
	20	65^{c-h}	396.30^{e-k}	37.65^{j-n}	5.25^{d-g}	2.935^{e-j}	1.193^{e-i}	4.129^{e-i}	45.45^{fgh}	54.37^{def}	62.58^{b-e}
	4	54.33^{l-o}	429.30^{a-f}	39^{j-m}	5.30^{d-g}	2.470^{pq}	0.969^{nop}	3.483^{pq}	54.60^{ab}	56.7^{bcd}	64.01^{a-d}
	5	52^{mno}	424^{a-g}	32.09^{o}	5.18^{d-h}	2.833^{g-m}	1.132^{g-l}	3.966^{h-m}	49.36^{de}	60.49^{a}	60.9^{d-k}
	6	50.66^{no}	421.60^{a-g}	37.92^{j-n}	6.42^{abc}	2.621^{l-q}	1.037^{k-o}	3.658^{m-q}	48.17^{ef}	61.98^{a}	62.02^{c-f}
	9	51^{mno}	461.30^{a}	36.65^{k-n}	6.21^{abc}	2.778^{i-n}	0.906^{p}	3.685^{l-q}	50.13^{cde}	61.48^{a}	60.23^{f-i}
12 dS m^{-1}	12	51.66^{mno}	444.30^{abc}	34.02^{no}	6.07^{a-d}	2.611^{m-q}	0.992^{m-p}	3.603^{opq}	51.19^{b-e}	53.77^{d-g}	66.56^{ab}
	15	56.66^{j-o}	423.60^{a-g}	36.15^{l-o}	6.65^{a}	2.745^{j-o}	1.087^{i-m}	3.832^{i-o}	48.17^{ef}	59.75^{abc}	58.53^{e-j}
	17	52.33^{l-o}	447.30^{ab}	35.82^{mno}	6.50^{ab}	2.621^{l-q}	1.038^{k-o}	3.659^{m-q}	55.23^{a}	60.81^{a}	64.94^{abc}
	19	58.66^{g-m}	439^{a-d}	41.74^{f-j}	6.86^{a}	2.471^{q}	0.943^{op}	3.414^{q}	52.24^{a-d}	61.78^{a}	6.30^{a-d}
	20	50o	400^{d-j}	34.87^{no}	6.33^{abc}	2.546^{opq}	1.001^{m-p}	3.548^{opq}	56.12^{a}	60.04^{ab}	67.72^{a}

Different letters in each column indicate significant differences according to LSD test ($p<0.05$).

3.2. Relative water content

Higher levels of salinity stress led to a more significant decrease in the relative water content (RWC) of various sugar beet genotypes compared to lower stress levels and the absence of salinity stress (Fig. 1). The maximum RWC (67.85%) was observed

in the control group (no salinity stress), while the minimum RWC (53.73%) occurred under the highest salinity stress (Fig. 1). Among cultivars, cultivar No. 5 exhibited the highest RWC (62.75%), which did not differ statistically from cultivars Nos. 15, 6, 9, 17, 19, and 20. Conversely, cultivar No. 4 displayed the lowest RWC (56.4%) (Supplementary Table S1).

Supplementary Table S1. Comparison of the mean effect of salinity stress on the relative water content and soluble sugars of some different sugar beet genotypes

CUS	Relative water content (%)	Soluble sugars (mg g^{-1} fw)
4	56.4^{b}	---
5	62.75^{a}	---
6	62.16^{a}	---
9	62^{a}	---
12	59.65^{ab}	---
15	61.66^{a}	---
17	61.76^{a}	---
19	61.45^{a}	---
20	62.12^{a}	---
SSR		
0	67.85^{a}	50.77^{d}
4	62.53^{b}	65.44^{c}
8	60.33^{b}	60.35^{b}
12	53.73^{c}	62.81^{a}

Different letters in each column indicate significant differences according to LSD test ($p<0.05$).

The reduction in leaf RWC under salinity stress is a common response to osmotic stress. This decrease can be attributed to diminished water absorption resulting from an elevated osmotic potential caused by soil salinity, which disrupts the balance between water uptake and transpiration, thereby lowering the plant's RWC (Malmir et al., 2021). Increased accumulation of ions, particularly sodium and chloride, due to restricted root systems or limited water availability in stressful conditions, can also contribute to reduced RWC (Rerhou *et al*., 2024). Similarly, Seymen et al. (2023) linked the decline in RWC under salinity stress to decreased water absorption by the plant due to a reduced osmotic potential. Wang et al. (2019) suggested that a high RWC might result from osmotic regulation through the production of compatible osmolytes like soluble sugars, proline, and glycine betaine. This can also be achieved by reducing water loss via stomatal closure or by enhancing water absorption through root expansion in the soil. Furthermore, Wang et al. (2019) attributed the increased RWC in sugar beet genotypes under salt stress to enhanced water absorption facilitated by increased proline accumulation and osmotic regulation.

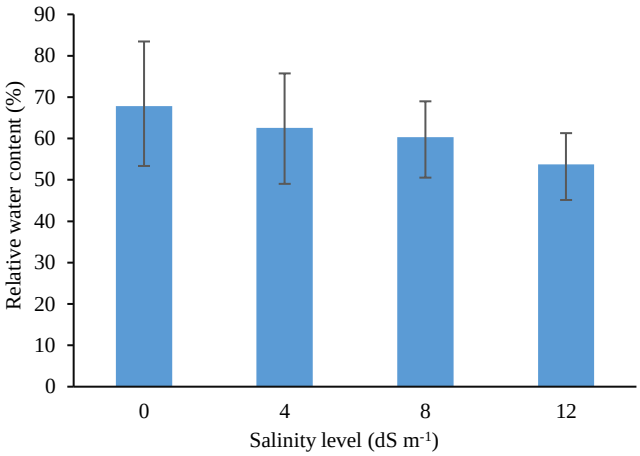

Figure 1. Effect of salinity stress on relative water content (RWC) of sugar beet cultivars.

3.3. Chlorophyll content

The lowest chlorophyll a, chlorophyll b, and total chlorophyll content (2.471, 0.906, and 3.414 mg g^{-1} FW, respectively) were observed in cultivars No. 19 and 9 under a salinity condition of 12 dS m^{-1} (Table 5; Fig. 2). Conversely, the highest content of these traits (3.289, 1.391, and 4.676 mg g^{-1}, respectively) was found in cultivars No. 15 and 4 under conditions with no salinity application (Table 5).

The reduction in chlorophyll content under salinity stress may be attributed to several factors: a decrease in essential elements like manganese, magnesium, nitrogen, and iron within the chlorophyll structure; the destruction of chlorophyll; increased leaf sensitivity to ethylene; and the accumulation of chloride ions (Zhang *et al*., 2020). Furthermore, under saline conditions, nitrate absorption is affected by direct competition from chloride and changes in the plasma membrane (Zhao *et al*., 2020), which can also contribute to decreased chlorophyll content.

Salinity reduces chlorophyll content by disrupting the balance of pigmented protein complexes, inducing chloroplast destruction, and increasing the activity of chlorophyll-degrading enzymes (Zhou *et al*., 2023). Studies have shown that salinity stress decreased chlorophyll in sugar beet genotypes by reducing root weight (Abbasi, 2020). Additionally, salinity-tolerant sugar beet genotypes maintained higher chlorophyll content than other cultivars by preserving their root structure under stress conditions (Abu-Ellail *et al*., 2021).

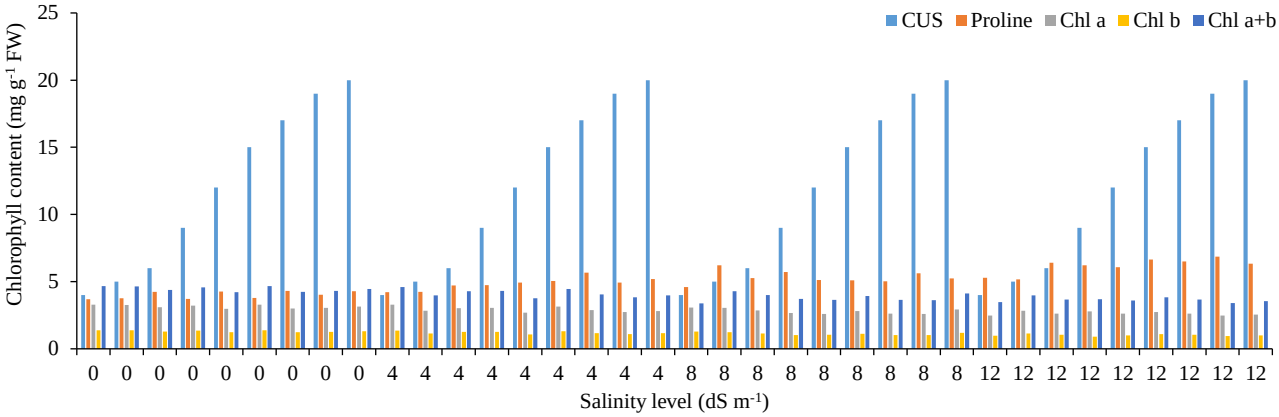

Figure 2. Effect of salinity stress on soluble protein content and proline concentration of different sugar beet cultivars

3.4. Electrolyte leakage

Mean data comparison revealed that electrolyte leakage in various sugar beet genotypes increased under salinity stress (Table 5; Fig. 3). The highest electrolyte leakage (461.3 mS cm^{-1}) was observed in cultivar No. 9 under 12 dS m^{-1} salinity, while the lowest (294.3 mS cm^{-1}) occurred in genotype No. 17 without salinity application (Table 5). This increase in electrolyte leakage under salinity stress is likely due to several factors: accumulation of sodium and chloride ions within cells at higher salinity levels, limiting water absorption by roots, ionic toxicity, increased osmotic pressure, and reduced cell membrane stability, all contributing to enhanced ion leakage (Abu-Ellail *et al*., 2020). Salt stress also reduces the membrane stability index by inhibiting carbonic anhydrase activity, damaging photosystem II, and decreasing chlorophyll synthesis (Alavilli *et al*., 2023). Furthermore, environmental stress appears to increase the production of reactive oxygen species (ROS) within cells, which reduces membrane stability and promotes the leakage of cytoplasmic materials, ultimately increasing electrolyte leakage (Alharbi *et al*., 2022). ROS induce lipid peroxidation in membranes and release potassium ions from the cell wall. Additionally, sodium ions competitively replace potassium at binding sites in the cell membrane; since potassium cannot perform its function, this leads to electrolyte leakage from the cell wall (AlKahtani *et al*., 2021). Aycan et al. (2023) similarly reported that salt stress elevates ROS production, including hydrogen peroxide, which enhances lipid peroxidation and ultimately increases cell membrane damage. Moreover, under salt stress, the decrease in relative leaf water content causes cell wrinkling, leading to a loss of cell membrane stability. Consequently, increased cell membrane permeability results in the leakage of intracellular contents (Bouras *et al*., 2021; El-Kady *et al*., 2021).

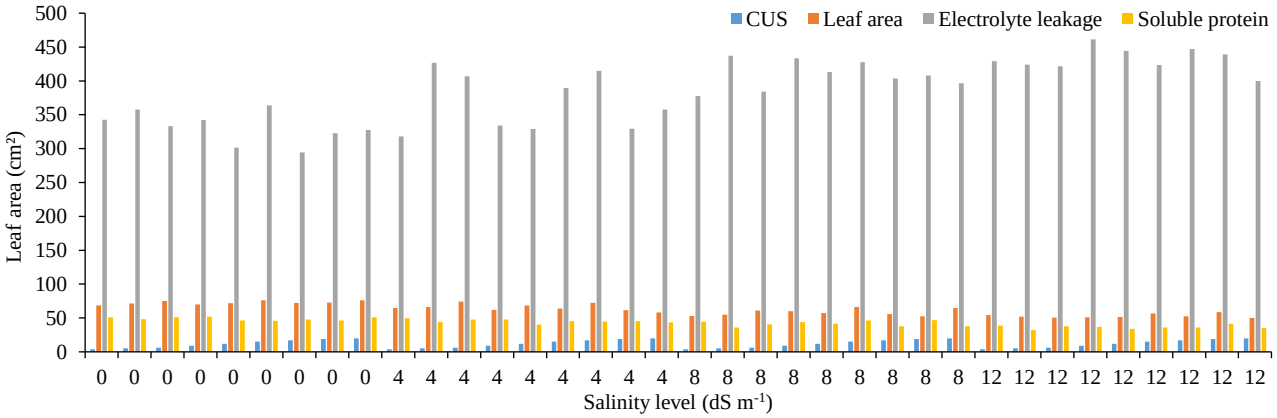

Figure 3. Effect of salinity stress on leaf area and electrolyte leakage of different sugar beet cultivars

3.5. Proline content

Mean comparison results indicated that the highest leaf proline content (6.86 µg g^{-1} FW) was observed in cultivar No. 19 under 12 dS m^{-1} salt stress. This was not statistically significantly different from cultivar No. 15 (Table 5; Fig. 6). Conversely, the lowest content (3.68 µg g^{-1} FW) was found in cultivar No. 4 under no salt stress conditions (Table 5). Dadkhah and Rassam

(2017), in their study on the effect of salinity stress on various sugar beet genotypes, noted that the accumulation of solutes in the root environment under saline conditions reduces plant water absorption. To counteract this, plants synthesize and accumulate compatible osmolytes such as soluble sugars, useful ions, and proline, enabling them to absorb more water (Geng *et al*., 2020). They also reported that salinity stress increases proline content, with the sugar beet cultivar (EC) exhibiting the highest proline content under such conditions (Gholipor *et al*., 2022). Given that salinity stress induces oxidative stress, leading to the formation of reactive oxygen species and stomatal closure, the increased proline content acts as a compatible osmolyte. Proline helps remove reactive oxygen species produced under environmental stress conditions, thereby protecting macromolecules (He *et al*., 2023). Furthermore, proline production is linked to soluble sugar production. One pathway for proline synthesis involves glutamate; as soluble sugar production increases, glutamate production also rises, intensifying proline synthesis (Hossain *et al*., 2021). In line with this, Hozayn et al. (2020) stated that salinity stress increases sugar beet leaf proline content through the disruption of protein synthesis. Other potential reasons for improved proline content under stressful conditions include increased activity of proline-synthesizing enzymes like ornithine aminotransferase, or inhibition of proline-catabolizing enzymes such as proline hydrogenase and proline oxidase (Khayamim, 2022).

3.6. Soluble sugars

A comparison of mean data revealed that a salinity stress of 12 dS m^{-1} increased the soluble sugar content by 71.23% compared to the control condition without salinity stress (Fig. 4). Under 12 dS m^{-1} salinity stress, sugar beet exhibited its highest soluble sugar content at 62.81 mg g^{-1} FW, whereas the lowest content, 50.77 mg g^{-1} FW, was observed in the absence of salt stress (Fig. 5).

Under salt stress, insoluble sugars (starch) are reportedly broken down into soluble sugars, which helps cells maintain osmotic potential and reduces dehydration risk (Khozaei *et al*., 2021). Furthermore, sugars accumulated during salt stress interact with cellular macromolecules, such as enzymes, to establish and stabilize cellular structures and their functions (Kumar *et al*., 2023). The accumulation of soluble sugars during stress can be attributed to the breakdown of insoluble sugars, growth cessation, or the synthesis of these compounds via non-photosynthetic pathways (Malmir *et al*., 2021).

Additionally, reduced water transfer to leaves and disrupted leaf transpiration rates are known to cause ultrastructural changes in cell organelles and alter the behavior of key enzymes involved in sugar metabolism (Abu-Ellail *et al*., 2020). By accumulating soluble sugars, plants maintain optimal carbohydrate reserves to support basic cellular metabolism under environmental stress (Rerhou *et al*., 2024). Consistent with these findings, Aycan et al. (2023) reported that salinity stress significantly increased soluble sugar content in sugar beet genotypes. Similarly, Gholipor et al. (2022) found that salinity stress treatments elevated soluble sugar content in sugar beet genotypes compared to conditions without salinity application.

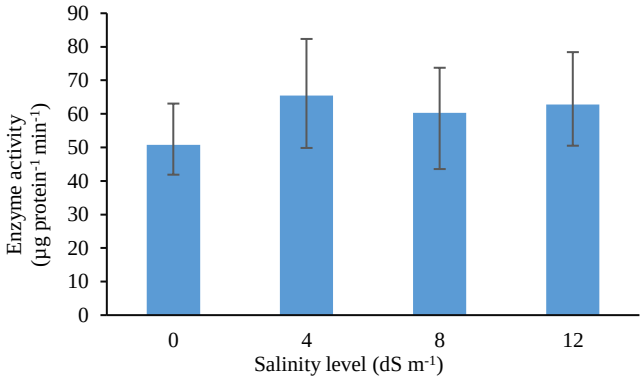

Figure 4. Changes in physiological and biochemical traits of sugar beet cultivars under different levels of salinity stress

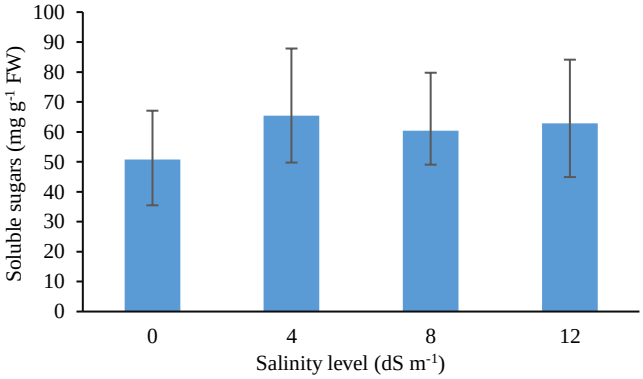

Figure 5. Effect of salinity stress on soluble sugar content of sugar beet leaves

3.7. Soluble protein content

Soluble protein content in sugar beet leaves decreased significantly under high salinity stress (Table 5; Fig. 6). The highest soluble protein content (51.80 mg ml^{-1}) was observed in cultivars No. 9 and 5 without

salt application, while the lowest (32.09 mg ml^{-1}) was found in cultivars No. 6 and 5 under 12 dS m^{-1} salinity conditions (Table 5). Salinity stress impacts nitrogen metabolism in plants, directly affecting the rate of protein and nucleic acid synthesis (Khozaei *et al*., 2021). Some researchers attribute the reduction in leaf soluble protein content to nitrogen accumulation caused by decreased nitrate reductase enzyme activity (Malmir *et al*., 2021). Rerhou et al. (2024) further suggest that this decrease is due to reduced protein synthesis, accumulation of free amino acids like proline, and increased activity of protein-degrading enzymes.

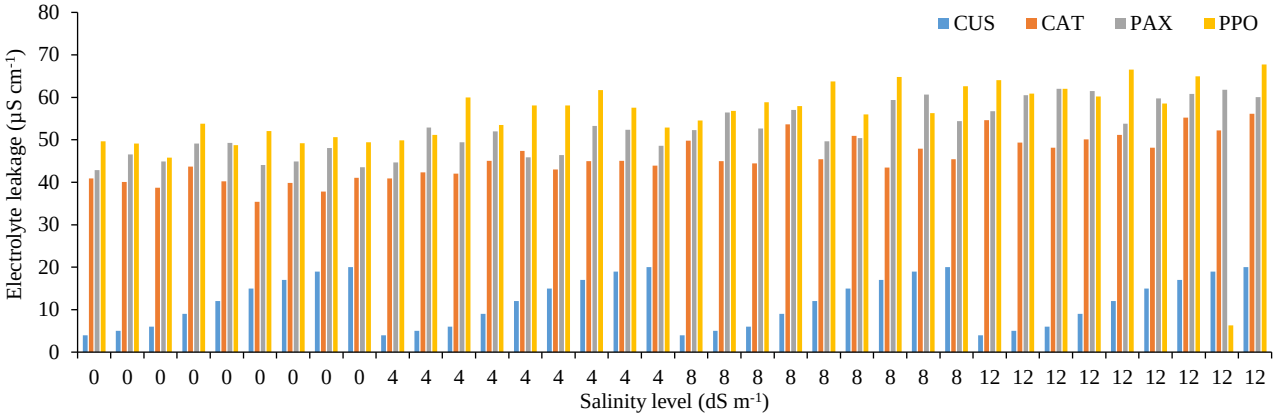

Figure 6. Effect of salinity stress on chlorophyll a, chlorophyll b, and total chlorophyll contents of different sugar beet cultivars

3.8. Antioxidant enzyme activity

According to the results, salt stress significantly increased the activity of antioxidant enzymes in various sugar beet genotypes (Table 5; Fig. 7). The highest catalase activity (52.6 µg protein min^{-1}) was observed in sugar beet cultivar No. 20 under 12 dS m^{-1} salt stress, showing no statistically significant difference from cultivar No. 17 (Table 5). Conversely, the lowest catalase activity (38.35 µg protein min^{-1}) was found in cultivar No. 15 without salt stress (Table 5). Similarly, the highest peroxidase activity (61.98 µg protein min^{-1}) occurred in sugar beet cultivar No. 6 under 12 dS m^{-1} salt stress, with no statistically significant difference from cultivars No. 9, 17, 19, and 20 (Table 5). The lowest peroxidase activity (42.87 µg protein min^{-1}) was recorded in cultivar No. 4 without salt stress (Table 5).

Regarding polyphenol oxidase activity, the highest level (67.72 µg protein min^{-1}) was noted in cultivar No. 20 under 12 dS m^{-1} salinity (Table 5). The lowest activity (45.78 µg protein min^{-1}) was observed in cultivar No. 6 without salinity stress (Table 5). Catalase enzyme plays a crucial role in detoxification by directly breaking down hydrogen peroxide, a toxic substance produced during various cellular mechanisms and natural reactions (El-Mageed *et al*., 2022). Its accumulation is detrimental to cells and tissues, necessitating immediate decomposition. Catalase utilizes hydrogen peroxide as a substrate, rapidly decomposing it to inhibit its destructive effects (El-Mageed *et al*., 2022). As one of the most important enzymes involved in neutralizing reactive oxygen species (ROS), catalase participates in detoxification when ROS levels increase under stress. It catalyzes the decomposition of H_2O_2 into water and oxygen in the peroxisome, protecting cells from oxidative damage caused by H_2O_2 and OH (Aycan *et al*., 2023).

Enzymes like catalase and peroxidase are essential in eliminating reactive oxygen species by reducing hydrogen peroxide to water (Gholipor *et al*., 2022). Peroxidase also contributes to stress responses in higher plants, participating in defense mechanisms, cross-linking of hydroxyproline-rich glycoprotein monomers and pectic polysaccharides in the cell wall, lignification, and cottonwood processes. This helps mitigate the destructive effects of environmental stresses such as salinity and drought (Dadkhah and Rassam, 2017).

Polyphenol oxidase enzymes are oxidoreductases involved in plant responses to abiotic stresses, including drought and salinity, and may contribute to the detoxification of reactive oxygen species (ROS) and the limitation of oxidative damage (Seymen *et al*., 2023). In the present study, the activities of catalase, peroxidase, and polyphenol oxidase generally increased under severe salinity, with the highest activities being observed at 12 dS m^{-1}. The highest catalase activity was recorded in genotype No. 20, whereas genotypes No. 6, 9, 17, 19, and 20 showed

comparatively high peroxidase activity under severe salinity. This response suggests that increasing salinity intensity was associated with an enhanced antioxidant defense capacity.

The physiological significance of this response becomes more evident when the antioxidant enzyme results are considered together with the changes in chlorophyll content and membrane permeability. Salinity imposes both osmotic and ionic stress, which can enhance ROS production and consequently increase oxidative pressure on cellular membranes and chloroplast structures. Excessive ROS accumulation can promote lipid peroxidation, impair membrane integrity, and accelerate the degradation of photosynthetic pigments. Therefore, the increased activities of catalase and peroxidase observed under severe salinity may help maintain cellular redox homeostasis by restricting the accumulation of hydrogen peroxide and other ROS. This mechanism could contribute to the preservation of chloroplast structure and photosynthetic pigments under saline conditions. In the present experiment, however, the activities of antioxidant enzymes were measured directly, whereas ROS accumulation and chloroplast damage were not directly quantified; therefore, the proposed relationship should be considered a physiological interpretation of the observed responses rather than a directly demonstrated molecular mechanism.

The differences among genotypes further support the possibility that antioxidant capacity contributes to differential salinity tolerance. Genotypes No. 6, 9, 17, and 19 exhibited relatively high antioxidant enzyme activities under 12 dS m^{-1} salinity, while chlorophyll content was substantially reduced in genotypes No. 19 and 9 under the same treatment. This finding indicates that antioxidant activation alone may not completely prevent pigment loss under severe salinity, but it may represent an important component of the defense response that limits oxidative injury. Conversely, the relatively high chlorophyll contents observed in genotypes No. 15 and 4 under non-saline conditions, together with their comparatively lower enzyme activities in the absence of stress, indicate that antioxidant enzymes are likely to be particularly important when oxidative pressure increases rather than under unstressed conditions. Thus, the maintenance of photosynthetic pigments under salinity is likely determined by the interaction of antioxidant defense, cellular water status, ion homeostasis, and osmotic adjustment rather than by antioxidant activity alone.

The accumulation of proline and soluble sugars observed in response to increasing salinity provides additional evidence for the involvement of osmotic adjustment in the response of sugar beet to saline conditions. In the present study, proline concentration reached 6.86 μg g^{-1} FW under 12 dS m^{-1} salinity, while soluble sugar content increased by 71.23% relative to the non-saline control. These compatible solutes can contribute to osmotic adjustment by helping maintain cellular water balance and protecting proteins and membranes against the destabilizing effects of reduced cellular water availability. Consequently, osmotic adjustment may act together with antioxidant defense to reduce the physiological consequences of salinity stress. The simultaneous increase in soluble sugars and proline and the decline in relative water content observed in the present study suggest that osmotic regulation was activated as a compensatory response to the increasing osmotic stress imposed by saline conditions.

Hormonal signaling may also participate in coordinating these responses. In particular, abscisic acid (ABA) is a major regulator of plant responses to osmotic stress and can contribute to stomatal regulation, water conservation, osmotic adjustment, and activation of antioxidant defense systems. Under saline conditions, enhanced ABA signaling may promote stomatal regulation and reduce excessive water loss while coordinating metabolic responses associated with the accumulation of compatible solutes and ROS-scavenging enzymes. Although ABA concentration and hormone-related gene expression were not measured in the present study, the observed changes in relative water content, proline, soluble sugars, and antioxidant enzyme activities are physiologically consistent with the involvement of osmotic and hormone-mediated stress responses.

Overall, the results indicate that salinity tolerance in sugar beet is not controlled by a single physiological trait but rather involves the coordinated activation of several defense mechanisms. Increased antioxidant enzyme activity may help control oxidative stress, while proline and soluble sugars contribute to osmotic adjustment and maintenance of cellular water balance. Together, these mechanisms may help preserve

membrane function and photosynthetic activity under saline conditions. The stronger antioxidant responses observed in several genotypes at 12 dS m^{-1} suggest that these genotypes possess a greater capacity to activate biochemical defense mechanisms when exposed to severe salinity. Nevertheless, because hormonal signaling, ROS accumulation, ion concentrations, and gene expression were not directly measured, these mechanisms should be considered plausible physiological explanations for the observed phenotypic and biochemical responses

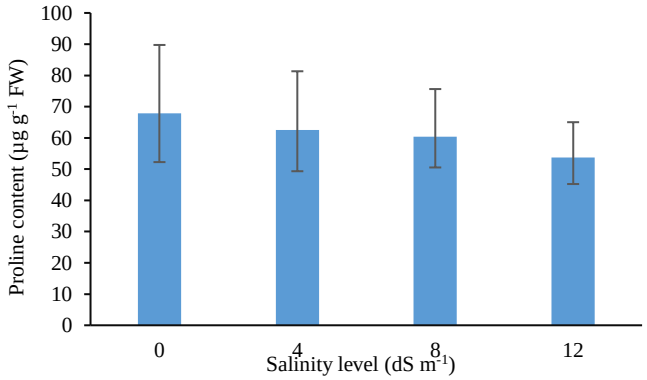

Figure 7. Effect of salinity stress on catalase (CAT), peroxidase (PAX), and polyphenol oxidase (PPO) activities of different sugar beet cultivars.

4. Conclusions

This research found that increased salinity stress elevated compatible osmolytes (proline, electrolyte leakage, and soluble sugars) and antioxidant enzyme activity (catalase, peroxidase, and polyphenol oxidase). Concurrently, salinity stress reduced chlorophyll a, chlorophyll b, total chlorophyll, relative water content, and leaf area in various sugar beet genotypes. Consequently, sugar beet genotypes 6, 9, 17, and 19 enhance their tolerance to high salinity stress by improving compatible osmolyte content, boosting antioxidant activity, and increasing photosynthetic pigment levels.

Conflict of interests

The authors declare that they have no recognized competing financial interests that could have seemed to impact the work reported in this paper. There are no conflicts of interest that appear for the manuscript submission.

Ethics approval and consent to participate

No humans or animals were used in the present research. The authors have adhered to ethical standards, including avoiding plagiarism, data fabrication, and double publication.

Consent for publications

All authors read and approved the final manuscript for publication.

Availability of data and material

All the data are embedded in the manuscript.

Authors' contributions

Actual experimentation, writing a review, and writing an original draft, were done by Pariya Nouri; Help in recording observation by Pariya Nouri: Help in writing and recording the observation in the manuscript by Sodabeh Jahanbakhsh, Salim Farzaneh: Statistical and data analysis by Saeid Heydarzadeh, Sodabeh Jahanbakhsh, Salim Farzaneh; Help in editing the manuscript by Saeid Heydarzadeh; Help in recording observation by Sodabeh Jahanbakhsh, Salim Farzaneh: Tabulation and data analysis by Pariya Nouri; Help in field study during irrigation by Sodabeh Jahanbakhsh, Salim Farzaneh; Chemical analysis done by Pariya Nouri: Help in writing and editing the manuscript by Saeid Heydarzadeh, Sodabeh Jahanbakhsh, Salim Farzaneh: Conceptualization and Supervision by Sodabeh Jahanbakhsh, Salim Farzaneh. All the authors agree to the submission of the manuscript.

Informed consent

The authors declare not to use any patients in this research.

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