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Strigolactone (GR24) Application Positively Regulates Photosynthetic Attributes, Stress-Related Metabolites and Antioxidant Enzymatic Activities of Ornamental Sunflower (*Helianthus annuus* cv. Vincent's Choice) under Salinity Stress

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Abstract: Strigolactones, a new group of phytohormones, are reported to improve plant tolerance to multiple abiotic stresses. A pot experiment was conducted to investigate the impact of synthetic strigolactone (GR24 at 0.001, 0.01 and 0.1 mg L^{-1}) application on ornamental sunflowers (*Helianthus annuus* cv. Vincent's Choice) grown under salt stress (150 mM NaCl). Salt stress was applied after 14 days, and SL was applied 25 days seed sowing. The results showed that amongst various GR24 concentrations, 0.01 mg L^{-1} proved to be superior, as it enhanced the photosynthetic rate (9.29%), transpiration rate (0.76%), stomatal conductance (77.5%), total soluble protein (0.55%) and K^+ (14.63% in roots; 14.87% in shoots) and Ca^{2+} (12.63% in roots; 11.48% in shoots) contents under control conditions. Similarly, the leaf turgor potential (Ψ_p), osmotic potential (Ψ_s) and free proline, glycinebetaine (GB), superoxide dismutase (SOD), catalase (CAT) and peroxide (POD) contents increased by 58.17, 89.95, 159.04, 101.54, 74.42, 175.68 and 53.62%, respectively, under salt stress conditions. The leaf water potential (Ψ_w) decreased (−0.14%) and the malondialdehyde (MDA) content increased (16.65%) when treated with the 0.001 mg L^{-1} GR24 level. Meanwhile, hydrogen peroxide (H_2O_2) and Na^+ concentrations in roots and shoots increased by 62.53%, 74.66% and 38.55% under saline conditions with a GR24 level of 0 mg L^{-1}. Regarding the plant biomass, a GR24 level of 0.01 mg L^{-1} with salt stress greatly decreased the root (−47.27% and −50.45%) and shoot (−44.79% and −59.42%) fresh and dry weights, respectively, compared to control conditions. These results reveal that exogenously applied GR24 might be an effective way to mitigate the perilous impacts of salt stress in ornamental sunflower production. It is suggested that the use of molecular techniques to study different processes in which GR24 could play a vital part in various commercial floricultural crops is extremely imperative and can open novel horizons for future investigations in this exhilarating field of plant hormones.

Keywords: antioxidants; floriculture; phytohormone; strigolactone; salt stress; water relation

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1. Introduction

Increased soil salinization significantly hampers crop growth and productivity, particularly in arid and semi-arid regions [1,2]. Globally, about 20% of the cultivated land has been reported to be salt-affected [3], which causes more than USD 12.5 billion in yearly losses due to crop productivity losses [4]. Salt-induced crop yield losses are largely attributed to specific ion toxicity, impaired CO_2 exchange, reduced photosynthetic performance and other physiological and metabolic consequences [5,6]. The enhanced production of reactive oxygen species (ROS) under salt stress also contributes to the oxidative stress load of crop plants [2]. To cope with salinity stress, plants have evolved different tolerance mechanisms, such as degrading proteins and nucleic acids. Plants also induce lipid peroxidation and the activation of enzymatic and non-enzymatic antioxidant defense systems for ROS removal and the maintenance of redox homeostasis [7,8].

Sunflower (*Helianthus annuus* L.) is the most vital crop for the production of oilseeds across the globe [3,9]. It is an extremely nutritive crop and a rich source of antioxidants, as well as polyunsaturated fatty acids [10]. Furthermore, the advancement of floriculture has also led to the use of sunflower as an ornamental plant. Thus, the ornamental sunflower is an extremely esteemed specialty cut-flower plant used for vase decorations in pots, for interior decoration and as garden plants due to its various colors and the liveliness of its inflorescence [11]. Flower growers are mainly interested in this crop due to its flower color, flower size, straight and long stem and decreased branching [12]. However, floricultural cut-flower crops are reported to be salt sensitive, and farmers have been apathetic to endangering the flower quality and economic output by growing them under saline soil conditions [13].

Phytohormone biosynthesis and signaling under abiotic stresses are well known to regulate plant growth and development [14,15]. Strigolactones (SLs), carotenoid-derived terpene lactones, were first isolated from a root culture solution of *Gossypium hirsutum* in the 1960s [16]. Later, in 2008, SLs were recognized as novel hormones that could suppress the generation of branches in higher plants [17]. GR24, a synthetic SL, is involved as a positive regulator in response to salt stress [18]. Under nutrient-deficient conditions, SLs released from the plant roots enhance the development of lateral roots and root hairs, which, in turn, improves nutrient uptake [2]. Simultaneously, SLs translocated to aboveground plant parts stifle lateral bud or branch generation and decrease the inorganic nutrient requirements of the branches [19,20]. Due to the minute concentrations of SLs in different plants, a series of SLs, such as GR5, GR7 and GR24, have been chemically manufactured, among which GR24 had the maximum activity [21]. GR24 application improved chlorophyll a and b contents as well as augmented the transpiration rate and stomatal conductance in tomato under salinity stress [22]. SLs proved to be an imperative signaling molecule required for the synthesis of photosynthetic pigments during salt stress [22]. SLs also significantly release the salt stress suppression by elevating SOD and POD activities in rice [2] and rapeseed [23]. SLs could also lessen salt stress by regulating the antioxidant system in tomato seedlings [22]. The foliar application of GR24 was previously reported to regulate growth responses in Arabidopsis [21] and sunflower [24] exposed to salt stress.

To overcome the negative impacts of salt stress on crop growth and productivity, plant scientists have developed different conventional and contemporary techniques to screen for salt-tolerant and salt-resistant varieties [25]. Previous studies quantified the implementation of tissue culture and other laboratory techniques for the screening of genotypes by germinating cells on severely salty media to pick tolerant cells, which redevelop salt-tolerant plant varieties [26]. Therefore, the current investigation was carried out to explore the impact of GR24 on photosynthesis, water relations, antioxidant enzymatic activities and mineral ion composition in ornamental sunflowers (*Helianthus annuus* cv. Vincent's Choice) under salinity stress.

2. Materials and Methods

2.1. Experimental Area, Seed Material and Layout

A pot experiment was conducted in a lath-house of the Old Botanical Research Area (31°24′ N, 72°09′ E, 300 m above average sea level) at the University of Agriculture, Faisalabad, Pakistan, to understand the interaction between selected levels of salinity and SLs. Sunflower seeds of Vincent's Choice (F1) were purchased from Sunny View Seed Company, Lahore, Pakistan. Pots were arranged according to a two-factor factorial completely randomized design (CRD) with four replications. There were eight pots in each treatment, and each pot contained 10.0 kg of sterilized canal sand. In pots, seeds were sown during the second week of September 2019. After germination, three seedlings of equal size were maintained in each pot until the harvesting of fully opened flowers (64 to 78 days after sowing). Essential nutrients were supplied by Hoagland's nutrient solution (full strength) equally to each pot [27].

2.2. Chemical Materials

Except for GR24, all chemicals were acquired from Sigma, Germany. The chemicals and their CAT numbers are as follows: BAP (Benzyl amino purine, CAT # 13151); NAA (Naphthalene acetic acid, CAT # 80862005); GR24 (obtained from the Department of Organic Chemistry, Radhoud University Nijmegen, The Netherlands); NBT (Nitroblue tetrazolium, CAT # 124823500); Salfosalicylic acid (CAT # 8006910100); 2,4-dichlorophenoxy acetic acid (CAT # D70724); Methionine (CAT # M9500); Toluene (CAT # 244511); Guaiacol (CAT # W253200); and Trichloroacetic acid (CAT # 8223420250). Strigolactone (GR24) was provided by Professor Dr. B. Zwanenburg, Department of Organic Chemistry, Radboud University Nijmegen, Holland.

2.3. Application of Salinity and Strigolactone (GR24)

Two salinity levels, i.e., non-saline and 150 mM NaCl, were applied two weeks after sowing. About 50 mM NaCl was applied thrice at three-day intervals to reach the 150 mM salt level. The potting sand was moistened daily by adding 250 mL of distilled water. Four SL (GR24) concentrations (0, 0.001, 0.01 and 0.1 mg L^{-1}) were foliar-applied (25 days after germination) twice at three-day intervals at 25 mL pot^{-1} during the vegetative stage of plants.

2.4. Measurement of Photosynthetic Attributes and Water Relations

An infrared gas analyzer (IRGA) (LCA-4, Analytical Development Company, Hoddesdon, UK) was used for the measurement of the photosynthetic rate (*A*), transpiration rate (*E*) and stomatal conductance (g_s). Water potential (Ψ_w) was determined with the help of a pressure chamber (Plant Moisture Stress (PMS) Instrument Company, Model 670, Albany, USA). Leaf tissues were frozen (at −80 °C) for two weeks, and leaf Ψ_w was extracted for the determination of osmotic potential (Ψ_s) with the help of a Wescor Vapor Pressure Osmometer (Model VAPRO 5520, El Cajon, CA, USA). Leaf turgor potential (Ψ_p) was calculated as $\Psi_p = \Psi_w - \Psi_s$.

2.5. Determination of Stress-Related Metabolites (Proline and Malondialdehyde)

Free proline contents in leaves were determined by following the protocol described by Bates et al. [28]. For this purpose, 500 mg leaf samples were extracted using 10 mL of 3% (*w*/*v*) sulfosalicylic acid (MP, Biomedicals, Inc., Irvine, CA, USA), and 2.0 mL of crushed filtered samples in a test tube was taken along with 2.0 mL of GAA (glacial acetic acid) and acid ninhydrin. This reaction was undertaken at 100 °C and completed in an ice-filled container. Toluene (4.0 mL) was added, and aliquots were vortexed. The OD of the filtrate was measured at 520 nm, while toluene was utilized as a blank, and proline was calculated.

Malondialdehyde (MDA) was determined by taking the extract of a fresh leaf sample (500 mg) in 5 mL of 1.0% (*w*/*v*) TCA (MP Biomedicals, de Kayserberg Illkirch, France). The homogenate was centrifuged at 20,000× *g* for 15 min (Model Sigma 3K30, Bremen, Germany). The supernatant (500 µL) was reacted with 2 mL of 0.5% TBA (2-thiobarbituric

acid) (Sigma-Alderich Chemie GmbH, Steinheim, Germany) in 20% TCA. At 100 °C, the leaf sample was subjected to a shaking water bath for 1 h. Afterwards, the reaction was stopped by cooling the samples in ice and centrifuging them at 1000× *g* for 10 min, and the OD of the filtrate was recorded at 532 and 600 nm.

2.6. Determination of Glycinebetaine (GB), Total Soluble Protein and Hydrogen Peroxide (H_2O_2)

Glycinebetaine (GB) was determined by placing 0.5 g of dry leaf material in 10 mL of toluene (0.5%) and keeping it overnight at 4 °C. About 1.0 mL of the filtrate was reacted with 1.0 mL of H_2SO_4. In a test tube, this extract (0.5 mL) was taken, 200 µL of a solution of KI_3 was added, and in a chiller, all contents were cooled. Ice-cooled deionized H_2O (2.8 mL) and 1–2 di-chloroethane (5.0 mL) were added. The organic layer absorbance was noted at λ 365 nm using a spectrophotometer. The GB concentration was verified with a curve by following Grieve and Grattan [29]. Furthermore, total soluble protein was determined by taking a fresh leaf sample (500 mg) and extracting it with 50 mM potassium phosphate (10 mL) buffer in an ice bath. The aliquot was centrifuged at 4 °C for 15 min at 10,000× *g*. The protein content in the extract was determined following Bradford [30]. Hydrogen peroxide (H_2O_2) was determined by taking 0.5 g of tissues of fresh leaves, which were crushed in a chilled mortar with 5 mL of 0.1% (*w*/*v*) trichloroacetic acid (TCA). The mixture was centrifuged at 12,000× *g* for 15 min. After vortexing, the OD of the blend was noted at 390 nm, and H_2O_2 was calculated by following the procedure explained by Velikova et al. [31].

2.7. Determination of Antioxidant Enzymatic Activities

Fresh leaf tissues (500 mg) were extracted in 10 mL of phosphate buffer (50 mM; pH 7.8). At 4 °C, the extract was centrifuged at 15,000× *g* for 10 min. The supernatant was separated and used for the determination of enzyme activities, i.e., superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT). For SOD, the photoreduction inhibition of nitro blue tetrazolium (NBT) was used by following the method described by Giannopolities and Ries [32]. Distilled water (100 µL), NBT (50 µL), methionine (100 µL), phosphate buffer at pH 7.6 (500 µL) and the sample extract (50 µL) were mixed in cuvettes that were kept for 20 min under light. SOD in the irradiated aliquot was read at 560 nm.

The activities of CAT and POD were calculated in accordance with the method described by Chance and Maehly [33]. The CAT reaction mixture (2 mL) containing phosphate buffer (50 mM) having a pH of 7.0 and H_2O_2 (5.9 mM) was taken, and the reaction was started by dissolving an aliquot (100 µL) of enzyme extract. The reduction in OD was recorded spectrophotometrically every 20 s for 2 min at 240 nm. For the determination of POD, 1.0 mL of the reaction mixture possessed 750 µL of phosphate buffer (50 mM; pH 5.0), 100 µL of H_2O_2 (40 mM), 100 µL of guaiacol (20 mM) and 100 µL of the extract of the enzyme. The OD of the reaction mixture was recorded every 20 s for 3 min at 470 nm.

2.8. Mineral Ion (Na^+, K^+ and Ca^{2+}) Quantification

Dry root and shoot samples (0.1 g) were digested in 2 mL of a digestion mixture (HNO_3 and $HClO_4$ in a ratio of 5:2) for 25 h. After cooling, 0.5 mL of perchloric acid was added to decolorize the mixture, and a final volume of 50 mL was made using distilled water. Sodium (Na^+), potassium (K^+) and calcium (Ca^{2+}) ions were quantified using a flame photometer (Jenway PFP 7, Cadmus, Chelmsford, England).

2.9. Statistical Analysis

All recorded data means were analyzed using the LSD test at a 5% probability level using STATISTIX software (version 8.3) and the analysis of variance (ANOVA) technique.

3. Results

3.1. Role of Exogenous GR24 Application in Photosynthetic Attributes and Water Relations of Sunflower under Salt Stress

The analysis of variance shows the mean squares of different physio-biochemical characteristics of ornamental sunflowers with numerous levels of GR24 under control and salinity stress conditions, indicating variable impacts of the treatments on the photosynthetic attributes and water relations of sunflower (Table 1). Furthermore, the imposition of salt stress significantly reduced the photosynthetic rate, transpiration rate and stomatal conductance at all GR24 levels. The highest reduction in the photosynthetic rate (−33.36%) under salt stress was recorded without GR24, while the transpiration rate and stomatal conductance under salt stress were reduced by −18.72% and −77.5%, respectively, at a GR24 concentration of 0.01 mg L^{-1} compared to control conditions (Figure 1). Statistically highly significant ($p < 0.001$) results were obtained for Ψ_w, Ψ_s and Ψ_p under salt stress compared to control conditions (Table 1). The application of all GR24 levels significantly enhanced Ψ_s and Ψ_p by up to 99.36% and 135.78%, respectively, whereas Ψ_w was reduced by −71.06%, −79.33% and −60.66% under saline conditions with 0.001, 0.01 and 0.1 mg L^{-1} GR24 applications, respectively (Figure 2).

Table 1. Analysis of variance (ANOVA) for different physiological and biochemical characteristics of ornamental sunflower with various GR24 levels (0, 0.001, 0.01 and 0.1 mg L^{-1}) under saline and non-saline conditions.

Source of Variation	Photosynthetic Rate (μmol CO_2 m^{-2} s^{-1})	Transpiration Rate (mmol H_2O m^{-2} s^{-1})	Stomatal Conductance (mmol m^{-2} s^{-1})	Ψ_w (−MPa)	Ψ_s (−MPa)	Ψ_p (−MPa)	Proline (μmol g^{-1} f.wt.)
Salt stress	424.86 **	2.12 **	17578.13 **	0.102 **	2.797 **	3.968 **	8173.56 **
GR24	94.55 **	0.03 ^{ns}	78.13 ^{ns}	0.003 **	0.161 **	0.124 **	1114.92 **
S × GR24	7.07 ^{ns}	0.05 *	936.46 **	5.736 **	0.063 **	0.056 **	577.219 **
Error	5.879	0.017	132.291	1.767	0.001	0.001	1.780
Source of variation	MDA (μmol g^{-1} f.wt.)	GB (μmol g^{-1} dry wt.)	TSP (mg m^{-1} f.wt.)	H_2O_2 (μmol g^{-1} f.wt)	SOD (Units mg^{-1} protein)	POD (Units mg^{-1} protein)	CAT (Units mg^{-1} protein)
Salt stress	93.213 **	2478.406 **	3.485 **	15292.74 **	1674.03 **	239.92 **	4368.37 **
GR24	142.011 **	1185.009 **	4.155 **	639.68 **	1194.86 **	2.71 **	659.46 **
S × GR24	14.346 **	173.373 **	0.453 **	110.83 **	188.41 **	0.42 ^{ns}	99.37 **
Error	2.888	2.054	0.066	2.783	5.076	0.141	9.178

The asterisks indicate statistically significant differences among the various treatments at probability level $p < 0.01$ according to least significant difference (LSD) test. Ψ_w: Water potential; Ψ_s: osmotic potential; Ψ_p: turgor potential; MDA: malondialdehyde; GB: glycinebetaine; TSP: total soluble protein; H_2O_2: hydrogen peroxide; SOD: superoxide dismutase; POD: peroxidase; CAT: catalase; ** highly significant; * significant; ^{ns} non-significant.

3.2. Effect of Exogenous GR24 Application on Stress-Related Metabolites (Free Proline and MDA) of Sunflower under Salt Stress

Stress-related metabolites showed statistically highly significant ($p < 0.01$) results under both growing conditions (Table 1). The highest proline content was recorded under saline conditions when GR24 was applied at 0.01 mg L^{-1}, followed by 0.1 mg L^{-1}. There were 159.04% and 173.56% increments in free proline contents under saline conditions, respectively (Figure 3). Parallel outcomes were also obtained for MDA values, which increased with the GR24 level under salt stress as compared to control conditions. The highest MDA content was recorded when the 0.001 mg L^{-1} GR24 level was applied under salt stress, whereas the minimum value was recorded under control conditions with 0.001 mg/L GR24 (Figure 3).

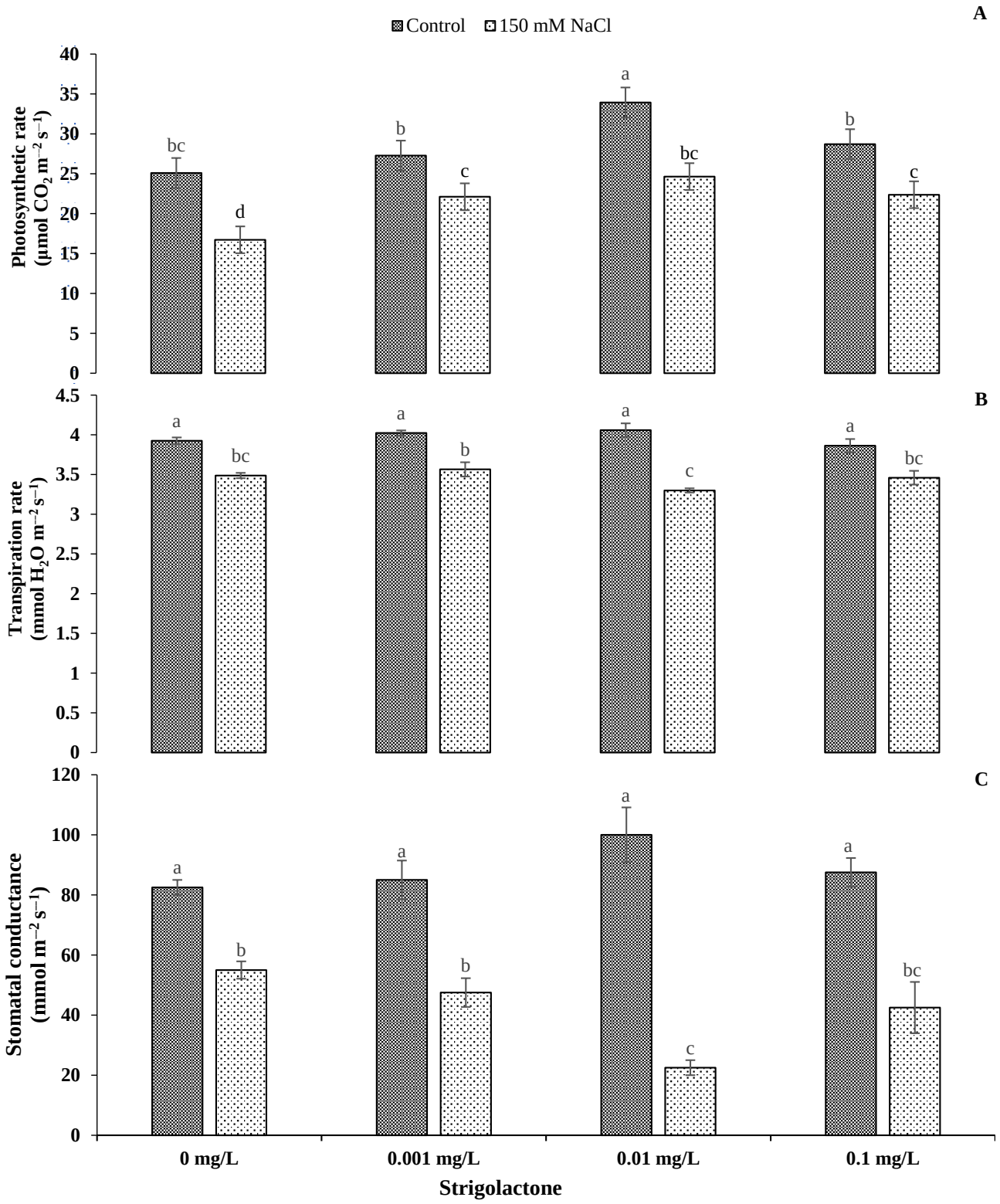

Figure 1. Impact of GR24 on physiological characteristics, i.e., (**A**) photosynthetic rate (*A*), (**B**) transpiration rate (*E*) and (**C**) stomatal conductance (g_s) of ornamental sunflower under control and salt stress conditions. Each vertical bar shows the mean of three replicates. Different letters indicate significant differences among treatments at $p \leq 0.01$ according to least significant difference test.

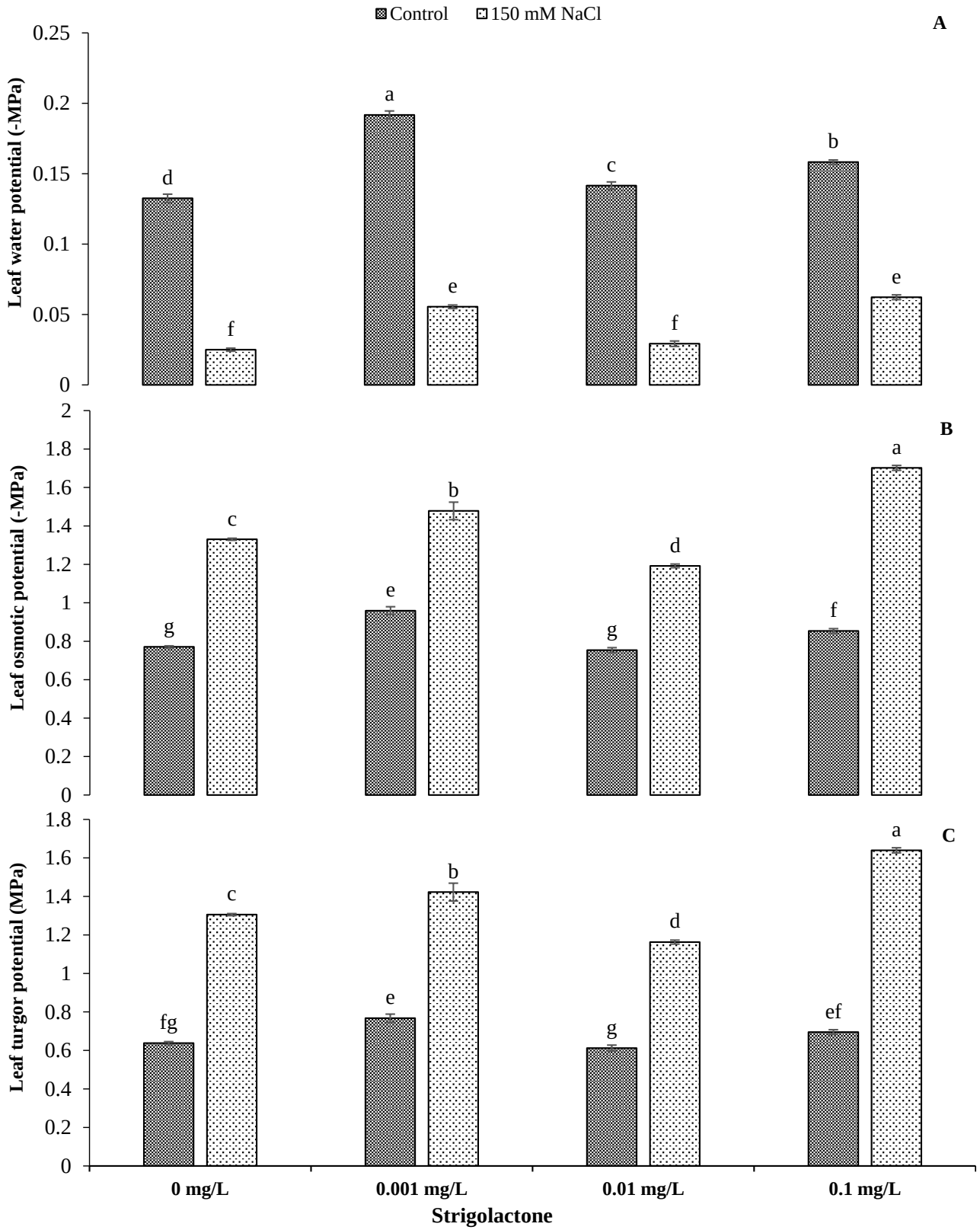

Figure 2. Impact of GR24 on leaf water potential (**A**), leaf osmotic potential (**B**) and leaf turgor potential (**C**) of ornamental sunflower under control and salt stress conditions. Each vertical bar shows the mean of three replicates. Different letters indicate significant differences among treatments at $p \leq 0.01$ according to least significant difference test.

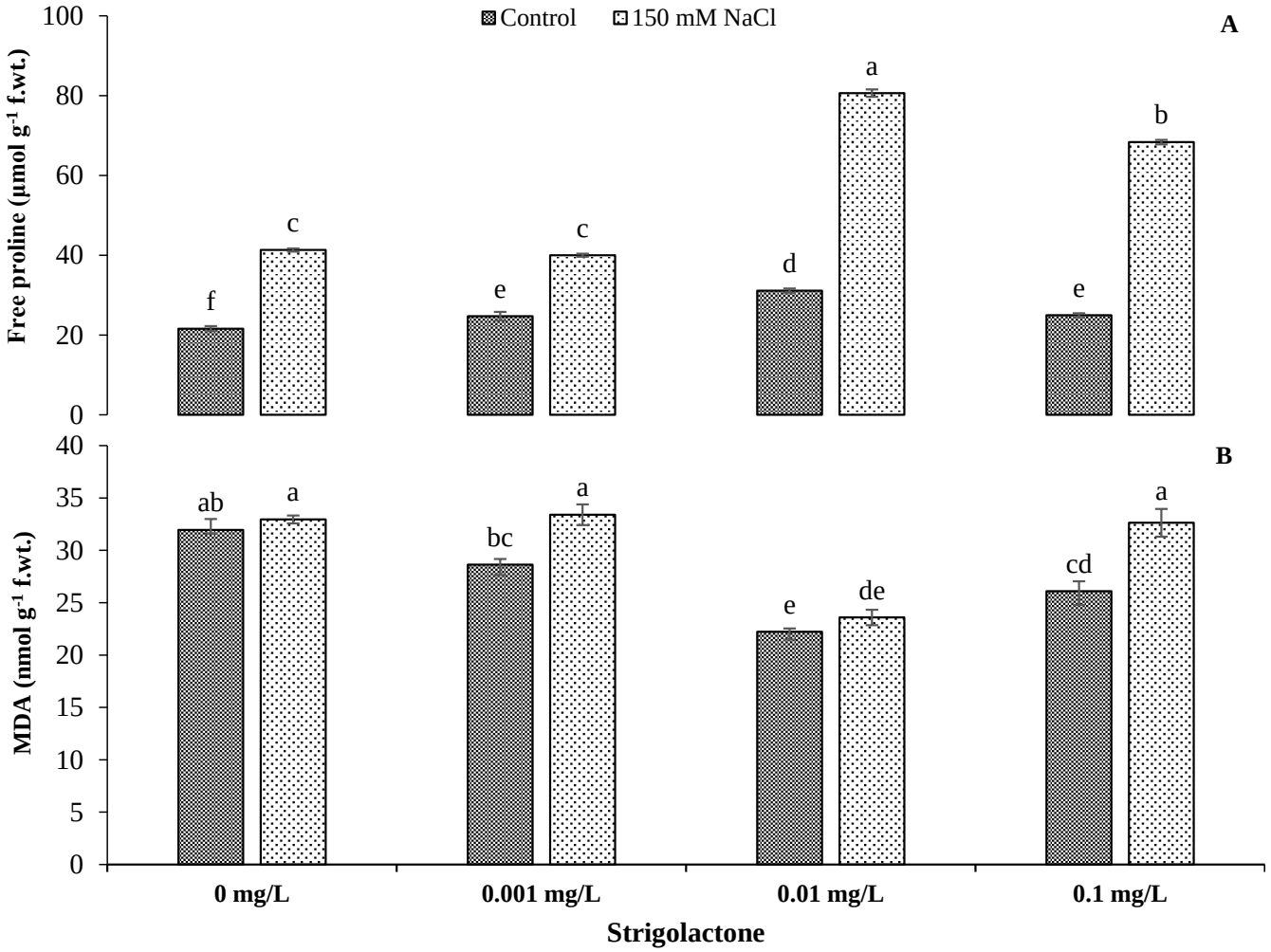

Figure 3. Impact of GR24 on free proline content (**A**) and malondialdehyde (**B**) in ornamental sunflower under control and salt stress conditions. Each vertical bar shows the mean of three replicates. Different letters indicate significant differences among treatments at $p \leq 0.01$ according to least significant difference test.

3.3. Impact of GR24 Application on GB, Total Soluble Protein and H_2O_2 under Salt Stress

Salt stress and GR24 showed a statistically highly significant ($p < 0.01$) impact on GB, total soluble protein and H_2O_2 (Table 1). The results showed increments in GB and H_2O_2 under saline conditions compared to the control with the application of GR24. The highest GB content was noted with the 0.01 mg L^{-1} GR24 concentration under salinity conditions. There was a 101.54% increase in the GB level compared to control conditions, whereas the lowest GB value was recorded under control conditions without GR24 application (Figure 4). Similar results were also noted for the H_2O_2 content with GR24 application under salt stress conditions. There were 70.61%, 53.03% and 62.66% increases in the H_2O_2 level under saline conditions with 0.001, 0.01 and 0.1 mg L^{-1} GR24 application, respectively (Figure 4). There was a significant drop in the total soluble protein content under salt stress conditions. The application of GR24 slightly improved the soluble protein level, but the highest value was recorded under control conditions. There was a 39.36% reduction in total soluble protein in the control treatment under salt stress (Figure 4).

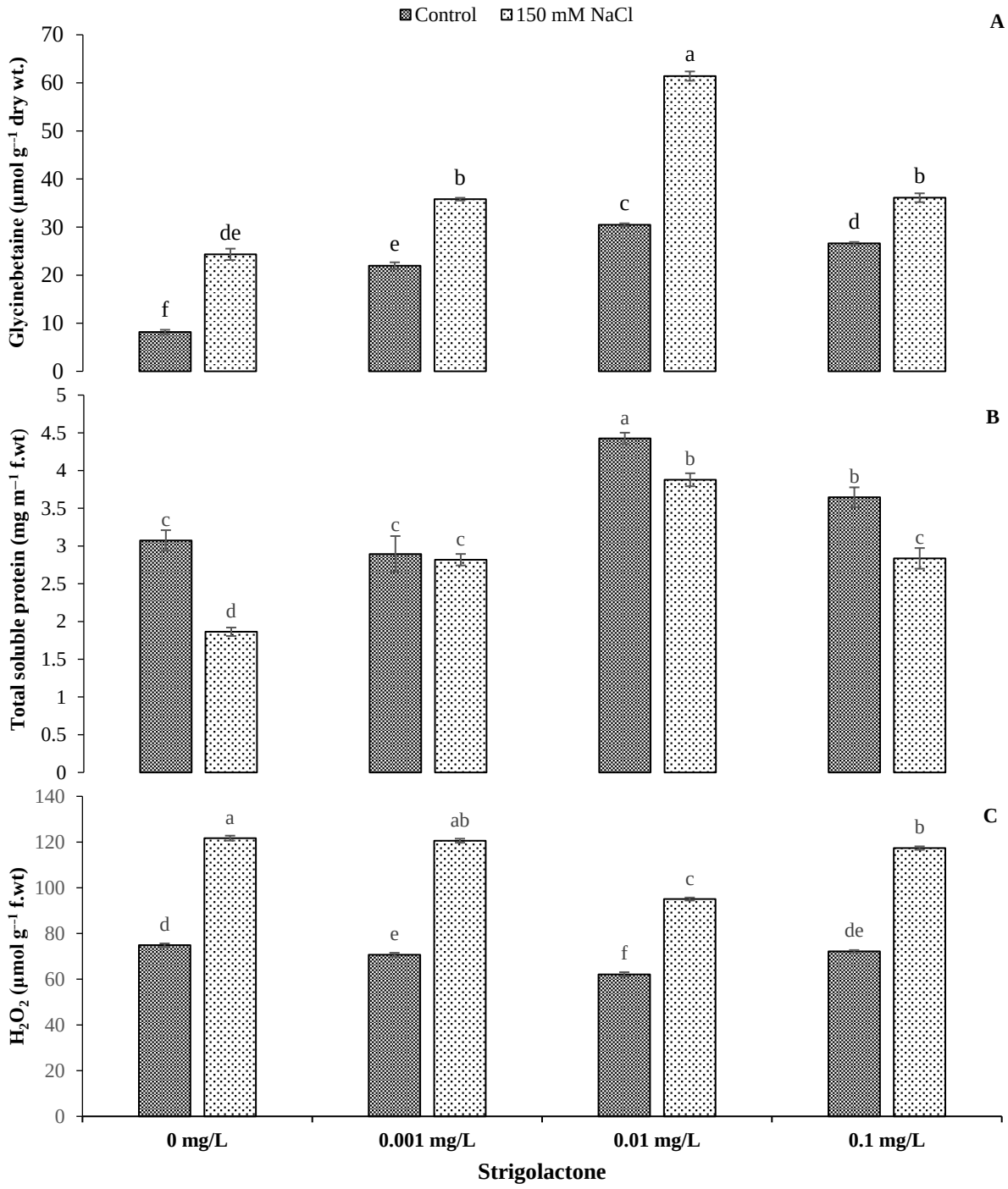

Figure 4. Impact of GR24 on glycinebetaine (**A**), total soluble protein (**B**) and hydrogen peroxide (**C**) of ornamental sunflower under control and salt stress conditions. Each vertical bar shows the mean of three replicates. Different letters indicate significant differences among treatments at $p \leq 0.01$ according to least significant difference test.

3.4. Impact of GR24 on Antioxidant Enzymatic Activities of Sunflower under Salinity Stress

A statistically highly significant ($p < 0.01$) effect of GR24 and salt stress was recorded for antioxidant enzymatic activities (Table 1). The application of salinity led to a notable enhancement of the activities of SOD, CAT and POD compared to the control treatment. GR24 applied at 0.01 mg L^{-1} showed the highest antioxidant values compared to other levels with salt stress conditions. There were 74.42%, 53.62% and 175.68% increases in SOD, CAT and POD contents under salt stress, respectively, compared to control treatments at

the said GR24 level. The lowest values of these observations were recorded under control conditions without GR24 application (Figure 5).

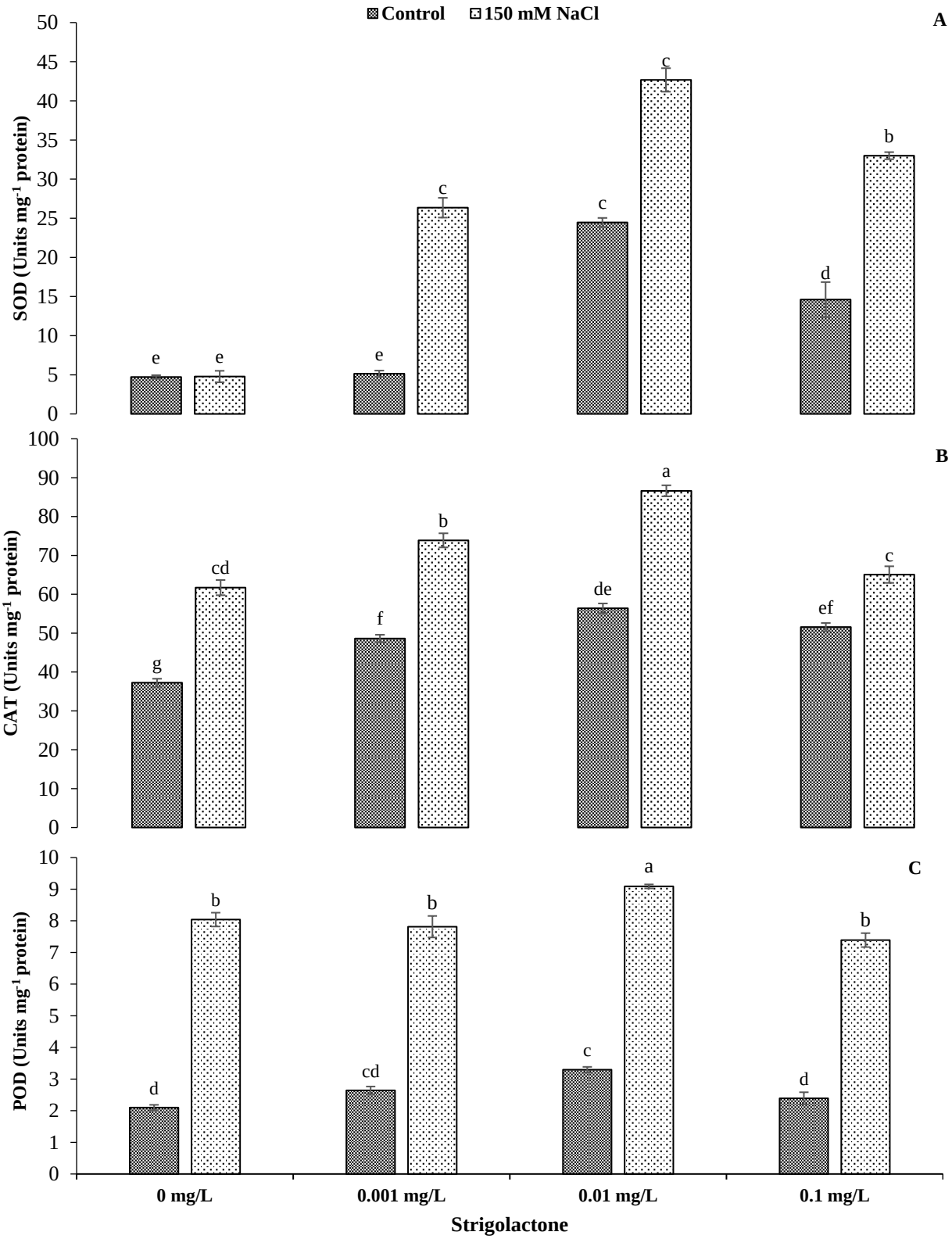

Figure 5. Impact of GR24 on antioxidant enzymatic activities, i.e., superoxide dismutase (**A**), catalase (**B**) and peroxide (**C**), in ornamental sunflower under control and salt stress conditions. Each vertical bar shows the mean of three replicates. Different letters indicate significant differences among treatments at $p \leq 0.01$ according to least significant difference test.

3.5. Impact of GR24 Application on Quantification of Mineral Ions in Roots and Shoots of Ornamental Sunflower under Salt Stress

The application of GR24 showed the elevation of Na^+ contents in the roots and shoots of ornamental sunflowers. A reducing trend was recorded in Na^+ content due to an increment in the GR24 level. There were 69.57%, 69.23% and 63.28% increases in root Na^+ contents and 34.56%, 32.46% and 29.74% increases in shoot Na^+ contents with GR24 applications at 0.001, 0.01 and 0.1 mg L^{-1}, respectively, under salinity stress conditions. A decreasing trend was found in K^+ and Ca^{2+} concentrations in both the roots and shoots of ornamental sunflowers under saline conditions. The greatest diminutions in K^+ contents in roots (−5.63% and −38.14%) and shoots (−5.63% and −36.29%) were recorded at 0.01 mg L^{-1}, whereas Ca^{2+} contents were reduced by −2.63% and −30% and −1.9% and 10.95% at the same GR24 level under control and saline conditions, respectively (Figure 6).

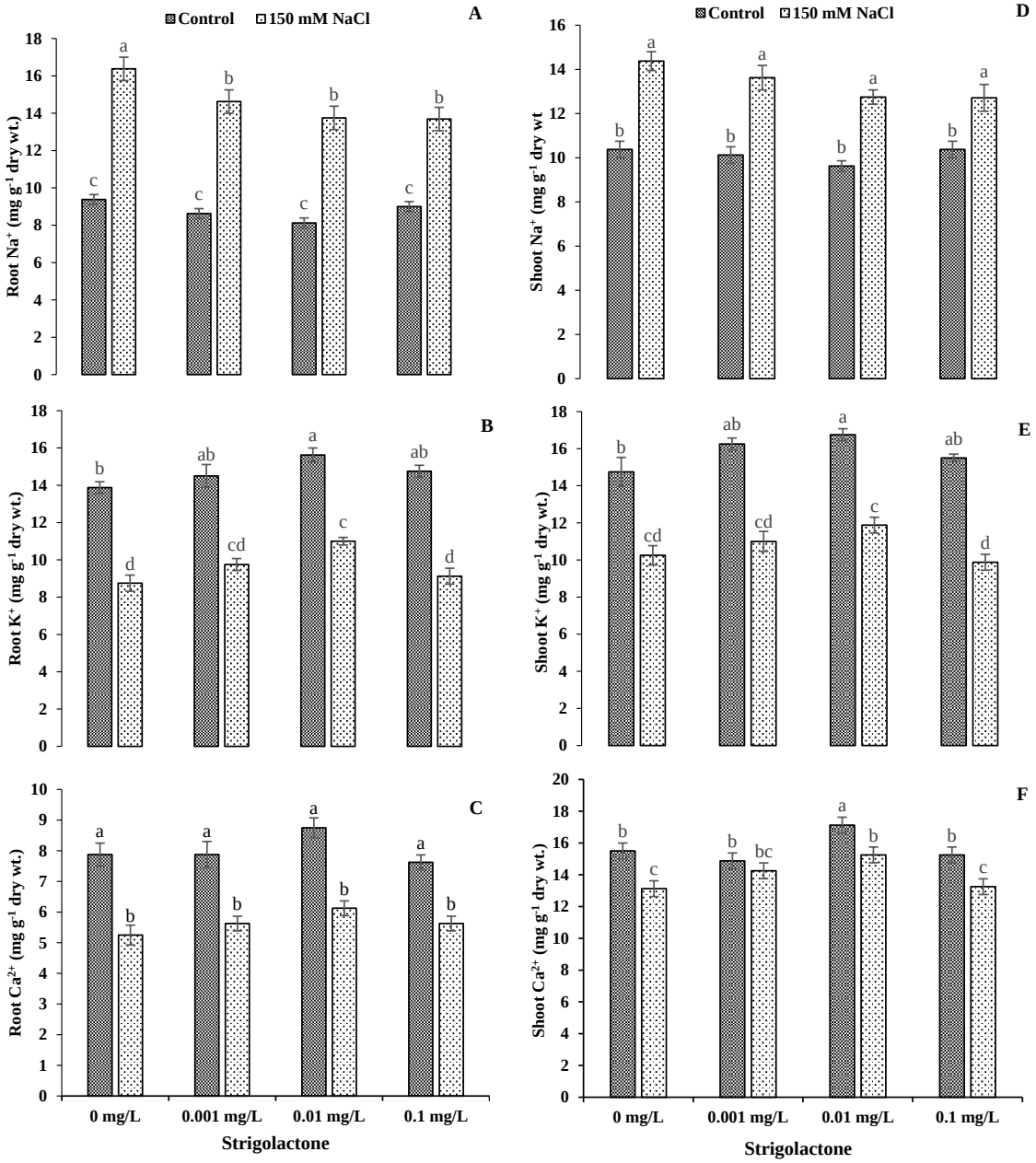

Figure 6. Impact of GR24 on root Na^+ (**A**), K^+ (**B**) and Ca^{2+} (**C**) and shoot (**D**), K^+ (**E**) and Ca^{2+} (**F**) ions of ornamental sunflower under control and salt stress conditions. Each vertical bar shows the mean of three replicates. Different letters indicate significant differences among treatments at $p \leq 0.01$ according to least significant difference test.

3.6. Impact of GR24 Application on Plant Biomass of Ornamental Sunflower under Salt Stress

Statistically, salt stress and GR24 showed highly significant ($p < 0.01$) impacts on root and shoot fresh and dry weights (Table 2). The results showed that the application of salt stress greatly reduced the fresh weights and dry weights of both plant parts (roots and shoots). The greatest fresh and dry weights were recorded when GR24 was applied at 0.01 mg L^{-1} under control and salt stress conditions. The lowest weights (fresh and dry) of roots and shoots were found under control conditions. There were −47.27% and −50.45% reductions in fresh and dry weights, respectively, in roots with a GR24 level of 0.01 mg L^{-1} compared to control conditions. A similar trend was also noted in shoots, where there were decreases of −40.79% and −59.42% in fresh and dry weights with the same GR24 concentration. Overall, there were reductions of −56.67% and −54.06% in fresh weight and −47.26% and −49.13% in dry weight with 0.001 and 0.1 mg L^{-1} GR24 levels in the roots of ornamental sunflowers. In shoots, reductions of −34.14% and −41.18% and −43.45% and −51.28% in fresh and dry weights were observed, respectively, at the same GR24 level compared to control conditions (Figure 7).

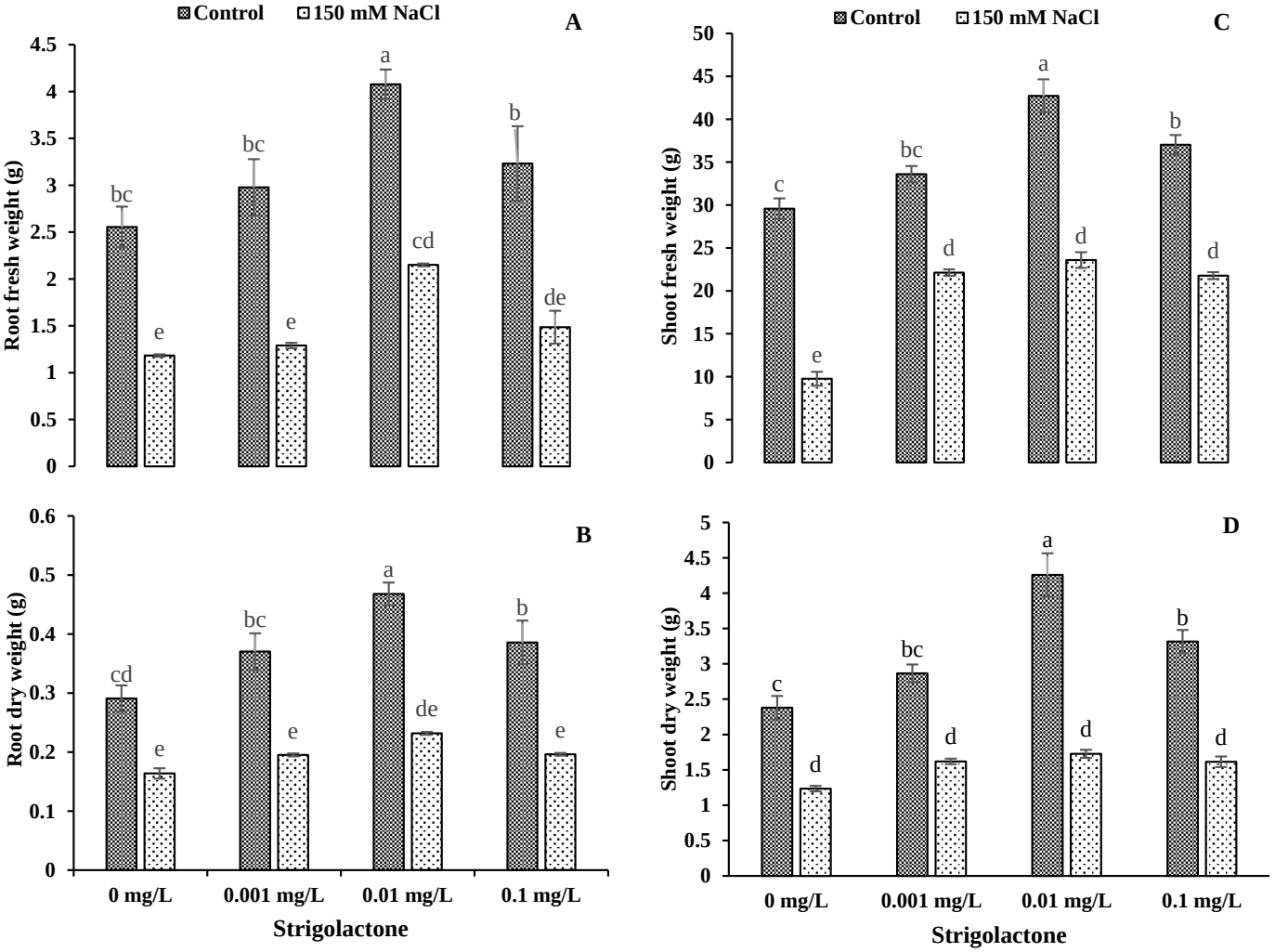

Figure 7. Impact of GR24 on root fresh weight (**A**), root dry weight (**B**), shoot fresh weight (**C**) and shoot fresh weight (**D**) of ornamental sunflower under control and salt stress conditions. Each vertical bar shows the mean of three replicates. Different letters indicate significant differences among treatments at $p \leq 0.01$ according to least significant difference test.

Table 2. Analysis of variance (ANOVA) for different mineral ions and plant biomass characteristics of ornamental sunflower with various GR24 levels (0, 0.001, 0.01 and 0.1 mg L^{-1}) under saline and non-saline conditions.

Source of variation	Root Na^+ (mg g^{-1} Dry wt.)	Root K^+ (mg g^{-1} Dry wt.)	Root Ca^{2+} (mg g^{-1} dry wt.)	Shoot Na^+ (mg g^{-1} Dry wt.)	Shoot K^+ (mg g^{-1} Dry wt.)	Shoot Ca^{2+} (mg g^{-1} Dry wt.)	Root fresh wt. (g)	Root dry wt. (g)	Shoot fresh wt. (g)	Shoot dry wt. (g)
Salt stress	300.125 **	202.507 **	45.125 **	99.757 **	205.031 **	23.632 **	22.680 **	0.264 **	2156.767 **	21.912 **
GR24	5.177 **	5.591 **	1.302 *	2.091 ^{ns}	5.718 **	6.716 **	2.294 **	0.020 **	258.936 **	1.944 **
S × GR24	0.729 ^{ns}	0.403 ^{ns}	0.187 ^{ns}	0.257 ^{ns}	0.468 ^{ns}	1.153 ^{ns}	0.106 ^{ns}	0.004 ^{ns}	29.849 **	0.797 **
Error	0.703	0.617	0.380	0.752	0.890	0.565	0.175	0.001	4.562	0.088

The asterisks indicate statistically significant differences among the various treatments at probability level $p < 0.01$ according to least significant difference (LSD) test. ** Highly significant; * significant; ^{ns} non-significant.

4. Discussion

The current investigation evaluated the role of GR24 in the physio-biochemical characteristics and biomass of the ornamental sunflower (*Helianthus annuus* cv. Vincent's Choice) under salinity stress to identify its most suitable quantity to diminish the drastic impacts of salt stress. It was observed that salinity stress drastically reduced the photosynthetic rate (*A*), transpiration rate (*E*) and stomatal conductance (g_s). Salinity stress mostly abolishes chlorophyll and decreases photosynthesis by limiting the rate of transpiration and stomatal conductance [18]. Salt stress not only overwhelms photosynthetic activity but also represses the plant's photosynthetic machinery. Salinity stress also disturbs cell organelles such as the chloroplast. The chloroplast is the site of most photosynthetic processes (PSI and PSII) and reactive oxygen species (ROS) generation [34]. Photosynthesis is the foundation of crop yield and quality in cropping systems [35]. The efficiency of the photosynthetic process is affected by salt stress, such as metabolic process changes or the limitation of stomata to CO_2 diffusion [36]. Similar findings were reported by Kausar and Shahbaz [37], who showed a lower photosynthetic rate and stomatal conductance due to salt stress even after GR24 application at different concentrations. These findings were contrary to the results of Zhang et al. [38], who observed increases in stomatal conductance, the rate of transpiration and photosynthesis in cucumber seedlings with exogenous GR24 application during salinity stress. This variation might be due to variations in environmental circumstances among plant species. Ma et al. [23] also noted that GR24 increased all photosynthetic attributes of *Brassica napus*. This could be due to increased sucrose synthase 2 (SUS2) and the decreased activity of kinases. SUS2 is responsible for the deprivation of sucrose [39]. The application of GR24 treatment enhanced the expression of SUS2 enzyme activity in rice [40].

The present study showed limited water relations due to the limited uptake of water and reduced solute potential. Similar results were also observed by Cha-um et al. [41], who observed negative water potential that limited plant growth and development after GR24 application. This decrease could be because of higher Na^+ and Cl^- ion accumulation in sunflower leaves. Contrary to the leaf water potential, leaf osmotic and turgor potentials were enhanced in our study under salt stress conditions. Similar findings were also noted by Sarwar and Shahbaz [24] in sunflower after GR24 treatment. The different effects of GR24 on water relation attributes indicate very intricate interactions among GR24 and other hormones [23]. Due to salinity stress, excessive ROS production has been noticed, which possesses lethal effects on the cellular organelles, causing decreased plant growth and development [42,43]. In the present experiment, remarkable increases in proline and MDA were detected. Similar findings were observed in sunflower [24] under salt stress conditions. When using GR24 (especially at 0.01 mg L^{-1}), there was a considerable elevation recorded in the proline content. Exogenously applied GR24 enhanced the free proline concentration in rapeseed, which mitigated the hindering effects of salt stress [23]. MDA is used to gauge the extent of oxidative impairment in stressed plants [44]. The results of the present study also showed higher MDA accumulation due to salt stress, indicating an elevation of lipid peroxidation in ornamental sunflowers. The higher MDA content due to GR24 application in salt stress plants demonstrated the strong positive impact of GR24 on protecting the membrane from stress damage [45]. Increased MDA levels under salt stress also indicated

that despite the presence of the antioxidant system mechanism, salt stress might still cause membrane lipid peroxidation in plant leaves [46]. Naveed et al. [47] also argued that higher stress-related metabolites show a defensive impact as well as plant support to alleviate the lethal role of salt stress.

To manage the deleterious ROS effects in plants, a defensive process is initiated for the tolerance to salts, which includes the compatible production of solutes such as GB [1,48]. In this research, a remarkable elevation of GB was observed under both saline and control conditions with the application of GR24. Under saline conditions, an osmotic modification occurred with the assistance of proteins by decreasing the osmotic potential [49]. The concentration of total soluble protein accumulation in plants differs from species to species [50]. In the present experiment, a reduction in total soluble protein was recorded. Similar results were also found by Zulfiqar et al. [51], where all levels of GR24 reduced the soluble protein content under control and saline conditions. Salt stress causes oxidative injury, particularly due to the enhanced production of H_2O_2 [52]. These ROS have harmful effects on plant tissues, which results in depressed plant growth [43]. Our results are also in line with this, as an elevation of H_2O_2 was found under saline conditions. Enhanced levels of H_2O_2 and others exert a defensive impact and support plants in alleviating perilous abiotic stress impacts [47]. A protective mechanism against ROS is induced in plants by different enzymatic and non-enzymatic antioxidants [53]. In advanced stages, the accrual of ROS tends to inactivate enzymes, degrade nucleic acids and oxidize proteins, which ultimately leads to cell death [54].

Enzymatic activities severely decrease due to stress imposed on plants under external abiotic conditions [2]. Saleem et al. [52] reported an increase in lipid peroxidation among different varieties of potatoes under NaCl exposure. Certainly, metabolite production activates plants to efficiently cope under abiotic stress conditions [55]. Likewise, in the current study, antioxidants (SOD, CAT and POD) were revealed to have boosted activities under salt stress conditions as compared to control conditions, whereas GR24 application enhanced the antioxidant enzymatic activities, especially at the 0.01 mg L^{-1} concentration. The significant augmentation of CAT, SOD and POD activities indicated that there is a positive regulatory effect on the scavenging of ROS produced by salt stress in plants [23]. This also indicated that GR24 could proficiently diminish superoxide free radicals resulting from saline stress, decreasing the cellular impairment caused by peroxidation by ROS for maintaining the proper development of rice seedlings [2]. As a kind of novel plant hormone, the impact of strigolactone GR24 on resistance to abiotic stressors has become an interesting research topic [56,57].

Tolerance to salinity stress in plants is observed because of ion accretion [53]. Tolerance to salts in plants is directly connected to the Na^+/K^+ ratio, which depends upon the nature of the plant species [58]. The present study revealed that GR24 increased the Na^+ contents in roots and shoots of ornamental sunflowers, whereas K^+ and Ca^{2+} decreased in both plant parts under salinity stress. Parallel results were also reported by Parveen et al. [48], in which elevated Na^+ ions in cells lessened the K^+ and Ca^{2+} ions in plant cells. The enhanced Na^+ ion levels in tissues under saline stress affect the characteristics of gas exchange, cytosolic enzyme activities and the development of plants [59]. Homeostasis and the attainment of major elements, i.e., K^+ and Ca^{2+}, were adversely affected by the accrual of Na^+ ions in the cells [24]. These findings confirmed the results of the present research on ornamental sunflowers, as well as previous research on quinoa [60] and wheat [34], under salt stress conditions. GR24 assisted in abating the harsh impacts [36] of salts by hoarding more K^+ and Ca^{2+} ions and minimizing the Na^+ ion content. The root is the main plant organ affected by salt stress conditions, and it affects the accumulation of ions and shoot growth [23]. The present results indicated that salt imposition severely decreased the accumulation of root and shoot biomass. The results are in line with the findings of Sarwar and Shahbaz [24] in sunflower and wheat [34]. Ionic toxicity and high osmotic stress along with ROS production could be the main cause of plant biomass reduction [61]. As noted, when GR24 was applied, it could move and transfer from roots into shoots and played a

regulatory role in plant responses to salt stress [18,45], and salt stress negatively affects the numerous metabolic processes occurring in plants [3].

5. Conclusions

Strigolactone (GR24) played a valuable part in coping with saline conditions; salt stress reduced the photosynthetic attributes (*A*, *E* and g_s) and leaf water potential, but GR24 exerted a regulatory impact under salinity. Among various GR24 levels in the current experiment, 0.01 mg L^{-1} led to significantly elevated photosynthetic characteristics (under control conditions), stress-related metabolites, antioxidant enzyme activities, root and shoot K^+ and Ca^{2+} ions and biomass (fresh and dry weights), while leaf osmotic and turgor potentials were greatest at 0.1 mg L^{-1}. Therefore, the current study offers evidence that exogenous GR24 application may play an important role in mitigating the adverse impacts of salt stress in ornamental sunflowers. Still, there are many gaps in the understanding of GR24 mechanisms and signaling that must be resolved for its sustainable application in agriculture. The diversification of SLs and their downstream signaling processes controlled by the favorable alleles of the genes involved and their identification would be a valuable asset to future breeding operations. A clear understanding of these aspects will open new horizons for plant resistance and improved crop yield.

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