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Innovative and sustainable catechin-based nanocomposites for enhancing salinity tolerance and secondary metabolite production in *Stevia rebaudiana* (Bertoni) Bertoni

Abazar Ghorbani [a,*], Ahlam Khalofah [b,c], Seyed Mehdi Razavi [d], Moxian Chen [a,e,**], Filippo Maggi [f]

[a] State Key Laboratory of Tree Genetics and Breeding, Key Laboratory of State Forestry and Grassland Administration on Subtropical Forest Biodiversity Conservation, the Southern Modern Forestry Collaborative Innovation Center, College of Life Sciences, Nanjing Forestry University, Nanjing 210037, China
[b] Biology Department, Faculty of Science, King Khalid University, P.O. Box 9004, Abha 61413, Saudi Arabia
[c] Research Center for Advanced Materials Science (RCAMS), King Khalid University, P.O. Box 9004, Abha 61413, Saudi Arabia
[d] Department of Biology, Faculty of Sciences, University of Mohaghegh Ardabili, Ardabil, Iran
[e] Clinical Laboratory, Shenzhen Children's Hospital, Shenzhen, China
[f] Chemistry Interdisciplinary Project (ChIP) Research Center, School of Pharmacy, University of Camerino, Camerino 62032, Italy

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ABSTRACT

Integrating innovative and sustainable nano-enabled technologies in agriculture has opened new avenues for enhancing crop resilience against abiotic stressors. Salinity stress is a major abiotic challenge that adversely affects medicinal plants' growth, productivity, and secondary metabolite synthesis, like *Stevia rebaudiana* (Bertoni) Bertoni. This study introduces a novel catechin-based nanocomposite synthesized using a bio-derived approach to improve stevia's resistance to salinity stress. Catechin, a potent antioxidant derived from natural sources, was chosen for its well-documented ability to neutralize oxidative stress and enhance plant defence mechanisms under abiotic stresses. Salinity stress significantly hampers stevia's growth and secondary metabolite production, which is crucial for its industrial exploitation. Catechin-based nanocomposites were synthesized using carboxymethyl cellulose as a biocompatible carrier, ensuring stability and effectiveness. The nanocomposites were characterized and tested at several concentrations (0, 0.01, and 0.1 mg/mL) on stevia plants under varying levels of NaCl (0, 50, and 100 mM). Salinity stress reduced plant growth, photosynthetic pigments, and the K/Na ratio while increasing oxidative stress markers like hydrogen peroxide and malondialdehyde. However, catechin-based nanocomposites improved these physiological and biochemical parameters, enhancing photosynthetic efficiency, antioxidant enzyme activity, and ion balance. Catechin nanocomposites showed protective effects on nitrogen and polyamine metabolisms, involved in stress defensive responses, while increasing levels of the valuable secondary metabolites stevioside and rebaudioside A. The research demonstrates the potential of utilizing bio-based catechin nanocomposites as a green solution in reducing salinity stress, thus showing a potentially viable means of improving resilience and commercial yields in stevia and other plants growing under stress conditions.

Abbreviations: Arg, Arginine; CA-NCs, Catechin-based nanocomposites; CAT, Catalase; CMC, carboxymethyl cellulose; DAO, Diamine oxidase; GOGAT, Glutamate synthase; GR, Glutathione reductase; GS, Glutamine synthetase; H_2O_2, Hydrogen peroxide; KAH, Kaurenoic acid 13-hydroxylase; MDA, Malondialdehyde; Met, Methionine; MSI, Membrane stability index; N, nitrogen; NiR, Nitrite reductase; PAO, Polyamine oxidase; POD, Peroxidase; Put, Putrescine; Reb A, Rebaudioside A; RWC, Relative water content; ROS, Reactive oxygen species; SOD, Superoxide dismutase; Spd, Spermidine; Spm, Spermine.

* Corresponding author.
** Corresponding author at: State Key Laboratory of Tree Genetics and Breeding, Key Laboratory of State Forestry and Grassland Administration on Subtropical Forest Biodiversity Conservation, the Southern Modern Forestry Collaborative Innovation Center, College of Life Sciences, Nanjing Forestry University, Nanjing 210037, China.
E-mail addresses: Ghorbani62a@gmail.com (A. Ghorbani), Cmx2009920734@gmail.com (M. Chen).

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1. Introduction

Salinity stress is accepted today as one of the most pressing problems the agricultural sector faces worldwide. It worsens plant growth as well as productivity. This stress results in a quantitative and qualitative lowering in the productivity of crops and medicinal plants, causing the degradation of soil structure and decreasing biodiversity (Ghorbani et al., 2018b; Hasanuzzaman and Fujita, 2022). Since almost a fifth of the global arable lands are directly impacted by salinity, and this percentage is going up due to climate change and misuse of water resources, proper research must be done to address this problem (Sarabandi et al., 2025). Prolonged salinity exposure generates osmotic and ionic stress in plants, impinging on various physiological and metabolic processes, which may affect the synthesis of secondary metabolites in medicinal and aromatic herbs (Stavi et al., 2021). However, the structural, qualitative, and functional disintegration is typical in plants that are less tolerant to salinity, as noted in this case, brews up water and nutritious materials, causing reduced growth and performance (Atta et al., 2023).

The metabolism of N and polyamines is vital in the plant's resistance systems against numerous stressors, including NaCl (Zhang et al., 2023; Ke et al., 2018; Ghorbani et al., 2023b), drought (Li et al., 2024), and heavy metal (Shah et al., 2022; Ghorbani et al., 2024b). N is a fundamental component of amino acids, the building blocks of proteins, including enzymes, and is essential for plant development (Saha et al., 2015). Under salinity, plants seek to improve their resilience by modulating the N absorption and distribution pattern and synthesizing protective substances like resistant proteins and antioxidants to regulate the ion balance and counter ion toxicity (Ke et al., 2018; Ghorbani et al., 2023b). Polyamines, relatively low-molecular-weight molecules, have been shown to participate in osmotic regulation, relieving oxidative stress and structural stability of the cell wall, among other valuable functions that plants may use to survive during salinity (Saha et al., 2015; Gill and Tuteja, 2010). Another adverse effect of salt stress on cellular metabolism and the health of plant cells is the accumulation of ROS and the resultant oxidative stress (Ghorbani et al., 2018a). The antioxidant defence mechanism is expected to lower the oxidative damage exerted on the plants due to salinity stress. Many authors have pointed out that controlling the nitrogen and polyamine metabolisms and improving the antioxidant defence under salinity stress could significantly increase the plant's adaptability (Atta et al., 2023; Ke et al., 2018; Ghorbani et al., 2023c).

Nanobiotechnology is a highly specialized branch of science that has tremendous potential in developing plant tolerance, allowing them to survive against diverse stressors, such as NaCl stress (Yang et al., 2024; Emamverdian et al., 2023). This technology allows for the development of bio-nanocomposites (bio-NCs) capable of reducing the deleterious impacts of environmental stress by enhancing nutrient acquisition efficiency, supporting plant defense, and enhancing adaptability traits (Ghorbani et al., 2024a). Their unique properties , especially the high surface area-to-mass ratio and precise release of active ingredients, position bio-NCs as efficient protective and supporting agents in plant systems (Yang et al., 2024; Ghorbani et al., 2024a). Catechin (CA), a major phenolic compound in tea leaves and other medicinal and aromatic herbs, plays an important role in defending plants against stress due to its ability to scavenge free radicals. The compound has potential in activating the defensive response as well as in rescuing the plant from the harmful impacts of environmental stressors by redox interaction with the specialized internal signaling components as well as with antioxidant molecules (Ahammed et al., 2023; Li et al., 2019). Though previous studies reported effective responses stimulated by exogenous treatment of CA in flooding (Yiu et al., 2011) and salinity (Yiu et al., 2012) stress, research on an NC involving CA and its effect in amplifying the defensive response in plants in saline conditions is vastly unexplored.

Recent studies have highlighted the importance of NCs due to their excellent stability, multi-functional nature, variety of morphological structures, defined size, optimized surface area, and enhanced solubility (Paul and Robeson, 2008). Nano-polymer carriers in the form of polymers, such as polyethylene glycol and polylactic glycolic acid, are used to carry a variety of substances for targeting specific cells, thus ensuring their controlled release and sustained release in the context of NC synthesizing technology (Jarosiewicz and Tomaszewska, 2003). However, naturally occurring polysaccharide polymers like cellulose are promising alternatives to the use of traditional polymers due to their non-toxic nature, ease of degradability, low price, and high degree of biocompatibility (Paul and Robeson, 2008; Arshneshin et al., 2023; Liu et al., 2024). The low reactivity of cellulose, together with its low solubility in organic and water-based solvents, limits its use in NC fabrication. On the other hand, carboxymethyl cellulose (CMC), a cellulose derivative, has polar carboxymethyl groups that are responsible for substantial water solubility and also show chelating properties. Such a feature makes CMC a good medium in the controlled release of diverse substances, not forgetting its water solubility (Pourmadadi et al., 2023). Furthermore, CMC contains carboxyl groups and hydroxyl groups that can be attacked by various reagents, resulting in stable nanopolymers. CMC is an advantageous alternative to produce bio-NCs that are not only inexpensive but environmentally friendly as well. It is suggested that these bio-NCs build up plants' stress resistance against environmental agents and also support their defensive mechanisms (Arshneshin et al., 2023; Pourmadadi et al., 2023).

Stevia rebaudiana (Bertoni) Bertoni, a member of the Asteraceae family, is a remarkably valuable medicinal herb also known as the sweet leaf. The plant contains natural sweetener molecules, about 30 times sweeter than sucrose, with no calorie value (Ghasemi-Omran et al., 2021; Patel and Navale, 2024), among which the major components are stevioside and rebaudiosides (Reb). Stevia sweeteners are used extensively as alternative sweeteners in many industries, such as food manufacturing, nutraceuticals, pharmaceutics, and beverage preparation (Patel and Navale, 2024). Empirical research has shown that glycosides from stevia have various health benefits, including the prevention of arteriosclerosis, improvement of insulin sensitivity, regulation of blood pressure, lowering of blood glucose levels in diabetics, and the inhibition of various cancers (Orellana-Paucar, 2023). While stevia has significant tolerance to saline conditions, it can suffer from the negative impacts of salinity on the secondary metabolite accumulation in the leaves (Ghasemi-Omran et al., 2021). With the growing demand for nature-based products as well as the need to minimize toxic chemical usage, sustainability, and ecologically friendly methods, developing a way of boosting stevia's tolerance in stressful conditions, such as salinity stress, is highly important. Apart from assessing its effects on salinity-stressed stevia, this study presents a novel approach for synthesizing and characterizing the first-ever nanocomposites based on catechin (CA-NCs). Apart from their regulatory enzymes, the study offers fresh insights into the function of bio-based NCs in modulating polyamine and nitrogen metabolism, supporting the antioxidant system, preserving Na/K homeostasis, and promoting glycoside molecule biosynthesis. The results confirm the possibility of using CA-NCs as a green and eco-friendly technique to raise plant tolerance under salt stress, a possible answer for sustainable agriculture.

2. Material and methods

2.1. Preparation and characterization of CA-NCs

2.1.1. Synthesis of CA-NCs

CMC polymer was utilized as a receptor in synthesizing CA-NCs, mixed with CA in a ratio of 1:2. CA was first dissolved in a beaker of 98 % ethanol, vortexed, and then sonicated at a temperature of 60°C for 5 min. After that, CMC was dissolved in distilled water, vortexed, and sonicated for 25 min at a temperature of 90°C. The CA solution was then placed in a burette, and the CMC mixture was added slowly, dropwise, under continuous vortexing. Lastly, the whole mixture was sonicated for

a further 5 min.

2.1.2. Purification and characterization

The resulting solution was centrifuged for 15 min at 8000 rpm. The supernatant was taken off and dried in a Petri dish; the precipitate was gathered. The microtubes received the dried yields for more experimental use. Fourier-transform infrared (FTIR) spectroscopy, dynamic light scattering (DLS), scanning electron microscopy (SEM), and thermogravimetric analysis (TGA techniques) characterized the properties of the produced CA-NCs.

2.2. Plant cultivation and treatment applications

Stevia rebaudiana cuttings from vegetative propagation with three fully mature leaves were obtained from Nisha Co., Sari, Iran, for this work. Water for ten days daily, followed by planting in pots packed with a mix of perlite and cocopeat (1:2 ratio) for these cuttings. Every five days, 1/2 Hoagland solutions were also used to feed the pots. The seedlings were grown at 25/22°C (day/night), 16 h light, and 75 % humidity under controlled conditions. Salinity treatments (NaCl, 50 and 100 mM) and CA-NCs treatments (0.01 and 0.1 mg/mL) were continuously applied to the plants by adding them to the 1/2 Hoagland solutions for 21 days, following 10 days. Each pot contained two plants, and each treatment was replicated 5 times (10 plants per treatment). Three weeks after the treatments began, sampling was conducted. The plant height was recorded, and the samples were transferred to − 80°C freezers for further analysis (Ghorbani et al., 2023a).

2.3. Photosynthetic parameters

2.3.1. Fv/Fm value

The Fv/Fm value of stevia leaves was assessed employing a DUAL PAM-100 Chl fluorometer (Heinz Walz, Ger) after a 30 min dark adaptation period.

2.3.2. Photosynthetic pigments

Acetone 80 % was used to extract the photosynthetic pigments from fresh stevia leaves. After centrifugation, the absorbance of the extracted solution was specified spectrophotometrically at 646, 670, and 470 nm (Arnon et al., 1948).

2.4. Oxidative stress markers

2.4.1. Membrane stability index (MSI)

After setting the leaf discs at 25°C in distilled water and the subsequent electrical conductivity (EC1) determination, the leaf discs were subjected to autoclaving for 20 min at 120°C. After acquiring EC2, MSI (%) = [1 − {C1/C2}] × 100.

2.4.2. Relative water content (RWC)

Following the leaf segments' preparation and weighing (W1), the segments were submerged in 25°C distilled water for 4 h. Afterwards, the weights of the leaf discs were measured (W2). The weight of the dried leaf pieces was then measured after drying at 78 °C (W3). RWC was calculated by RWC = [{W1 − W3}/{W2 − W3}] × 100 (Browne et al., 2020).

2.4.3. Hydrogen peroxide (H_2O_2)

A leaf extract obtained with a 0.1 % trichloroacetic acid extraction buffer was used to assay H_2O_2. The measurement was conducted at 390 nm, based on the reaction with KI (Alexieva et al., 2001).

2.4.4. Malondialdehyde (MDA)

By extracting stevia leaves with a 20 % trichloroacetic acid solution, the MDA content was quantified as per the process of Heath and Packer (1968) with readings at 532 and 600 nm.

2.5. Antioxidant enzyme activity

2.5.1. Enzyme extraction

A phosphate buffer (0.05 M) solution containing PVP (1 %) and EDTA (1 mM) was used to extract antioxidant enzymes. The supernatants served as enzyme extracts for assessing antioxidant enzyme activities.

2.5.2. Antioxidant enzyme activity

The activity of catalase (CAT) was quantified by reading the mixture of phosphate buffer (25 mM, pH 6.8) with the enzyme extract and H_2O_2 (10 %) at 240 nm, as per Aebi (1983). A mixture of enzymatic extract, guaiacol (16 mM), potassium phosphate buffer (100 mM, pH 6.0), and H_2O_2 (10 %) was used to determine POD activity based on the quantification of produced tetraguaiacol at 470 nm (Hemeda and Klein, 1990).

By monitoring the 50 % inhibition of nitroblue tetrazolium reduction at 240 nm in the mixture of enzymatic extract, H_2O_2 (10 mM), and phosphate buffer (25 mM, pH 6.8), the activity of superoxide dismutase (SOD) was quantified as per Giannopolitis and Ries (1977). The activity of glutathione reductase (GR) was determined by quantifying the absorbance at 340 nm over 1 min in a mixture containing enzymatic extract, NADPH (0.5 mM), phosphate buffer (0.1 mM, pH 7.5) containing $MgCl_2$ 6.25 mM, and GSSG (10 mM) (Ghorbani et al., 2018b).

2.6. Polyamine metabolism

2.6.1. Polyamine biosynthetic enzyme activity

Enzyme extracts were prepared from fresh leaves using a mixture of dithiothreitol (5 mM) and 100 mM phosphate buffer (pH 6.5). Subsequently, the activity of diamine oxidase (DAO) and polyamine oxidase (PAO) was quantified as per Asthir et al. (2002). For DAO, a mixture of 50 U CAT, 50 mM potassium phosphate buffer (pH 7.6), o-aminobenzaldehyde (1 %), and enzyme extract was used, and for PAO, a mixture of 50 U CAT, 50 mM potassium phosphate buffer (pH 6.0), o-aminobenzaldehyde (1 %), 10 mM spermidine (Spd), and enzyme extract was employed. The readings were taken at 430 nm.

2.6.2. Free polyamines

Stevia leaves were extracted with 5 % (v/v) $HClO_4$, and after centrifugation, sodium hydrate (2 N) and benzoyl chloride were added to the supernatants, followed by another centrifugation. Subsequently, the supernatants were mixed with saturated NaCl and diethyl ether, and after centrifugation, the diethyl ether phase was evaporated from the supernatants, and methanol was added to the residues. Then, HPLC (Waters, Milford, MA, USA) was used to analyze polyamines, and for quantification, a spectrofluorometric method with excitation and emission wavelengths of 365 and 550 nm was employed (Naka et al., 2010).

2.6.3. Methionine and arginine

Arginine and methionine levels were measured in stevia leaves using o-phthalaldehyde for derivatization. Fresh stevia leaves were crushed with 0.1 M hydrochloric acid. After centrifugation, the resulting liquids were combined with o-phthalaldehyde and assessed with an HPLC instrument equipped with an RF-20AXS fluorescence detector (Tokyo, Japan). The compounds were separated using a 4.6 × 250 mm ODS Spheri 5 column (5 μm, GL Science Inc., Torrance, CA, USA) and a 15 × 3.2 mm guard column (Shim-Pack, Tokyo, Japan) (Ghorbani et al., 2023b; Noctor and Foyer, 1998).

2.7. Nitrogen metabolism analysis

2.7.1. Nitrate and N concentration

Dried stevia leaves were extracted with 2 % acetic acid and mixed with powdered zinc, sulfanilamide, citric acid, N-1-naphthyl ethylenediamine dihydrochloride, and manganese sulfate. The nitrate

amount was specified as per Singh (1988) at 540 nm. The N contents were specified using the micro-Kjeldahl method on dried stevia leaves.

2.7.2. Nitrate reductase (NR) and nitrite reductase (NiR) activity

First, the enzymatic extract was obtained from fresh stevia leaves by mixing with a cysteine (5 mM), 100 mM potassium phosphate buffer (pH 7.6), EDTA (2 mM), and PVP (0.5 %). By adding the enzymatic extract to a mixture of 0.1 M potassium phosphate buffer (pH 7.0), 0.4 mM $NaNO_2$, 4.3 mM $Na_2S_2O_4$, and 2.3 mM methyl viologen for NiR enzyme, and a mixture of 100 mM potassium phosphate buffer (pH 7.5), 0.5 M zinc acetate, 0.14 mM NADH, 7 mM KNO_3, and 10 mM $MgCl_2$ for NR enzyme, and reading them at 540 nm, the activities of the NiR and NR enzymes were quantified as per the protocol of Debouba et al. (2006).

2.7.3. Glutamine synthetase (GS) and glutamate synthase (GOGAT) activity

Reaction mixtures were prepared through the combination of a solution containing 50 mM potassium phosphate buffer (pH 7.5), 2 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 3.58 M ethylene glycol, 10 mM KCl, and 14 mM β-mercaptoethanol, whereby GOGAT activity was determined at 340 nm (Groat, and Vance, 1981). Conversely, a reaction solution was created by the combination of 50 mM Tris-HCl buffer at pH 7.2, 50 mM glutamine, 20 mM Na_3AsO_4, 13 mM hydroxylamine, 1 mM ADP, 20 mM $MgCl_2$, 0.5 M HCl, and 0.2 M $FeCl_3$, for the determination of GS activity at 540 nm (Agbaria et al., 1998).

2.8. Na and K levels

The Na and K accumulation levels in dried stevia leaves were specified by PFP7 flame photometry (Jenway, UK).

2.9. Essential oils and diterpene glycosides

The essential oil contents from dried leaves were estimated using a Clevenger apparatus. The diterpene glycosides were extracted by homogenizing dried leaves with hot water (80°C) for 3 h and purifying the resulting solution using a mixture of $CaCl_2$ and sulphate (5:3), following the method described by Yang et al. (2011). The supernatant obtained after centrifugation at 12,000 g for 10 min was then diluted with distilled water and filtered using a 1 μm filter. HPLC analysis was performed using two Grace Alltima 5 μm C18 columns connected in series (10 mm path length; 250 × 4.6 mm ID) with UV detection at 200 nm. The HPLC system included a SIL-HTc autosampler and two LC-20AT pumps supplied by Shimadzu, Deurne, Belgium. The solvent flow rate was maintained at 1.0 mL min^{-1}. Rebaudioside A, with a purity exceeding 99 %, served as the external standard (Ghasemi-Omran et al., 2021).

2.10. Gene expression

Total RNA from fresh stevia leaves was prepared by Invitrogen TRIzol (Carlsbad, USA). After cDNA synthesis with Thermo Fisher (Waltham, USA) reverse transcription kits, qPCR processes were performed using the C1000 thermal cycler (Bio-Rad, Hercules, USA) and 2X Thermo Scientific SYBR Green Master Mix kits (Waltham, USA). The relative expression of diterpene glycoside biosynthetic genes was determined using three independent replicates and the $2^{-\Delta\Delta CT}$ method with the *Actin* gene as the internal reference gene. The primer sequences of the internal reference gene and those of other genes are listed in Table S1.

2.11. Statistical analyses

Data analysis was performed with SAS (V9, Cary, USA). Using Duncan's test, the means (± SD) obtained from five independent replicates for morphological and physio-biochemical traits and three independent replicates for gene expression were compared at the 5 % probability level. Principal component analysis (PCA) was run through Origin-Lab Software to distinguish the effects of CA-NCs and salinity. The relationships between the variables were visualized by examining trait correlations (Pearson's correlation coefficient) and generating a heatmap using GraphPad Prism.

3. Results and discussion

3.1. Fabrication and characterization of CA-NCs

The DLS analyses revealed an average size of 6.8 ± 0.05 nm for NCs particles, approving its nanoscale identity and structure. The quantity of particles with a size of 6.9 nm exceeds that of other particles (Fig. 1A). The successful production and characterization of the CA-NCs validate their future use as bio-nanocarriers, pointing toward substantial scope for improving the stress tolerance of plants. Characterization through DLS confirmed the nanoscale properties of the CA-NCs, giving a particle diameter of around 6.8 nm, consistent with previous observations in similar bio-nanocarriers, where particle sizes generally varied from 5 nm up to a maximum range of around 20 nm (Arshneshin et al., 2023; Sepehry Javan et al., 2024). The dominance of particles with a size around 6.8 nm further supports the effectiveness of the used production process in yielding uniformly minute nanoparticles, a key consideration in ensuring bioavailability, in addition to nanocarriers' interaction with plant cells (Ghorbani et al., 2024a).

The analysis with SEM provided further insight into the morphology of CA-NCs, indicating that the particles are quasi-spherical to polyhedral in shape, with all the dimensions being below 50 nm (Fig. 1B). The shape and size uniformity of the particles are of paramount importance, as it determines the surface area, hence affecting the further interactions of the nanoparticles with biological systems, in this case, their potential to penetrate and distribute in plant tissues (Ghorbani et al., 2024a). Similar observations have been reported by previous studies where organic materials were used for the synthesis of nanoparticles, specifically the bioactive compound cinnamic acid (Sepehry Javan et al., 2024).

The FTIR spectra of CA indicated two distinct peaks at 3405 and 1610 cm^{-1} related to hydroxyl stretching and carbonyl absorption vibrations, respectively. However, in the CA-NCs, relevant peaks were slightly shifted, demonstrating an intermolecular hydrogen bond between CMC and CA that formed CA-NCs. The interactional observations evidenced a strong encapsulation of catechin in the CMC matrix, supporting the stable formation of nanocomposites (Ghorbani et al., 2024a). During the production of CA-NCs, the frequency involved in the carbonyl group (C═O) of CA reduced from 1610 cm^{-1} to 1607 cm^{-1}, indicating the formation of hydrogen bonds between CMCs and CA. The decrease in frequency implies stretching in the internal carbonyl bond due in turn to a non-covalent interaction in the form of a hydrogen bond between the hydroxyl group of CMCs and the carbonyl group of CA. Also, the possible existence of hydrogen bond formation between CMCs and CA is signified by a frequency shift in the hydroxyl group, from 3405 cm^{-1} for CA to 3404 cm^{-1} in CA-NCs (Fig. 1C, D). These non-covalent associations are responsible for maintaining the structural stability in the NCs against diverse environmental conditions, such as salinity stress (Haydar et al., 2023). The spectral differences between CA (Fig. 1C) and CA-NCs (Fig. 1D), particularly the shifts in –OH and C═O bands, are clearly distinguishable, supporting the formation of hydrogen bonds and the successful encapsulation of CA within the nanocomposite structure.

The TGA curve of CA shows a major weight loss event starting at approximately 170°C, which corresponds to the thermal decomposition of CA. This decomposition indicates the breakdown of organic compounds in the CA, leading to significant weight loss (Fig. 2A). For CMC, the TGA curve shows initial weight loss below 110°C due to the loss of adsorbed water. A significant weight loss event begins at around 250°C,

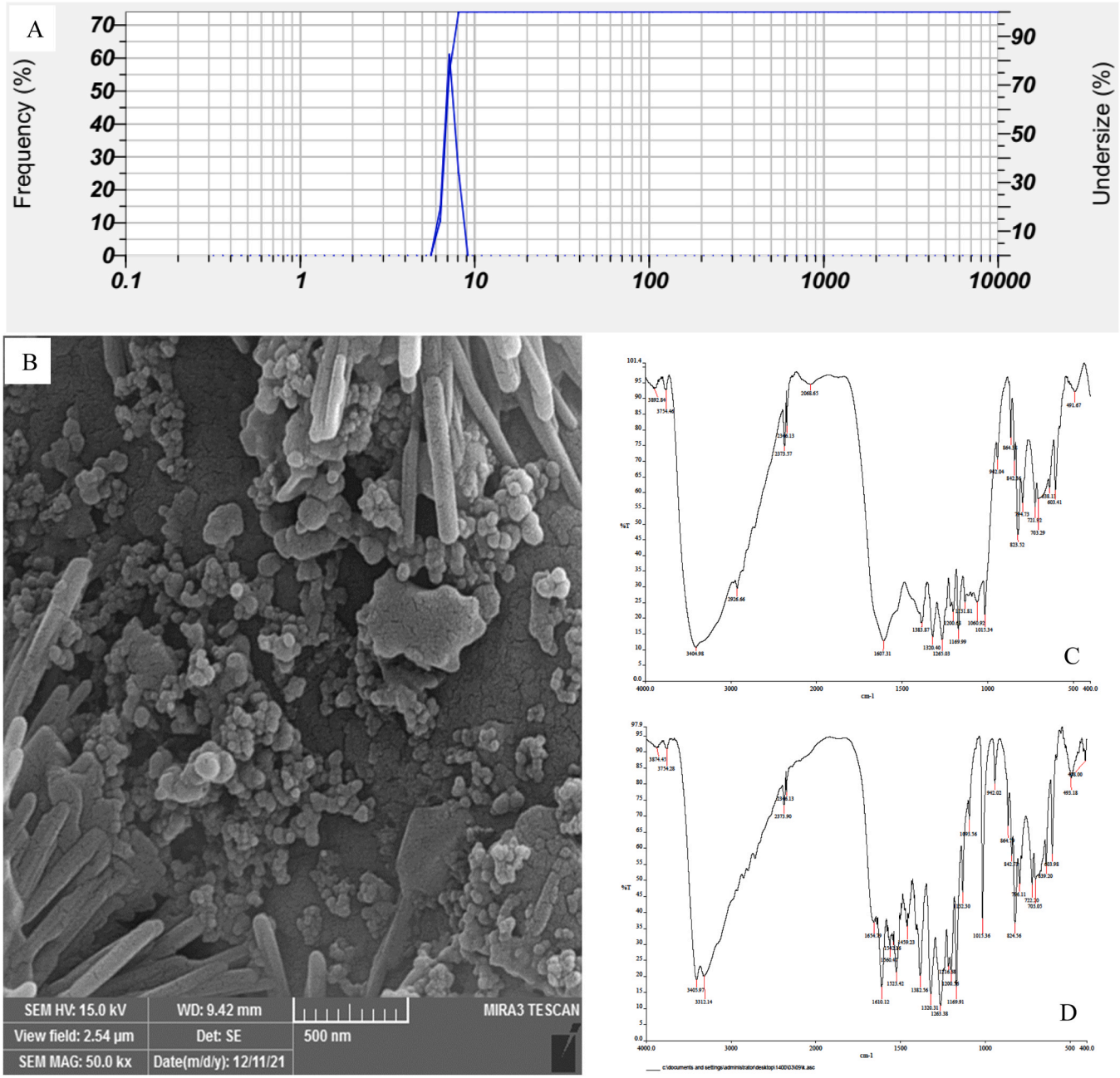

Fig. 1. The Dynamic Light Scattering (A) and Scanning Electron Microscopy (B) analyses of CA-NCs and Fourier-transform infrared (FT-IR) spectra of CA (C) and CA-NCs (D).

corresponding to the thermal degradation of the CMC polymer backbone. This exercise shows the thermal stability and degradation profile of the pure CMC polymer (Fig. 2B). CA-NC's TGA reveals two phases of weight loss. The first stage is a slight weight loss occurring below 110°C, which can be ascribed to the loss of moisture or other volatile chemicals. Roughly 200°C to 515°C marks the second, far larger weight loss, which corresponds to the decomposition of the organic components inside the NCs (Fig. 2C). Unlike the weight loss pattern of pure CA, this one shows the effective creation of a new composite material. Such thermal activity indicates that CA-NCs have better thermal stability than pure CA and CMC, probably because of the interaction between CA and CMC that enhances the general structural integrity of the NCs. The structural and chemical stability of CA-NCs is fully understood by combining DLS, SEM, FTIR, and TGA data. These findings confirm that the fabricated NCs are structurally intact and chemically stable, making them a viable candidate to improve plant tolerance to salinity stress (Haydar et al., 2023).

3.2. Morphological and photosynthetic apparatus attributes

Under non-salinity conditions, although the application of CA-NCs did not significantly affect the total dry weight, the 0.01 mg/L treatment resulted in a 5.4 % rise in the height of stevia over the control. Additionally, mild and severe salinity reduced stevia's height by 22.3 and 43.8 %, respectively. This reduction in total dry weight was 24.3 and 47.8 %, respectively, over the control stevia. In contrast, the CA-NCs induced a concentration-dependent increase in both morphological parameters of stevia compared with salinity alone (Table 1). A significant decrease in the Fv/Fm index and photosynthetic pigments was recorded with increasing salinity levels, with the most pronounced

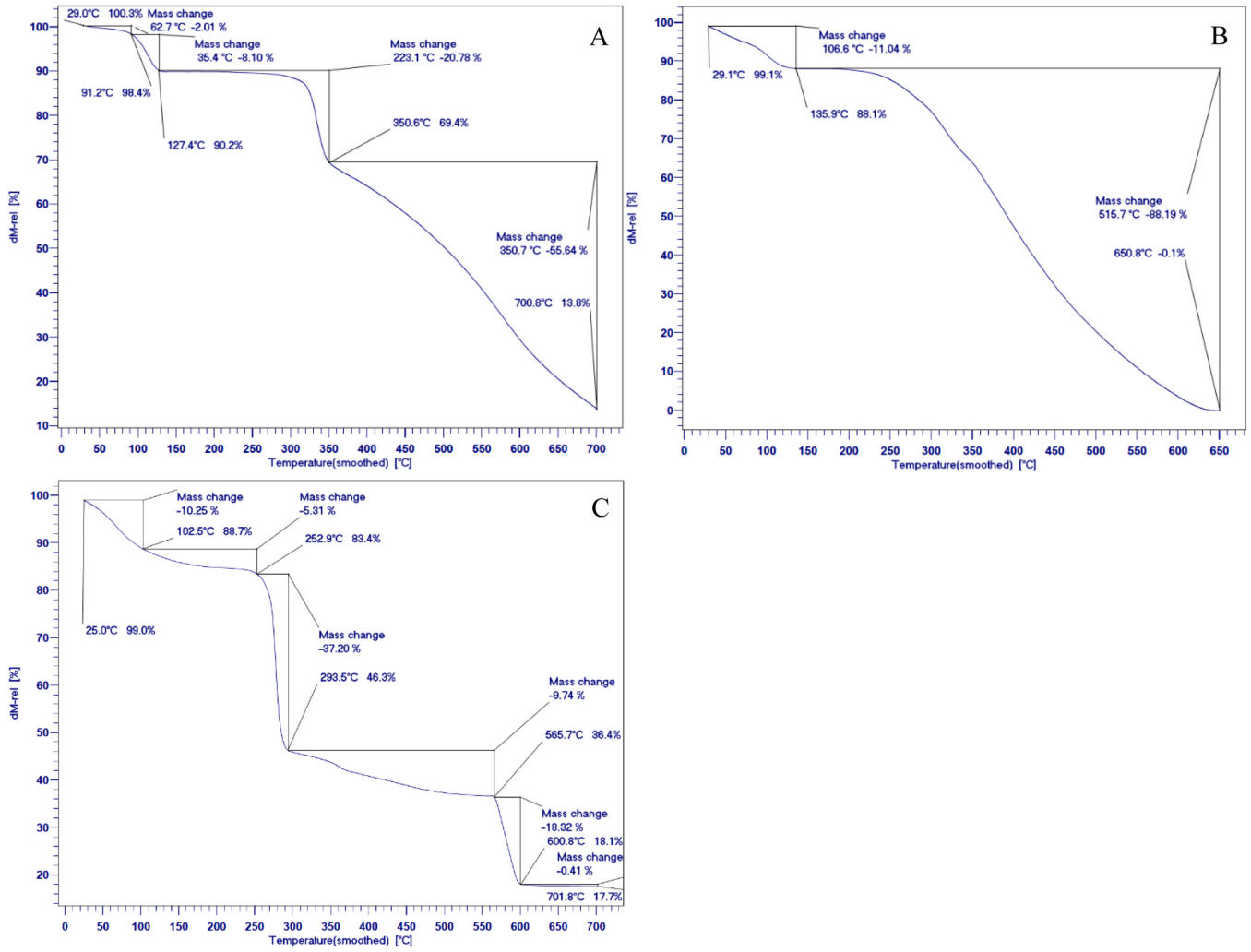

Fig. 2. TGA graph of CA (A), CA-NCs (B), and CMC (C).

Table 1
Effects of catechin nanocomposite (CA-NCs) at different concentrations (0, 0.01, and 0.1 mg/mL) on growth and photosynthetic traits of stevia under different salinity levels (non-salinity: 0 mM NaCl, mild-salinity: 50 mM NaCl, and severe-salinity: 100 mM NaCl).

		Height (cm)	TDW (g)	Chl a	Chl b	Car	Fv/Fm
Non-salinity	0 CA-NCs	33.45 ± 1.27^{b}	17.73 ± 0.45^{ab}	3.01 ± 0.18^{a}	1.23 ± 0.04^{a}	0.348 ± 0.023^{a}	0.738 ± 0.014a
	0.01 CA-NCs	33.31 ± 1.35^{ab}	17.93 ± 0.42^{ab}	3.16 ± 0.23^{a}	1.26 ± 0.06^{a}	0.354 ± 0.015^{a}	0.737 ± 0.018^{a}
	0.1 CA-NCs	$35.25 + 1.31^{a}$	18.21 ± 0.63^{a}	3.08 ± 0.19^{a}	1.24 ± 0.07^{a}	0.342 ± 0.024^{a}	0.738 ± 0.019^{a}
Mild-salinity	0 CA-NCs	26.01 ± 0.96^{e}	13.42 ± 0.39^{d}	1.92 ± 0.17^{c}	0.80 ± 0.04^{d}	0.293 ± 0.011^{c}	0.669 ± 0.016^{cd}
	0.01 CA-NCs	28.93 ± 0.71^{d}	14.95 ± 0.36^{c}	2.45 ± 0.12^{b}	1.00 ± 0.08^{c}	0.325 ± 0.013^{ab}	0.693 ± 0.012^{bc}
	0.1 CA-NCs	31.55 ± 1.37^{c}	17.10 ± 0.52^{b}	2.68 ± 0.13^{b}	1.12 ± 0.05^{b}	0.335 ± 0.016^{a}	0.716 ± 0.013^{ab}
Severe-salinity	0 CA-NCs	18.82 ± 0.63^{g}	09.26 ± 0.46^{f}	1.18 ± 0.11^{d}	0.58 ± 0.03^{e}	0.196 ± 0.015^{e}	0.597 ± 0.016^{e}
	0.01 CA-NCs	24.21 ± 0.74^{f}	12.45 ± 0.53^{e}	1.86 ± 0.13^{c}	0.75 ± 0.03^{d}	0.255 ± 0.014^{d}	0.645 ± 0.010^{d}
	0.1 CA-NCs	28.42 ± 0.60^{d}	15.66 ± 0.61^{c}	2.47 ± 0.15^{b}	0.97 ± 0.05^{c}	0.302 ± 0.014^{bc}	0.686 ± 0.013^{c}

Values (means ± SD) followed by the same letter are not significantly different ($p < 0.05$; Duncan test).

reduction occurring under severe salinity. In contrast, the CA-NCs restored all examined photosynthetic apparatus attributes in salinity-stressed plants, with the recovery being more pronounced in the 0.1 mg/L CA-NCs treatment (Table 1). One of the most significant impacts of salt on plants can be the negative effect on photosynthetic pigments and disruption of the photosynthetic apparatus (Nanehkaran et al., 2024; Sepehry Javan et al., 2024). Salt stress, especially severe salinity, reduced photosynthetic pigments and the Fv/Fm index, decreasing stevia biomass and growth. Such salinity-induced effects had been previously observed in stevia plants (Ghasemi-Omran et al., 2021; Esmaielpour et al., 2021). The main reasons for salt toxicity affecting the photosynthetic apparatus and plant growth are cited as high Na accumulation, disruption of ionic balance, secondary stresses (physiological drought and oxidative stress), and disruption in essential plant metabolic processes (Atta et al., 2023; Ghorbani et al., 2023b; Sha et al., 2024). Several reports have indicated that CA can significantly affect plant adaptation to various stressors, including salinity (Yiu et al., 2012; Ahammed et al., 2018). However, the precise nature of this role has not been well formulated. Therefore, for the first time, an attempt has been made to develop a CA-based NCs to address the aspects of this role more accurately. CA-NCs significantly reduced the adverse effects of salinity on photosynthetic pigments, leading to improved performance of this

vital system, which was supported by enhanced biomass and growth. Li et al. (2019) demonstrated that CA improved the adaptability of tomato seedlings under salt toxicity by alleviating oxidative stress and photoinhibition by strengthening the antioxidant defence system dependent on Respiratory Burst Oxidase Homolog1. Similarly, Nalina et al. (2018) indicated a positive correlation between CA accumulation levels and water balance during drought stress, indicating the function of this compound in regulating plant osmotic potential under stress conditions. Therefore, our results confirmed that the CA-based NCs can improve stevia plants' adaptability and growth under moderate and high salinity, possibly through enhancing the antioxidant system and adjusting osmotic potential.

3.3. K/Na homeostasis

Moderate and severe salinity resulted in a 73.4 and 254.8 % increase in leaf Na and a 31 and 42.4 % decrease in leaf K compared with the leaves of control plants. In contrast, CA-NCs treatments had the opposite effects on K and Na accumulation in the leaves, with Na levels decreasing and K levels increasing (Fig. 3A, B). Therefore, following the results from the alterations in leaf K and Na levels, salinity stress reduced the K/Na ratio, while CA-NCs improved it (Fig. 3C). The increase in Na uptake is one of the most significant direct impacts of NaCl on plants, which, by disrupting the absorption and transport of nutrients, particularly K, induces adverse effects on the plant's vital activities and

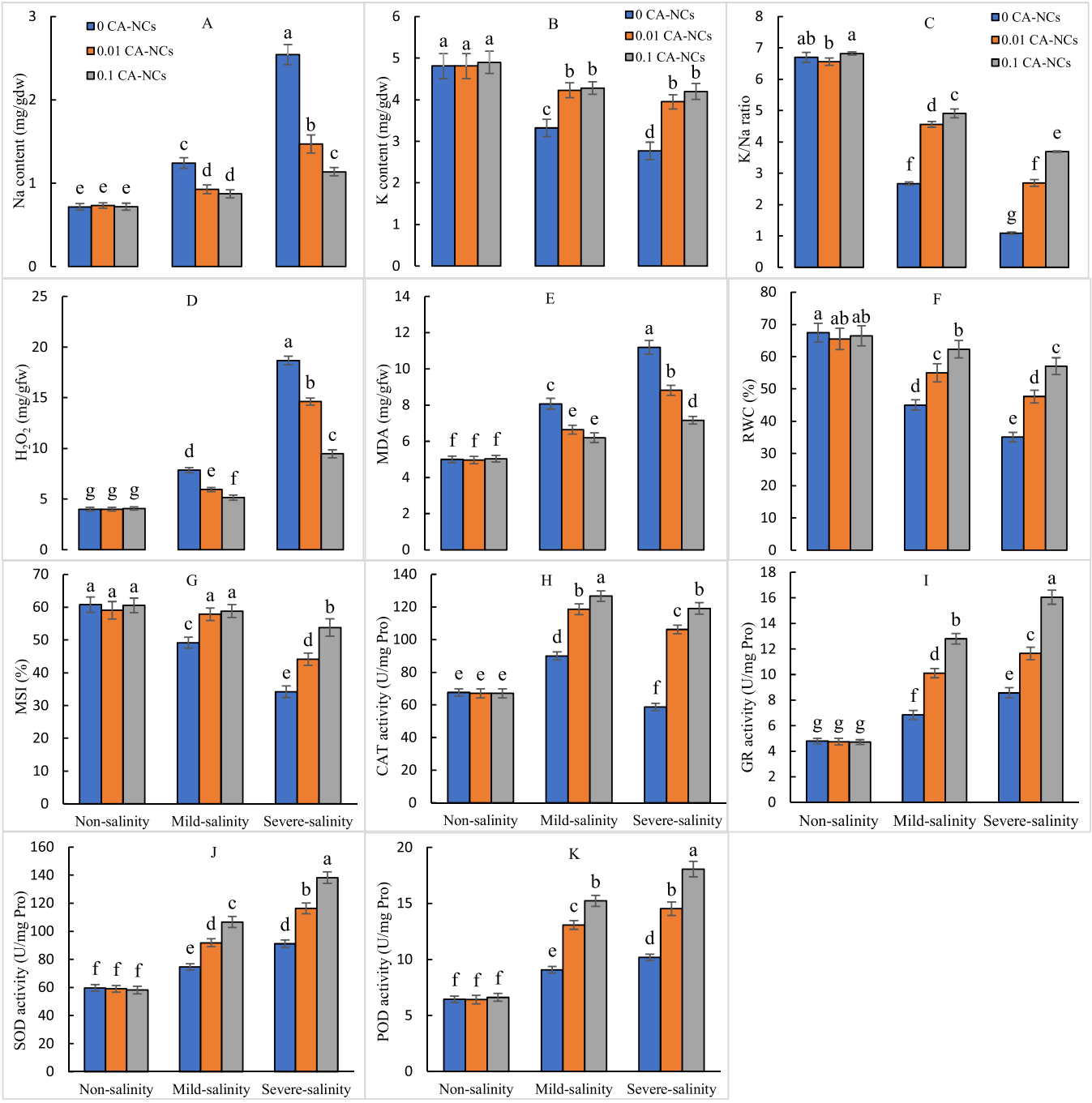

Fig. 3. Effects of catechin nanocomposite (CA-NCs) at different concentrations (0, 0.01, and 0.1 mg/mL) on K/Na homeostasis (A-C), oxidative stress markers (D-G), and activity of antioxidant enzymes (H-K) in stevia leaves under different salinity levels (non-salinity: 0 mM NaCl, mild-salinity: 50 mM NaCl, and severe-salinity: 100 mM NaCl). Values (means ± SD) followed by the same letter are not significantly different ($p < 0.05$; Duncan test).

metabolisms (Hasanuzzaman and Fujita, 2022). By affecting Na and K's absorption, salinity significantly decreased the K/Na ratio in leaves. Comparable findings on the rise in Na uptake and the disturbance of the K/Na ratio have also been reported in various plant species (Ghorbani et al., 2018b; Ghasemi-Omran et al., 2021). High Na levels in the rhizosphere can impact nutrient uptake systems by influencing cell membrane selectivity or competitive interactions, inducing ionic imbalance. Therefore, maintaining the K/Na balance can play a potential role in stevia's adaptation to salinity. However, applying CA-NCs restored the leaf K/Na homeostasis by lowering Na accumulation and elevating K levels at both salinity levels. Supporting our results, Yiu et al. (2012) demonstrated that applying CA improved K/Na homeostasis in *Capsicum annuum* L. under salinity, accompanied by enhanced Ca uptake. Therefore, our findings confirmed that CA-NCs can strongly contribute to stevia's adaptation to salinity by maintaining the K/Na balance. Nevertheless, investigating the relationship between Ca and the activity or expression of transporters involved in K and Na uptake and transport could provide more comprehensive and precise insights into the mechanism of action of CA-NCs.

3.4. Antioxidant defence machinery

Moderate and severe salinity caused a 2- and 4.7-fold increase in H_2O_2 and a 1.6- and 2.3-fold increase in MDA in stevia leaves. In contrast, the CA-NCs significantly reduced both H_2O_2 and MDA at both salinity levels. Among these treatments, the highest inhibitory effect on oxidative stress markers was observed at 1 mg/L CA-NCs (Fig. 3D, E). Applying moderate and severe salinity on stevia resulted in a 33.3 and 48 % increase in RWC and a 19.1 and 43.7 % increase in MSI, respectively, in the leaves over non-salinity plants. In contrast, adding CA-NCs at both salinity levels induced a significant recovery effect on both measured traits compared with the corresponding stresses alone (Fig. 3F, G). Moderate salinity significantly increased CAT activity by 33.2 %, while severe salinity significantly decreased it by 13.2 % compared with control plants. At both moderate and severe salinity levels, CA-NCs significantly enhanced CAT activity, with the highest increase observed during the 1 mg/L CA-NCs treatment (Fig. 3H). The boost in the activity of GR, SOD, and POD was observed in a concentration-dependent manner during salinity in the leaves of stevia. In contrast, over the salinity alone, the CA-NCs significantly enhanced the activity of all enzymes, with the highest enhancement achieved under the simultaneous treatment of severe salinity and 1 mg/L of CA-NCs (Fig. 3I, J, K). Oxidative stress and osmotic dehydration are among the main consequences of salinity in plants, which, individually or in combination, cause irreversible effects on various plant parts and processes (Hasanuzzaman and Fujita, 2022). Our results, showing an increase in H_2O_2 and a decrease in RWC, confirm this issue by demonstrating damage to plasma membranes through increased accumulation of MDA and decreased MSI. Similar effects of salinity on stevia (Ghasemi-Omran et al., 2021; Sheikhalipour et al., 2021) and other plants (Sepehry Javan et al., 2024; Haydar et al., 2023) have been reported in previous studies. It has been well established that enhancing the antioxidant machinery can potentially mitigate the toxic levels of radicals resulting from stressful situations (Ranjbar et al., 2023). However, when the CA-NCs were applied, the activity of antioxidant enzymes was significantly induced, indicating an enhancement of the antioxidant system mediated by the CA-NCs. In fact, by causing the activity of antioxidant enzymes and returning H_2O_2 levels to normal, these polyphenol-based NCs led to more excellent stability of plasma membranes under salinity. Similar effects have been reported for NCs containing polyphenols (like quercetin) on the antioxidant system under salinity (Arshneshin et al., 2023). Yiu et al. (2012) also demonstrated that applying exogenous CA reduced oxidative stress in salt-stressed cucumber plants by improving the activity of CAT, POD, SOD, and ascorbate peroxidase enzymes. Another report showed that CA, by enhancing the antioxidant defence system, reduced the production of ROS under salinity (Li et al., 2019), indicating the strengthening role of CA on the antioxidant machinery. Additionally, as a polyphenol, CA can directly act as an antioxidant and neutralize ROS (Ahammed et al., 2023). Therefore, our results confirmed that the CA-NCs are highly effective in mitigating salinity-induced oxidative stress.

3.5. Polyamine metabolism

Applying moderate and severe salinity resulted in a 22.8 and 61.9 % decrease in Spd and a 24.3 and 36.8 % decrease in spermine (Spm) in stevia leaves, respectively, over control plants. Although applying CA-NCs under normal conditions did not significantly affect the concentration of these polyamines, it significantly increased both polyamines in stevia leaves under moderate and severe salinity (Fig. 4A, B). Severe salinity significantly decreased leaf putrescine (Put) levels by 8 % over control. In contrast, treatments with 0.01 and 0.1 mg/L CA-NCs increased leaf Put levels by 23 and 33.3 % during moderate salinity and by 46.9 and 58.5 % during severe salinity, respectively (Fig. 4C). Salinity stress and the application of CA-NCs induced different effects on the total leaf polyamine content, such that salinity reduced their accumulation in leaves. At the same time, CA-NCs increased their levels under different salinity levels (Fig. 4D). Both salinity levels significantly increased methionine and arginine levels, with the highest increases under severe salinity, showing 47.3 and 63.2 %, respectively, over the control plants. In contrast, CA-NCs, in a concentration-dependent manner, reduced the leaf levels of both amino acids in stevia plants under both salinity levels (Fig. 4E, F). The salinity stress applied to stevia plants significantly increased the PAO and DAO activities over the control stevia plants. In contrast, the application of CA-NCs reduced the activity of both enzymes. At both salinity levels, both enzymes' highest and lowest activities were observed in the salinity treatment alone and the salinity+ 0.01 CA-NCs treatment, respectively (Fig. 4G, H). Polyamines are a group of compounds whose roles in germination, fruit ripening, aging, abscission, tissue lignification, pollination, and so on are well known. On the other hand, the role of these compounds as signalling molecules and inducers of the defence system against environmental stresses, especially salinity, has also been reported. A plant's ability to regulate polyamine metabolism may directly correlate with its adaptability to NaCl toxicity (Ke et al., 2018; Saha et al., 2015). Salinity stress caused a decrease in polyamine accumulation in stevia leaves, with a concomitant increase in the activity of the enzymes involved in their catabolism and the buildup of their synthetic precursors. This observation implies a change in the pathway of biosynthesis of polyamines, resulting in reduced precursor utilization followed by their buildup. This could be due to the effect of salinity-induced oxidative stress on polyamine biosynthetic enzymes or the toxic effect of sodium ions (Saha et al., 2015). Also, low availability of precursors within the polyamine biosynthesis pathway may lead to reduced polyamine contents under stress conditions (Borromeo et al., 2023). Consistent with this, similar results were noted in stevia (Ghorbani et al., 2023b) and wheat (Saha et al., 2015) under salinity stress. On the other hand, treatment with CA-NCs caused a rise in polyamine contents in the leaf. These results were accompanied by lower contents of arginine and methionine, suggesting these precursors were diverted from their usual biosynthetic routes for the production of polyamines. Given the role of CA as a polyphenol with the potential to counteract oxidative stress (Yiu et al., 2012; Ahammed et al., 2018), one would consider its properties in enhancing polyamine biosynthesis. Its effect in maintaining K/Na homeostasis (Li et al., 2019) could also be a contributing factor for this increase in polyamine biosynthesis. Therefore, the use of CA-NCs, by modifying polyamine biosynthesis and increasing their buildup in stevia leaf, improved the ability of the plant to protect its leaf from salinity-induced toxicity.

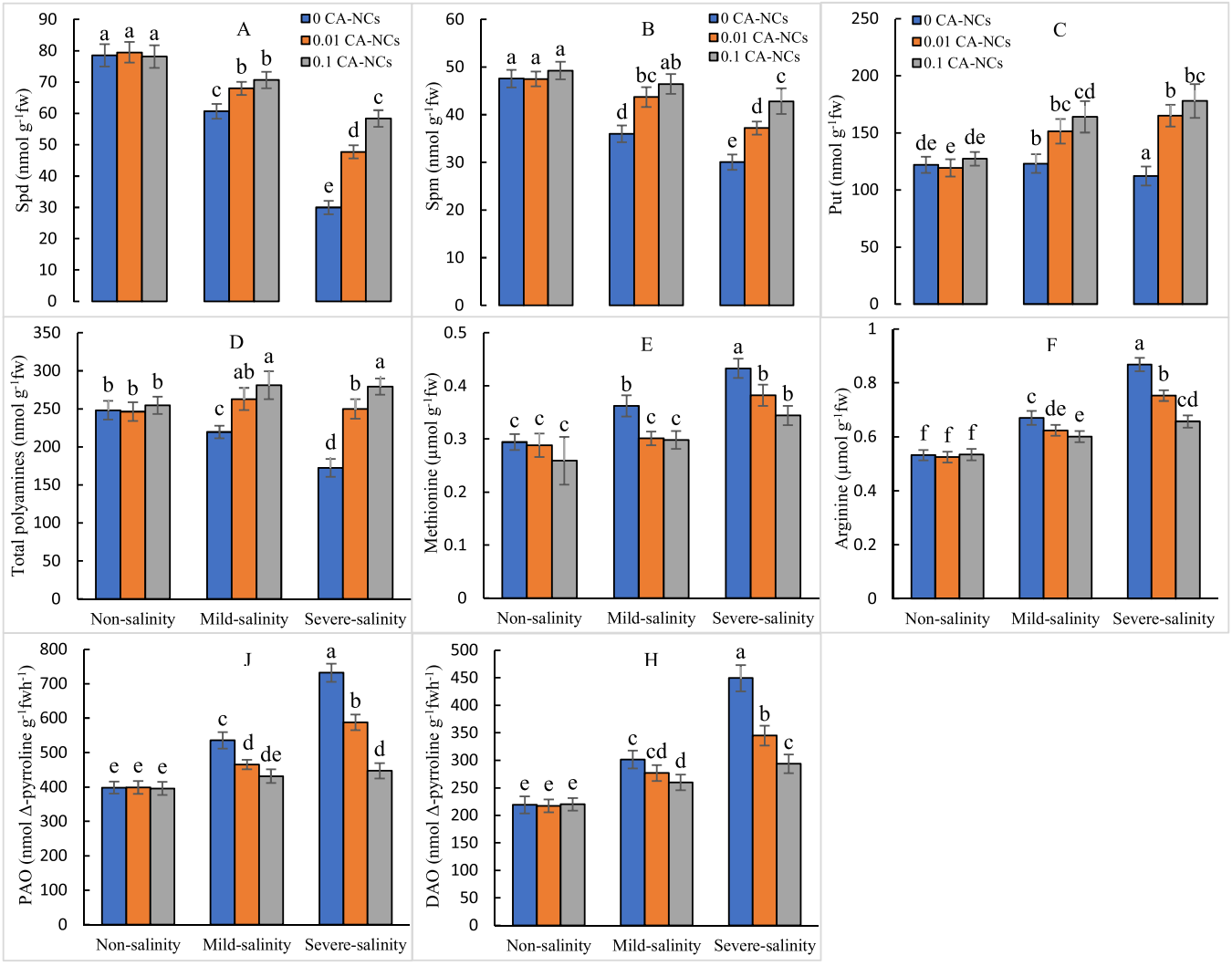

Fig. 4. Effects of catechin nanocomposite (CA-NCs) at different concentrations (0, 0.01, and 0.1 mg/mL) on the levels of spermidine (Spd, A), spermine (Spm, B), putrescine (Put, C), total polyamines (D), methionine (E), and arginine (F), as well as the activity of polyamine oxidase (PAO, J) and diamine oxidase (DAO, H) in stevia leaves under different salinity levels (non-salinity: 0 mM NaCl, mild-salinity: 50 mM NaCl, and severe-salinity: 100 mM NaCl). Values (means ± SD) followed by the same letter are not significantly different ($p < 0.05$; Duncan test).

3.6. Nitrogen metabolism

With increasing salinity stress, the N and NO_3 content experienced a significant drop in stevia leaves. The lowest levels of these compounds were recorded during severe salinity, with a decrease of 51 and 52.4 %, respectively, over the control. In contrast, exposure to CA-NCs significantly enhanced the level of both compounds in salinity-stressed plants, reaching their highest levels under salinity with 0.1 mg/L of CA-NCs (Fig. 5A, B). NR activity experienced 32 and 44.7 % reductions under moderate and severe salinity, respectively, while NiR activity decreased 27 and 54.9 %, respectively. In contrast, CA-NCs restored the activity of both enzymes. During 1 mg/L CA-NCs treatment, their activity reached the highest level under both salinity levels (Fig. 5C, D). Similarly, a salinity level-dependent decrease in GS and GOGAT activity was observed over the control stevia plants, with the highest reductions being 55.9 and 58.6 %, respectively. In contrast, CA-NCs stimulated the activity of both under both salinity levels in a concentration-dependent manner (Fig. 5E, F). N is an essential nutrient for plant growth and development, defence responses, and various signalling pathways helping plants to adapt to environmental stresses (Sheikhalipour et al., 2021; Mohammadbagherlou et al., 2025). Salinity is one of the external stimuli affecting N metabolism by altering N uptake and the activity of N metabolism-involved enzymes. Salinity in stevia plants reduced leaf accumulation of N and NO_3. It significantly decreased the activity of various enzymes implicated in N metabolism, indicating a disruption in the N uptake and metabolism processes. These detrimental effects could be due to the direct toxicity of high Na levels and ROS generated by salinity (Nazir et al., 2023; Ghorbani et al., 2023c). Similar findings have been reported in other plants, including rice (*Oryza sativa* L.) and stevia. Zhang et al. (2023) indicated that salinity reduces plant yield by decreasing N and NO_3 contents and damaging the N metabolism pathway. However, it is well established that maintaining the dynamics of N uptake and metabolism plays a significant role in enhancing plant adaptation under salinity (Nazir et al., 2023), which is why it has recently attracted considerable attention. In contrast, CA-NCs significantly improved the N uptake and metabolism process in stevia leaves, supported by the results from applying other nanomaterials (Khatoon et al., 2024; Yang et al., 2024). Various reports have confirmed that maintaining the dynamics of N metabolism under salinity stress mainly enhances adaptation; however, the role of NCs containing a polyphenolic compound in this context has been less explored. Therefore, it can be suggested that applying CA-NCs, through the provision of CA and considering the antioxidant role of this polyphenol, improves the performance of N metabolism enzymes by mitigating the toxic impacts of

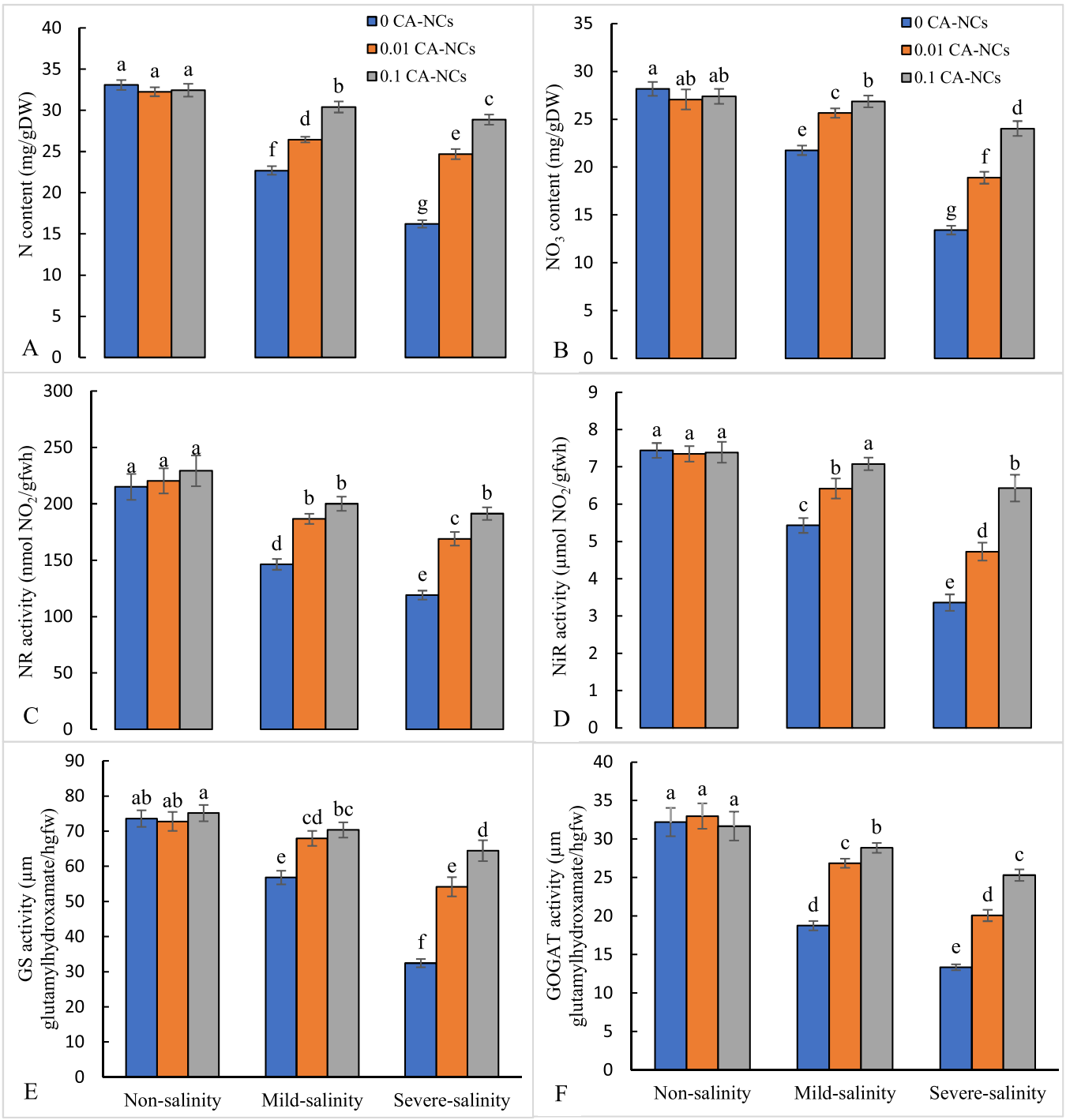

Fig. 5. Effects of catechin nanocomposite (CA-NCs) at different concentrations (0, 0.01, and 0.1 mg/mL) on the level of N (A) and NO_3 (B), as well as the activity of NR (C), NiR (D), GS (E), and GOGAT (F) in stevia leaves under different salinity levels (non-salinity: 0 mM NaCl, mild-salinity: 50 mM NaCl, and severe-salinity: 100 mM NaCl). Values (means ± SD) followed by the same letter are not significantly different ($p < 0.05$; Duncan test).

ROS (Ahammed et al., 2023). On the other hand, the CA-based NCs, by reducing Na uptake and maintaining membrane potential and ion homeostasis in the cytoplasm (Li et al., 2019), can stabilize the N uptake system in plants and preserve the dynamics of N metabolism under salinity stress. Nevertheless, more detailed information from molecular studies on the regulatory effects of CA-NCs on genes involved in this pathway can expand our understanding of the defence processes induced by these NCs.

3.7. Glucoside compounds, essential oil content, and relative expression of glycoside biosynthetic genes

Moderate salinity reduced the stevioside content in the leaves by 22.2 %, while severe salinity did not cause a significant change. CA-NCs had varying effects on the stevioside content under different salinity levels. In moderate salinity, stevioside levels were decreased, whereas, under severe salinity, the leaf accumulation of steviosides was elevated (Fig. 6A). The leaves experienced a 22.8 and 41 % increase in Reb A under moderate and severe salinity, respectively, over the control

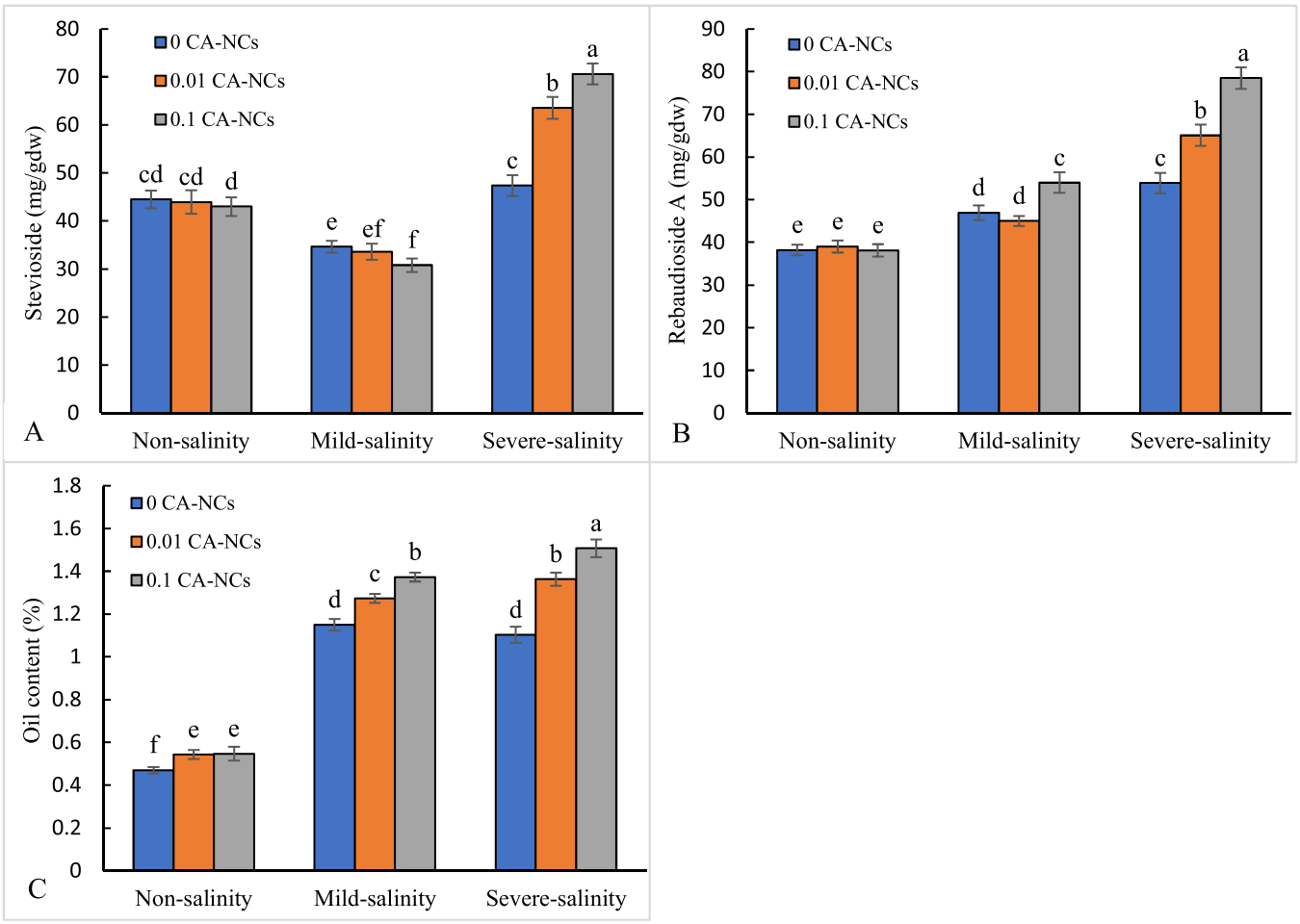

Fig. 6. Effects of catechin nanocomposite (CA-NCs) at different concentrations (0, 0.01, and 0.1 mg/mL) on the level of stevioside (A), rebaudioside A (B), and oil (B) in stevia leaves under different salinity levels (non-salinity: 0 mM NaCl, mild-salinity: 50 mM NaCl, and severe-salinity: 100 mM NaCl). Values (means ± SD) followed by the same letter are not significantly different ($p < 0.05$; Duncan test).

leaves. Conversely, the 0.1 mg/L CA-NCs treatment resulted in a 15.1 % increase in Reb A under moderate salinity. In contrast, the 0.01 and 0.1 mg/L CA-NCs treatments led to a 20.8 and 45.6 % increase in Reb A under severe salinity (Fig. 6B). Salinity stress, both alone and in combination with CA-NCs, significantly elevated the leaf essential oil content over the control leaves. The highest oil content was recorded in the treatment with 0.1 mg/L CA-NCs combined with severe salinity (Fig. 6C).

Moderate and severe salinity significantly reduced the relative expression of *KAH* and *UGT74G1* over the control. In contrast, both levels of CA-NCs boosted the expression of both genes at both salinity levels (except for a concentration of 0.01 mg/L CA-NCs in *UGT74G1* expression under moderate salinity) (Fig. 7A, B). Moderate and severe salinity significantly increased the relative expression of the *UGT76G1*, while only severe salinity significantly increased the expression of the *UGT85C2*. In contrast, at both salinity levels, the expression of both genes was notably enhanced by both CA-NCs levels (Fig. 7C, D).

Given the significance of secondary metabolites in medicinal plants' biological activities, enhancing or improving the bioactive content is one of the most critical current efforts focused on the stevia plant. This plant is particularly valued in the pharmaceutical industry, especially for non-caloric diabetes medications, due to its glycoside compounds, including stevioside and Reb A (Patel and Navale, 2024; Ceunen and Geuns, 2013). Salinity led to a notable rise in Reb A and a slight drop in stevioside in the leaves, which correlates with the increased expression of *UGT85C2* and *UGT76G1* and the decreased expression of *UGT74G1* and *KAH*. The results indicate that stevia, under salinity, tends to synthesize and accumulate more Reb A, which may be directly linked to the function of this compound in coping with salt toxicity. The increase in glycosidic sugars, particularly Reb A, along with essential oil content in the stevia plant, has also been previously reported during salinity (Ghorbani et al., 2023b; Sheikhalipour et al., 2021) and drought (Lahijanian et al., 2023) stresses. On the other hand, the osmoprotectant function of diterpene glycosides, particularly Reb A, as an osmolyte has been mentioned (Ceunen and Geuns, 2013), which could explain its increase in this study as a response to salinity-induced water stress (Cantabella et al., 2017). Therefore, the stevia plants increased the accumulation of glycosidic sugars, especially Reb A, to enhance their adaptation to secondary water stress under salinity conditions. Interestingly, the application of CA-NCs, by upregulating the expression of all four genes linked to the biosynthesis of glycosidic sugars, led to an enhancement in the leaf level of stevioside and Reb A under salinity, which could be of particular significance from a food and pharmaceutical industry perspective. Although the effects of bio-compounds-containing NCs on stevia have rarely been studied, other NPs, especially metallic NPs, have shown potential as an effective and efficient method for enhancing stress tolerance and improving the quality of Stevia metabolites under salinity (Sheikhalipour et al., 2021). The inductive effect of NPs on the synthesis and accumulation of secondary metabolites in other medicinal herbs, like lemon verbena (*Aloysia citrodora* Paláu) (Ghanbari and Azizi, 2023) and basil (*Ocimum basilicum* L.) (Sepasi et al., 2024), has been previously documented. Further studies have shown that NPs can stimulate the biosynthetic enzymes controlling secondary metabolites in medicinal plants,

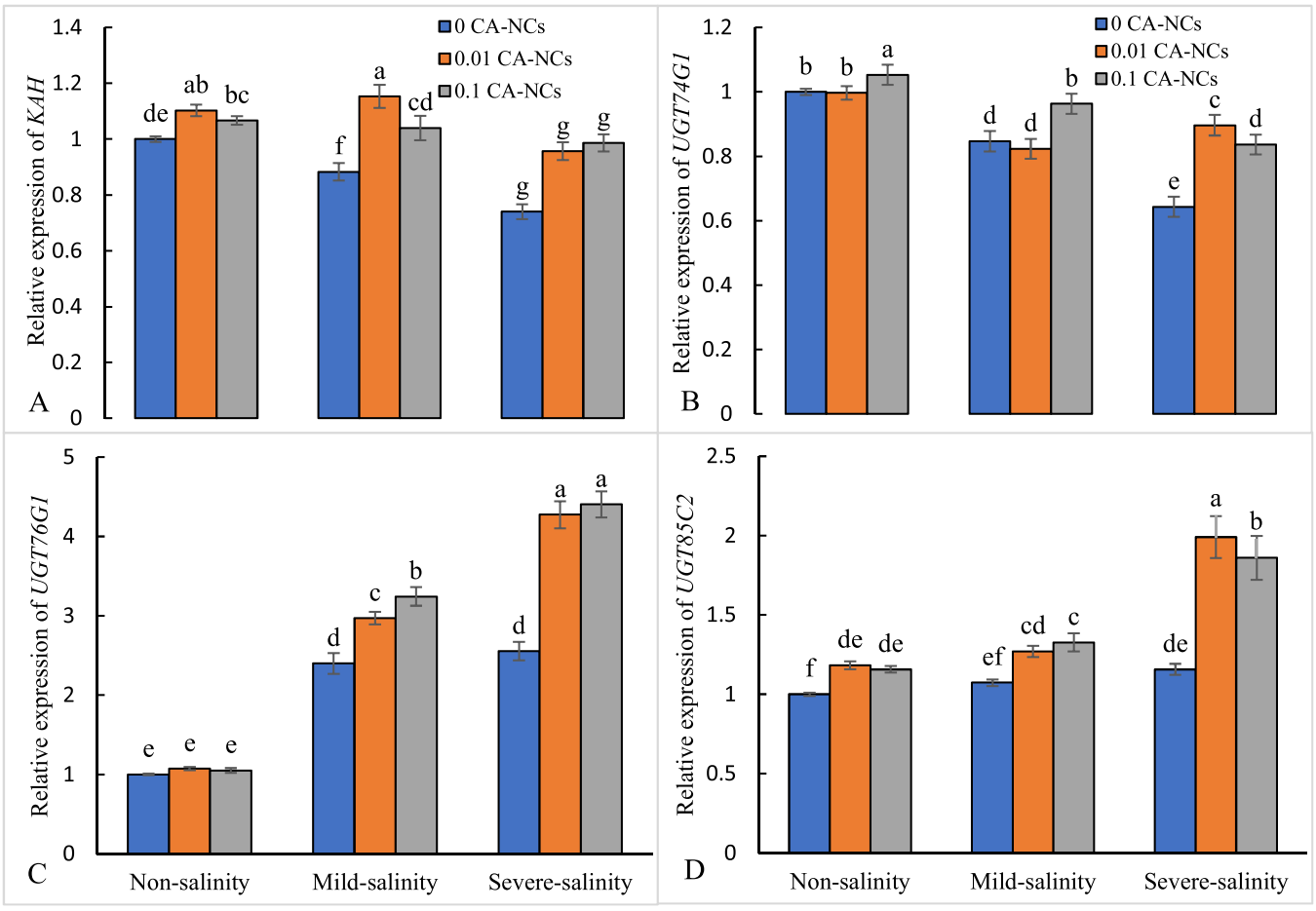

Fig. 7. Effects of catechin nanocomposite (CA-NCs) at different concentrations (0, 0.01, and 0.1 mg/mL) on the relative expression of KAH (A), *UGT74G1* (B), *UGT76G1* (C), and *UGT85C2* (D) in stevia leaves under different salinity levels (non-salinity: 0 mM NaCl, mild-salinity: 50 mM NaCl, and severe-salinity: 100 mM NaCl). Values (means ± SD) followed by the same letter are not significantly different ($p < 0.05$; Duncan test).

therefore enhancing them by means of the biosynthetic route (Li et al., 2020; Rajaee Behbahani et al., 2020). By activating the biosynthetic route of diterpene glycosides, our findings show that the manufactured CA-NCs can promote the buildup of secondary metabolites in stevia under salinity. This increase in secondary metabolites, along with improved stress tolerance and stevia growth, underlines its potential application in industry and suggests it as an effective method to cultivate this crop in saline areas. On the other hand, the elevated levels of these glycosides, particularly Reb A, under CA-NCs and salinity treatments underscore the potential of this compound as an effective defence mechanism for enhancing plant's adaptability under stress conditions.

3.8. Correlation and PCA analysis

The Pearson correlation results indicated that morphological and photosynthetic traits positively correlated with the K/Na ratio, N metabolism traits, RWC, MSI, Spm, and Spd, and negatively correlated with Na, oxidative stress markers, DAO, PAO, Arg, and Met. A positive correlation was also observed between Na with polyamine metabolism and oxidative stress markers and N and NO_3 content with N metabolism enzymes (Fig. 8). The inference from the PCA analysis indicated that the examined traits fall into three main groups, capturing 91.27 % of the variability in the original data. The first group is associated with growth traits, N metabolism, K/Na ratio, photosynthetic apparatus, Spm, Spd, MSI, and RWC. The second cluster, on the other hand, is correlated with Na, PAO, DAO, Put, arginine, methionine, and markers of antioxidant stress. The third cluster, by contrast, relates to antioxidant enzymes and diterpenoid glycosides (Fig. 9). The data obtained by PCA and correlation analysis suggest that treatment with CA-NCs could significantly improve the salinity tolerance of stevia plants. By enhancing the K/Na ratio, preventing oxidative stress, and upregulating the activity of antioxidant enzymes, CA-NCs help maintain cellular integrity and photosynthetic efficiency. Furthermore, the PCA results show that features related to N metabolism and ionic balance are important for the plant's response to salinity. By reducing the harmful effects linked with the buildup of Na ions and activating the plant's defenses, the results of this study suggest that CA-NCs can maintain plant performance in stressful environmental conditions. Finally, these results could represent a valuable source of information for the development of new agro-managerial approaches designed to increase the tolerance of medicinal plants such as stevia towards environmental stress factors. Future studies could address the molecular mechanism involved, as well as the use of similar nanoparticles in other crop plants subject to a variety of stress conditions.

4. Conclusion

This work clearly proved that the new CA-NCs, created for the first time using a bio-derived method, greatly improve the salinity tolerance of stevia. Using CMC as a biocompatible carrier, the CA-NCs were created, guaranteeing the stability and efficacy of the NCs and offering an eco-friendly substitute for conventional synthetic materials. NCs produced by the creative synthesis technique had outstanding qualities including a small and consistent particle size that helped them interact with plant tissues. By enhancing important physiological and biochemical factors—such as the K/Na ratio, photosynthetic efficiency,

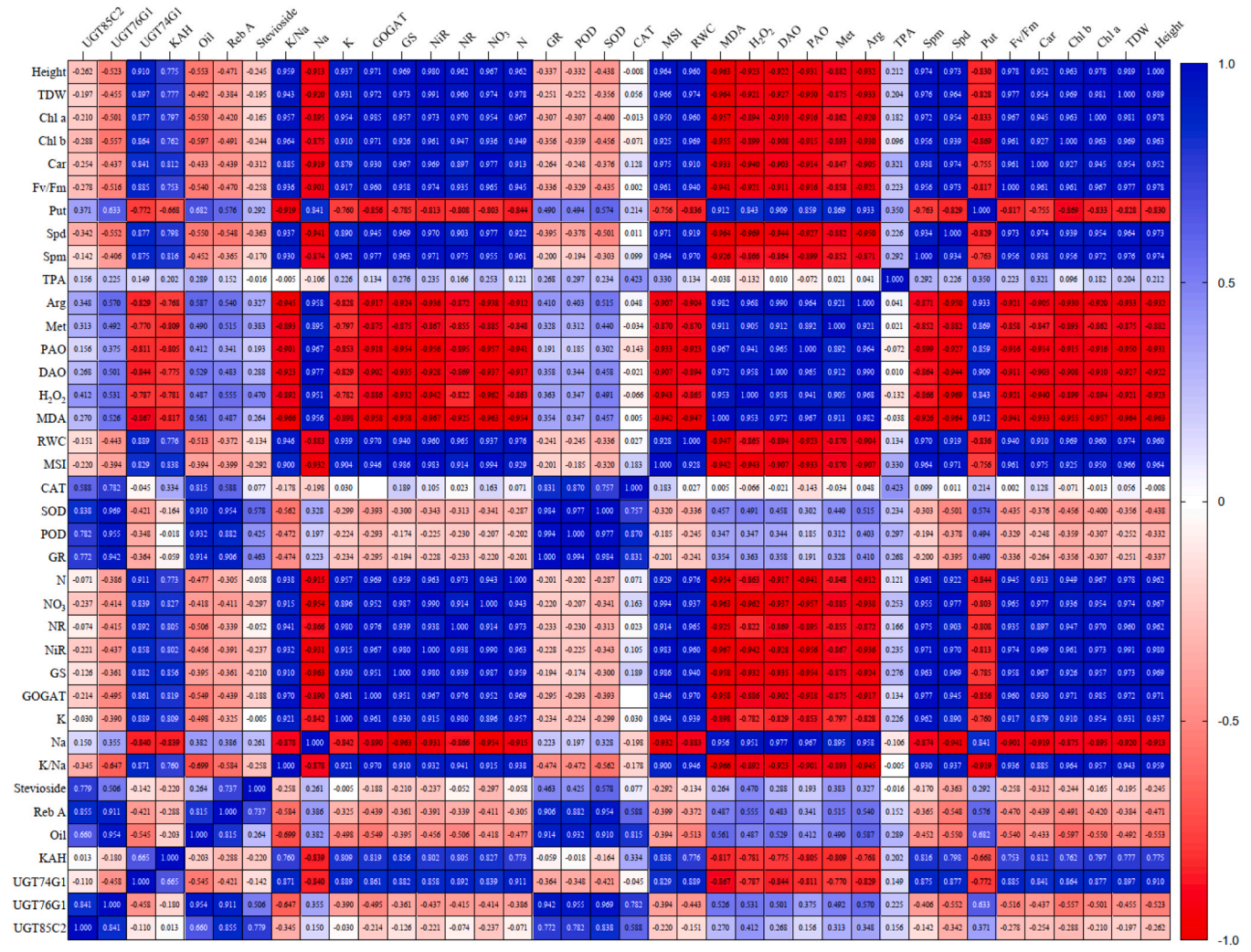

Fig. 8. The Pearson correlation analysis heatmap for the impact of different salinity levels and CA-NCs application on all parameters of the stevia plant.

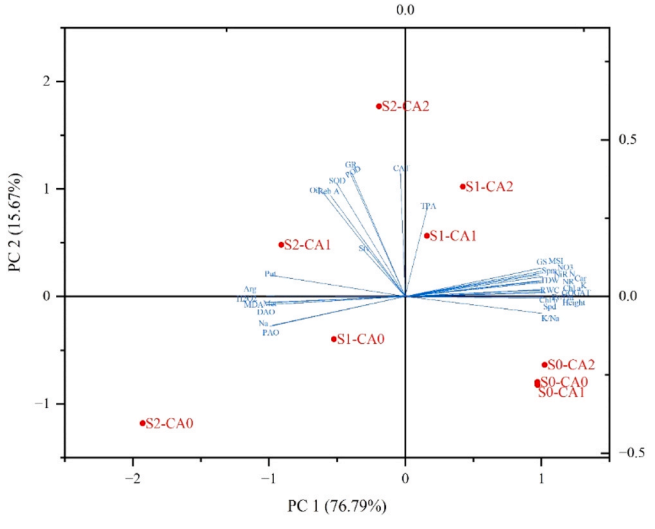

Fig. 9. The principal component analysis for the impact of different salinity levels and CA-NCs application on all parameters of the stevia plant.

and antioxidant enzyme activity—applying these CA-NCs reduced the negative consequences of salinity. With CA-NCs playing a key role in changing these characteristics, correlation and PCA studies underlined even more that N metabolism, ionic balance, and oxidative stress management qualities are vital for the adaptive response of the plant to salinity.

Furthermore, this study found that CA-NCs promote plant growth and development under salinity and increase the concentration of desirable secondary metabolites, including Reb A and stevioside, essential for the stevia medicinal and commercial value of . The effective application of this bio-derived NC provides a strong instrument for improving the resilience of medicinal plants in saline soils and opens new paths for sustainable agriculture.

Although the physiological and biochemical reactions to CA-NCs under salinity stress were thoroughly investigated, the molecular mechanisms behind these effects remain uncharted. CA-NC-mediated stress tolerance's exact pathways require more research, especially transcriptomic and proteomic studies. The research was also done under controlled conditions; field trials are required to validate the effectiveness and long-term influence of CA-NCs in actual agricultural environments. The possible buildup of NCs in plant tissues and their impact on soil microbial communities also call for more investigation to guarantee their environmental safety.

Future studies should seek to clarify more the molecular mechanisms propelling the noted impacts of CA-NCs and investigate their use over a wider variety of crops and environmental stresses. Their effective use in agricultural practices will also depend on the long-term influence of CA-NCs on soil ecology and plant health. By means of a strong basis, this work gives creative ideas to increase crop tolerance to abiotic stressors,

supporting more efficient and sustainable agricultural systems.

CRediT authorship contribution statement

Chen Moxian: Writing – review & editing, Supervision, Resources, Project administration, Conceptualization. **Maggi Filippo:** Writing – review & editing, Data curation, Conceptualization. **Razavi Seyed Mehdi:** Resources, Methodology, Formal analysis, Data curation, Conceptualization. **Khalofah Ahlam:** Visualization, Validation, Resources, Investigation. **Ghorbani Abazar:** Writing – review & editing, Writing – original draft, Software, Methodology, Formal analysis, Data curation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.indcrop.2025.121134.

Data availability

Data will be made available on request.

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