

EXOGENOUS TREHALOSE IMPROVES GROWTH, FLOWERING, OSMOTIC ADJUSTMENT AND ANTIOXIDANT DEFENSE OF CHRYSANTHEMUM UNDER SALINITY STRESS

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Abstract

Salinity is among the main factors that restrict the cultivation of chrysanthemum, because the excess of NaCl influences the dynamics of water, nutrient uptake, photosynthesis and oxidative balance, which compromises the growth of the plant and the quality of the flowers. The present study aimed to explore if exogenous trehalose could mitigate the detrimental effects of salinity on chrysanthemum (*Chrysanthemum indicum* L.) under greenhouse conditions. Plants were treated with four different concentrations of NaCl (0, 50, 100, and 150 mM) and four different concentrations of trehalose (0, 5, 10, and 20 mM). The parameters quantified were vegetative growth, flowering, relative water content (RWC), photosynthetic pigments, osmolytes, oxidative stress markers, antioxidant enzymes and mineral accumulation. Higher salinity levels decreased plant height, shoot and root weight, chlorophyll content, relative water content, flower size and number of flowers, and delayed flowering. Salinity increased proline, soluble sugars, H₂O₂ and malondialdehyde (MDA) and enhanced activities of SOD, CAT and APX. Salinity induced a significant increase in the accumulation of sodium ion (Na⁺) while the concentration of potassium ion (K⁺) and K⁺/Na⁺ ratio decreased. These responses were greatly reduced by trehalose, with 10 mM generally being the most effective dose. Addition of 10 mM trehalose at 100 and 150 mM NaCl enhanced biomass, chlorophyll content, relative water content and flowering efficacy, while decreasing H₂O₂ and MDA accumulation, increasing antioxidant activity, reducing Na⁺ levels and partially restoring K⁺ homeostasis. These results suggest that trehalose has a role in the coordination of osmotic adjustment, oxidative stress protection and ionic homeostasis. To our knowledge, this is the first report supporting the potential use of exogenous trehalose as a biostimulant to improve chrysanthemum growth under saline irrigation

Key words: *Chrysanthemum indicum* L.; Salinity; NaCl; Trehalose; Osmotic adjustment; Antioxidant enzymes; Ion homeostasis; Flowering.

Introduction

Chrysanthemum, generally referred to as *Chrysanthemum indicum* L., is one of the world's most important ornamental plants. The economic value is dependent on the blooming capacity of the plant, the vegetative growth, petal size, quantity of flowers and the overall quality of the plant. Salinity might be one of the parameters that determine these features, particularly in plants irrigated with saline solutions, or in soils where salt accumulates over time with an increasing concentration. Chrysanthemum is a moderately salt stress-responsive plant with high diversity across cultivars and species (Bandurska *et al.*, 2022; Vanlalruati *et al.*, 2023). They found that the response to salt stress was not particularly high in most cultivars. An increase in the salt content in "Palisade White" plants resulted in a significant build-up of sodium and chloride ions in the leaf that inhibited photosynthesis, stomatal conductance and transpiration (Bandurska *et al.*, 2022). The suppression of growth was dosage dependant. diverse cultivars of chrysanthemum exhibited diverse responses to 150 mM sodium chloride in terms of osmolytes and mineral levels. A chosen set of genotypes also performed better than other genotypes (Vanlalruati *et al.*, 2023). The extent of salt damage was the fundamental element affected by the capacity of each chrysanthemum to

maintain water balance, ionic balance and metabolic function under stress, researchers discovered.

The three separate consequences of the water salinity on chrysanthemum are ionic toxicity, oxidative stress and osmotic pressure (Rahi & Singh, 2011). It may stop cells from dividing and suppress the development of plants. During prolonged exposure, oxidative stress and Na⁺ and Cl⁻ accumulation increase, whereas K⁺ and Ca²⁺ concentrations decline. For example, salinity stress increased sodium and chloride ions in the leaves of *C. × grandiflorum* 'Palisade White'. In contrast, the K⁺ balance and proline build-up appear to be able to maintain the physiological function even in case of persistent stress (Bandurska *et al.*, 2022). Vanlalruati *et al.*, (2023) revealed the greater osmolyte accumulation and K⁺/NaCl ratio in NaCl tolerant chrysanthemum cultivars on independent screening studies which have been appropriately executed. Sodium chloride build-up may alter the growth of chrysanthemum and reduce the concentration of chlorophyll and restrict the absorption of mineral resources. Vanlalruati *et al.*, (2021) results appear to indicate a strong connection between salinity and efficiency of photosynthesis and absorption of nutrients by plants. Under salt stress, they found imbalance in the tissue water content, photosynthetic pigment concentration and the optimal ratio of K⁺ and Na⁺ in chrysanthemum.

In recent years, molecular techniques have greatly contributed to the knowledge of the susceptibility of chrysanthemum to salt stress. Transgenic plants overexpressing the transcription factor *CmHSFA4* showed enhanced salt tolerance via decreased Na^+ accumulation, maintenance of K^+ levels, increased antioxidant defense mechanisms and increased expression of ion transporters *CmSOS1* and *CmHKT2* (Li *et al.*, 2017). Recently, *CmCIPK8* has been demonstrated to be an important component of the salt response network. Ding *et al.*, (2022) reported that salinity stress promoted the expression of *CmCIPK8*, leading to the higher chlorophyll and relative water content, reduced ROS and membrane lipid peroxidation, improved antioxidant activity, and increased K^+/Na^+ and $\text{Ca}^{2+}/\text{Na}^+$ ratios. (Wang *et al.*, 2024). The regulatory complex *CmHSFA4-CmMYBS3-CmTPL* inhibited *CmMYB121*, hence improving salt tolerance via regulating the antioxidant and ion transport activities. *CiHY5* was shown to be involved in hormonal signaling, photosynthesis and carbohydrate metabolism to improve salt tolerance of *Chrysanthemum indicum*. Wang *et al.*, (2025) demonstrated that the enhancement was caused by *CiHY5*. The results indicated that salt tolerance in chrysanthemum is not controlled by a single protective mechanism but by a systematic regulating process comprising a series of different mechanisms. These include ion transport processes, ROS metabolism, photosynthesis, carbohydrate metabolism and transcriptional response.

One of the most important discoveries has been the identification of mechanisms that contribute to the protection of cells and the integrity of DNA. Recently, it was revealed that salt stress may induce the synthesis of *WEE1* in *Chrysanthemum lavandulifolium*, and *CIWEE1* was proposed to be involved in regulation of cell cycle and DNA repair. Wang *et al.*, (2025) demonstrated that the increased salt tolerance of *CIWEE1* was correlated with the up-regulation of *CINHX*, *CIHKT*, *CICBL* and *CIDREB2A*. In addition, proteome analysis showed that redox activity, glucose and glutathione metabolism and several stress-related pathways in chrysanthemum were impacted by saline stress. The findings show that chrysanthemum response to salinity is a complex mechanism including osmoregulation, antioxidant defense, ion transport, carbohydrate metabolism and cell repair.

Biostimulants are chemicals that may act on many processes simultaneously and the rising quantity of knowledge permits to estimate these recent biostimulants. In such a context, trehalose seems to be a very promising candidate for a suitable solute and signalling metabolite. Exogenous trehalose may alleviate osmotic adjustment, cell membranes, and proteins, photosynthetic activity, and antioxidant capacity under the effect of abiotic stress (Nawaz *et al.*, 2022; Ibrahim, 2025). There is lack in literature regarding the effects of trehalose on chrysanthemum. Trehalose affects a number of physiological processes associated with salt control in plants in general. They include water transport, control of ROS, preservation of membrane integrity and glucose levels. Trehalose may affect proline, soluble sugars, antioxidant enzymes and K^+/salt ratio in Chrysanthemum. However, no publication has shown the direct proof of exogenous trehalose in salt-stressed chrysanthemum.

The salinity is a highly important factor when working with ornamental horticulture, as salinity management is required for flowering and vegetative growth (Ruati *et al.*, 2021; Bandurska *et al.*, 2022; Vanlalruati *et al.*, 2023). Salt stress has a major impact on the biomass of chrysanthemum plants, the efficiency of photosynthetic activities and the mineral balance of their bodies. The present study intends to investigate the effect of exogenous trehalose on chrysanthemum under the rising levels of NaCl during greenhouse experiment. The present study was conducted to study the effect of trehalose on plant growth and flowering and relative water content, photosynthetic pigments, osmolyte accumulation, oxidative stress, antioxidant activity and mineral balance. It was hypothesized that trehalose might reduce salt stress via osmotic adjustment, retention of photosynthetic pigments, boosting scavenging of ROS and optimal K^+/Na^+ ratios. This may allow plants to survive and flourish in high salt conditions. Trehalose might potentially protect the pigments needed in photosynthesis.

Materials and Methods

Plant material and growth conditions: Uniform 10 cm rooted chrysanthemum (*Chrysanthemum indicum* L.) cuttings of similar size and developmental stage were used. Plants were transplanted individually into plastic pots containing a well-drained substrate consisting of peat moss, perlite and washed sand at a ratio of 2:1:1 (v/v/v). The substrate was characterized before planting for pH, electrical conductivity and basic nutrient status.

Plants were maintained in a controlled greenhouse at King Saud University under natural photoperiod supplemented when necessary to provide approximately 14 h light per day. Day/night temperature was maintained at approximately 25/18°C, with relative humidity maintained between 60 and 70%. Plants were irrigated with half-strength Hoagland nutrient solution until establishment. The experimental treatments were initiated after plants had developed a stable root system and several fully expanded leaves.

Experimental design and treatments: The experiment followed a factorial completely randomized design consisting of four NaCl concentrations and four trehalose concentrations. Salinity treatments were NaCl at 0, 50, 100, and 150 mM and four different concentrations of trehalose (0, 5, 10, and 20 mM). Trehalose was dissolved in distilled water and applied to the soil as a drench according to the treatment schedule. The same volume of solution was applied to all treatments to ensure consistency among treatments. Sodium chloride was introduced gradually over several days, with the concentration progressively increased until the designated treatment concentrations of 50, 100, and 150 mM were reached. This gradual acclimation was used to minimize the risk of osmotic shock. This was continued until the required concentrations were attained. Control plants were treated with the same quantity of water with no NaCl or trehalose. This concentration range was selected because previous work has indicated that trehalose may reduce salinity-induced growth inhibition and ~10 mM may be a beneficial

concentration, but higher concentrations can become supra-optimal (Chang *et al.*, 2014). The plants were maintained under the different treatments until they flowered at maturity.

Growth measurements: Plant height was measured from the surface of the substrate to the growth apical point using a ruler. After the experiment branches and roots were separated and new masses were immediately weighted. The plant material was then oven-dried at 70°C to constant mass and weighed to calculate shoot and root dry weight.

Flowering measurements: Flowers were recorded during the experiment. The days to blooming were recorded as days from treatment beginning to the first flower at the designated stage of anthesis. At full bloom, flower diameter was measured using a digital caliper. At the conclusion of the experiment the total number of flowers per plant was recorded. Flower fresh and dry weights may also be assessed as another indication of commercial flower quality. The inclusion of flowers was justified as salt reduces both vegetative growth and reproductive performance. The enhancement of flowering rather than biomass alone is especially essential in decorative chrysanthemum production.

Relative water content: The relative water content was estimated according to Barrs & Weatherley (1962). Immediately after sampling, fresh weight (FW) of fully inflated leaves was weighed and the leaves were floated in distilled water until they attained full turgidity, wiped gently and measured again for turgid weight (TW). Then, the samples were dried at 70°C to constant weight to obtain dry weight (DW).

RWC was calculated as:

$$\text{RWC (\%)} = [(\text{FW} - \text{DW}) / (\text{TW} - \text{DW})] \times 100$$

Photosynthetic pigments: Fresh leaf tissue was extracted with 80% acetone for chlorophyll a, chlorophyll b and total chlorophyll. Absorbance was determined spectrophotometrically at respective wavelengths and pigment concentrations were estimated (Lichtenthaler & Wellburn, 1983; Al-Yafsi *et al.*, 2026). At the same time, carotenoid concentration was measured.

Proline determination: Free proline was calculated following Bates *et al.*, (1973) and Elansary *et al.*, (2026b). Fresh leaf material was ground in 3% aqueous sulfosalicylic acid and filtered. The extract was mixed with acid ninhydrin and glacial acetic acid and incubated in boiling water bath. After cooling, the chromophore was extracted with toluene and the absorbance was measured at 520 nm. The proline concentration was determined by use of a standard curve produced using L-proline.

Soluble sugar determination: Fresh leaf tissue was extracted with aqueous ethanol for total soluble sugars. The sugar concentration was measured using the anthrone technique with glucose as the standard (Elhindi, 2026). Results are provided on a fresh weight basis.

Determination of H₂O₂ and lipid peroxidation: The concentration of hydrogen peroxide was measured spectrophotometrically following Elansary *et al.*, (2026). Lipid peroxidation was determined from the concentration of malondialdehyde using the thiobarbituric acid reaction according to Heath & Packer (1968) and absorbance was measured at 532 and 600 nm. The MDA concentration was reported as nmol g⁻¹ fresh wt.

Antioxidant enzyme assays: Fresh leaves were homogenized in suitable phosphate buffer including EDTA and antioxidant stabilizing components under cold circumstances. For enzyme tests, the supernatants of the centrifuged homogenates were employed. SOD activity was determined as suppression of photochemical reduction of nitroblue tetrazolium according to Elansary *et al.*, (2026a). CAT activity was measured as the rate of degradation of H₂O₂ and APX activity was measured as the decrease in absorbance due to ascorbate oxidation (Moussa *et al.*, 2026; Elansary *et al.*, 2026b). The activity of the enzymes were expressed per protein basis. Soluble protein content was measured by the Bradford technique (Bradford, 1976).

Mineral analysis: Dried leaf samples were crushed to a fine powder and acid digested. Sodium, K⁺ and Ca²⁺ concentrations were evaluated by atomic absorption spectrophotometry. The ionic balance degree under salinity was estimated using the K⁺/Na⁺ and Ca²⁺/Na⁺ ratios. The maintenance of K⁺ and limitation of Na⁺ buildup are of particular relevance in chrysanthemum, since recent studies have found variable Na⁺ exclusion and K⁺ retention as significant components of salt tolerance across genotypes (Wang *et al.*, 2025).

Statistical analysis

The experiment followed a factorial completely randomized design consisting of four NaCl concentrations (0, 50, 100, and 150 mM) and four trehalose concentrations (0, 5, 10, and 20 mM). The effects of NaCl and trehalose concentration evaluated. When significant differences were detected, means were separated using Fisher's least significant difference (LSD) test at $p \leq 0.05$. The statistical analysis was performed using SPSS (2025).

Results

Trehalose improved vegetative growth and flowering under salinity: Development of chrysanthemum was significantly influenced by salinity (Table 1). Plant height, shoot fresh weight, shoot dry weight and root dry weight were decreased progressively with increasing NaCl concentration from 0 to 150 mM. Maximum inhibition was seen at 150 mM NaCl where plants without treatment were about 46% shorter in plant height and 56% lower in shoot dry weight as compared to unstressed control. Trehalose significantly ameliorated the growth inhibition caused by salt at several salinity levels, with 10 mM generally producing the strongest response. The response was concentration-dependent, with 10 mM frequently providing the most benefit. The plant height increased from 43.1 to 53.2 cm and shoot dry weight from 5.34 to 6.78 g with 10

mM trehalose at 100 mM NaCl. The relative values at 150 mM NaCl were 44.6 cm and 5.49 g, compared to 33.7 cm and 3.87 g. Salinity also delayed the flowering and reduced the diameter and number of flowers. The time to flowering in untreated plants with 150 mM NaCl was around 91.5 days compared with 72.4 days in the non-saline control. Under 150 mM NaCl, the application of 10 mM trehalose reduced days to flowering to 83.4 from 91.5 days and an increased n flower diameter from 5.42 to 6.37 cm and number of flowers from 3.1 to 4.3 flowers plant⁻¹. The results observed with 20 mM trehalose were mostly similar but not better than those with 10 mM trehalose, suggesting that 10 mM was close to the optimum dosage of trehalose under the experimental conditions of this investigation.

Trehalose maintained photosynthetic pigments: The concentrations of chlorophyll a, chlorophyll b, total chlorophyll and carotenoids of chrysanthemum were

progressively decreased with increasing NaCl concentration (Table 2). The reduction was more pronounced at 100 and 150 mM NaCl and lowest pigment amounts were detected in plants treated with 150 mM NaCl without trehalose. Chlorophyll a, chlorophyll b, total chlorophyll and carotenoids decreased to 0.76, 0.33, 1.09 and 0.23 mg g⁻¹ FW, respectively at this salinity level. Application of trehalose greatly ameliorated salt-induced pigment losses. The best improvement was generally found at 10 mM trehalose, where chlorophyll a, chlorophyll b, total chlorophyll and carotenoids were enhanced at all saline levels tested. For example, at 150 mM NaCl, 10 mM trehalose increased total chlorophyll from 1.09 to 1.65 mg g⁻¹ FW and carotenoids from 0.23 to 0.32 mg g⁻¹ FW. The results indicated that exogenous trehalose was helpful in maintaining the photosynthetic pigment system of chrysanthemum under saline conditions.

Table 1. Effect of salinity and trehalose application on vegetative growth and flowering of chrysanthemum.

NaCl (mM)	Trehalose (mM)	Plant height (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)	Days to flowering	Flower diameter (cm)	Flowers plant ⁻¹
0	0	62.4 ± 1.8 a*	48.6 ± 1.5 a	8.72 ± 0.31 a	3.21 ± 0.14 a	72.4 ± 1.3 d	8.42 ± 0.18 a	7.8 ± 0.4 a
0	5	64.1 ± 1.6 a	51.3 ± 1.7 a	9.18 ± 0.28 a	3.38 ± 0.12 a	70.8 ± 1.2 d	8.65 ± 0.17 a	8.1 ± 0.3 a
0	10	65.0 ± 1.7 a	52.4 ± 1.6 a	9.36 ± 0.30 a	3.44 ± 0.13 a	69.6 ± 1.1 d	8.78 ± 0.16 a	8.4 ± 0.4 a
0	20	64.6 ± 1.5 a	51.8 ± 1.8 a	9.29 ± 0.27 a	3.41 ± 0.15 a	70.1 ± 1.2 d	8.71 ± 0.18 a	8.3 ± 0.3 a
50	0	54.2 ± 1.5 c	39.7 ± 1.4 c	7.12 ± 0.25 c	2.73 ± 0.11 c	76.8 ± 1.4 cd	7.56 ± 0.17 c	6.4 ± 0.3 c
50	5	58.7 ± 1.4 b	44.1 ± 1.5 b	7.91 ± 0.26 b	2.98 ± 0.12 b	74.1 ± 1.3 d	7.91 ± 0.16 bc	6.9 ± 0.3 bc
50	10	60.8 ± 1.5 b	46.7 ± 1.3 b	8.35 ± 0.29 b	3.16 ± 0.13 ab	72.6 ± 1.2 d	8.18 ± 0.15 ab	7.3 ± 0.3 ab
50	20	59.9 ± 1.6 b	45.8 ± 1.4 b	8.18 ± 0.27 b	3.09 ± 0.12 b	73.2 ± 1.3 d	8.07 ± 0.16 ab	7.2 ± 0.3 ab
100	0	43.1 ± 1.3 e	29.8 ± 1.2 e	5.34 ± 0.21 e	2.12 ± 0.10 e	83.7 ± 1.6 b	6.48 ± 0.15 e	4.7 ± 0.3 e
100	5	49.5 ± 1.4 d	34.8 ± 1.3 d	6.21 ± 0.22 d	2.43 ± 0.11 d	80.1 ± 1.5 c	6.92 ± 0.16 d	5.4 ± 0.3 d
100	10	53.2 ± 1.5 cd	37.9 ± 1.4 cd	6.78 ± 0.24 cd	2.65 ± 0.12 cd	77.6 ± 1.4 b	7.32 ± 0.17 cd	5.9 ± 0.3 cd
100	20	52.1 ± 1.4 cd	37.0 ± 1.3 cd	6.63 ± 0.23 cd	2.58 ± 0.11 cd	78.2 ± 1.5 c	7.21 ± 0.16 cd	5.8 ± 0.3 cd
150	0	33.7 ± 1.2 g	21.5 ± 1.0 g	3.87 ± 0.18 g	1.51 ± 0.08 g	91.5 ± 1.8 a	5.42 ± 0.14 g	3.1 ± 0.2 g
150	5	39.8 ± 1.3 f	26.9 ± 1.1 f	4.82 ± 0.19 f	1.83 ± 0.09 f	87.2 ± 1.7 ab	5.91 ± 0.15 f	3.7 ± 0.2 f
150	10	44.6 ± 1.4 ef	30.7 ± 1.2 e	5.49 ± 0.21 e	2.08 ± 0.10 e	83.4 ± 1.6 b	6.37 ± 0.15 e	4.3 ± 0.3 f
150	20	43.3 ± 1.3 ef	29.8 ± 1.1 e	5.34 ± 0.20 e	2.02 ± 0.09 e	84.1 ± 1.6 b	6.24 ± 0.16 e	4.1 ± 0.2 f

*Values are means (n=5) ± SD. Different letters within each column indicate significant differences at p≤0.05

Table 2. Effect on photosynthetic pigments.

NaCl (mM)	Trehalose (mM)	Chlorophyll a (mg g ⁻¹ FW)	Chlorophyll b (mg g ⁻¹ FW)	Total chlorophyll (mg g ⁻¹ FW)	Carotenoids (mg g ⁻¹ FW)
0	0	1.82 ± 0.06 ^a	0.76 ± 0.03 ^a	2.58 ± 0.08 ^a	0.46 ± 0.02 ^a
0	5	1.88 ± 0.05 ^a	0.79 ± 0.02 ^a	2.67 ± 0.07 ^a	0.48 ± 0.02 ^a
0	10	1.93 ± 0.05 ^a	0.82 ± 0.03 ^a	2.75 ± 0.08 ^a	0.51 ± 0.02 ^a
0	20	1.90 ± 0.06 ^a	0.80 ± 0.03 ^a	2.70 ± 0.09 ^a	0.50 ± 0.02 ^a
50	0	1.57 ± 0.05 ^b	0.65 ± 0.02 ^b	2.22 ± 0.07 ^b	0.40 ± 0.02 ^b
50	5	1.70 ± 0.05 ^{bc}	0.70 ± 0.03 ^{bc}	2.40 ± 0.07 ^{bc}	0.44 ± 0.02 ^{bc}
50	10	1.78 ± 0.04 ^c	0.74 ± 0.02 ^c	2.52 ± 0.06 ^c	0.47 ± 0.02 ^c
50	20	1.73 ± 0.05 ^{bc}	0.72 ± 0.02 ^{bc}	2.45 ± 0.07 ^{bc}	0.45 ± 0.02 ^{bc}
100	0	1.31 ± 0.04 ^d	0.55 ± 0.02 ^d	1.86 ± 0.05 ^d	0.34 ± 0.01 ^d
100	5	1.47 ± 0.05 ^{de}	0.61 ± 0.02 ^{de}	2.08 ± 0.06 ^{de}	0.39 ± 0.02 ^{de}
100	10	1.58 ± 0.04 ^e	0.67 ± 0.02 ^e	2.25 ± 0.06 ^e	0.42 ± 0.02 ^e
100	20	1.52 ± 0.05 ^{de}	0.64 ± 0.02 ^{de}	2.16 ± 0.07 ^{de}	0.40 ± 0.02 ^{de}
150	0	0.76 ± 0.03 ^f	0.33 ± 0.01 ^f	1.09 ± 0.04 ^f	0.23 ± 0.01 ^f
150	5	1.02 ± 0.04 ^g	0.43 ± 0.02 ^g	1.45 ± 0.05 ^g	0.28 ± 0.01 ^g
150	10	1.17 ± 0.04 ^h	0.48 ± 0.02 ^h	1.65 ± 0.05 ^h	0.32 ± 0.01 ^h
150	20	1.10 ± 0.04 ^{gh}	0.46 ± 0.02 ^{gh}	1.56 ± 0.05 ^{gh}	0.30 ± 0.01 ^{gh}

Values are means (n=5) ± SD. Different letters within each column indicate significant differences at p≤0.05

Table 3. Water relations and osmotic adjustment.

NaCl (mM)	Trehalose (mM)	RWC (%)	Proline ($\mu\text{mol g}^{-1}$ FW)	Soluble sugars (mg g^{-1} FW)
0	0	91.8 $\pm$ 1.5 ^a	2.84 $\pm$ 0.15 ^a	38.6 $\pm$ 1.4 ^a
0	5	92.6 $\pm$ 1.4 ^a	3.02 $\pm$ 0.14 ^{ab}	40.1 $\pm$ 1.3 ^a
0	10	93.1 $\pm$ 1.3 ^a	3.18 $\pm$ 0.16 ^{ab}	41.5 $\pm$ 1.5 ^a
0	20	92.8 $\pm$ 1.4 ^a	3.10 $\pm$ 0.15 ^{ab}	40.8 $\pm$ 1.4 ^a
50	0	86.4 $\pm$ 1.6 ^b	4.76 $\pm$ 0.21 ^c	45.2 $\pm$ 1.6 ^b
50	5	89.2 $\pm$ 1.5 ^c	5.42 $\pm$ 0.23 ^d	49.6 $\pm$ 1.7 ^c
50	10	90.8 $\pm$ 1.4 ^{cd}	5.96 $\pm$ 0.24 ^e	52.1 $\pm$ 1.8 ^d
50	20	89.9 $\pm$ 1.5 ^c	5.71 $\pm$ 0.22 ^{de}	50.8 $\pm$ 1.6 ^{cd}
100	0	78.3 $\pm$ 1.8 ^e	7.28 $\pm$ 0.28 ^f	53.7 $\pm$ 1.9 ^e
100	5	83.7 $\pm$ 1.7 ^f	8.16 $\pm$ 0.30 ^g	59.4 $\pm$ 2.0 ^f
100	10	86.9 $\pm$ 1.5 ^g	8.94 $\pm$ 0.32 ^h	63.8 $\pm$ 2.1 ^g
100	20	85.1 $\pm$ 1.6 ^{fg}	8.61 $\pm$ 0.31 ^h	61.7 $\pm$ 2.0 ^{fg}
150	0	67.5 $\pm$ 2.0 ^h	10.42 $\pm$ 0.36 ⁱ	61.8 $\pm$ 2.1 ^h
150	5	76.8 $\pm$ 1.8 ⁱ	11.86 $\pm$ 0.39 ^j	69.5 $\pm$ 2.3 ⁱ
150	10	81.4 $\pm$ 1.7 ^j	13.12 $\pm$ 0.42 ^k	74.8 $\pm$ 2.4 ^j
150	20	79.2 $\pm$ 1.8 ^{ij}	12.64 $\pm$ 0.40 ^{jk}	72.3 $\pm$ 2.2 ^j

*Values are means (n=5) $\pm$ SD. Different letters within each column indicate significant differences at $p \leq 0.05$

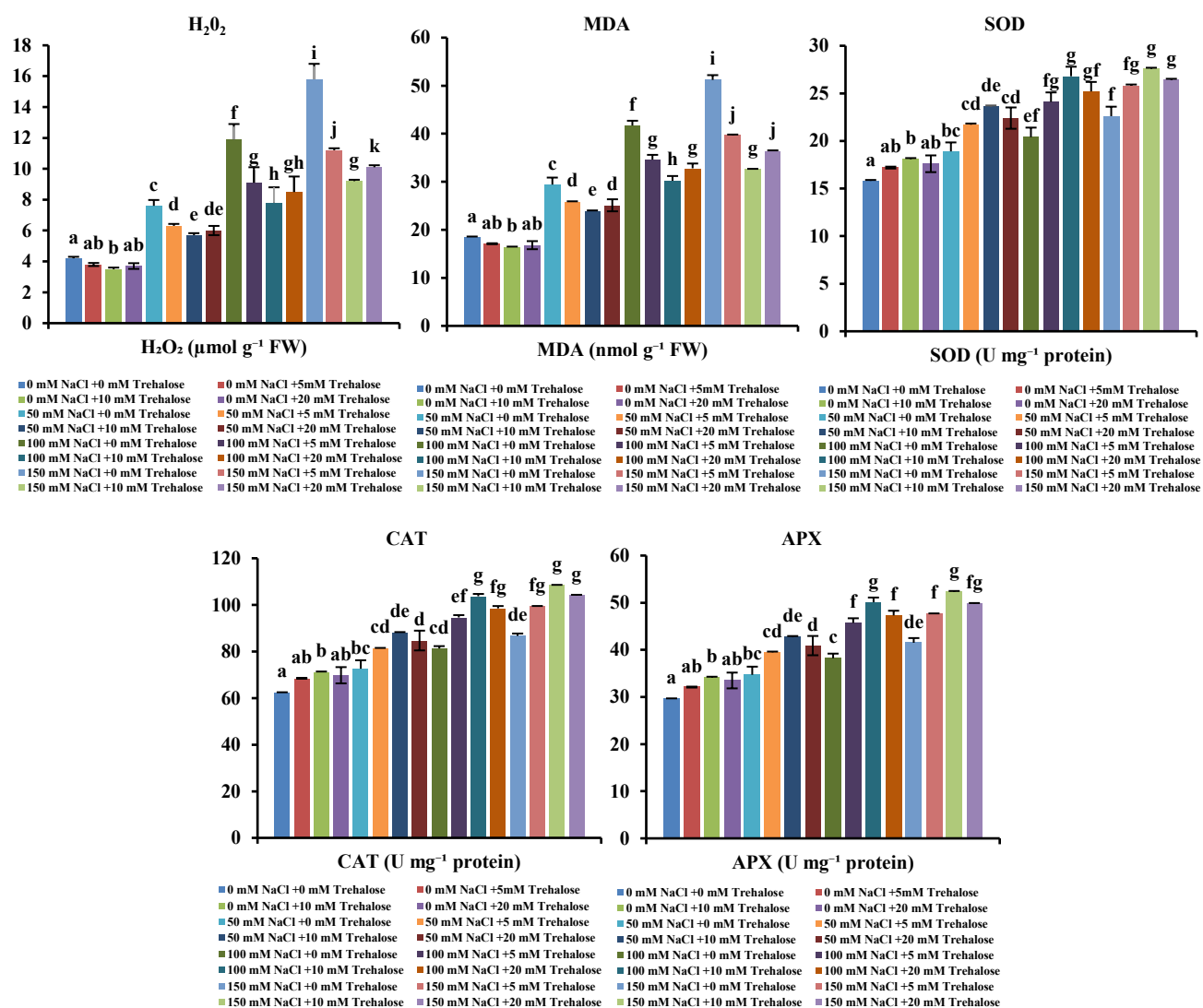


Fig. 1. Oxidative stress and antioxidant enzymes of chrysanthemum as affected by NaCl and trehalose treatments. Values are means $\pm$ SD (n = 5). Values followed by different letters are significantly different at $p \leq 0.05$ according to Fisher's LSD test.

Trehalose improved water status and osmotic adjustment: The relative water content (RWC) of chrysanthemum was significantly decreased with the increase of NaCl concentration, while the accumulation of proline and soluble sugar was increased (Table 3). RWC declined significantly from 91.8% in control plants to 67.5% at 150 mM NaCl while contents of proline and soluble sugars increased significantly from 2.84 to 10.42 $\mu\text{mol g}^{-1}$ FW and from 38.6 to 61.8 mg g^{-1} FW, respectively. Application of trehalose significantly alleviated the reduction in RWC caused by salt and further enhanced accumulation of osmolyte. The best response was generally obtained with 10 mM trehalose. Under 150 mM NaCl, trehalose 10 mM increased RWC to 81.4% and increased proline and soluble sugars to 13.12 and 74.8 $\mu\text{mol g}^{-1}$ FW and mg g^{-1} FW, respectively. The results indicate that trehalose improved the water status of plants under salinity and promoted osmotic adjustment by enhancing the accumulation of compatible solutes.

Trehalose reduced oxidative damage and enhanced antioxidant activity: Salinity strongly increased the accumulation of H_2O_2 and MDA (Fig. 1). The highest concentrations were observed in plants exposed to 150

mM NaCl without trehalose, reaching about 15.8 $\mu\text{mol H}_2\text{O}_2 \text{ g}^{-1}$ FW and 51.2 nmol MDA g^{-1} FW. Trehalose significantly reduced these oxidative stress indicators. At 150 mM NaCl, 10 mM trehalose reduced H_2O_2 to 9.2 $\mu\text{mol g}^{-1}$ FW and MDA to 32.6 nmol g^{-1} FW. Conversely, salinity increased the activities of antioxidant enzymes. The activities of SOD, CAT and APX were further enhanced by trehalose. At 150 mM NaCl, 10 mM trehalose increased SOD activity from 20.4 to 27.6 units, CAT from 81.3 to 108.5 units and APX from 38.2 to 52.4 units compared with untreated saline plants.

Trehalose improved ionic balance: Salinity greatly enhanced build-up of Na^+ and lowered K^+/Na^+ ratio (Fig. 2). The Na^+ concentration rose to around 19.7 mg g^{-1} DW in untreated plants while K^+ concentration decreased to 14.8 mg g^{-1} DW at 150 mM NaCl. Application of 10 mM trehalose decreased Na^+ buildup to about 14.2 mg g^{-1} DW and elevated K^+ to 21.7 mg g^{-1} DW. Thus, the K^+/Na^+ ratio was enhanced from 0.75 to 1.53 at 150 mM NaCl. These data suggest that trehalose improved development via osmotic adjustment and also maintained mineral homeostasis under saline circumstances.

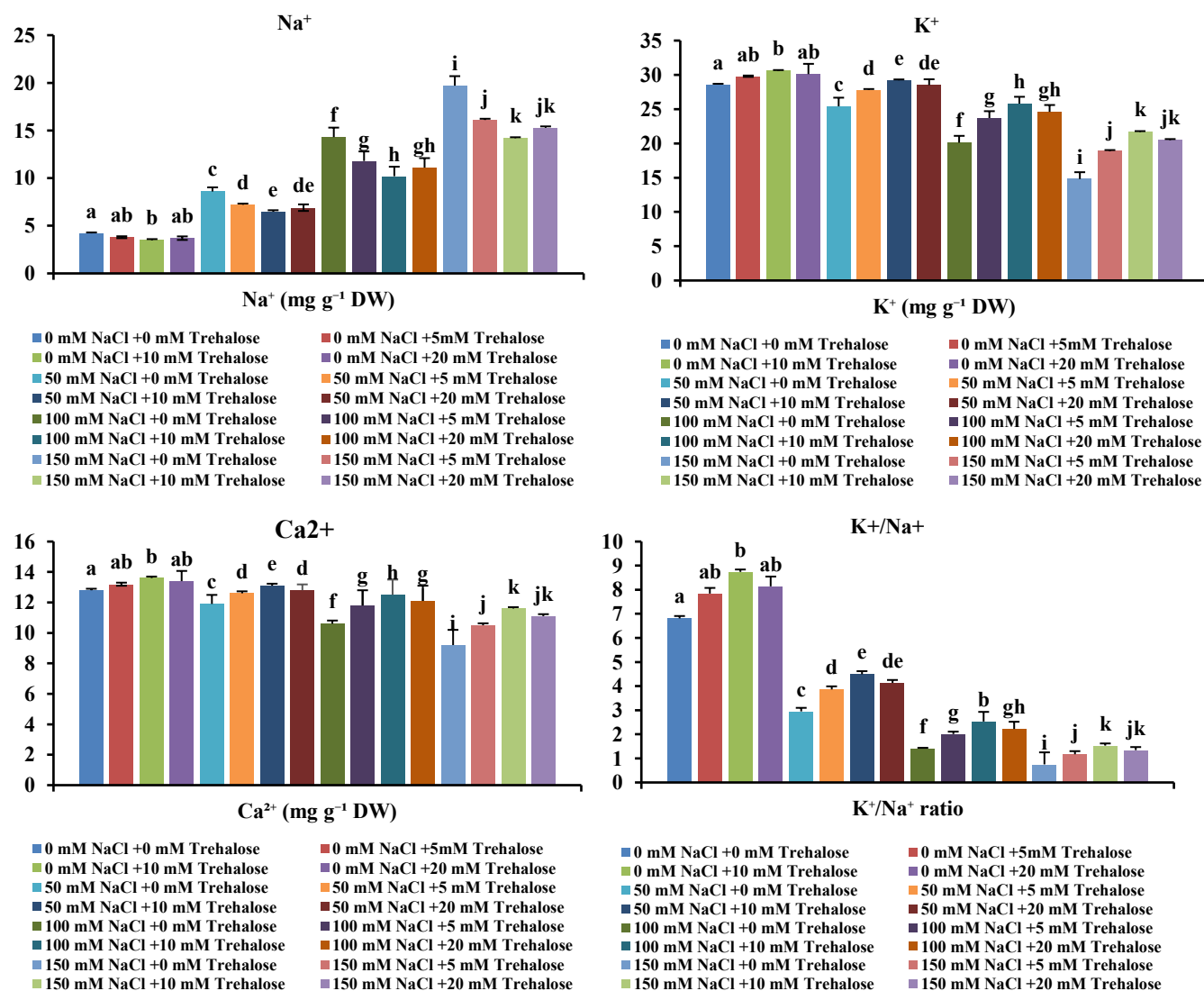


Fig. 2. Mineral composition of chrysanthemum as affected by NaCl and trehalose treatments. Values are means $\pm$ SD ($n = 5$). Values followed by different letters are significantly different at $p \leq 0.05$ according to Fisher's LSD test.

Discussion

Salinity markedly reduced chrysanthemum growth and flowering: The findings reveal that salt has an essential inhibitory effect on the growth of chrysanthemum. The plant height, shoot biomass and root biomass decreased gradually with the rise of NaCl. Such effects have been described in experiments using chrysanthemum. Bandurska *et al.*, (2022) found that the greater the salt level in the solution, the more significant the dose-dependent decrease in the growth of “Palisade White”. Ruati *et al.*, (2021) reported decrease in plant growth and the photosynthetic characteristics in presence of sodium chloride. Growth of 10 different chrysanthemum cultivars was studied at 150 mM NaCl. Vanlalruati *et al.*, (2023) reported considerable reduction in the growth and in the yield-related parameters due to salt stress. However, the degree of response was different according to the cultivars. These findings are in agreement with the available data and also suggest that the development of chrysanthemum is strongly reliant on the capacity of the plants to tolerate the osmotic and ionic components of salinity.

This decline in flowering in salt environment is especially important for chrysanthemum since the commercial production of chrysanthemum relies not only on biomass but also on quality of the flower. Results obtained in this research proved that the increase in concentration of NaCl leads to delay in flowering, decrease in floral diameter, reduction in number of flowers. This excessive salinity inhibited the growth of the plant in both vegetative and reproductive capacities. The delay in flower time might be attributed to the change in mineral nutrition, poor water relations and hindered photosynthetic carbon absorption. Bandurska *et al.*, (2022) reported that increased salt exposure reduced photosynthetic activity, stomatal conductance and transpiration in chrysanthemum ‘Palisade White’ and this indicate that salt stress may have detrimental effects on the reduction of the photosynthetic and the growth in the current study. Vanlalruati *et al.*, 2023 also reported comparable decreases in chlorophyll and growth patterns in chrysanthemum types exposed to 150 mM NaCl. Chrysanthemum, previous screening studies have shown that the increase in the salinity to 150 mM NaCl leads to comparable results. Better growth and mineral balance were found in certain cultivars which tolerated 150 mM NaCl (Vanlalruati *et al.*, 2023). These studies on Chrysanthemum also confirm the genotype-dependent response. They showed variations in salt tolerance in terms of buildup of Na⁺, retention of K⁺, metabolism of proline and the antioxidant activity. The level of protection that trehalose may provide to chrysanthemum may also depend on the genetic background of the cultivar examined.

Trehalose improved growth under saline conditions: NaCl substantially reduced the development of Chrysanthemum, which was considerably improved by trehalose. Highest salt tolerance was seen generally at 10 Mm, but 20 mM did not often provide any further advantage. This implies an appropriate range of trehalose concentrations and the increased dose beyond this range does not necessarily enhance salt tolerance. The increased growth parallels the known role of trehalose in stabilizing cells and controlling osmosis under stress (Nawaz *et al.*,

2022). There is no previous study on chrysanthemum illustrated the physiological mechanisms related to trehalose stress tolerance. Chrysanthemum plants grown under salt stress generally demonstrate a reduction in RWC, chlorophyll and growth and an increase in proline, soluble sugars and antioxidant activities (Bandurska *et al.*, 2022; Vanlalruati *et al.*, 2023). Thus, trehalose might augment the physiological processes that chrysanthemum naturally deploys to combat salt stress. The response is likewise much in line with the present molecular findings. Under salt stress, *CmCIPK8*-overexpressing chrysanthemum plants showed higher RWC, chlorophyll content, soluble sugar and proline accumulation and improved antioxidant activity than wild-type plants (Ding *et al.*, 2022). These responses are similar to the physiological changes seen following trehalose application in the present study. This does not imply that trehalose acts via *CmCIPK8* but raises the possibility that the two treatments might converge on similar physiological processes of water status, osmotic adjustment, ROS detoxification and ion homeostasis.

Trehalose protected photosynthetic pigments: Salinity caused a significant decrease in chlorophyll a, chlorophyll b, total chlorophyll and carotenoids. Stress of NaCl also induced the same loss of color in chrysanthemum. Decreases in chlorophyll and photosynthetic effectiveness of salt-treated chrysanthemum were reported by Ruati *et al.*, (2021). Long-term salinity caused major physiological limitations at higher salt concentrations, affecting photosynthetic gas exchange as reported by Bandurska *et al.*, (2022). The fall in pigment composition is consistent with the known salt sensitivity of chrysanthemum. Trehalose somewhat prevented the loss of color, particularly with 100 and 150 mM NaCl. The chlorophyll retention would be directly related to enhanced biomass and improved flowering of the treated plants. The photosynthetic pigments allow plants to sustain higher photosynthetic capability and hence to continue the production of carbon skeletons and energy for growth and reproductive development. In recent years molecular studies have supplied further support for this idea. Recently, *CiHY5* was discovered to increase the salt tolerance of *Chrysanthemum indicum* by regulating hormone signalling, photosynthesis and carbohydrate metabolism (Xia *et al.*, 2025). This is noteworthy because it shows that photosynthesis and carbon metabolism are not only downstream processes of salt damage in chrysanthemum but rather major players in the salt-response network. Trehalose is a metabolite and signalling molecule of carbohydrate nature that might possibly cross-talk with numerous actions. Future experiments using chrysanthemums should clearly explore this option.

Trehalose improved water status and osmotic adjustment: The results indicated the salt had a profound effect on the water interactions that took place in chrysanthemum in a profound manner as the RWC reduced with increasing concentrations of NaCl. Bandurska *et al.*, (2022) found that treatment to 'Palisade White' to salt led in measurable decreases in RWC and changes in growth and photosynthesis. Recently, Ding *et al.*, (2022) shown that an expanding body of evidence supports the control of water status as an important component of salt tolerance in chrysanthemum in plants. Salt stress increased the root water content in *CmCIPK8*-overexpressing chrysanthemum plants to a considerably larger extent than in wild-type plants.

In the high salinity it was obviously evident that the RWC increased a lot. It is speculated that the response was due to its osmoprotective role and the impact on the metabolism of appropriate solutes. The concurrent rise in proline and soluble sugars in the plants was an indicator that the effect of trehalose on osmotic adjustment response was more apparent in the treated plants. This was found in the current study as the trehalose treated plants were more resistant.

Another distinguishing feature of the chrysanthemum is the high amount of proline that it contains. Results of Bandurska *et al.*, (2022) indicated that the increase in proline was due to the higher salt tolerance of 'Palisade White' over a longer length of time. Vanlalruati *et al.*, (2023) observed a substantial difference in the quantity of proline accumulated by various chrysanthemum cultivars at 150 mM NaCl concentration. In this manner, excess build-up of proline during trehalose application may be due to the amplification of a natural chrysanthemum protective mechanism rather than a reaction to the biostimulant treatment.

Trehalose reduced oxidative stress and enhanced antioxidant protection:

The high build-up of H₂O₂ and MDA during salt stress suggested that oxidative damage constituted a significant part of salinity injury. ROS control has been frequently shown to be important during salt stress in chrysanthemum experiments. For example, *CmHSFA4-OE* plants accumulated lower levels of H₂O₂ and superoxide and exhibited more intense SOD, APX and CAT activities than wild-type plants under salinity (Li *et al.*, 2018). It was shown recently that *CmCIPK8* enhances chrysanthemum salt tolerance via lower ROS formation, more antioxidant activity and decreased membrane lipid peroxidation (Ding *et al.*, 2022). Therefore, decrease the of H₂O₂ and MDA in trehalose-treated plants is an essential protective response. Trehalose-treated plants showed reduced ROS and higher SOD, CAT and APX activities. This combination shows that trehalose increased the ability of chrysanthemum to detoxify ROS before they caused significant damage to membranes. The response is similar to the antioxidant phenotype found in the lines of genetically modified chrysanthemum. The overexpression of *CmHSFA4* in chrysanthemum resulted in increased salt tolerance, which was correlated with reduced ROS build-up and improved antioxidant enzyme activity (Li *et al.*, 2018). Similarly, salt stressed *CmCIPK8* overexpressing plants showed decreased ROS and increased SOD, CAT and APX activities (Ding *et al.*, 2022). The comparisons show that antioxidant reinforcement is a major component of chrysanthemum salt tolerance and that trehalose may induce a physiological response similar to that of more genetically resistant plants.

Trehalose improved Na⁺/K⁺ homeostasis: In the current investigation, a considerable improvement in K⁺ / Na⁺ ratio was shown. Higher concentration of NaCl caused considerable buildup of Na⁺ and decrease of K⁺ concentration. Trehalose might somewhat rescue these oscillations. In several individual studies ionic homeostasis was identified as a major predictor of salt tolerance (Van Zelm *et al.*, 2020). The maintenance of K⁺ level and the avoidance of Na⁺ generation were very important for chrysanthemum. Comparable results of this equilibrium had been described in faba beans (Lamlom *et al.*, 2026) and

sweet potatoes (Huang *et al.*, 2026). Li *et al.*, (2018) found that the overexpression of *CmHSFA4*, up-regulated expression of *CmSOS1* and *CmHKT2* in transgenic chrysanthemums under salt stress, which resulted to decreased accumulation of sodium ions and improved retention of potassium ions. This also complements the ongoing study of *CmHSFA4-CmMYB33-CmTPL*. Wang *et al.*, (2024) demonstrated that this regulatory complex suppressed *CmMYB121* and activated antioxidant and ion transport responses. Meanwhile, overexpression of *CmMYB121* increased the sensitivity to salt stress. The improvement of K⁺/Na⁺ balance after trehalose treatment was consistent with one of the most documented physiological feature of salt tolerance in chrysanthemum. The influence of trehalose on the Na⁺ and K⁺ build-up is not known. It may also function indirectly by improving the water status and the metabolic activity of the plants. It may also change the function or expression of ion transporters. *CIWEE1* overexpression led to up-regulation of *CINHX*, *CIHKT*, *CICBL* and *CIDREB2A* in *C. lavandulifolia* under salt stress (Wang *et al.*, 2025). Which makes the former all the more important. Future studies on trehalose should include the expression of *SOS1*, *NHX*, *HKT* and *CBL/CIPK* genes.

The improvement in flowering strengthens the horticultural significance of trehalose:

One of the important strengths of the present study was that the response was assessed not only by vegetative growth but also by flowering. Salinity negatively affected floral size, flower number and flowering speed, which were somewhat recovered by trehalose. The commercial aim in chrysanthemum production is to create uniform plants which bloom at the expected time and produce flowers of a suitable size and quantity. One reason for the improvement in flowering may be that the improvement is due to the combination of physiological effects of trehalose. The improved RWC would facilitate cell growth and reproductive development, elevated chlorophyll which would help in carbon absorption, elevated antioxidant defense, prevent cellular damage and improved K⁺/Na⁺ balance. All these effects together would make the conditions are more favourable for plants to flourish. Thus, flowering enhancement should be seen as the consequence of many interacting defensive mechanisms rather than a distinct response. This integrated viewpoint is supported by a recent research on chrysanthemums. Improved growth and physiological activity of salt tolerant genotypes is possible due to the coordinated management of ion balance, osmolytes and antioxidant defenses (Vanlalruati *et al.*, 2023; Wang *et al.*, 2025). The present findings indicate that that trehalose may be able in part to mimic this physiological behavior in chrysanthemum under salt stress.

Practical implications and future research: From a production perspective, the response obtained with 10 mM trehalose is encouraging because the treatment improved several characteristics that are directly relevant to chrysanthemum quality. Future work should compare foliar and root-zone application of trehalose, determine the persistence of the response and evaluate its effectiveness under saline irrigation water rather than only NaCl solutions. Because chrysanthemum genotypes differ markedly in salt tolerance, cultivar-dependent responses should also be evaluated (Bandurska *et al.*, 2022; Vanlalruati *et al.*, 2023; Wang *et al.*, 2025).

A particularly promising direction would be to combine trehalose with molecular measurements. Quantifying *CmSOS1*, *CmHKT*, *CmNHX*, *CmCIPK*, *CIWEE1* and *CiHY5*, together with photosynthetic measurements and ROS imaging, could reveal whether trehalose activates pathways already known to contribute to chrysanthemum salt tolerance. Such an approach would provide a stronger mechanistic basis for using trehalose as a biostimulant and could distinguish a true signalling effect from a general improvement in plant nutritional or water status.

Proposed mechanism of trehalose-mediated salinity tolerance in chrysanthemum:

Initially, salinity restricts water availability to roots because of osmotic stress and subsequently causes Na^+ accumulation, K^+ homeostasis disruption, damage of photosynthetic pigments and ROS production. These changes ultimately result in reduced vegetative growth and reduced reproductive development. Similar interaction between water relations, ion balance, photosynthesis and oxidative stress has been documented in salt stressed chrysanthemum (Bandurska *et al.*, 2022; Vanlalruati *et al.*, 2023; Ding *et al.*, 2022). Trehalose seems to interfere with the cascade of stress responses at several levels. The higher RWC and the accumulation of proline and soluble sugars at first suggest that trehalose promoted osmotic adjustment and helped to maintain cellular hydration. This is notably true for chrysanthemum, where salinity tolerant plants showed a better water status and accumulated more appropriate solutes (Bandurska *et al.*, 2022; Vanlalruati *et al.*, 2023). The improved water status might subsequently stimulate cell development and photosynthetic activity which may explain the greater plant height and biomass seen following trehalose treatment. Secondly, trehalose improved the antioxidant system. The simultaneous increase in SOD, CAT and APX activities and reduction in H_2O_2 and MDA imply that the treated plants were more efficient in controlling the generation of ROS. This mechanism is in keeping with previous results in chrysanthemum where an improved salt tolerance was correlated with a higher antioxidant capacity. Overexpression of *CmHSFA4* in salt challenged plants revealed reduced ROS content and increased antioxidant enzyme activity, while overexpression of *CmCIPK8* likewise increased antioxidant protection and lowered membrane lipid peroxidation (Li *et al.*, 2018; Ding *et al.*, 2022). The present results thus suggest that trehalose may promote an antioxidant defense system that is already an important component of salt tolerance in chrysanthemum. Third, the improved K^+/Na^+ ratio suggests that trehalose decreased the ionic toxicity. Na^+ accumulation is one of the basic limits of chrysanthemum function in salty environments, yet K^+ is essential for enzyme activity, osmotic regulation and cellular metabolism. Recent studies on chrysanthemum have identified many molecular elements involved in this process, including *CmCIPK8*, *CmHSFA4*, and genes associated with Na^+ transport and sequestration (Li *et al.*, 2018; Ding *et al.*, 2022; Wang *et al.*, 2024). Thus, the greater K^+/Na^+ ratio following treatment with trehalose provides a strong physiological explanation for the superior growth and flowering of the treated plants. The graphical model also includes a photosynthetic protection between the ameliorated water and ionic condition and the final growth response. The preservation of chlorophyll and carotenoids under salinity would allow the treated plants to sustain better photosynthetic activity and

carbohydrate production. This may be particularly important for flowering; as reproductive development requires a steady supply of assimilates. Recent studies in *Chrysanthemum indicum* have demonstrated that *CiHY5* is involved in salt tolerance by interacting with photosynthesis, hormone signalling and carbohydrate metabolism, further emphasizing the close relationship between salt tolerance and carbon uptake in chrysanthemum (Xia *et al.*, 2025).

It is also possible that trehalose interacts with the wider molecular stress response network recently found in chrysanthemum. *CmCIPK8*, *CmHSFA4*–*CmMYBS3* and *CIWEE1* have been implicated in a variety of activities including ion homeostasis, ROS regulation and cellular protection under salt stress (Ding *et al.*, 2022; Wang *et al.*, 2024; Wang *et al.*, 2025). It remains to be seen if these pathways are directly modulated by trehalose. Nevertheless, the physiological phenotype triggered by trehalose is quite similar to the responses observed in genetically salt tolerant chrysanthemum lines, strongly suggesting that these pathways might be promising targets for future mechanistic studies. Finally, the graphical model gives a multi-level mechanism of action of trehalose in chrysanthemum. Trehalose seems to act not just as an osmoprotectant but also as a coordinator of a coordinated response that involves water conservation, osmolyte accumulation, photosynthetic protection, ROS detoxification and ionic homeostasis. The joint advancement of both systems provides a plausible explanation for the observed persistence of vegetative growth and flowering in saline conditions. However, this concept has to be treated as a working hypothesis until it is shown by molecular studies that trehalose directly regulates the corresponding ion-transport, antioxidant, carbohydrate and stress-signaling pathways.

Salinity induces osmotic stress, Na^+ accumulation, K^+ depletion, photosynthetic pigment loss and oxidative damage, resulting in reduced growth and flowering. Trehalose application, particularly at 10 mM, enhances osmotic adjustment, maintains leaf water status and photosynthetic pigments, stimulates SOD, CAT and APX activities, reduces H_2O_2 and MDA accumulation, and improves K^+/Na^+ homeostasis. These coordinated responses contribute to improved plant growth, earlier flowering, greater flower diameter and increased flower number under saline conditions.

Conclusion

Salinity strongly inhibited chrysanthemum growth, hydration status, photosynthetic pigments and blooming, and increased oxidative stress and Na^+ build-up. Exogenous trehalose, especially 10 mM, eased these effects by increasing RWC, encouraging accumulation of proline and soluble sugar, retaining chlorophyll and carotenoids, increasing activities of SOD, CAT and APX, decreasing accumulation of H_2O_2 and MDA, and improving K^+/Na^+ balance. These physiological changes associated with higher plant biomass, bigger flower, more flowers and decreased flowering delay. The results indicate that trehalose has potential as a biostimulant for chrysanthemum production where saline irrigation or salt accumulation limits crop performance. The strongest future direction is to determine whether the physiological

benefits of trehalose are associated with regulation of chrysanthemum ion-transport genes, carbohydrate metabolism and recently identified salt-response pathways such as *WEE1*, *HY5*, *CIPK*, *NHX* and *HKT*. Greenhouse trials using commercial saline irrigation water and long-term field experiments are also needed to establish the optimum application concentration, timing and method.

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