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ORIGINAL PAPER

Improving Naomi Mango Trees Capability to Withstand Salt Stress Using Some Plant Growth Regulators

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Abstract

Salt stress is a significant abiotic stress factor that negatively impacts plant growth and productivity. This effects growth and productivity of salt-sensitive crops. One promising approach to improving plants capability to withstand salt stress is the use of plant growth regulators, which have shown great potential in enhancing the ability of plants to withstand stress. Thus, the objective of this study is to provide useful information about the influence of melatonin (MT) or brassinosteroid (BR) spraying on vegetative growth, physiological attributes, and productivity of mango trees under salt stress. A field experiment was conducted on Naomi mango trees irrigated with salty well water. Trees were treated with MT and BR at the concentrations of (0.025 mM, 0.05 mM, and 0.1 mM) each. The design used to establish the experiment was completely random sectors, each treatment consisted of five replicates. Indicated that MT at 0.05 and 0.1 mM and BR at 0.05 mM treatments significantly improved vegetative growth, chlorophyll content, and tree productivity under salinity stress. The treatments also enhanced fruit quality by increasing total soluble solids and reducing acidity. Additionally, the application regulators increased the activity of antioxidant enzymes and proline content. Furthermore, the potassium and calcium content of the leaves increased while sodium and chlorine decreased, contributing to better stress tolerance. In conclusion, the use of melatonin and brassinosteroid mitigates the adverse effects of salinity stress on mango trees, leading to improved growth, productivity, and fruit quality. Since such treatments have increased the trees' tolerance to salt stress, it is advisable to be applied as an agronomic practice in mango trees grown under salt stress.

Keywords Naomi · Mango · Salt stress · Abiotic stress · Brassinosteroid · Melatonin · Enzyme

1 Introduction

Mango (*Mangifera indica*), a member of the family Anacardiaceae, is one of the world's major tropical and subtropical fruits. It is widely cultivated for its delicious fruit and economic value. It is considered the sixth largest fruit crop globally and the second most farmed tropical fruit. In Egypt, mango is the third-largest crop after citrus and grapes. However, numerous biotic and abiotic stressors frequently such as salinity, low and high temperatures and nutrition problems, threaten mango trees which can negatively impact their growth and productivity. Salt stress have a negative impacts on plants (Mahouachi 2018; Mubarak et al. 2021; Elgendy et al. 2024) specifically mango tress which are salt-sensitive. orchards producing low output or death because of higher salty levels in soil or irrigation water.

Researchers are investigating several methods to increase the stress tolerance of mango plants in order to overcome these difficulties. One promising approach is the use of plant growth regulators, which have shown great potential in enhancing the ability of plants to withstand stress (El-Bially et al. 2018, 2022a, b). The natural activators of growth and development in the plant life cycle are known as phytohormones or plant growth regulators (Abass et al. 2024; El-Ziat et al. 2024; Shaaban et al. 2025). Not only do they keep plants healthy, but they also contribute significantly to

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stress-reduction systems (Saudy et al. 2021a, 2023a). Plant hormone-induced signal transduction cascades are crucial for the body’s reaction to abiotic stress (Verma et al. 2016; Hassan et al. 2024; Ansabayeva et al. 2025; Mohamed et al. 2025). Thus, the aims of this study were to improve the ability of mango trees to withstand stress by using melatonin (MT) and brassinosteroid (BR). Melatonin (N-acetyl-5-methoxytryptamine, MT) has primordially been identified as an important animal hormone related to various biological processes, such as antioxidant mechanisms, which is generally distributed among various plant tissues (Himanshu et al. 2024). Many fruits and vegetables contain endogenous MT, which is important for the ripening and post-harvest processes of these foods. Reactive oxygen species (ROS) in plants can be scavenged by MT, which has been demonstrated to possess antioxidant qualities. Additionally, it has been shown to increase the activity of both non-enzymatic and antioxidant enzymes, lowering oxidative stress in plants (Nasser et al. 2022; El-Sayed et al. 2022; Tawfik et al. 2022; El-Beltagi et al. 2023). Through the increase of antioxidant enzymes, non-enzymatic antioxidants, and enzymes involved in oxidized protein repair, exogenous MT administration eliminates excess ROS (Ramadan et al. 2025a, b). Meanwhile, MT’s control of gene expression and interactions with other phytohormones, such as ethylene, have recently been described (El-Yazied et al. 2022). Gao et al. (2016) reported that brassinosteroids (BR) are a group of plant hormones that have been found to regulate various physiological processes, including stress responses. Studies have shown that the exogenous application of BR can improve the drought tolerance of mango trees by enhancing the synthesis of stress-related proteins and antioxidants, reducing water loss through transpiration, and maintaining cellular integrity under drought conditions. BR also promotes root growth and improves the efficiency of water uptake, thereby enhancing the ability of mango trees to withstand drought stress. Numerous plant growth and development activities, such as cell division and expansion, etiolation, reproduction, and abiotic stress tolerance, are regulated by BR (Thussagunpanit et al. 2015; Planas-Riverola et al. 2019). BR are involved in many aspects of plant growth and development, such as vascular differentiation, cell division, elongation, reproductive development, and resistance to abiotic and pathogen.

Our study hypothesis was that the application of MT or BR could improve the ability of mango trees to withstand salt stress. Bearing the above in mind, the objective of this study is to provide useful information about the influence of MT or BR spraying on vegetative growth, physiological attributes, and productivity of mango trees.

2 Materials and Methods

2.1 Experimental Design and Field Transplantation

The field experiment took place in a private mango orchard located at the desert land of Giza Governorate, Giza, Egypt (30°15’44.3"N 30°45’59.7"E), during 2023 and 2024 seasons on Naomi mango trees grafted by top working from five years old on ten years old Zibda mango trees that budded on Succari rootstock. The average height of the trees was about 4 m, planted at 5*5 m space in a sandy soil and irrigated with drip irrigation system (EC = 3.40 dSm^{-1}). The trees were fertilized with the recommended fertilization program of 300 N, 96 P_2O_5, and 240 K_2O kg/ha/year distributed during the growing season. Micronutrients (Fe, Mn, and Zn) were provided at 300, 150, and 100 gm, respectively, as chelated fertilizers by foliar spray. The water and soil analysis presented in Tables (1 and 2). The design used to establish the field experiment was completely random sectors. Each treatment consisted of five replicates. The selected trees were healthy and as uniform as possible in growth. The treatments involved spraying with MT, at 0.025 mM, 0.05mM, and 0.1mM and BR, at 0.025 mM, 0.05mM, and 0.1mM compared to the control treatment (well water). Fifteen liters of spraying solution were prepared with the addition of the surfactant (Tween 20) at a rate of 3 ml 15 L^{-1} for each treatment. Spraying the experiment trees was in the early morning or before sunset time to avoid the high temperature time, using a 20 L hand-held sprayer, and plants were sprayed to completely cover the plant foliage. Five sprays were carried out every two months, starting from early February to late October in each season.

2.2 Measurements

2.2.1 Vegetative Growth

Shoot number, shoot length, shoot thickness, and leaf number were measured. Leaf area (cm^2): The leaf area was measured in the leaves of the selected branches in August according to Ghoreishi et al. (2012) by Eq. 1.

$$LA = 0.2452 * ((L * w) * N) \tag{1}$$

Table 1 Chemical analysis of well water on the site

pH	Electrical conductivity (dS m^{-1})	soluble cations (meq L^{-1})				Soluble anions (meq L^{-1})			
		Ca^{++}	Mg^{++}	Na^{+}	K^{+}	CO_3^{-}	HCO_3^{-}	SO_4^{-}	Cl^{-}
8.3	3.40	10.0	1.4	20.0	0.12	----	0.9	10.0	20.0

L: length of longest leaf; W: width of widest leaf; N: number of leaves.

SPAD reading: Three to four leaves of each selected branch were used in SPAD reading. Minolta SPAD-502 was used three times in different points for each leaf. Afterwords, the average to these readings were calculated according to Shah et al. (2017).

2.2.2 Leaf Pigments

As for the chlorophylls and carotenoids analyses, 0.1 g of fresh leaves were taken from the top (the first expanded leaf) of the plant. Total chlorophyll and carotenoids were extracted by grinding the tissue with a mortar and pestle using 10 ml N, N dimethyl formamide according to Wellburn (1994). The resulting extracts were incubated in the dark fridge overnight. The chlorophyll and carotenoids concentrations were measured using a spectrophotometer (Mapada UV 1200). The absorbance of the solution was recorded at 470, 647 and 664 nm for chlorophyll a, chlorophyll b and carotenoids, respectively. The concentration of the total chlorophyll was calculated via gathering chlorophyll a and chlorophyll b.

2.2.3 Tree Productivity

At the harvest stage, corresponding to 120 days after full bloom at the end of the second week of August (Rungpichayapichet et al. 2017). The number of fruits per tree was counted then the fruits weighed to determine the total yield per tree via Eq. 2.

$$\text{Total yield per tree} = \text{number of fruits per tree}$$

$$^{*}\text{average of fruit weight} \qquad (2)$$

2.2.4 Fruit Quality

Ten fruits were chosen at random to measure the fruit's length, diameter, shape, pulp weight, and peel thickness. Total soluble solids (TSS) were measured using a hand refractometer (HR-110) in biochemical analysis of fruits, and titratable acidity (TA) was noted as citric acid, the primary organic acids in mangos using 5 milliliters of juice for titration according to AOAC (1995), The TSS: TA ratio, or maturity index, was computed. Ten grams of pulp were treated with 3% oxalic acid, and an aliquot was titrated against 2,6-dichlorophenolindophenol to measure the amount of vitamin C present. Vitamin C levels were stated in milligrams per 100 milliliters (Abou El-Nasr et al. 2025).

Table 2 Physical and hemical properties of soil on the site

Sand%	Silt%	Clay%	Soil texture	pH	Electrical conductivity (dS m^{-1})	soluble cations (meq L^{-1})				Soluble anions (meq L^{-1})			
						Ca^{++}	Mg^{++}	Na^{+}	K^{+}	CO$_3^{-}$	HCO$_3^{-}$	SO$_4^{-}$	Cl^{-}
93.3	0.2	6.5	Sandy	7.0	2.70	9.0	7.0	13.0	0.3	----	1.2	5.9	18.8

2.2.5 Oxidative Stress Markers

2.2.5.1 Polyphenol Oxidase Activity (PPO) By monitoring the rise in the absorbance measurement at 420 nm caused by the synthesis of the benzoquinone molecule, the activity of the polyphenol oxidase enzyme (IU mL^{-1} enzyme) was determined. Oktay et al. (1995) In short, 1 g of leaf material was crushed in 4 mL of extraction buffer, which was made up of 0.1 M phosphate buffer with a pH of 7, 0.1 mM EDTA, and 1% polyvinylpyrrolidone (PVP) The homogenates underwent a 15-minute chilling centrifugation. The supernatant was collected and used for the assays of enzymatic activities. The enzyme activity was determined by taking 2.3 mL of a phosphate buffer 0.6 mL of 0.1 M catechol was added after the (pH 6.5, 0.1 M) solution, and 0.1 mL of the enzymatic extract was added last. A change of 0.001 per minute in absorbance per milliliter of enzyme extract was considered one unit of enzyme activity.

2.2.5.2 Peroxidase Activity (POD) Peroxidase activity was measured based on how well it could change guaiacol into tetraguaiacol according to Polle et al. (1994). In short, 4 mL of extraction phosphate buffer was used to grind 1 g of leaf material. 2.9 mL of 100 mM phosphate buffer (pH 7.0), 20.1 mM guaiacol, 10 mM H_2O_2, and 0.1 mL of the enzyme extract were all present in the reaction mixture. When H_2O_2 was added, the absorbance increased by three minutes at 470 nm.

2.2.5.3 Proline Concentration Proline concentration was determined according to the method of Troll and Lindsley (1955). Fresh leaf samples (0.5 g) were ground and homogenized with one volume of 100 mM sodium phosphate buffer (pH 6.0). The samples were centrifuged for 10 min at 16.000 x g. The reaction mixture contained 200 µl of the supernatant and 1 ml of ninhydrin solution (2.5 g dissolved in 100 ml of ortho-phosphoric acid, acetic acid, and water 15: 60: 25. V: V: V). The reaction proceeded for 1 h in boiling water bath and the developed dye was extracted with 1 ml of toluene and measured by the spectrophotometer at 515 nm by using a spectrophotometer (Mapada UV 1200). The proline concentration was determined by the standard curve of L-proline.

2.2.6 Leaf Mineral Content

Leaf mineral content was determined as follows: six leaves taken from 6–7-month-old non-fruiting branches from the middle sampled from all directions from each tree in late September were collected in each season according to Chadha et al. (1980). The samples were digested with H_2SO_4 and H_2O_2. A flame photometer was used to measure potassium and sodium (Asch et al. 2022). Calcium concentrations were determined using EDTA Titration, following the procedure outlined by (Tucker and Kurtz 1961), Cl^- content was measured via titration with silver nitrate using the Mohr's method, described by Belcher and Macdonald (1957).

2.3 Statistical Analysis

replicates for each treatment. For all statistical analyses of the various features, the analysis of variance (ANOVA) approach was used. Comparisons of means were performed by Komorowski et al. (2016) $p \leq 0.05$, multiple range test. A SAS program was used to statistically examine the data using the analysis of variance. The RStudio program with "tidyverse" package in the R software environment was used to create a heatmap, which offered insightful information.

3 Results

3.1 Vegetative Growth

The treatments had an impact on Naomi mango trees' vegetative growth when they were subjected to salinity stress. The treatments influenced the number and length of branches growing during the growth cycles of the trees, as well as the average number of leaves on the branches, as shown in Table (3). Additionally, the treatments affected the leaf area, leaf dry matter, and SPAD readings as shown in Table (4). The treatment with MT at concentrations of 0.05 and 0.1 mM recorded the highest values for the average number of branches, especially in the second season, surpassing the control by 103.29 and 142.01%, and the length of branches by 28.77 and 56.05%, respectively. It was followed closely by the treatment with BR at 0.025 and 0.05 mM. Meanwhile, the lowest values for the average number and length of branches resulted from control treatment and 0.1 mM BR. On the other hand, there was no effect of the treatments on the branch thickness in both seasons. For leaf area, leaf dry matter, and SPAD readings, the highest increases (45.26, 7.15, and 14.29%, respectively) were obtained by 0.05 mM BR compared to the control in the second season. Furthermore, 0.1 mM MT gave the best results for leaf area and SPAD readings that amounted to 25.34 and 18.61% greater than the control.

Table 3 Effect of melatonin and brassinosteroid concentrations on shoot number, shoot length, thickness of shoot, and number of leaves for Naomi Mango trees grown under salt stress in 2023 and 2024 seasons

treatments	Shoot number	Shoot length (cm)	Thickness of shoot (cm)	Number of leaves
2023				
Control	12.00 ± 1.00^{b}	28.00 ± 1.73^{ab}	7.20 ± 0.79^{a}	19.67 ± 0.58^{cd}
Melatonin 0.025 mM	14.33 ± 1.53^{ab}	39.67 ± 4.51^{ab}	8.88 ± 0.68^{a}	27.33 ± 2.52^{ab}
Melatonin 0.05 mM	14.67 ± 1.53^{ab}	38.67 ± 8.50^{ab}	8.63 ± 1.50^{a}	27.67 ± 1.15^{a}
Melatonin 0.1 mM	16.33 ± 1.53^{a}	27.33 ± 0.58^{ab}	7.07 ± 0.31^{a}	$21.67 \pm 2.08^{b-d}$
Brassinosteroid 0.025 mM	14.33 ± 1.15^{ab}	26.00 ± 4.58^{b}	7.50 ± 0.95^{a}	17.00 ± 0.01^{d}
Brassinosteroid 0.05 mM	14.00 ± 1.00^{ab}	41.33 ± 6.35^{a}	7.87 ± 0.61^{a}	20.33 ± 2.52^{cd}
Brassinosteroid 0.1 mM	11.33 ± 1.15^{b}	32.67 ± 7.23^{ab}	7.27 ± 0.76^{a}	$23.00 \pm 3.61^{a-c}$
2024				
Control	10.33 ± 1.15^{e}	22.00 ± 1.00^{b}	9.07 ± 0.12^{a}	15.00 ± 0.01^{d}
Melatonin 0.025 mM	13.67 ± 2.08^{de}	26.00 ± 2.65^{b}	9.13 ± 0.91^{a}	20.67 ± 1.53^{bc}
Melatonin 0.05 mM	21.00 ± 1.73^{ab}	28.33 ± 3.06^{ab}	10.40 ± 0.36^{a}	23.67 ± 1.53^{ab}
Melatonin 0.1 mM	25.00 ± 3.00^{a}	34.33 ± 3.21^{a}	9.47 ± 0.25^{a}	26.33 ± 1.53^{a}
Brassinosteroid 0.025 mM	19.67 ± 2.08^{bc}	23.00 ± 1.00^{b}	9.23 ± 1.08^{a}	18.67 ± 1.15^{cd}
Brassinosteroid 0.05 mM	$18.67 \pm 1.15^{b-d}$	27.00 ± 3.00^{b}	9.57 ± 0.51^{a}	20.00 ± 2.00^{bc}
Brassinosteroid 0.1 mM	$15.33 \pm 1.15^{c-e}$	26.00 ± 1.00^{b}	9.63 ± 0.12^{a}	15.67 ± 0.58^{d}

According to the Tukey test, means that do not share the letters for each variable in each column differ significantly at $p \leq 0.05$

Table 4 Effect of melatonin and brassinosteroid concentrations on leaf area, leaf dry matter, and SPAD reading for Naomi Mango trees grown under salt stress in 2023 and 2024 seasons

Treatments	Leaf area (cm^2)	Leaf dry matter	SPAD reading
2023			
Control	18.90 ± 0.36^{b}	$40.64 \pm 2.70^{a-c}$	28.93 ± 2.56^{d}
Melatonin 0.025 mM	22.42 ± 2.98^{ab}	48.81 ± 5.85^{a}	41.33 ± 1.53^{ab}
Melatonin 0.05 mM	24.76 ± 1.61^{a}	$36.67 \pm 3.70^{a-c}$	42.43 ± 0.51^{ab}
Melatonin 0.1 mM	24.87 ± 0.82^{a}	$41.37 \pm 7.55^{a-c}$	44.60 ± 0.69^{a}
Brassinosteroid 0.025 mM	21.85 ± 1.27^{ab}	30.06 ± 6.09^{c}	36.57 ± 0.38^{c}
Brassinosteroid 0.05 mM	26.65 ± 2.52^{a}	32.86 ± 5.99^{bc}	40.33 ± 1.53^{b}
Brassinosteroid 0.1 mM	24.52 ± 1.06^{b}	45.33 ± 3.36^{ab}	39.43 ± 0.74^{bc}
2024			
Control	16.26 ± 1.56^{b}	44.34 ± 2.29^{ab}	31.70 ± 2.00^{c}
Melatonin 0.025 mM	18.05 ± 1.74^{b}	44.57 ± 2.16^{ab}	37.87 ± 0.84^{ab}
Melatonin 0.05 mM	20.11 ± 2.12^{ab}	37.81 ± 4.66^{b}	38.00 ± 2.03^{ab}
Melatonin 0.1 mM	20.38 ± 1.54^{ab}	42.31 ± 1.05^{ab}	37.60 ± 0.95^{ab}
Brassinosteroid 0.025 mM	20.11 ± 0.93^{ab}	43.98 ± 1.61^{ab}	40.63 ± 0.84^{a}
Brassinosteroid 0.05 mM	23.62 ± 2.69^{a}	47.51 ± 4.48^{a}	36.23 ± 1.37^{ab}
Brassinosteroid 0.1 mM	18.17 ± 0.70^{b}	46.19 ± 3.43^{ab}	35.40 ± 2.36^{c}

According to the Tukey test, means that do not share the letters for each variable in each column differ significantly at $p \leq 0.05$

3.2 Leaf Pigments

The findings for chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids are shown in (Fig. 1a, b, c and d respectively). The use of MT and BR resulted in a notable increase in chlorophyll a, chlorophyll b, and total chlorophyll except for the 0.1 mM concentration of BR. The lowest values of 0.59, 0.26, 0.85 and 0.20 were observed in untreated trees (control) conditions in the first season, respectively. While the values were 0.53, 0.22, 0.75 and 0.20, respectively in the second season.

3.3 Tree Productivity

The treatments affected the tree's production, which was established by the quantity of fruits per tree, the average weight of the fruits, and the yield per tree (Fig. 2a, b and c respectively). There was no significant difference among treatments in fruit weight, except that the control treatment had the lowest values. However, the number of fruits, and consequently the tree's yield, was influenced by the different treatments. The treatments with MT at 0.1 and BR at 0.05 resulted in the highest values in both seasons. The treatment with MT at 0.05 showed positive results only in the second season.

3.4 Fruit Physical and Chemical Characteristics

Physical variables such as peel, seed, and flesh weights were measured (Fig. 3a, b and c respectively). TSS%, acidity%, TSS/acid ratio, and Vitamin C were determined as chemical characteristics (Fig. 4a, b, c and d respectively). Regarding

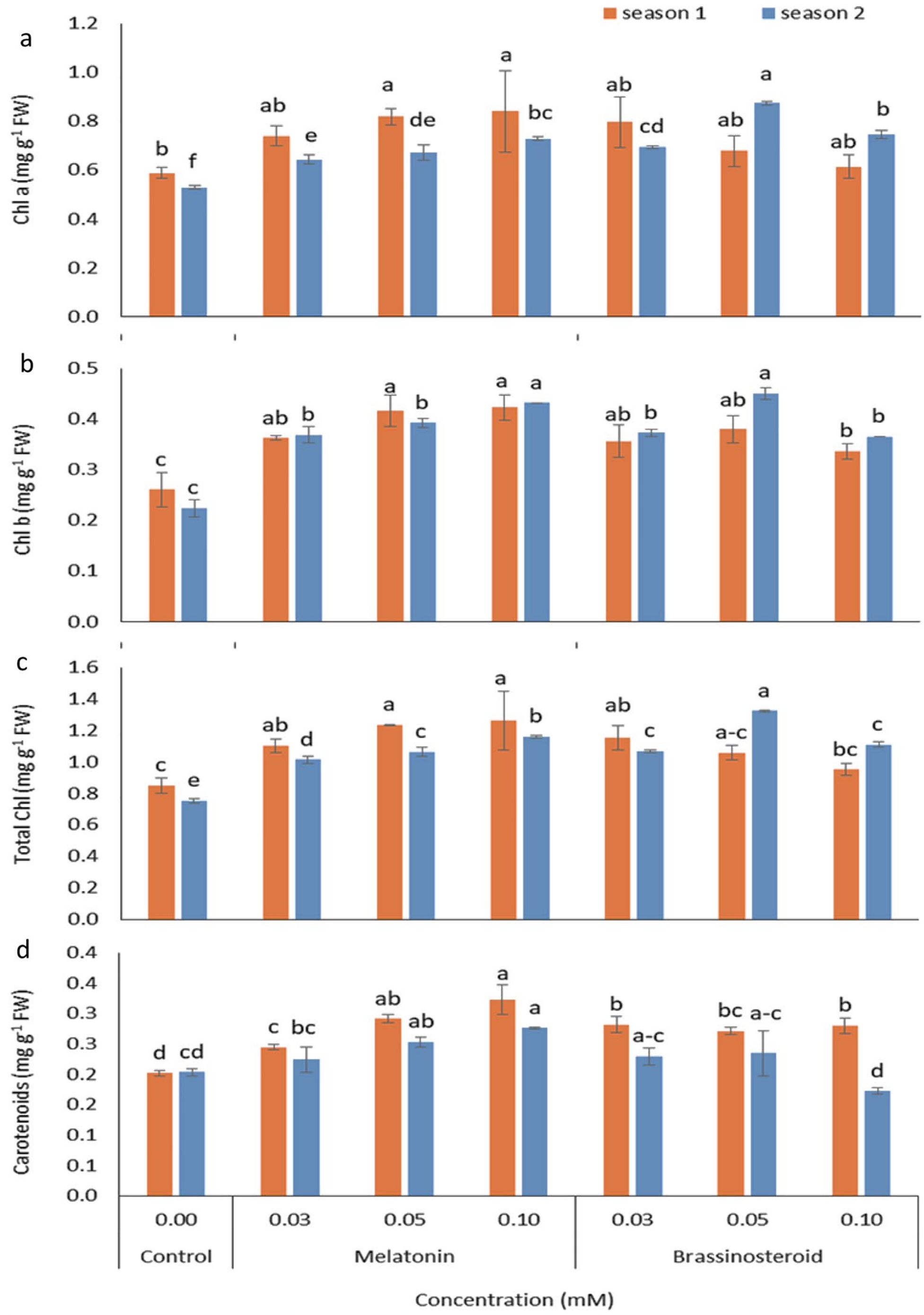


Fig. 1 Effect of melatonin and brassinosteroid concentrations on leaf pigments (**a**) chlorophyll a (**b**) chlorophyll b (**c**) total chlorophyll (**d**) carotenoids for Naomi mango trees grown under salt stress in season 1 (2023) and season 2 (2024). According to the Tukey test, means that do not share the letters for each variable in each bar differ significantly at $p \leq 0.05$

the physical properties, there was no clear trend in the effect of treatments on Peel weight in both seasons. Although the seed weight was lower in control treatment compared to the other treatments, the control treatment had the lowest flesh weight. The treatments of 0.05- and 0.025-mM BR and 0.025, 0.05- and 0.1-mM MT showed the highest values of flesh weight. Regarding chemical characteristics, TSS was affected by treatments. The highest values of TSS were obtained by 0.025 mM BR followed closely by 0.025, 0.05 and 0.1-mM MT. The results suggest that the treatments used may have lowered the acidity level in the fruits compared to the untreated trees. the acidity level of mango fruits decreased in trees that were treated with MT at 0.1 mM followed by MT at 0.05 and BR at 0.025 mM. However, the highest acidity level was found in the fruits of the control trees. As for the TSS/acid ratio, treatment MT at 0.1mM resulted in the highest values, closely followed by treatments of BR at 0.025 and 0.05 mM. The lowest values

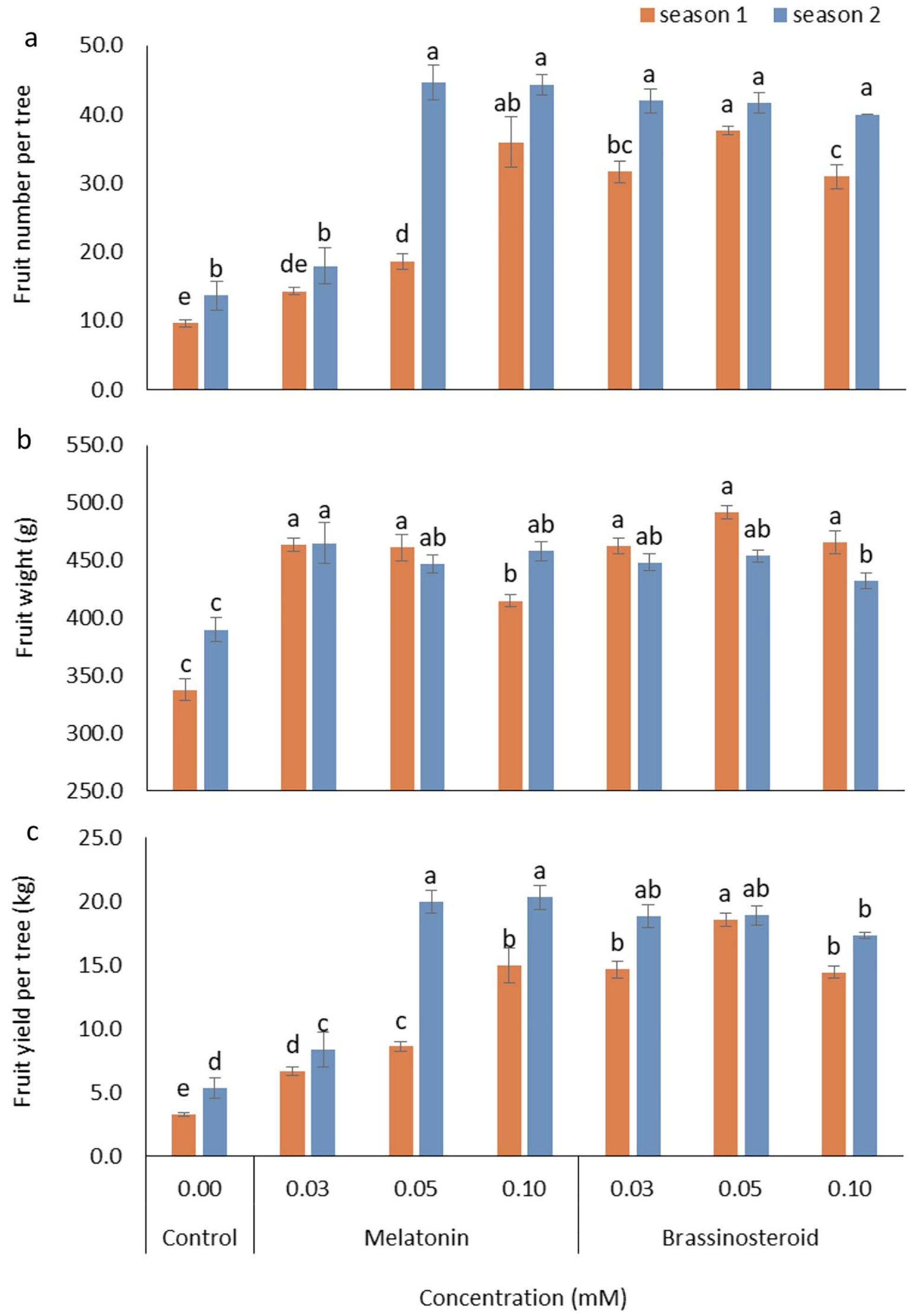


Fig. 2 Effect of melatonin and brassinosteroid concentrations on tree productivity (**a**) fruit number per tree (**b**) fruit wight (**c**) fruit yield per tree for Naomi mango trees grown under salt stress in season 1 (2023) and season 2 (2024). According to the Tukey test, means that do not share the letters for each variable in each bar differ significantly at $p \leq 0.05$

were observed in control and BR at 0.1 mM. On the other hand, the vitamin C content in the fruits was not affected by the treatments.

Exploring the association between applied treatments and tree productivity and fruit quality parameters studied is pivotal for providing valuable insights. Utilizing the heatmap and hierarchical clustering, studying parameters including fruit number per tree, fruit weight, yield per tree, flesh weight, TSS, TSS/acid ratio, and Vit C, grouped the applied treatments into distinct clusters at season 2023 (Fig. 5a) and season 2024 (Fig. 5b). It was observed that the applied BR with a concentration of 0.05 mM, followed by 0.0.25, and MT with a concentration 0.1 mM, demonstrated favorable performance across all evaluated parameters. Conversely, the untreated control exhibited unfavorable performance (Fig. 5).

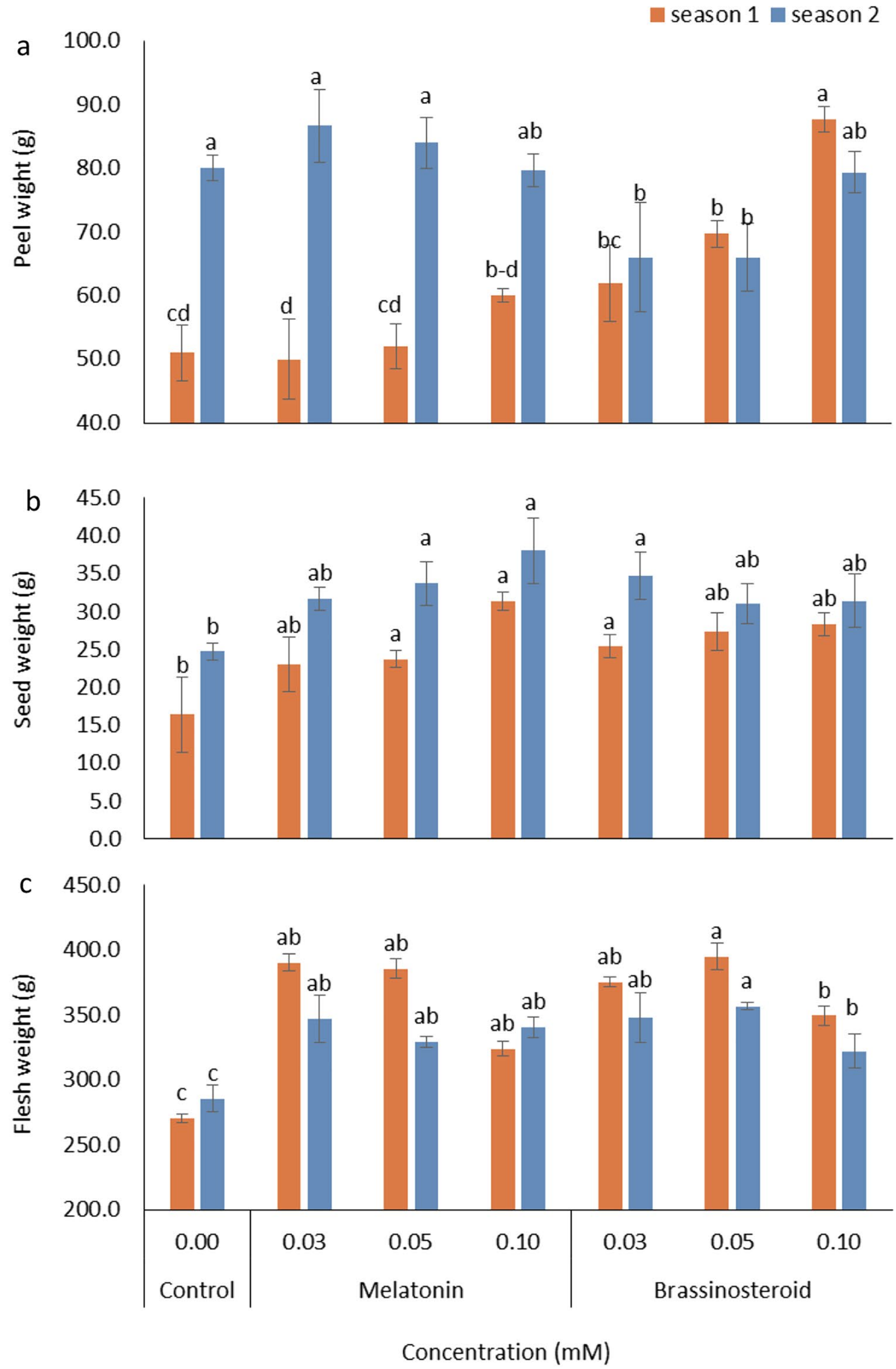
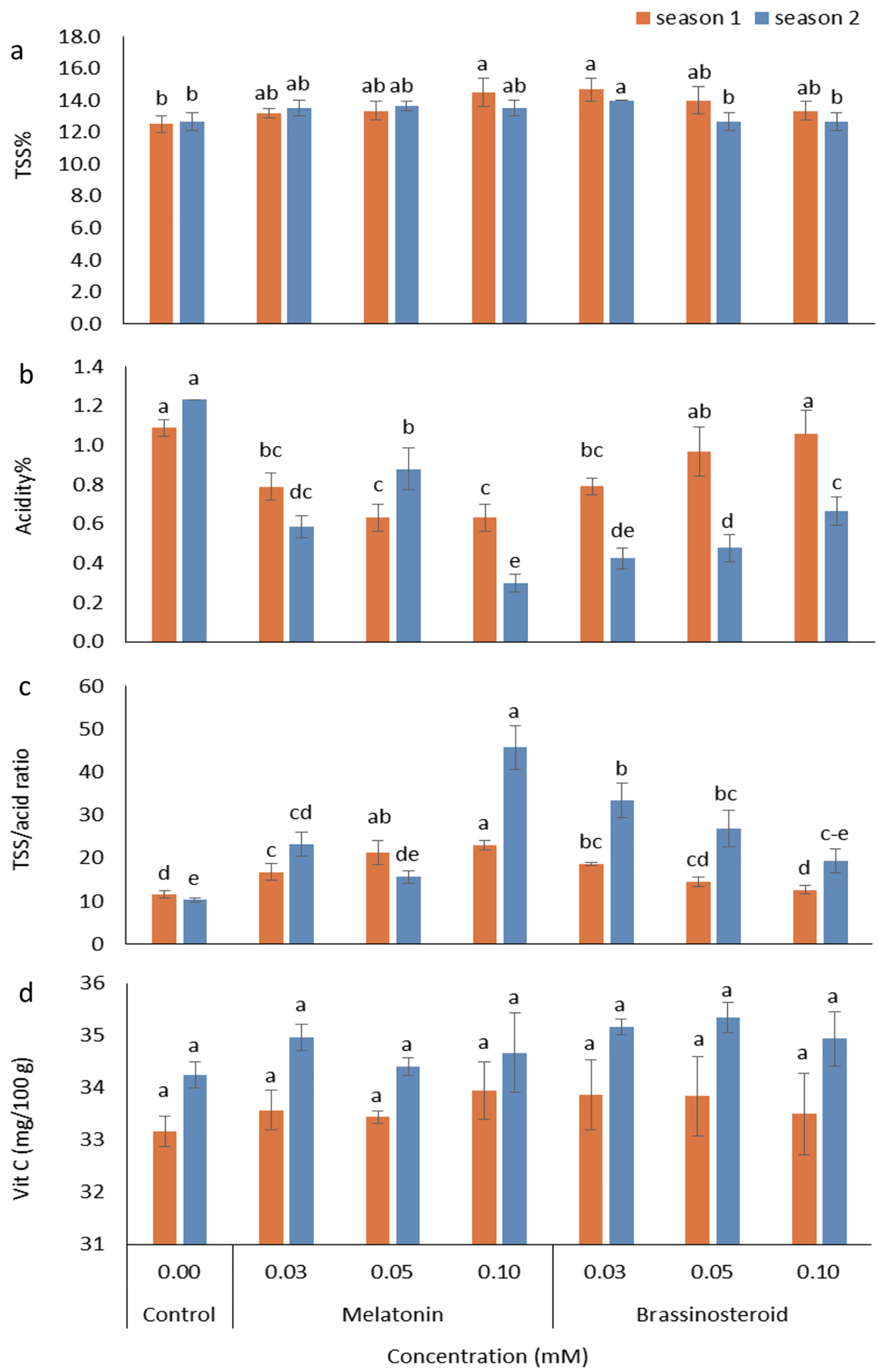


Fig. 3 Effect of melatonin and brassinosteroid concentrations on fruit physical characteristics (**a**) peel wight (**b**) seed weight (**c**) flesh weight for Naomi mango trees grown under salt stress in season 1 (2023) and season 2 (2024). According to the Tukey test, means that do not share the letters for each variable in each bar differ significantly at $p \leq 0.05$

3.5 Oxidative Stress Markers

As anticipated, mango plants exposed to abiotic stress were irrigated with water at EC = 3.40 dSm^{-1} which results in an increase in the number of free radicals in the plants. MT and BR were sprayed to enhance PPO activity, POD activity and proline content (Fig. 6a, b and c respectively). The treatment produced the PPO enzyme's maximum activity. by MT at 0.1 mM and 0.05 mM, respectively in the two seasons (Fig. 6a). The activity of enzyme POD increased when trees were treated with MT at 0.1mM (Fig. 6b). As for the proline content of the leaves, the highest content was found

Fig. 4 Effect of melatonin and brassinosteroid concentrations on fruit chemical characteristics (**a**) total soluble solids (TSS%) (**b**) acidity% (**c**) TSS/acid ratio (**d**) vitamin C for Naomi mango trees grown under salt stress in season 1 (2023) and season 2 (2024). According to the Tukey test, means that do not share the letters for each variable in each bar differ significantly at $p \leq 0.05$



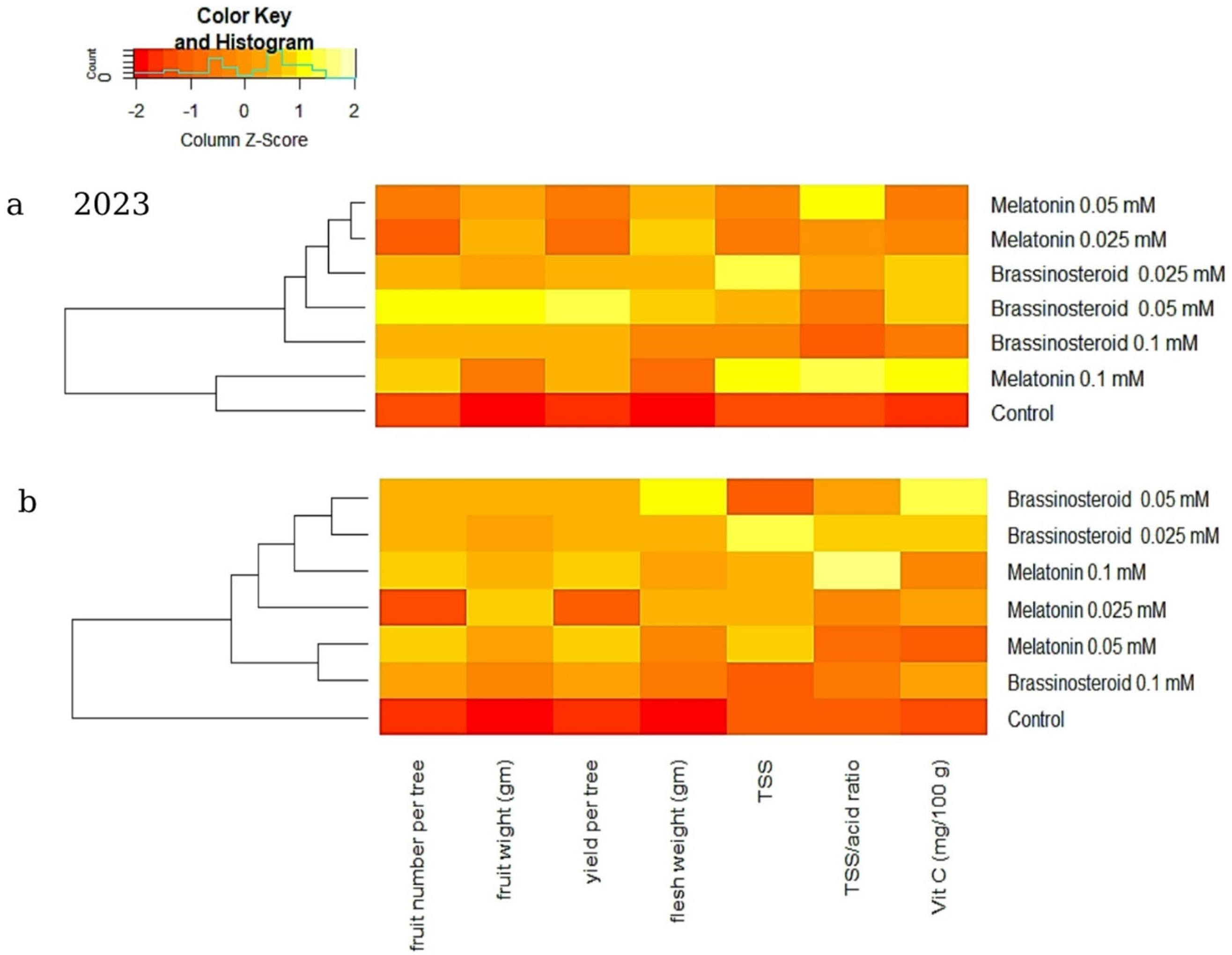


Fig. 5 Heatmap for the evaluated parameters of mango plants treated with different concentrations of melatonin and brassinosteroid in (**a**) 2023 and (**b**) 2024 seasons. Parameters in Yellow color and red color reveal high and low values for the corresponding parameters, respectively

in the MT -treated plants' leaves at 0.1mM and BR at 0.05 mM (Fig. 6C).

3.6 Leaf Mineral Content

The leaf contents of sodium, chloride, potassium and calcium of mango *cv*. Naomi were affected by MT treatment and BR (Fig. 7a, b, c and d respectively). The control had the highest sodium and chloride concentration readings. However, MT treatment at 0.1 mM and 0.05 mM, respectively and BR at 0.05 mM helped reduce the leaf content of sodium and chloride by (38.46, 44.78 respectively) and increasing K^+ and Ca^{++} content by (29.64, 41.38 respectively).

4 Discussion

The growth and yield of mango trees are greatly impacted by salinity stress. Salinity stress can lead to reduce growth in mango trees due to osmotic stress, which limits water uptake and causes physiological drought stress (Ataya et al. 2025). This could result in growth stunt and leaf area reduction. High salinity levels can cause nutritional imbalances in crop plants by affecting the uptake of essential nutrients like nitrogen, phosphorus, and potassium (Salem et al. 2021; Abd El-Mageed et al. 2022; Shaaban et al. 2023). This can lead to deficiencies and negative impact overall tree health. Excessive salt ions, particularly sodium and chloride, could be accumulated in the soil and plant tissues, leading to ion toxicity (Saudy et al. 2023b; Emam et al. 2025). This can cause leaf burns, chlorosis, and necrosis, further reducing the tree's productivity. Mango trees that experience salinity stress may develop oxidative stress, which can result in the

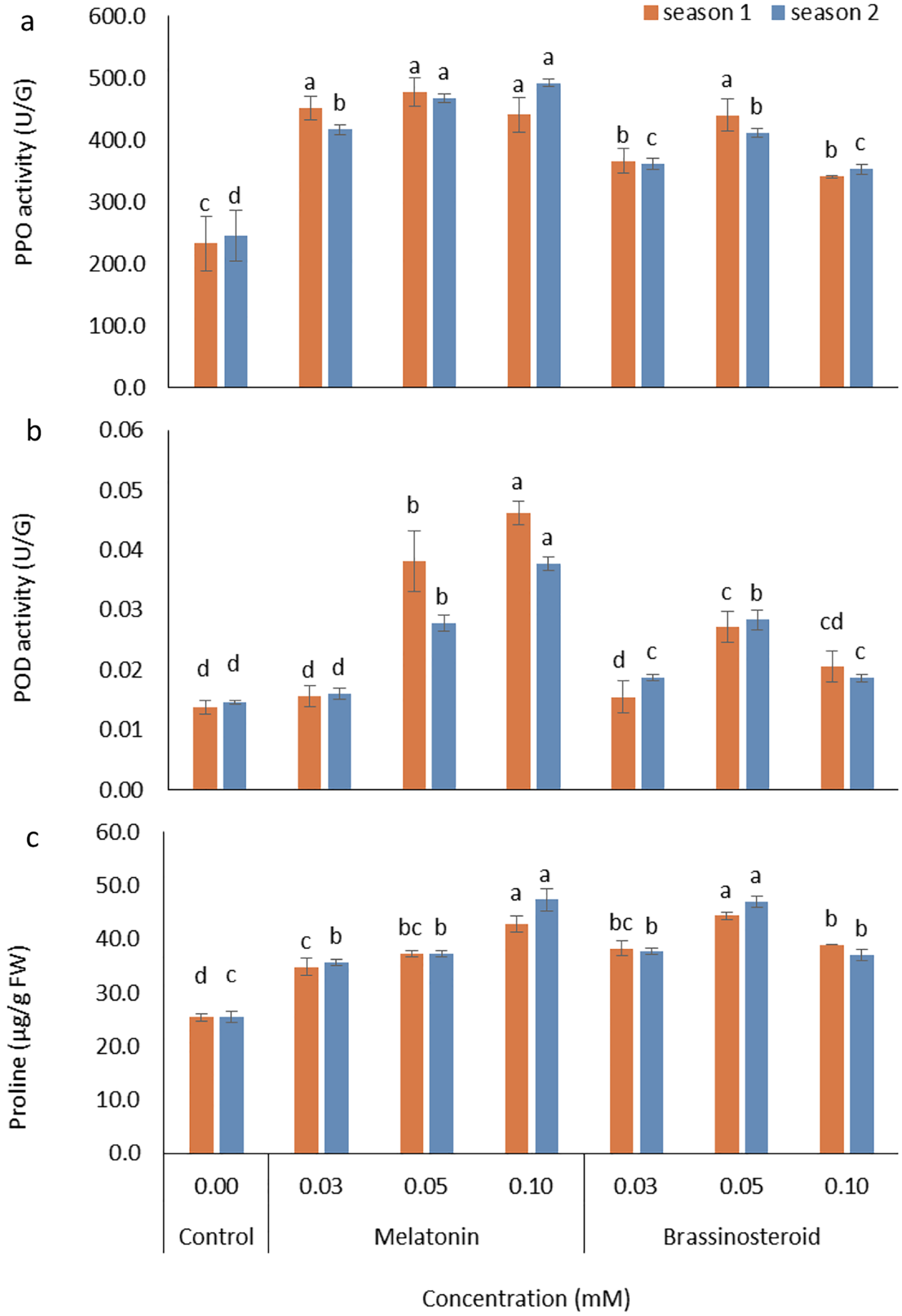


Fig. 6 Effect of melatonin and brassinosteroid concentrations on oxidative stress markers (**a**) Polyphenol Oxidase activity (PPO) (**b**) Peroxidase activity (POD) (**c**) proline concentration for Naomi mango trees grown under salt stress in season 1 (2023) and season 2 (2024). According to the Tukey test, means that do not share the letters for each variable in each bar differ significantly at $p \leq 0.05$

generation of ROS that can harm cellular components. As well, photosynthesis and other essential activities may be hampered due to salinity (Helal et al. 2024; Ramadan et al. 2024). Fruit yield and quality can be significantly reduced by the combined impacts of oxidative stress, ion toxicity, nutritional imbalance, and osmotic stress (Elshiekh et al. 2025).

In general, all MT and BR foliar treatments boosted the growth of vegetation in comparison to control except for BR at 0.1mM. The best results for vegetative growth were for trees treated with MT at 0.05 and 0.1mM and BR at 0.05mM, which were represented in the shoot number, shoot length, leaf area, leaf dry matter, and SPAD reading. The reduction in these variables related to leaf area and dry mass is attributed to decreased cellular activities such as

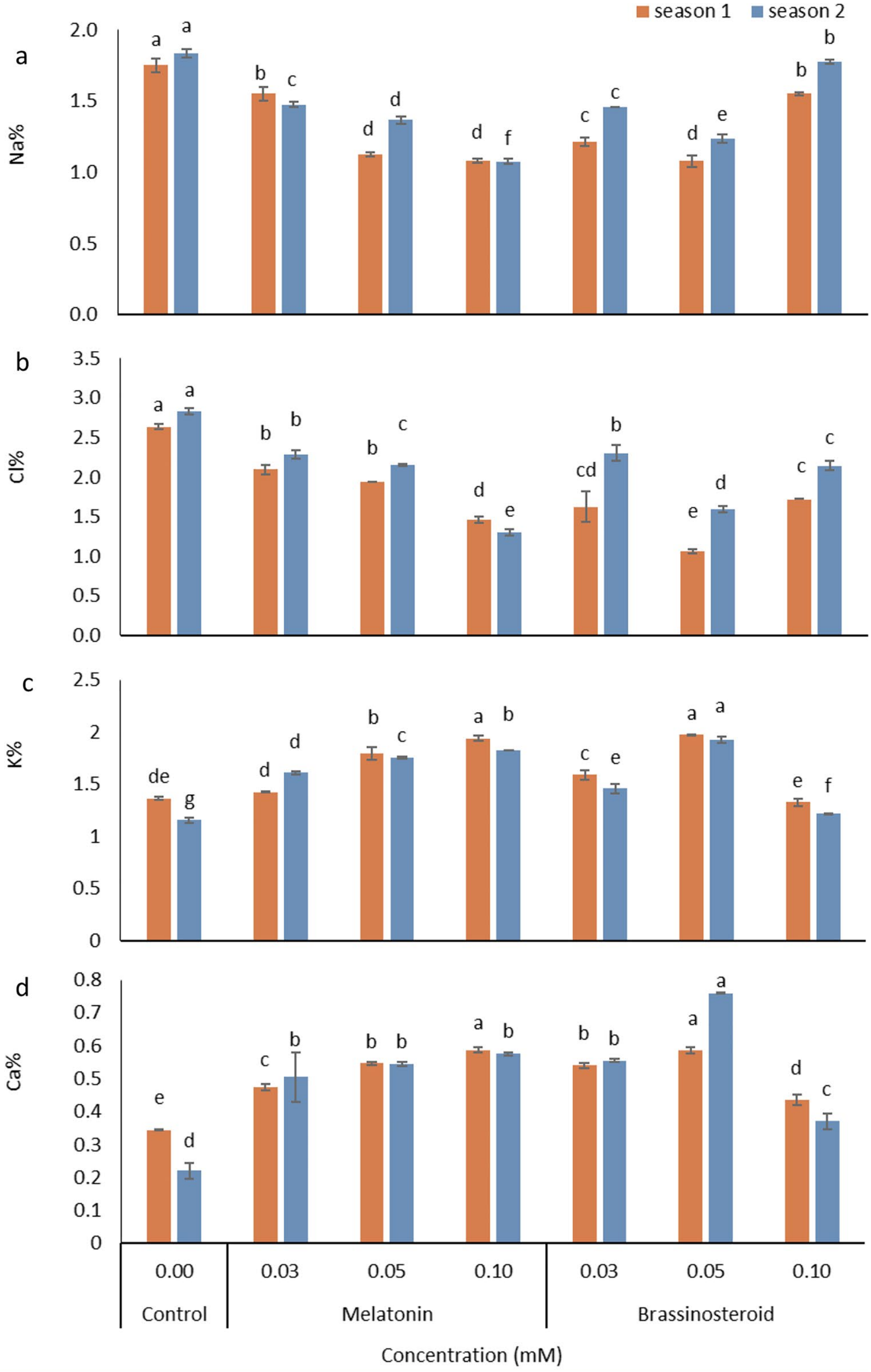


Fig. 7 Effect of melatonin and brassinosteroid concentrations on leaf mineral content (**a**) Na (b) Cl (**c**) K (**d**) Ca for Naomi mango trees grown under salt stress in season 1 (2023) and season 2 (2024). According to the Tukey test, means that do not share the letters for each variable in each bar differ significantly at $p \leq 0.05$

photosynthesis and respiration. Consequently, the osmotic effect leads to salt accumulation in the soil, compromising the plant's absorption of water and nutrients, thereby diminishing cell growth and leaf surface (Ali et al. 2024a). Radish plant development and production are less negatively impacted by salt stress when MT is applied exogenously. The most effective way to lessen the symptoms of salt stress is to apply 0.5 mM of MT (Ribeiro et al. 2024). MT is crucial in controlling the development and vegetative growth of crops under various abiotic stressors (song et al. 2024).

Steroid hormones known as BR are essential for the growth and development of plants. They are essential in controlling the number of physiological and developmental processes, including floral transition, stem cell maintenance, cell division and growth, and the elongation of diverse cell types. Moreover, BR plays a role in stomata formation and the regulation of responses to biotic and abiotic stressors (Manghwar et al. 2022). BR hormone promotes the growth of plant organs above and below ground. This is evidenced by the dwarf appearance of plants lacking BR. Recent research indicates that BR activity affects growth stages differently depending on the context and concentration of the hormones. Additionally, BR signaling factors are involved in various signaling pathways that regulate growth and development, such as cell elongation, shoot branching, stomata formation, lateral root formation, and xylem differentiation (Singh and Savaldi-Goldstein 2015). BR may regulate stress responses to heat, cold, salt, and drought by interacting with other hormones like gibberellin, ethylene, abscisic acid, and jasmonic acid. Exogenous EBR pretreatment has been shown to increase grapevines' levels of ABA, GA, auxin, and GA to combat dryness (Gao et al. 2024). In study on tomato, salinity stress induced growth reduction was mitigated to considerable levels due to BR at 1µM application (Ahanger et al. 2020).

Growing mango plants under salinity stress significantly decreased the levels of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids in the leaves. Salinity stress can push the photosynthetic apparatus to produce excessive ROS, leading to rapid degradation of leaf pigments, triggering leaf senescence, and eventually resulting in leaf cell death (Ali et al. 2021). These effects might stem from MT's ability to regulate the transcription of genes related to leaf pigments, protect photosynthetic proteins, enhance antioxidant systems, and activate the xanthophyll cycle (Ali et al. 2024b; Helal et al. 2024) In relation to BR impact on plant pigments content in leaves. treatment with 0.2 mg L^{-1} BR helped increasing chlorophyll levels, enhancing photosynthetic efficiency in tea leaves (Zhang et al. 2024), chlorophyll a, b, and carotenoid contents in tomatoes (Jia et al. 2021), and rice seedlings under Gamma Radiation Stress (Lu et al. 2024).

Salt stress significantly impacts the productivity of mango plants. High salt concentrations in the soil can lead to reduced growth and yield in mango plants due to the negative effects of salinity on water uptake, nutrient balance, and overall plant health (Zuazo et al. 2004; Yadav et al. 2019). Treatments MT at 0.1 and 0.05mM and BR at 0.05mM led to an improvement in yield by increasing the number of fruits on the trees, with fruit weight slightly higher than other treatments. Applying a 0.1 mM MT treatment increased pomegranate fruit production (Medina-Santamarina et al. 2021). This boost in harvest can be attributed to MT's ability to minimize fruit abscission typically caused by environmental factors such as wind or rain during the active growth phase. Additionally, MT is known to enhance plants' resilience to abiotic and biotic stress (Medina-Santamarina et al. 2021; Ali et al. 2024b). BR interacts with other plant growth regulators to orchestrate physio-biochemical responses, improving photosynthetic efficiency, enzymatic activities, and overall plant tolerance to salt stress (Shah et al. 2024). (Almutairi et al. 2023) In an experiment to improve the productivity of mango trees, it was found that the application of BR at 0.45 mM on mango trees greatly raised the fruit set percentage, fruit yield. Additionally, when putrescence and spermidine were administered to date palm *cv.* Kabkab at concentrations of 0.1 mM and 1.0 mM, it clearly increased fruit yield while also reducing the proportion of fruit drops compared to untreated trees (Tavakoli and Rahemi 2014).

Fruit quality and productivity showed that plants treated with MT at 0.05 and 0.1 mM and BR at 0.05 mM fared better under salt stress because the trees were superior to the other therapies in terms of health. MT pre-ripening therapies improve the final quality of the fruit (Himanshu et al. 2024). The MT treatment (500 µM, day 124 post-anthesis) on the apple tree significantly advanced the ethylene production and increased the fruit size, weight, sugar content, and firmness (Verde et al. 2022). Mango fruit quality, including the amount of chlorophyll in the fruit peel, pulp quality, TSS percentage, reducing sugars, and TSS–TA ratio, increased with its application of BR at 0.45 mM (Almutairi et al. 2023). On date palm trees cv. Zaghloul, the exogenous administration of 1 mM of BR during the phases of cell division and elongation markedly increased the fruit content from dry matter, ash, and soluble solids (Talaat et al. 2023).

Markers of oxidative stress were measured by measuring PPO, POD activity, and proline content. Increased PPO and POD activity reduces free radicals within plant cells resulting from abiotic stress by modifying the metabolism of ROS associated with oxidative stress relatively (Hadid et al. 2023; Mansour et al. 2025). Roughly 1–2% of oxygen absorbed is converted into ROS, such as hydrogen peroxide (H_2O_2), superoxide radical ($O_2^{\bullet-}$), singlet oxygen ($^1O_2$), and hydroxyl radical ($\bullet OH^-$) (Nasser et al. 2022; Bahnasy et al. 2025). Salt-tolerant mango cultivars accumulate higher levels of proline, glycine betaine, and total sugars to counter osmotic stress (Schmutz 2000; Laxmi et al. 2021). Exogenous MT treatment has been found to increase POD activity in cherry tomato fruit (Li et al. 2019). It also enhanced POD and CAT activities in broccoli florets (Zhu et al. 2018). In a previous study, the application of exogenous MT was shown to boost POD and SOD activities in sweet cherries (Wang et al. 2019). The increase in POD and CAT activities was effective in reducing the harmful effects of $O_2^{\bullet-}$ and

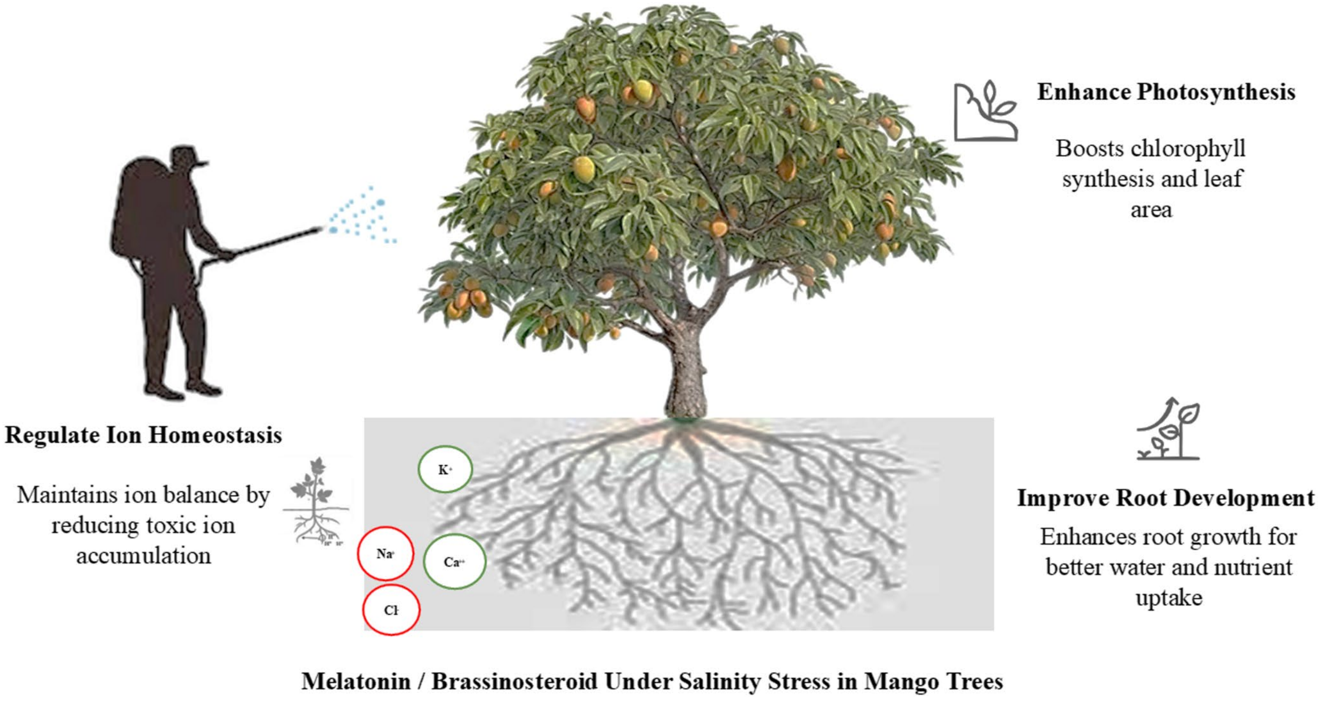


Fig. 8 Simplified model for the suggested effects of melatonin and brassinosteroid, as a foliar application, on mango trees exposed to salt stress

H_2O_2, which decreased in grape berries (Nasser et al. 2022). In melon seedlings under cold stress, MT administration further raised the amounts of proline and soluble protein. (Zhang et al. 2017). Application of 0.05, 0.1 and 1 µM MT concentration significantly reduced the proline content in the cherry rootstock (Sarropoulou et al. 2012).

Regarding ions homeostasis, it has been reported that unfavorable conditions negatively impact nutrient uptake and utilization, thereby affecting plant metabolism (Saudy et al. 2020, 2021b; Abd–Elrahman et al. 2022; Ali et al. 2024a; Lasheen et al. 2024). Cell dryness, stomata closure, and a decrease in CO_2 levels within the photosynthetic cell are caused by the high concentration of Cl^- and Na^+ ions inside the cells, as well as a low K^+/Na^+ ratio, which affects plant enzyme and membrane performance (Ramadan et al. 2023, 2025a; Abdo et al. 2024). MT enhances the absorption of potassium, which counteracts sodium and reduces its absorption through the roots (Maola et al. 2023). The MT foliar application raised the element contents of *Moringa oleifera* plants under drought-stress especially K^+ and Ca^{++} (Sadak et al. 2020). Ca is the most effective divalent and stabilizes cell membrane under abiotic stress via mediating membrane association such as the reduction of ion leakage, uptake of ions and amino acids, and maintenance of the configuration of enzyme binding sites in cells (Fu et al. 2006). Plants may produce K^+ to seal their stomata and reduce water loss through transpiration in response to an increase in salt intake. But stomata closing can hinder CO_2 intake, which will ultimately impact plant growth and photosystem function (Song et al. 2024).

Eventually, the suggested mechanisms effects of melatonin and brassinosteroid as a foliar application on mango trees exposed to salt were simplified in Fig. 8.

5 Conclusions

The treatments of melatonin and brassinosteroid helped improve the vegetative growth of the Naomi mango trees irrigated with salty water. That was reflected in the productivity of the tree and the physical and chemical properties of the fruits. This is done through playing a role in regulating vital processes within plants under stress conditions. It increased the plant's proline content as well as the enzyme activity of polyphenol oxidase and peroxidase, which helped eliminate reactive oxygen species and reduce their harmful effects. Also, the melatonin and brassinosteroid treatments helped increase the absorption of potassium and calcium, which counteracted the absorption of toxic sodium and chlorine. Therefore, spraying Naomi mango with melatonin at concentrations of 0.05 mM or 0.1 mM and brassinosteroid at 0.05 mM are recommended practices to obtain better yield and productivity of salt-stressed mango.

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editing, A.M., M.N., M.S. and N.M.; supervision, N.M. All authors have read and agreed to the published version of the manuscript.

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Declarations

Competing Interests There is no conflict of interest in the present study.

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