

Article

Effect of Plant Growth Regulators on the Physiological Response and Yield of *Cucumis melo* var. *inodorus* Under Different Salinity Levels in a Controlled Environment

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Abstract

The objective of this study was to evaluate the physiological, biochemical, and productive effects of the foliar application of bioregulators, based on auxin, cytokinin, and gibberellic acid, on yellow melon, cultivar DALI[®], plants subjected to different salinity levels in a protected environment to simulate Brazil's semi-arid conditions. The experiment was conducted using a completely randomized block design, in a 4 × 3 factorial scheme, with four salinity levels (0, 2, 4, and 6 dS m⁻¹) and three doses of the bioregulator, Stimulate[®] (0%, 100%, and 150% of the recommended dose), with six weekly applications. The physiological variables (chlorophyll *a* fluorescence and gas exchange) and biochemical parameters (antioxidant enzyme activity and lipid peroxidation) were evaluated at 28 and 42 days after transplanting, and the agronomic traits (fresh fruit mass, physical attributes, and post-harvest quality) were evaluated at the end of the experiment. The results indicated that salinity impaired the physiological and productive performance of the plants, especially at higher levels (4 and 6 dS m⁻¹), causing oxidative stress, reduced photosynthesis, and decreased yield. However, the application of the bioregulator at the 100% dose mitigated the effects of salt stress under moderate salinity (2 dS m⁻¹), promoting higher CO₂ assimilation rates of up to 31.5%, better water-use efficiency, and reduced lipid peroxidation. In addition, the fruits showed a greater mass of up to 66%, thicker pulp, and higher soluble solids (> 10 °Brix) content, making them suitable for sale in the market. The 150% dose did not provide additional benefits and, in some cases, resulted in inhibitory effects. It is concluded that the application of Stimulate[®] at the recommended dose is effective in mitigating the effects of moderate salinity, up to ~3 dS m⁻¹, in yellow melon crops; however, its effectiveness is limited under high salinity conditions, requiring the use of complementary strategies.

Keywords: melon; bioregulators; gas exchange; oxidative stress; salinity stress



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1. Introduction

Salinity is one of the main abiotic stresses, limiting the growth and productivity of agricultural crops in various regions of the world, particularly in semi-arid areas, where

irrigation water quality often shows high electrical conductivity [1]. It is estimated that more than 900 million hectares of agricultural land are affected by salinization in the world, resulting in annual economic losses exceeding USD 27 billion [1,2]. Melon cultivation (*Cucumis melo* L.) holds significant economic importance and is widely practiced in regions susceptible to salinization, such as northeastern Brazil [3].

Melon, belonging to the Cucurbitaceae family, is a widely cultivated horticultural crop with notable global economic and nutritional importance. Among its botanical varieties, *C. melo* var. *inodorus* includes the widely consumed yellow melon types, such as ‘Goldex’ and ‘Valéria’. Globally, melon cultivation occupies over 1.2 million hectares, with major producers including China, Turkey, Iran, and Brazil. In Brazil, especially in the semi-arid northeast, melon is a key export commodity, contributing significantly to regional income and employment. Beyond its economic relevance, melon fruits are rich in vitamins (A and C), antioxidants, and bioactive compounds, such as carotenoids and flavonoids, which support immune function and reduce oxidative stress, reinforcing their importance in a health-promoting diet.

Salt stress affects multiple physiological processes in plants, leading to changes in nutrient uptake and translocation, osmotic imbalance, the accumulation of toxic ions, reduced photosynthesis, and excessive production of reactive oxygen species (ROS), which causes cellular damage and impairs plant growth [2,3]. Among the main physiological responses observed are reductions in leaf area, stomatal conductance, and the photosynthetic rate, as well as changes in the K^+/Na^+ ratio in leaf tissues, an essential strategy to mitigate the toxic effects of Na^+ [3].

ROS, such as the superoxide anion ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($\bullet OH$), cause oxidative damage to lipids, proteins, and nucleic acids, leading to cellular dysfunction and impaired growth. To counteract ROS damage, plants activate antioxidant defense systems, including enzymatic mechanisms (e.g., superoxide dismutase [SOD], catalase [CAT], guaiacol peroxidase [GPX], and peroxidase [POD]) and non-enzymatic antioxidants (e.g., ascorbic acid, carotenoids, phenolic compounds, flavonoids, soluble sugars, and proline). These metabolites play key roles in osmotic adjustment, membrane stabilization, and ROS detoxification, and are widely used as physiological indicators of salt stress tolerance.

In this context, the exogenous application of plant growth regulators has proven to be a promising practice to alleviate the deleterious effects of salinity by promoting physiological adjustments that favor ionic homeostasis and redox balance, in addition to stimulating antioxidant and osmoprotective mechanisms [4,5]. The coordinated action of phytohormones can modulate tolerance to salt stress by regulating processes such as stomatal closure, the maintenance of plasma membrane integrity, and calcium signaling [6].

In regard to melon cultivation, studies have shown that genotypes more tolerant to salinity can maintain higher water-use efficiency, preserve higher photosynthetic rates, and achieve better productivity under saline conditions [3,7]. Examples of yellow melon genotypes that have demonstrated greater tolerance to salinity include *Goldex*, *AF-682*, *Hales Best Jumbo*, *Valéria*, and *Torreón*. Previous investigations using the bioregulator formulation, *Stimulate*[®], have demonstrated beneficial effects under abiotic stress conditions; however, few studies have explored its impact on melon plants under salinity stress in a controlled environment. Therefore, this study offers a novel contribution by evaluating the combined physiological and productive responses of yellow melon to *Stimulate*[®] application under different salinity levels during protected cultivation.

Given this scenario, this research hypothesizes that the exogenous application of bioregulators can mitigate the adverse effects of salinity by promoting beneficial physiological changes that support the maintenance of crop growth and development. To test

this hypothesis, the present study aimed to evaluate the physiological effects of the foliar application of bioregulators on yellow melon plants grown in a greenhouse and irrigated with different salinity levels.

2. Materials and Methods

2.1. Experiment Location

The experiment was conducted from February to April 2018 in a greenhouse at the Teaching, Research, and Extension Farm of São Manuel (22°44' S, 47°34' W, and 750 m above sea level), which belongs to the Faculty of Agricultural Sciences at São Paulo State University, Botucatu Campus, and in the Plant Physiology II Laboratory as part of the Department of Botany at São Paulo State University (UNESP), also located on the Botucatu Campus.

The region's climate is classified as humid subtropical mesothermal, with a dry season during winter [8].

A protected arch-type structure was used, with the following characteristics: 50 m in length, 7 m in width, and 3 m in height, covered with a 150 micron thick additive low-density polyethylene film, and enclosed on the sides with 75% shade cloth.

2.2. Evaluated Hybrid Characteristics

The hybrid evaluated in this study was a yellow-type cultivar, DALI[®], developed by Sakata Seeds Sudamérica Ltd. This cultivar has an average yield of 32 t ha⁻¹, reaching up to 40 t ha⁻¹. The fruits have white flesh, a small internal cavity, slightly wrinkled yellow skin, an average weight ranging from 1.4 to 2 kg, and a soluble solids content above 10 °Brix, traits that provide excellent commercial quality. Additionally, the cultivar shows moderate resistance to powdery mildew races 1 and 2 and has a production cycle ranging from 65 to 73 days [9]. Although not classified as a salt-tolerant genotype, field observations and company reports indicate that DALI[®] demonstrates relatively stable performance under moderate salinity conditions (up to 3.5 dS m⁻¹), maintaining acceptable fruit quality and physiological integrity.

The choice of this hybrid is justified by its widespread use among Brazilian producers, especially in semi-arid regions, due to its high market acceptance, yield stability, and fruit quality.

2.3. Soil Preparation, Seedlings, and Transplanting

The soil used is classified, according to Embrapa [10], as a dystrophic Red Latosol, with a sandy texture. The soil samples were collected from the 0 to 20 cm depth layer (Table 1), and based on the analysis results, soil correction was carried out prior to planting.

Table 1. Results of the analysis of the soil used in the experiment.

pH	Ca	Mg	Al + H	CTC	O.M.	P _{resin}	K	Zn	Cu	Mn	B	V
CaCl ₂	-----Cmol _c dm ³ -----				dm ³		-----mg dm ³ -----					%
4.2	16	4	44	65	11	2	1	0.1	0.3	0.6	0.21	32

O.M. = organic matter; V = base saturation.

The seedlings were produced by Sakata Seeds Sudamérica Ltd at the company's Experimental Farm in Bragança Paulista, SP, and donated to this research 30 days after sowing. For seedling production, plastic trays with 128 cells, measuring 18.5 × 19.0 × 11.0 cm in width, length, and depth, respectively, were used. The commercial substrate Carolina Soil[®] II was used, composed of sphagnum peat, expanded vermiculite, organic agro-industrial class A waste, dolomitic limestone, agricultural gypsum, and traces of NPK fertilizers, with a pH of 5.5 ± 0.5, an EC of 0.4 ± 0.3 mS cm⁻¹, and a density of 145 kg m⁻³.

The experimental unit consisted of one plant grown in a pot, with a volume capacity of 14.3 L. The soil was corrected by the addition and homogeneous mixing of dolomitic limestone with a Relative Power of Total Neutralization (PRNT) of 90%. The pots were filled with a mixture of 75% corrected soil and 25% Carolina Soil® II substrate, in a 3:1 ratio. The spacing used was 0.5 m between pots and 1.20 m between rows. Transplanting was carried out on February 6, 2018, 15 days after sowing.

2.4. Electrical Conductivity (ECa) of Irrigation Water

To obtain water with the desired levels of electrical conductivity, sodium chloride (NaCl, non-iodized) was added to water from the local supply system (Table 2) (the water used on the experimental farm originates from a natural spring located on the property).

Table 2. Results of the analysis of the water used in the experiment.

pH	E.C.	N	P	Ca	Mg	K	S	Mn	Zn	Cu	Fe	B
	dS m ⁻¹		mg L ⁻¹									
7.09	0.00125	13.0	0.21	23.94	3.63	2.0	<DL	<DL	<DL	<DL	0.04	<DL

E.C. = electrical conductivity; DL = detection limit = 0.01.

The amount of NaCl added was calculated based on the initial electrical conductivity of the water, following the methodology proposed by Richards [11], where: ECa—electrical conductivity of the water, expressed in dS m⁻¹; NaCl required (mg L⁻¹) = 640 × (desired ECa—initial ECa).

Salinity was calculated based on the electrical conductivity of the solution, where the total dissolved salts (TDSs), in milligrams per liter, equals the EC, in deciSiemens per meter (dS m⁻¹), multiplied by the constant 640 mg L⁻¹. Generally, the higher the salt concentration in a solution, the greater its capacity to conduct electricity [11].

2.5. Experimental Design and Treatments

The experiment was conducted using a randomized complete block design (RCBD), arranged in a 4 × 3 factorial scheme, totaling 12 treatments with three replicates, resulting in 36 experimental plots. Each plot consisted of five plants, totaling a population of 180 plants. The treatments were defined by the combination of two factors: the level of irrigation water salinity and the foliar application dose of the plant growth regulator.

The salinity factor was tested at four levels, with an irrigation water electrical conductivity of 0, 2, 4, and 6 dS m⁻¹. The bioregulator factor was applied at three doses: 0% (no application), 100% (500 mL ha⁻¹, corresponding to the recommended dose for the crop), and 150% (750 mL ha⁻¹).

The plant growth regulator used was Stimulate®, a commercial formulation manufactured by Stoller®, containing a mixture of plant hormones, namely kinetin (a cytokinin) at 0.009% (m/v), gibberellic acid (GA₃) at 0.005% (m/v), and indole-3-butyric acid (an auxin) at 0.005% (m/v), according to the manufacturer's specifications.

The treatments were as follows: T1—control (no salinity, no bioregulator); T2—no salinity + bioregulator (100%); T3—no salinity + bioregulator (150%); T4—salinity (2 dS m⁻¹) without bioregulator; T5—salinity (2 dS m⁻¹) + bioregulator (100%); T6—salinity (2 dS m⁻¹) + bioregulator (150%); T7—salinity (4 dS m⁻¹) without bioregulator; T8—salinity (4 dS m⁻¹) + bioregulator (100%); T9—salinity (4 dS m⁻¹) + bioregulator (150%); T10—salinity (6 dS m⁻¹) without bioregulator; T11—salinity (6 dS m⁻¹) + bioregulator (100%); and T12—salinity (6 dS m⁻¹) + bioregulator (150%).

The salinity treatments were initiated on the 13th day after transplanting and maintained continuously until the end of the crop cycle. Foliar applications of the bioregulator

began seven days after the start of saline irrigation. A total of six weekly applications were carried out using a pressurized manual CO₂ backpack sprayer, operating at a pressure of 0.3 kgf cm⁻² and a flow rate of 0.2 L min⁻¹. The spray volume ranged from 400 to 800 L ha⁻¹, adjusted according to the phenological stage of the plants. To prevent spray drift between treatments, a plastic curtain was installed as a barrier.

2.6. Irrigation System

The irrigation system was managed independently for each salinity treatment. Each of the four salinity levels was supplied by a 310 L polyethylene reservoir, equipped with a negative suction or flooded-type pumping system, that is, with the pump shaft positioned below the suction reservoir level. Water pumping was carried out by a peripheral monoblock electric pump, model Xkm, with a power rating of 0.5 HP.

The irrigation system was equipped with pressure and flow control valves, operating continuously at 1 BAR, with the pressure monitored using a glycerin-filled pressure gauge. The system was automated through the use of a timer that injected the saline solution into each operational unit's irrigation system. Water filtration was performed using 200 mesh screen filters made of interwoven steel wires. Saline solution homogenization was ensured by a hydraulic agitator, which was activated by the return flow of pumped water into the reservoir.

The irrigation system was built with low-density polyethylene (LDPE) pipes, PN 30, 16 mm in diameter, connected to self-compensating drippers with a flow rate of 4.0 L h⁻¹, spaced 0.50 m apart, matching the plant spacing used in the experiment.

The saline solution was applied daily via irrigation water. The irrigation depth was sufficient to replenish the soil moisture to field capacity and was adjusted based on the average soil water tension, obtained through readings from tensiometers installed at a depth of 15 cm. Water tension was measured using a digital tensiometer (Sondaterra, Piracicaba, SP, Brazil). A total of 24 tensiometers were used, distributed uniformly among the treatments. The irrigation volume was calculated based on the soil water retention curve, considering a 3:1 soil-to-substrate ratio, and specific tensiometric readings for each salinity level. Readings were taken daily in the morning, and irrigations were performed immediately afterward.

2.7. Plant Management and Cultural Practices

The plants were individually trained and supported using plant ties, attached to 2.5 m bamboo stakes, to avoid compromising fruit production and quality.

Side shoot removal (pruning) was carried out weekly. Fruit thinning was performed to retain only one fruit per plant, which was supported with mesh netting and ribbons until harvest. Pollination occurred naturally, facilitated by opening the greenhouse in the morning and avoiding agrochemical applications during pollinator visitation periods.

Pest and disease control was performed preventively and/or curatively, depending on the incidence of biotic stress. Spraying was preferentially carried out in the late afternoon to avoid interference with pollinator activity.

Fertigation was conducted according to the nutritional requirements of melon during protected cultivation, based on crop developmental stages. The recommendations followed were guided by the IAC Technical Bulletin Number 196 [12] for the region of Santa Cruz do Rio Pardo, São Paulo (SP), Brazil.

2.8. Variables Analyzed

2.8.1. Chlorophyll *a* Fluorescence

Chlorophyll *a* fluorescence measurements were performed using a fluorometer coupled to the LI-6400 system, following the saturation pulse method [13] and adopting the

nomenclature recommended by Baker [14]. Under actinic light, the following parameters were recorded, namely the maximum fluorescence (F_m'), steady-state fluorescence (F'), and minimum fluorescence in the light-adapted state (F_o'), allowing the calculation of the following variables [14]: the maximum quantum efficiency of PSII (F_v/F_m), representing the quantum yield of the photochemical phase of photosynthesis; the quantum efficiency of the antenna (F_v'/F_m'), representing the efficiency of excitation capture by open PSII reaction centers; the photochemical quenching coefficient (qP), reflecting carbon-related photosynthetic metabolism; the non-photochemical quenching coefficient (qNP), representing all other forms of energy dissipation, mainly heat; and the apparent electron transport rate (ETR), as described by Schreiber et al. (1986) [15].

2.8.2. Gas Exchange

Gas exchange measurements were carried out using an open-system infrared gas analyzer (IRGA, model LI-6400, LI-COR), equipped with CO_2 and water vapor sensors.

The measurements were based on the difference in the CO_2 and water vapor concentrations between the reference air (chamber without leaf) and sample air (chamber with leaf), enabling the calculation of the CO_2 assimilated and water vapor released through the stomata.

Six weekly measurements were taken, starting at 15 days after transplanting, selecting one plant per treatment. The PSII quantum efficiency was measured at 4:00 a.m., and the gas exchange measurements were performed between 8:30 and 11:30 a.m.

The following gas exchange variables were evaluated: CO_2 assimilation rate (A), transpiration rate (E), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i). These parameters were calculated using the equipment's data analysis software, based on the general gas exchange equation. The water-use efficiency (WUE) was determined using the A/E ratio, and the carboxylation efficiency (CE) was determined using the A/C_i ratio.

2.8.3. Antioxidant Defense

Two samplings of the leaves were performed for enzymatic and lipid peroxidation analyses, at 28 and 42 days after transplanting. The leaves were placed in plastic bags, wrapped in aluminum foil, immediately frozen in liquid nitrogen to halt biochemical activity, and stored in an ultra-freezer at $-70^\circ C$.

The enzymatic extraction followed the protocol described by Kar and Mishra [16]. The samples were ground in a cryogenic mill (Spex 6700-230 freezer/mill, Spex Industries, Edison, NJ, USA), at 1100 rpm for 5 min, until ultrafine particles were obtained, and kept frozen at $-196^\circ C$ in liquid nitrogen. The powdered tissue was stored in 15 mL Falcon tubes. For each sample, 300 mg of frozen fresh leaf tissue was homogenized in 4 mL of ice-cold 0.1 M potassium phosphate buffer (pH 6.8). The homogenate was centrifuged at $10,000 \times g$ for 10 min at $4^\circ C$, and the supernatant was aliquoted into microtubes and stored at $-20^\circ C$.

The soluble protein content was quantified according to Bradford [17]. The reaction mixture contained 100 μL of enzymatic extract and 5000 μL of Bradford reagent. After 15 min at room temperature, the absorbance was read at 595 nm. A 1% casein solution was used for the standard curve, and the protein content was expressed in mg of protein per gram of fresh matter.

The superoxide dismutase (SOD) activity was determined, based on the enzyme's ability to inhibit nitroblue tetrazolium (NBT) photoreduction [18]. The reaction included 50 μL of extract, 13 mM of methionine, 75 μL of NBT, 100 nM of EDTA, 2 μM of riboflavin, and 3.0 mL of 50 mM potassium phosphate buffer (pH 7.8). The reaction was initiated by exposing the tubes to fluorescent light (15 W) at $25^\circ C$ for 5 min. Absorbance was measured

at 560 nm. One SOD unit was defined as the enzyme amount required to inhibit NBT photoreduction by 50%. The specific activity was calculated considering the percentage inhibition, sample volume, and soluble protein concentration ($\mu\text{g } \mu\text{L}^{-1}$).

The catalase (CAT) activity was measured by monitoring the hydrogen peroxide decomposition at 240 nm [19]. A 50 μL aliquot of crude extract was added to 950 μL of 50 mM potassium phosphate buffer (pH 7.0), containing 12.5 mM of H_2O_2 . The change in absorbance was recorded over 80 s, and the enzymatic activity was calculated using the molar extinction coefficient of $39.4 \text{ mM}^{-1} \text{ cm}^{-1}$. The specific activity ($\mu\text{Kat } \mu\text{g}^{-1} \text{ protein}$) was calculated based on the protein concentration.

The peroxidase (POD) activity was determined according to the method outlined by Teisseire and Guy [20]. The reaction mixture (1.0 mL total) contained 30 μL of diluted extract (1:10 in extraction buffer), 50 mM of potassium phosphate buffer (pH 6.5), 20 mM of pyrogallol (1,2,3-benzenetriol), and 5 mM of H_2O_2 . The reaction proceeded for 5 min at room temperature. Purpurogallin formation was monitored at 430 nm, and the activity was calculated using its molar extinction coefficient ($2.5 \text{ mM}^{-1} \text{ cm}^{-1}$), expressed in $\mu\text{mol purpurogallin min}^{-1} \text{ mg}^{-1}$ of protein.

Lipid peroxidation (MDA) was assessed using three hundred milligrams of frozen leaf tissue in liquid nitrogen, using a cryogenic mill, and homogenized in 5 mL of 0.25% thiobarbituric acid (TBA), prepared in 10% trichloroacetic acid (TCA). The homogenate was incubated in a water bath at 90 °C for 60 min, cooled to room temperature, and centrifuged at $10,000 \times g$ for 15 min at 25 °C. The absorbance of the supernatant was measured at 560 and 600 nm, and lipid peroxidation was quantified using the molar extinction coefficient of MDA ($155 \text{ mM}^{-1} \text{ cm}^{-1}$), with the results expressed as nmol MDA g^{-1} of fresh weight [21].

2.8.4. Fruit Production and Post-Harvest Attributes

The fruits were identified and harvested for analysis. In the laboratory, the fresh fruit weight was measured using a digital scale (in grams); the mesocarp thickness was measured on both sides of the fruit using a digital caliper (in mm); and the epicarp thickness was also measured with a digital caliper (in mm).

For the physicochemical analyses, 10 g of fruit pulp was ground and homogenized. The following parameters were determined: pH, through a direct reading from the homogenized pulp solution, using a pH meter (Digital DMPH-2) [22]; soluble solids content (SS), using a digital refractometer (Palette PR-32, ATAGO brand, Itabashi, Tokyo District, Japan), with automatic temperature compensation [23], with the results expressed in °Brix; and titratable acidity (TA), expressed as grams of citric acid per 100 g of pulp, determined by titrating 5 g of homogenized pulp diluted to 100 mL with distilled water, using a standardized 0.1 N sodium hydroxide solution and phenolphthalein as an indicator [24].

The SS/TA ratio (*Ratio*) was calculated as the ratio between the soluble solids and titratable acidity [25].

Three fruits per plot were selected for the evaluation of the weight loss, soluble solids, and pH. The fruits were stored on a bench at room temperature.

Pulp firmness was measured using a penetrometer (INSTRUTHERM, model PTR 00, São Paulo, Brazil), equipped with an 8 mm diameter conical plunger. The firmness values obtained in pounds were converted to Newtons (N) by multiplication with the conversion factor 4.45 [26].

2.9. Statistical Analysis

The results were subjected to analysis of variance (ANOVA), and the treatment means were compared using Tukey's test at 5% probability, with the aid of SISVAR® software [27].

The homogeneity of variance among the treatments was verified using Levene's test, conducted using SAS 9.2 statistical software.

3. Results

3.1. Physiological and Biochemical Variables in the Two Evaluation Periods

At 28 days after transplanting (DAT), the quantum efficiency of Photosystem II showed no significant differences among the treatments, regardless of the salinity level or the application of bioregulator, with values ranging from 0.84 to 0.97 electrons quantum⁻¹. Similarly, no significant differences were observed for the antenna quantum efficiency, photochemical quenching coefficient, non-photochemical quenching coefficient, or apparent electron transport rate, which remained unchanged across the treatments (Figure 1).

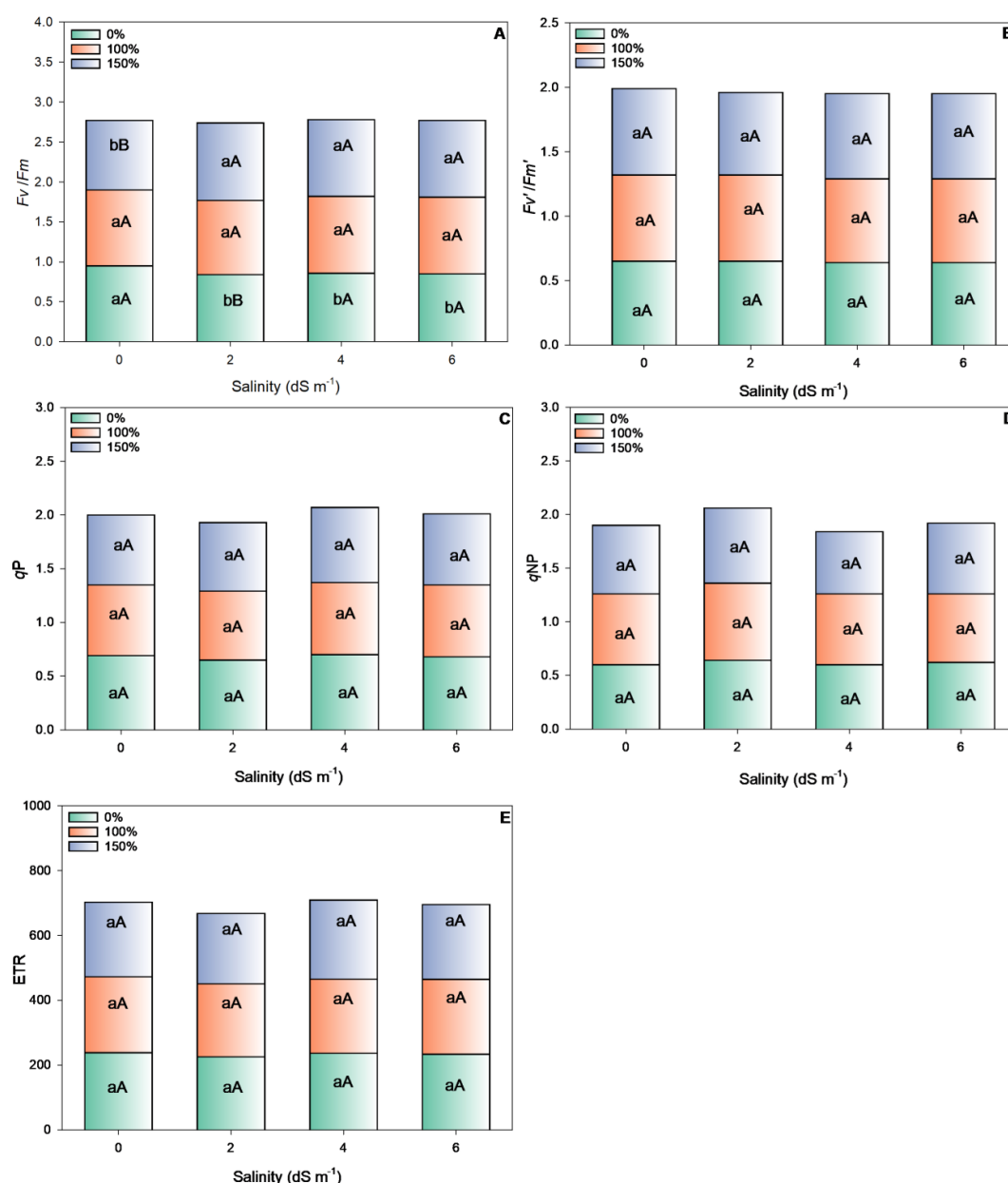


Figure 1. Potential quantum efficiency of PSII— F_v/F_m (A); antenna quantum efficiency— F_v'/F_m' (B); photochemical quenching coefficient— q_P (C); non-photochemical quenching coefficient— q_{NP} (D); apparent electron transport rate—ETR (E) in yellow melon plants under different salinity levels and plant growth regulator doses at 28 days after transplanting. Means followed by the same letter, lowercase in columns and uppercase in rows, do not differ significantly according to Tukey's test ($p \leq 0.05$).

Plants treated with the 100% dose of the bioregulator and irrigated with water at 2 dS m^{-1} had the highest CO_2 assimilation rate ($44.83 \mu\text{mol m}^{-2} \text{ s}^{-1}$); however, this was not significantly different from the plants irrigated with 2 dS m^{-1} without the bioregulator, but was 31.5% superior to the plants under the highest salinity level at 28 DAT (Figure 2A).

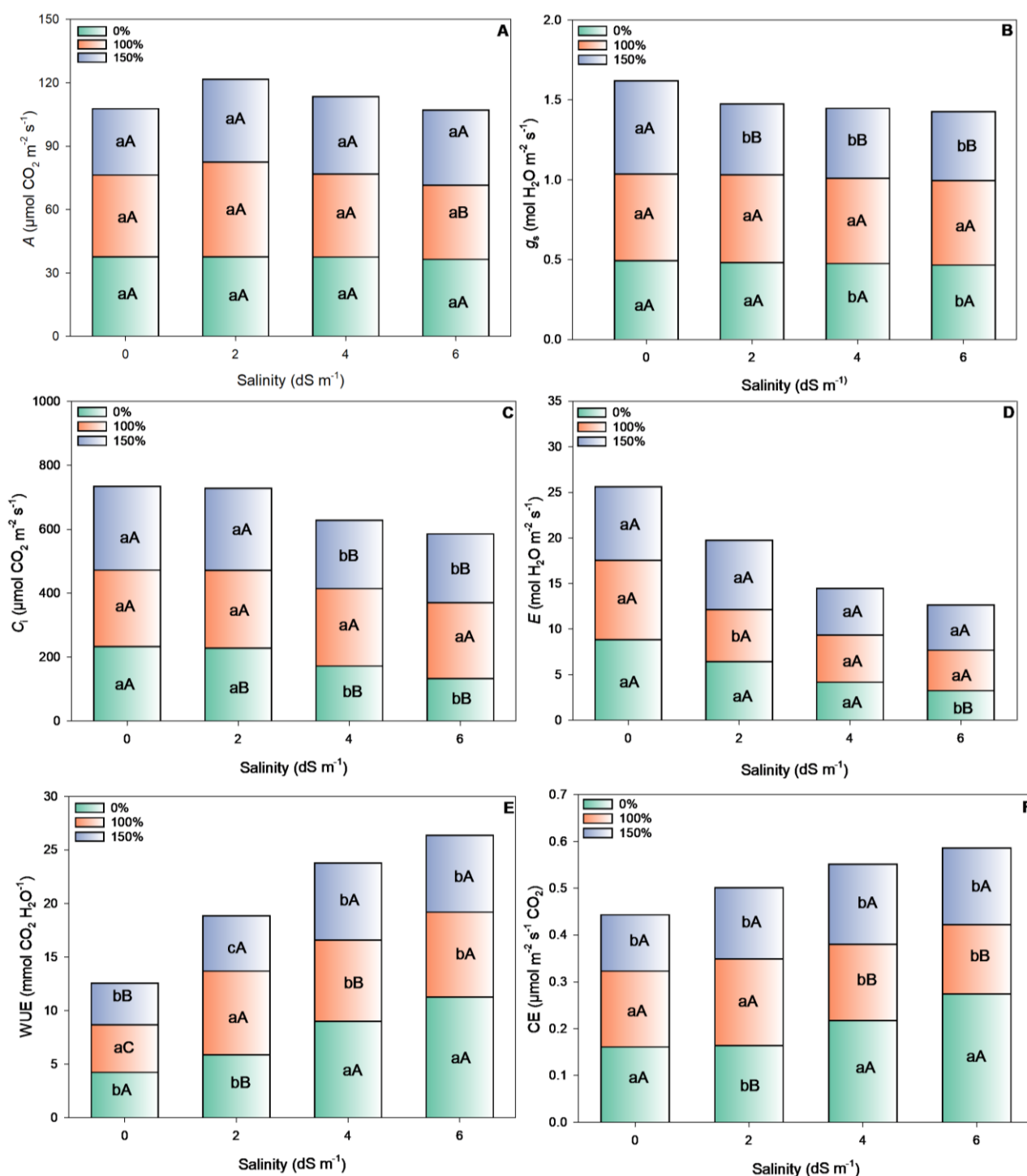


Figure 2. Gas exchange parameters: CO_2 assimilation rate— A (A); stomatal conductance— g_s (B); intercellular CO_2 concentration— C_i (C); transpiration rate— E (D); water-use efficiency—WUE (E); and carboxylation efficiency—CE (F) in yellow melon plants under different salinity levels and foliar application of plant growth bioregulator at 28 days after transplanting. Means followed by the same letter, lowercase in columns and uppercase in rows, do not differ significantly according to Tukey's test ($p \leq 0.05$).

At the same time point, the 100% dose of bioregulator promoted increased stomatal conductance across all the salinity levels, with significantly higher values compared to the 150% dose at 2, 4, and 6 dS m⁻¹ (Figure 2B). A lower internal CO₂ concentration (C_i) was observed in the plants treated with the 150% doses of the bioregulator under 4 and 6 dS m⁻¹, compared to those treated with the 100% doses under the same salinity conditions (Figure 2C). Transpiration was the lowest in the plants treated with the 100% doses of the bioregulator and irrigated with saline water at 2 dS m⁻¹ (Figure 2D).

The water-use efficiency (WUE) was higher in the melon plants treated with the recommended dose of growth bioregulator and irrigated with saline water at 2 and 6 dS m⁻¹ (Figure 2E). Similarly, the carboxylation efficiency (CE) was superior in the plants treated with the 100% dose of the bioregulator and irrigated at 2 dS m⁻¹ (Figure 2F).

At 28 DAT, the oxidative stress analysis revealed a progressive increase in lipid peroxidation with increasing salinity, with the highest levels observed at 4 and 6 dS m⁻¹ and the lowest levels observed in the plants treated with the 100% dose of the bioregulator and irrigated with 2 dS m⁻¹ (Figure 3A). The antioxidant enzyme activities (SOD, CAT, and POD) were also elevated in the plants subject to these treatments (Figure 3B–D).

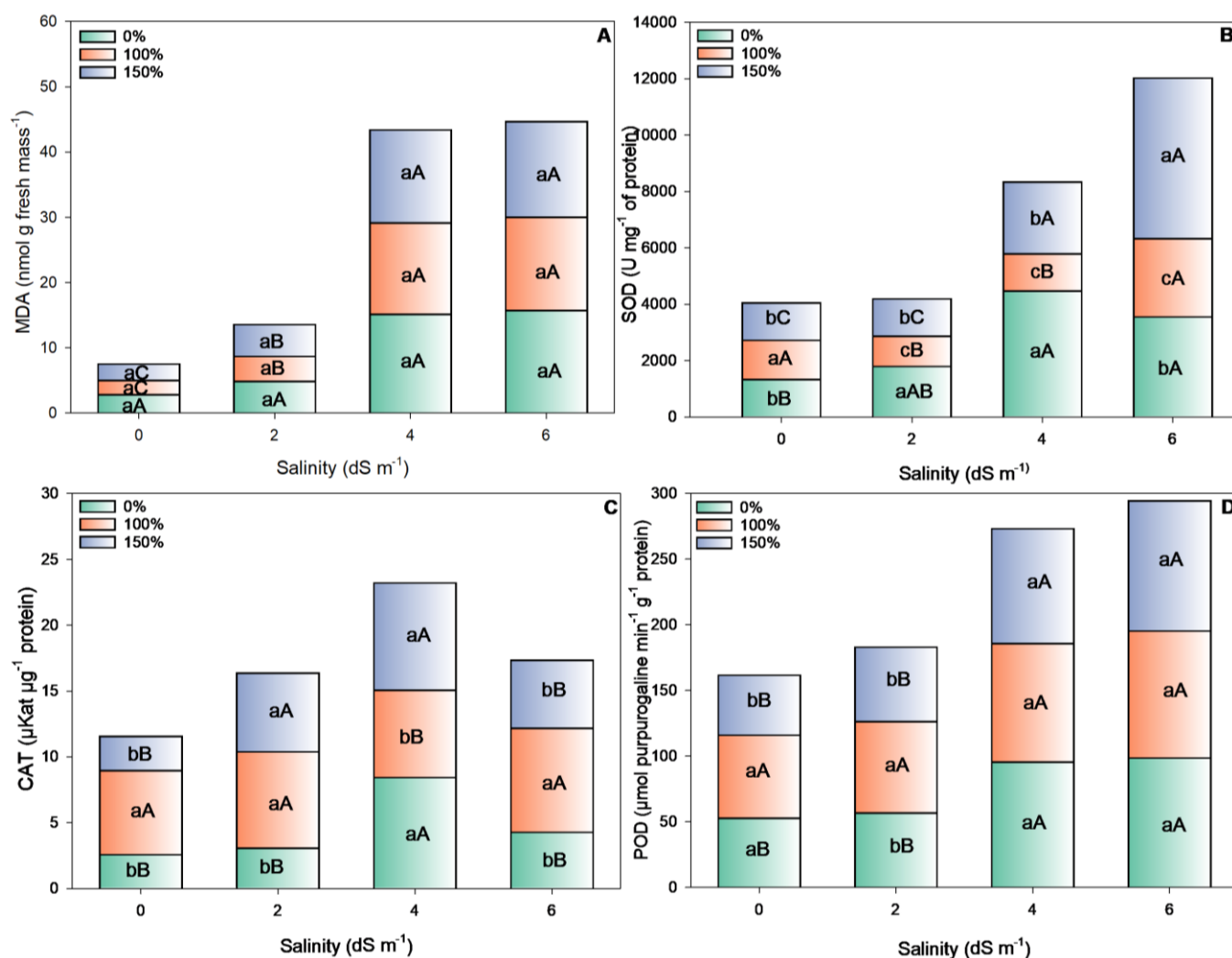


Figure 3. Oxidative stress: lipid peroxidation—MDA (A); activity of superoxide dismutase—SOD (B), catalase—CAT (C), and peroxidase—POD (D) in yellow melon plants under different salinity levels and foliar application of bioregulator at 28 days after transplanting. Means followed by the same letter, lowercase in columns and uppercase in rows, do not differ significantly according to Tukey's test ($p \leq 0.05$).

At 42 DAT, most chlorophyll *a* fluorescence parameters were not significantly affected by the treatments (Figure 4).

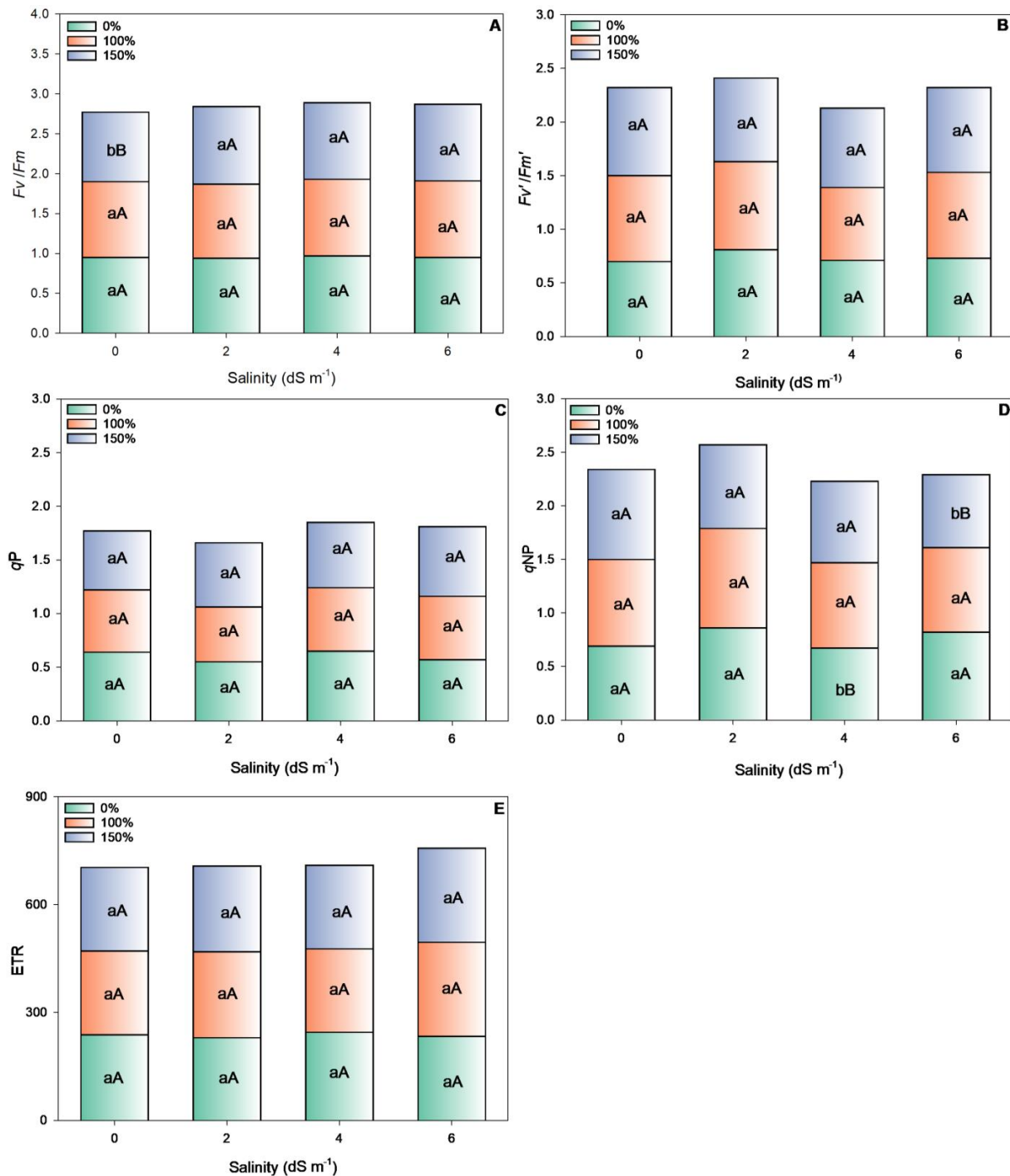


Figure 4. Potential quantum efficiency of PSII— F_v/F_m (A); antenna quantum efficiency— F_v'/F_m' (B); photochemical quenching coefficient— qP (C); non-photochemical quenching coefficient— qNP (D); apparent electron transport rate—ETR (E) in yellow melon plants under different salinity levels and plant growth bioregulator doses at 42 days after transplanting. Means followed by the same letter, lowercase in columns and uppercase in rows, do not differ significantly according to Tukey's test ($p \leq 0.05$).

The CO₂ assimilation rate was not significantly affected by the treatments at 42 DAT (Figure 5A). However, the recommended dose of the bioregulator increased the stomatal conductance at 2 and 6 dS m⁻¹ (Figure 5B). A lower internal CO₂ concentra-

tion was observed in the plants treated with the 150% dose of the bioregulator under 2 and 6 dS m⁻¹ (Figure 5C). The transpiration rate was not significantly affected by the treatments (Figure 5D).

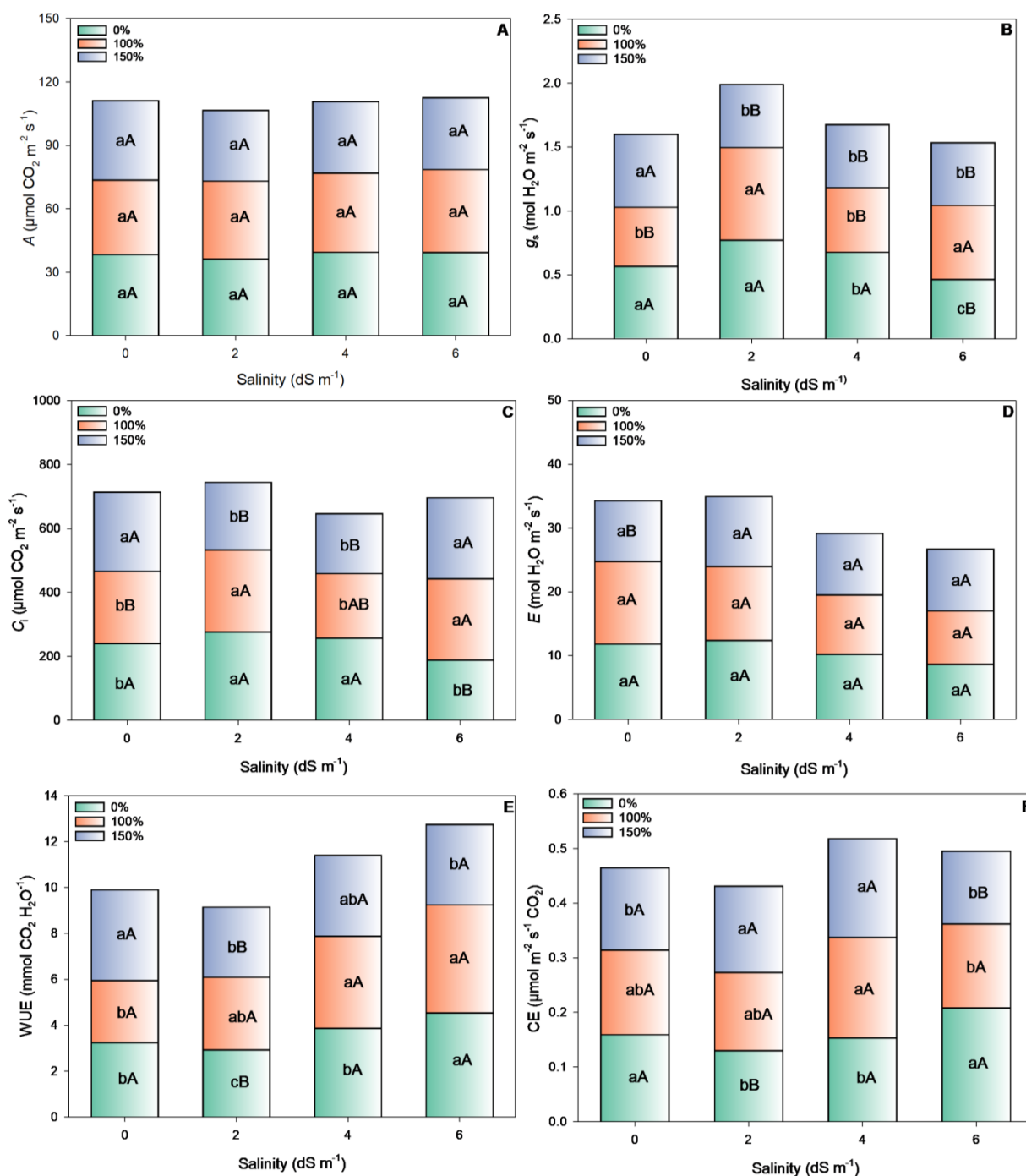


Figure 5. Gas exchange parameters: CO₂ assimilation rate— A (A); stomatal conductance— g_s (B); intercellular CO₂ concentration— C_i (C); transpiration rate— E (D); water-use efficiency— WUE (E); and carboxylation efficiency— CE (F) in yellow melon plants under different salinity levels and foliar application of plant growth bioregulator at 42 days after transplanting. Means followed by the same letter, lowercase in columns and uppercase in rows, do not differ significantly according to Tukey's test ($p \leq 0.05$).

At 42 DAT, the water-use efficiency was higher in plants treated with the 100% and 150% dose of the bioregulator under higher salinity levels (Figure 5E). The carboxylation efficiency was also greater with these doses, but only under 4 dS m⁻¹ irrigation (Figure 5F).

Lipid peroxidation was higher in the plants exposed to the highest salinity levels at 42 DAT (Figure 6A). The SOD activity was lower in the plants treated with the 100% and 150% dose of the growth bioregulator under 4 dS m⁻¹ salinity (Figure 6B). The CAT activity decreased with increasing salinity, particularly under the 150% bioregulator dose (Figure 6C). The POD activity was lower in the plants treated with the 100% bioregulator dose under 0, 2, and 4 dS m⁻¹ salinity (Figure 6D).

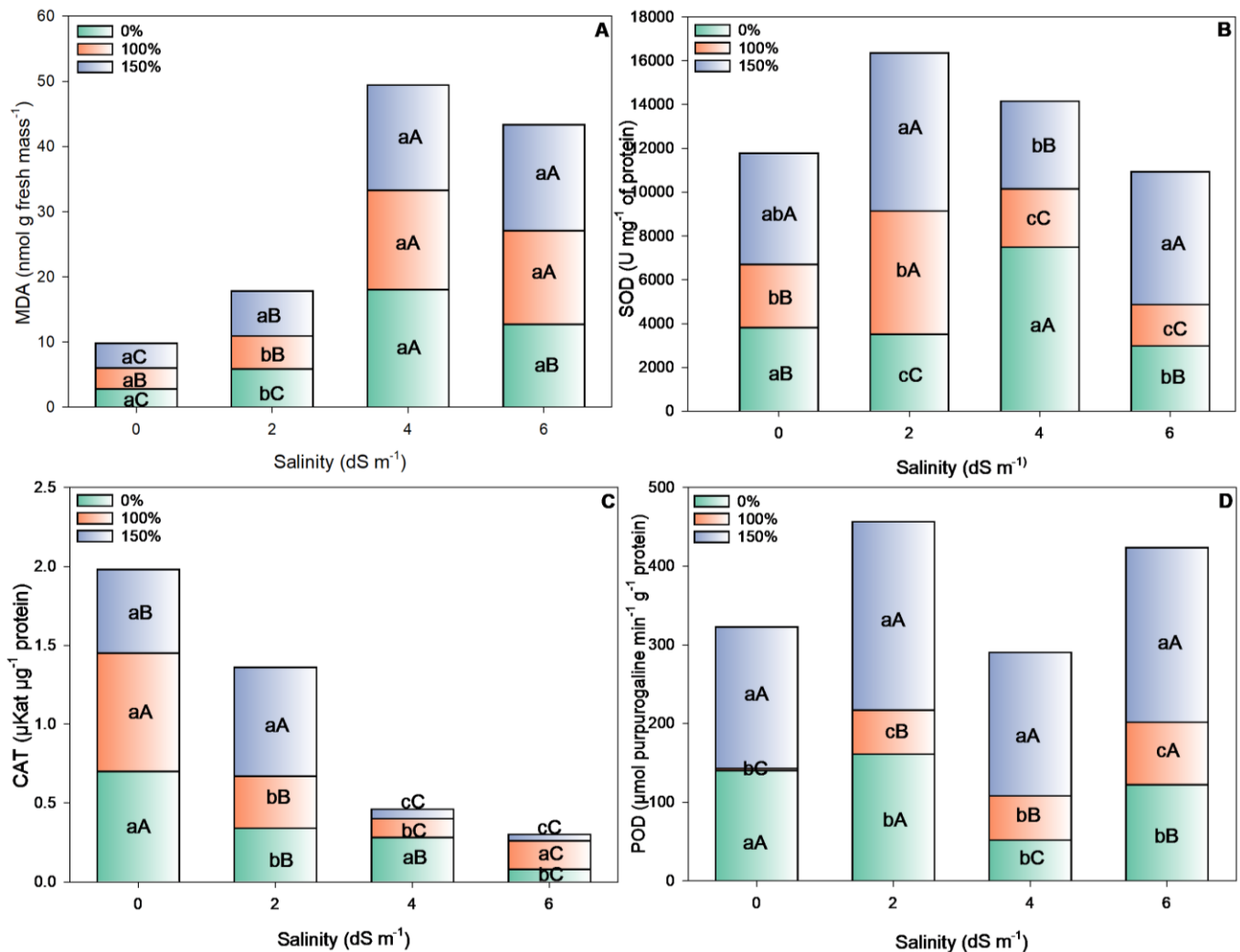


Figure 6. Oxidative stress: lipid peroxidation—MDA (A); activity of superoxide dismutase—SOD (B), catalase—CAT (C), and peroxidase—POD (D) in yellow melon plants under different salinity levels and foliar application of bioregulator at 42 days after transplanting. Means followed by the same letter, lowercase in columns and uppercase in rows, do not differ significantly according to Tukey's test ($p \leq 0.05$).

3.2. Morphological and Productive Variables

The fresh fruit mass was maximized in plants under 2 dS m⁻¹ salinity with 100% bioregulator application, with no significant difference compared to the 150% dose under the same condition, but 66% higher than the treatment without Stimulate application®. In the absence of salinity, the 100% dose also resulted in a higher fruit mass than the control and the 150% dose (Figure 7A).

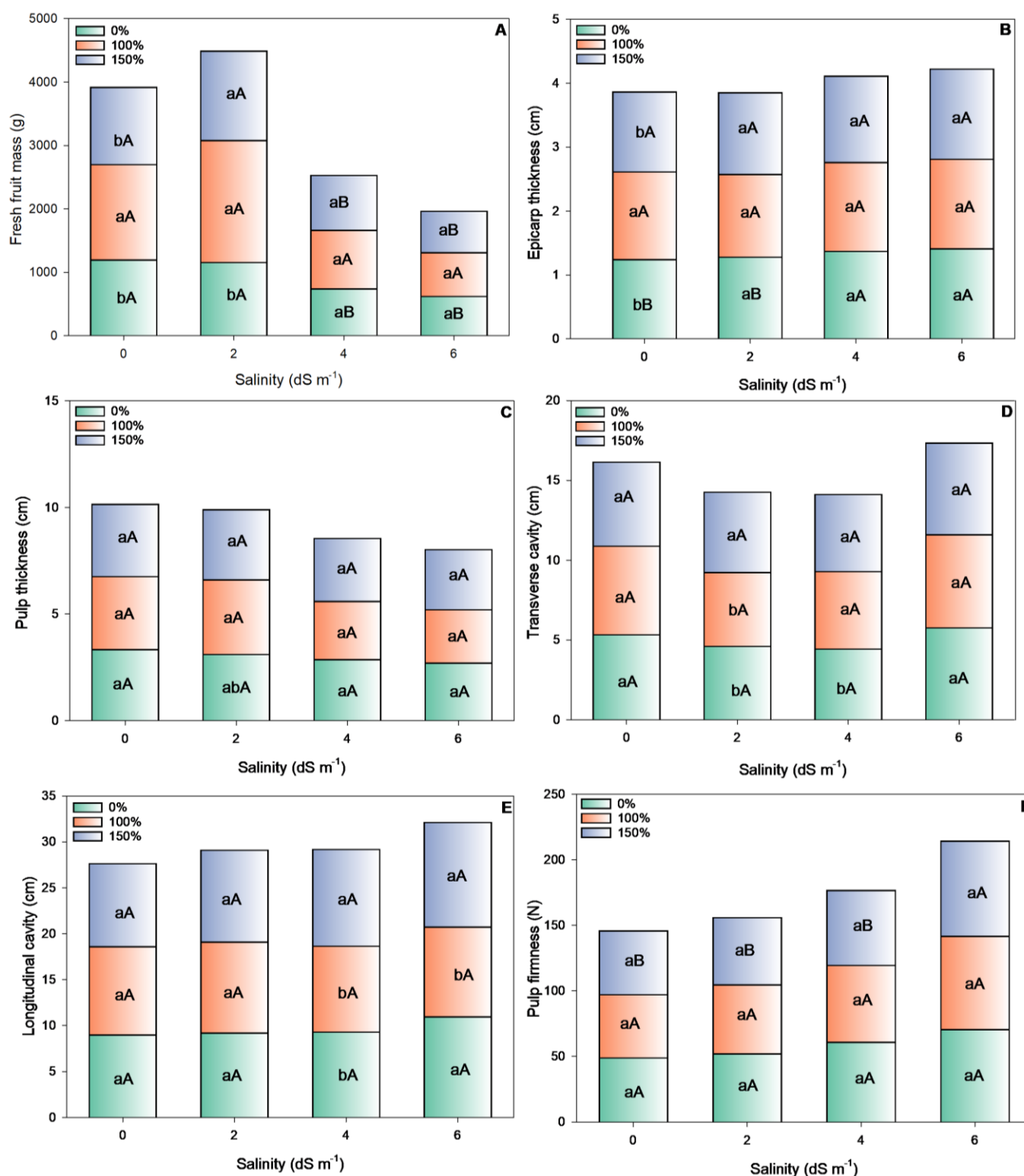


Figure 7. Fresh fruit mass (A), epicarp thickness (B), pulp thickness (C), transversal locule diameter (D), longitudinal locule diameter (E), and fruit firmness (F) of yellow melon under different salinity levels and foliar application of bioregulator. Means followed by the same letter, lowercase in columns and uppercase in rows, do not differ significantly according to Tukey's test ($p \leq 0.05$).

The epicarp thickness was greater in fruits from plants grown under 6 dS m⁻¹ salinity (Figure 7B). The pulp thickness was highest at up to 2 dS m⁻¹, but declined significantly at higher salinity levels. No significant differences were observed between the bioregulator doses (Figure 7C). The transverse fruit cavity was greater in fruits from plants treated with

the 100% or 150% dose of the bioregulator and irrigated with 2 or 4 dS m⁻¹ saline water (Figure 7D), while the longitudinal cavity was greater under the 150% dose bioregulator application at the highest salinity levels (Figure 7E). The pulp firmness tended to increase with higher salinity, although no statistical differences were observed between the bioregulator doses (Figure 7F).

The soluble solids content remained above 10 °Brix in regard to all the treatments, except under 6.0 dS m⁻¹ salinity. However, no significant differences were found among the treatments (Figure 8A). Fruits cultivated under 2.0 and 4.0 dS m⁻¹ salinity with 100% foliar application of the bioregulator showed higher titratable acidity (Figure 8B). Conversely, the lowest *Ratio* values (SS/TA) were observed with the 100% and 150% bioregulator doses under 6.0 dS m⁻¹ irrigation (Figure 8C). The fruit pH was not affected by the salinity levels, but increased significantly with the application of the 100% and 150% bioregulator dose (Figure 8D).

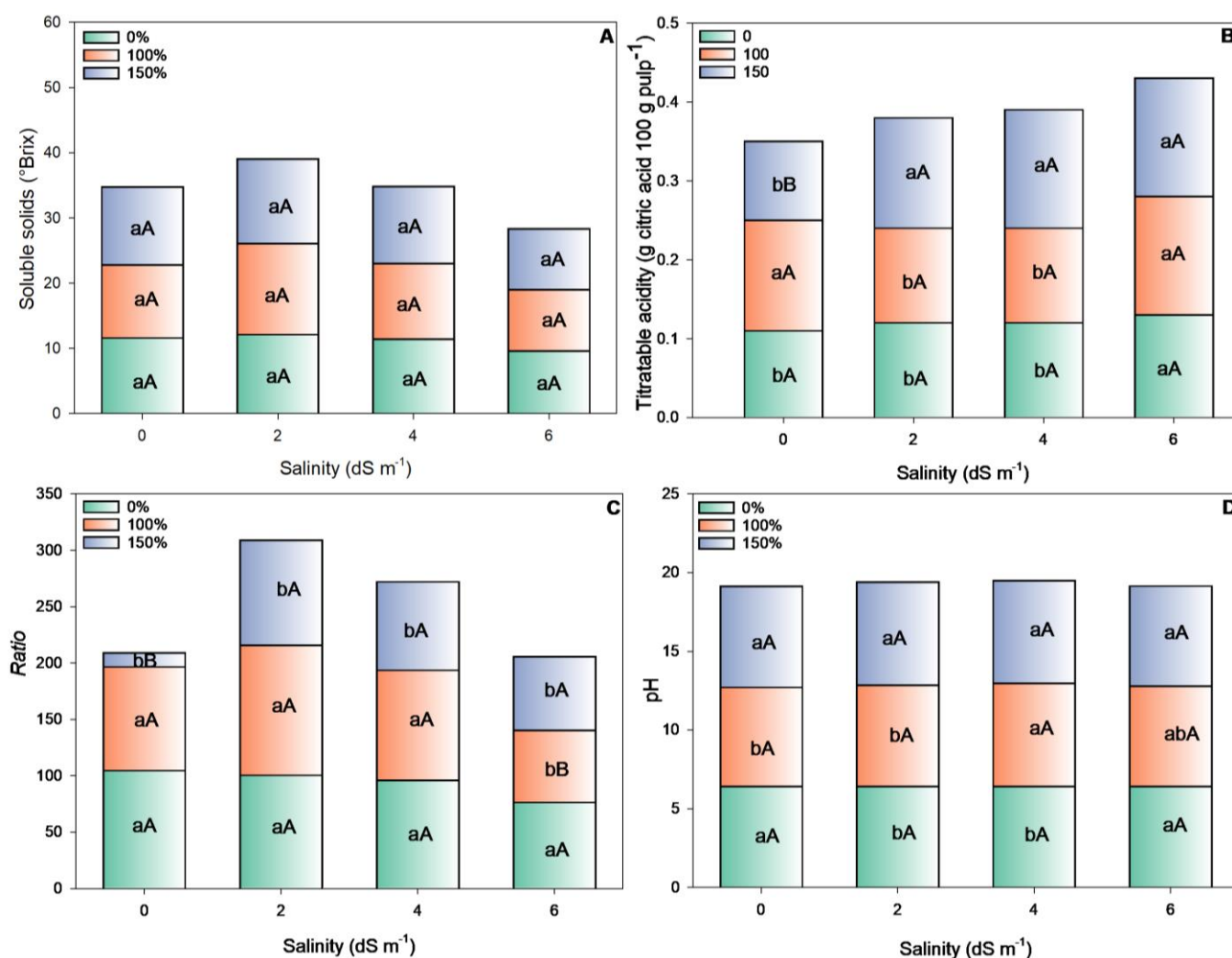


Figure 8. Soluble solids content (A), titratable acidity (B), soluble solids to acidity ratio—*Ratio* (C), and pH (D) of yellow melon fruits under different salinity levels and foliar application of bioregulator. Means followed by the same letter, lowercase in columns and uppercase in rows, do not differ significantly according to Tukey's test ($p \leq 0.05$).

4. Discussion

Salinity is widely recognized as one of the main abiotic factors limiting agricultural productivity, acting through osmotic and ionic effects that negatively impact nutrient uptake, cellular water potential, and photosynthetic metabolism [1,2]. The results obtained

in this study confirm these deleterious impacts on melon plants, but also demonstrate that the exogenous application of plant growth regulators is an effective strategy to mitigate the effects of salt stress, especially under moderate salinity conditions.

The commercial formulation composed of auxin (indole-3-butyric acid), cytokinin (kinetin), and gibberellic acid (GA₃) played a relevant role in modulating the physiological responses of melon to increased electrical conductivity of irrigation water. The results indicate that the foliar application at 100% of the recommended dose of the bioregulator improved the photosynthetic performance of melon plants, particularly under moderate salinity, as evidenced by the maintenance of the CO₂ assimilation rate, stomatal conductance, and carboxylation efficiency, as well as by the enhanced water-use efficiency.

The beneficial effect of bioregulators can be attributed to the synergistic action of their hormonal components. Auxin, due to its ability to activate H⁺-ATPase pumps and promote cell elongation via the cell wall loosening mechanism, contributes to root growth maintenance under stress, thereby enhancing water and nutrient uptake [4]. Cytokinin, in turn, acts positively on cell division and in regard to the preservation of photosynthetic apparatus integrity by delaying salt-induced leaf senescence through the repression of chlorophyll degradation genes and the enhancement of antioxidant enzyme activity [6]. Gibberellic acid (GA₃) is known for stimulating the biosynthesis of enzymes involved in reserve mobilization and for modulating the expression of genes related to growth and cell expansion, even under adverse conditions [2].

Furthermore, the maintenance of stomatal opening in bioregulator-treated plants under moderate salinity may be explained by the functional antagonism between cytokinins/gibberellins and abscisic acid (ABA), the main hormone involved in stomatal closure under salt stress [1,6]. The presence of cytokinin and GA₃ likely modulated endogenous ABA levels or sensitivity, promoting a favorable hormonal balance for maintaining photosynthesis and regulating transpiration [28–30].

From a productive standpoint, the application of the bioregulator contributed to the maintenance of the fruits' soluble solids content and pH, with more expressive responses observed at the 100% dose under intermediate salinity. This response may be associated with reduced membrane degradation and preserved sugar metabolism, resulting from the decreased oxidative damage and improved osmotic homeostasis conferred by the hormonal treatment [5].

On the other hand, applying 150% of the recommended dose did not lead to further gains and, for some variables, resulted in inferior performance compared to the 100% dose, suggesting a potential dose-dependent inhibitory effect. This response is consistent with the concept of a hormonal response window, in which supra-optimal doses may cause a hormonal imbalance and negative feedback to growth signaling [4]. In such scenarios, excess levels of applied hormones, such as auxin, cytokinin, and gibberellin, can interfere with the synthesis, signaling, or sensitivity of other endogenous hormones, such as abscisic acid (ABA) and ethylene, leading to antagonistic interactions [29]. These hormonal imbalances may downregulate growth-promoting pathways or overstimulate stress signaling networks, ultimately compromising plant performance. Thus, the negative response observed with the 150% dose may reflect a threshold beyond which exogenous hormone application becomes counterproductive due to antagonism or toxicity. A more detailed understanding of these hormonal interactions and their thresholds is crucial for optimizing the use of bioregulators under stress conditions, such as salinity.

It is important to emphasize that, although the growth regulator was effective in attenuating the effects of moderate salinity, its capacity to mitigate stress was limited under high salinity levels (6 dS m^{−1}), wherein pronounced oxidative stress, a decline in enzymatic activity, and a reduction in fruit quality occurred. This underscores the importance

of adjusting hormonal doses according to the stress intensity and, most importantly, selecting more tolerant genotypes for cultivation in environments with greater water and salt restrictions.

In view of the above, it is evident that the foliar application of plant growth regulators at the recommended dose is effective in improving the physiological performance and productivity of melon under moderate salinity, representing a promising strategy for semi-arid regions. However, the limited response under higher salinity levels highlights the need for further studies, including the evaluation of different cultivars and hormonal combinations, to more deeply elucidate the underlying mechanisms.

5. Conclusions

Foliar application of plant growth regulators at the recommended dose of 500 mL ha^{−1} (100%) proved to be an effective strategy to mitigate the effects of moderate salinity in melon plants, promoting significant improvements in physiological performance, the antioxidant balance, and fruit quality attributes. Additionally, it supported the maintenance of photosynthetic activity, reduced oxidative damage, and contributed to increased productivity and post-harvest quality.

Under higher salinity levels (4 and 6 dS m^{−1}), the benefits conferred by the bioregulator was limited, highlighting the need for complementary strategies, such as the use of more salt-tolerant genotypes or the combination of multiple regulators involving optimized formulations.

It is, therefore, recommended to adopt the application of Stimulate® under mild-to-moderate salinity conditions, especially in regard to protected melon cultivation systems. Future research should explore the responses of different cultivars to this technology, assess potential cumulative effects over successive crop cycles, and investigate the molecular mechanisms underlying tolerance induced by plant growth regulators.

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