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ARTICLE

Water Stress Mitigation in Melon: Effectiveness of Stress Attenuating Agents and Selection of Tolerant Cultivars

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ABSTRACT: Semiarid regions are frequently affected by low water availability, which hinders the development of horticultural species such as melon (*Cucumis melo* L.). In this context, techniques that enhance drought tolerance are essential for more effective crop management. This study aimed to evaluate the tolerance and antioxidant activity of different melon cultivars using seed pre-treatment with stress-attenuating agents. The experiment was conducted in two stages, both arranged in a completely randomized design with four replicates of 50 seeds. In the first stage, a 3 × 5 factorial scheme was used, combining three levels of water deficit (0.0, −0.15, and −0.3 MPa) and five melon cultivars ("Dali", "Premier", "Supreme", "Imperial 45", and "Asturia"). The second stage consisted of the two previously selected cultivars (one sensitive and one tolerant), subjected to combinations of water deficit and attenuating agents: T1 = 0.0 MPa (control), T2 = −0.15 MPa (water deficit), T3 = −0.15 MPa + hydropriming (12 h), T4 = −0.15 MPa + gibberellic acid, T5 = −0.15 MPa + ascorbic acid, T6 = −0.15 MPa + salicylic acid, and T7 = −0.15 MPa + hydrogen peroxide. In the first stage, morphological and biochemical variables were evaluated. In the second stage, the same variables were analyzed, along with citrulline content, hydrogen peroxide and malondialdehyde levels, and the activity of superoxide dismutase, catalase, and ascorbate peroxidase. Overall, salicylic acid mitigated the effects of water stress on germination, seedling length, and dry mass in the cultivar Dali. For the sensitive cultivar (Imperial 45), hydrogen peroxide reduced the production and accumulation of H_2O_2, mainly through the action of the enzymatic antioxidant system, resulting in improved germination performance under water deficit.

KEYWORDS: *Cucumis melo* L.; Cucurbitaceae; drought tolerance; pregerminative treatments; antioxidants

1 Introduction

The melon (*Cucumis melo* L.), a member of the Cucurbitaceae family, is a crop of substantial economic relevance and is widely cultivated worldwide, including in the Brazilian Northeast [1]. Its fruits are notable in human nutrition for their flavor and aroma, as well as for their nutritional and therapeutic properties [2,3]. Brazil ranks fifth among global melon producers [4], with most production concentrated in the Northeast. In this semiarid region, limited water availability and poor water quality are persistent challenges for growers, compromising key agronomic and physiological traits crucial for crop performance [5]. Low water availability is a major constraint for crop development, especially during germination [6]. Different cucurbit species combine morphological, physiological, and molecular responses that contribute to drought tolerance, such as enhanced root growth, citrulline accumulation, and the expression of drought-responsive genes [7].

Under water-deficit conditions, plants may experience oxidative stress, characterized by increased production of reactive oxygen species (ROS), including hydrogen peroxide (H_2O_2) and other highly reactive molecules generated as by-products of respiratory and photosynthetic metabolism [8]. Excessive ROS production is harmful to cellular structures such as the plasma membrane [9]. Accordingly, enzymatic and non-enzymatic responses, including the accumulation of osmolytes and antioxidant enzymes, play essential roles in plant adjustment to drought-induced stress.

Within the context of seed technology, the use of attenuating agents to mitigate harmful effects associated with water deficit during germination is a promising strategy [10]. Regulatory compounds such as salicylic, ascorbic, and gibberellic acids have gained prominence for activating plant defense mechanisms against the deleterious effects of water scarcity, thereby enhancing stress tolerance [11,12].

A widely accepted understanding of drought tolerance is that multiple traits are required to prevent biochemical or physiological damage during water scarcity [13]. Thus, efforts aimed at developing selection and breeding tools, along with biochemical and molecular investigations, are essential for identifying markers capable of indicating plants that maintain productivity even under unfavorable environmental conditions.

Accordingly, the hypothesis is that treating melon seeds with attenuating agents enhances plant tolerance to water deficit during the early stages of development by activating antioxidant mechanisms and inducing physiological and biochemical markers associated with stress resistance. Therefore, the objective of this study was to evaluate the effect of stress-attenuating agents on germination, early development, osmotic adjustment, and antioxidant activity in melon cultivars under water-deficit conditions.

2 Material and Methods

The study was conducted at the Seed Analysis Laboratory (LAS) of the Department of Agronomic and Forestry Sciences (DCAF) at the Federal Rural University of the Semiarid Region (UFERSA), Mossoró, RN, Brazil. In the first stage, two melon cultivars—one tolerant and one sensitive to water scarcity—were selected, as well as the level of water deficit to be used in the following phase. In this subsequent phase, the activity of the enzymatic antioxidant system was evaluated, along with the effects of pre-germination treatments with attenuating agents and the subsequent exposure of seeds to water deficit.

2.1 Stage I

A completely randomized experimental design was used in a factorial scheme, with four replications of 50 seeds. The first factor corresponded to water-deficit levels (H = 0.0; H1 = −0.15; H2 = −0.3 MPa), and

the second to five melon cultivars (C1 = Dali; C2 = Supreme; C3 = Imperial 45; C4 = Asturia; C5 = Premier). The seeds were donated by the companies Sakata Seed Sudameria and Isla Seeds.

Seeds were sown on two sheets of paper towel and covered with a third, moistened with PEG 6000 solutions or distilled water at osmotic potentials of 0, −0.15, and −0.3 MPa [14]. The volume of solution used corresponded to twice the dry weight of the paper [15]. The paper sheets were then rolled, placed in transparent plastic bags, and incubated in a germination chamber at 25°C with an 8 h photoperiod. The following variables were assessed:

1. Germination (G): number of normal seedlings counted on the eighth day after sowing, expressed as a percentage [15];
2. Germination speed index (GSI): daily counts of normal seedlings according to Maguire [16];
3. Root length (RL) and shoot length (SL): at the end of the germination test, 10 normal seedlings per replication were randomly measured using a centimeter-graduated ruler, with results expressed in cm seedling^{-1};
4. Root dry mass (RDM) and shoot dry mass (SDM): seedlings used for length measurements were separated into root and shoot, placed in Kraft paper bags, and dried in a forced-air oven at 65°C. Samples were weighed on a precision balance (0.0001 g), with results expressed in mg seedling^{-1}.

The remaining fresh material from each replication was frozen in liquid nitrogen (−196°C) and stored in an ultrafreezer (−80°C). Samples were then manually macerated to obtain crude extract, which was divided into triplicates containing approximately 0.2 g of macerated material. Each sample was placed in a hermetically sealed plastic tube containing 3 mL of 60% ethanol (v/v), heated in a water bath at 60°C for 20 min, and centrifuged at 10,000 rpm at 4°C for 10 min. The supernatant was collected for the determination of the following variables:

1. Total soluble sugars (TSS): determined by the anthrone method [17], using glucose as the standard, with results expressed in μmol GLU g^{-1} fresh mass;
2. Total free amino acids (TFAA): determined by the acid ninhydrin method [18], using a glycine standard curve, with results expressed in μmol GLY g^{-1} fresh mass;
3. Free proline (PRO): determined according to Bates; Waldren; Teare [19], with results expressed in μmol PRO g^{-1} fresh mass.

2.2 Stage II

This stage followed a 2 × 7 factorial arrangement with four replications of 50 seeds. The first factor consisted of two cultivars (one sensitive and one tolerant), and the second consisted of the combinations between water deficit and seed pre-treatments with attenuating agents: T1 = 0.0 MPa (control); T2 = −0.15 MPa (water deficit); T3 = −0.15 MPa + hydropriming (12 h); T4 = −0.15 MPa + gibberellic acid (50 mg L^{-1}); T5 = −0.15 MPa + salicylic acid (50 mg L^{-1}); T6 = −0.15 MPa + ascorbic acid (50 mg L^{-1}); T7 = −0.15 MPa + hydrogen peroxide (15 mM).

Preliminary tests defined the exposure period and concentration of each attenuator. The imbibition period (12 h) was established for both cultivars based on the imbibition curve (Fig. S1).

The analyses conducted in the second stage were the same as in the previous stage, with the addition of citrulline quantification, antioxidant enzyme activity, and oxidative stress markers. After biometric assessments of normal seedlings from each replication, they were used for extract preparation.

For citrulline (CIT) extraction, 0.5 g of fresh tissue was macerated in 1.5 mL of 96% ethanol (v/v). Extracts were heated to 100°C for complete ethanol evaporation, and the residues were rehydrated with

1.5 mL of distilled water, vortexed, and centrifuged for 10 min (5000 rpm) at 24°C. The supernatant was collected and stored at −20°C. Citrulline quantification followed the method of Knipp and Vašák [20], based on a standard curve prepared with L-citrulline and absorbance readings at 540 nm. Results were expressed as μmol CIT g^{-1} fresh mass.

For the determination of hydrogen peroxide (H_2O_2) and malondialdehyde (MDA), crude extracts were obtained from approximately 0.2 g of plant material frozen in liquid nitrogen (−196°C) and macerated with 2 mL of trichloroacetic acid (TCA, 0.1%, m/v) and 20% (m/m) polyvinylpolypyrrolidone (PVPP) for 1 min. After homogenization, samples were centrifuged at 10,000 rpm, 4°C, for 5 min.

The activity of superoxide dismutase, catalase, and ascorbate peroxidase was assessed in extracts obtained from plant material macerated in liquid nitrogen together with 20% (m/v) PVPP. Subsequently, approximately 0.5 g of the macerated material was solubilized in 3 mL of potassium phosphate buffer (100 mM, pH 7.5), supplemented with 1 mM EDTA (ethylenediaminetetraacetic acid) and 3 mM DTT (dithiothreitol). Aliquots were transferred to separate tubes and centrifuged at 10,000 rpm, 4°C, for 10 min. The supernatant was collected cold (kept on ice) and stored in cooled Eppendorf tubes (−35°C) until enzymatic assays.

The following determinations were performed:

1. Hydrogen peroxide (H_2O_2): obtained according to Alexieva et al. [21], using potassium iodide, with spectrophotometric readings at 390 nm. Results were expressed as μmol H_2O_2 g^{-1} fresh mass.
2. Lipid peroxidation: measured following Heath and Packer [22], based on the production of malondialdehyde (MDA), which reacts with thiobarbituric acid (TBA). Absorbance was recorded at 535 and 600 nm, and results expressed as μmol MDA g^{-1} fresh mass.
3. Superoxide dismutase activity (SOD): measured based on the adaptation of Giannopolitis and Ries [23]. After light exposure, samples were analyzed spectrophotometrically at 560 nm. Each sample was processed in triplicate, and results expressed as U SOD mg $protein^{-1}$, with one unit defined as the amount of enzyme required to inhibit formazan formation by 50% per gram of protein.
4. Catalase activity (CAT): determined following the protocol adapted by Azevedo et al. [24], based on Havir and McHale [25]. The reaction was monitored in a spectrophotometer at 240 nm and 25°C, with absorbance measured at time zero and after 60 s. Results were expressed as μmol min^{-1} mg $protein^{-1}$, considering that one unit of catalase consumes 1 μmol H_2O_2 per mg protein in 1 min at pH 7.5.
5. Ascorbate peroxidase activity (APX): determined using the method of Nakano and Asada [26], modified by Koshiba [27]. APX activity was measured by monitoring ascorbate oxidation in a spectrophotometer at 290 nm, at 30°C, for 60 s. Results were expressed as μmol min^{-1} mg $protein^{-1}$, with one unit defined as the conversion of 1 μM ascorbic acid to monodehydroascorbate per minute.

2.3 Statistical Analysis

Data obtained in Stage I were subjected to analysis of variance using the F test ($p < 0.05$). Main effects and interactions were evaluated by the Scott–Knott means clustering test ($p < 0.05$). Cluster analysis was performed using Ward's method and Euclidean distance as the measure of dissimilarity, with PAST 4 software. Data from Stage II were analyzed by ANOVA via the F test ($p < 0.05$), and means were grouped using the Scott–Knott test at 5% significance. Data analyses for Stages I and II were conducted using SISVAR [28].

3 Results

3.1 Stage I

Analysis of variance indicated a significant interaction between melon cultivars and levels of water deficit, evidencing distinct responses of the cultivars to increasing water restriction (Table 1).

Table 1: Germination (G), germination speed index (GSI), root length (RL), shoot length (SL), root dry mass (RDM), shoot dry mass (SDM), total soluble sugars (TSS), total free amino acids (TFAA), and free proline (PRO) of melon cultivars (*Cucumis melo* L.) as a function of water deficit.

Cultivars	G (%)			GSI			RL (cm seedling $^{-1}$)		
	0.0 MPa	−0.15 MPa	−0.30 MPa	0.0 MPa	−0.15 MPa	−0.30 MPa	0.0 MPa	−0.15 MPa	−0.30 MPa
"Dali"	92aA	77bB	18aC	11.41aA	6.89aB	1.33aC	6.72aB	14.05aA	10.35aB
"Supreme"	94aA	61cB	19aC	9.29bA	5.45bB	1.33aC	7.23aB	13.31aA	9.46aB
"Imperial 45"	85aA	22eB	1bC	7.95cA	1.67dB	0.04bC	6.96aB	12.02aA	8.13aB
"Asturia"	87aA	43dB	4bC	10.71aA	3.81cB	0.27bC	6.96aB	10.78aA	11.28aA
"Premier"	94aA	89aA	8bC	11.66aA	7.40aB	0.58bC	7.89aB	12.51aA	8.67aB
Cultivars	**SL (cm seedling $^{-1}$)**			**RDM (mg seedling $^{-1}$)**			**SDM (mg seedling $^{-1}$)**		
	0.0 MPa	−0.15 MPa	−0.30 MPa	0.0 MPa	−0.15 MPa	−0.30 MPa	0.0 MPa	−0.15 MPa	−0.30 MPa
"Dali"	5.04cA	2.24aB	1.41aC	2.42cC	4.10aA	3.15aB	4.97aA	5.21aA	4.74aA
"Supreme"	5.73bA	1.94aB	1.17aC	3.50bB	4.50aA	2.79aC	4.70aA	4.79aA	5.12aA
"Imperial 45"	4.07dA	2.48aB	0.33bC	4.45aA	3.95aA	1.50bB	2.29cB	2.74cB	3.36cA
"Asturia"	4.95cA	2.20aB	0.90aC	3.45bB	4.32aA	1.75bC	3.89bB	4.34bB	4.94aA
"Premier"	6.82aA	1.28bB	1.09aB	3.25bA	3.75aA	2.19bB	4.11bA	4.52bA	4.32bA
Cultivars	**TSS (mg g FM^{-1})**			**TFAA (μmol g FM^{-1})**			**PRO (μmol g FM^{-1})**		
	0.0 MPa	−0.15 MPa	−0.30 MPa	0.0 MPa	−0.15 MPa	−0.30 MPa	0.0 MPa	−0.15 MPa	−0.30 MPa
"Dali"	5.55aB	4.00cB	12.98aA	83.83aA	83.15eA	79.22bA	9.60aA	10.13bA	2.10aB
"Supreme"	5.84aC	44.59aA	16.33aB	80.99aB	220.07aA	84.84bB	9.33aB	12.30aA	2.58aC
"Imperial 45"	11.25aB	22.97aA	18.94aA	66.31bC	101.46dB	120.43aA	7.35bA	2.22dB	2.62aB
"Asturia"	5.54aB	16.82bA	9.61aB	69.54bB	183.99bA	66.41cB	7.78bA	6.97cA	2.67aB
"Premier"	4.22aB	26.39aA	11.73aB	60.65bC	152.84cA	71.60cB	6.66bA	3.25cB	1.83aB

Note: Means followed by uppercase letters indicate differences among water restriction levels within each cultivar, whereas lowercase letters indicate differences among cultivars within each water deficit level, according to the Scott–Knott test at 5% probability.

Water deficit significantly reduced germination and germination speed index in all melon cultivars, with increasing severity as osmotic potential decreased. At −0.15 MPa, Premier and Dali cultivars showed superior germination performance, whereas Imperial 45 exhibited high sensitivity even under moderate stress. At −0.3 MPa, a pronounced reduction in germination potential was observed across all cultivars.

Root growth was stimulated under moderate water deficit (−0.15 MPa), exceeding the control treatment, and remained higher even under more severe stress, although to a lesser extent, regardless of cultivar. In contrast, shoot length and shoot dry mass were negatively affected by increasing water restriction, with cultivar-dependent responses, highlighting Imperial 45 as the most sensitive and Dali, Supreme, and Asturia as more tolerant.

Biochemical analyses revealed a significant interaction between cultivars and water deficit levels, with increased accumulation of soluble sugars and amino acids under stress conditions. Proline accumulation was cultivar-specific, being more pronounced in the Supreme cultivar under moderate deficit, while other cultivars maintained levels similar to the control, indicating distinct physiological strategies for water stress adaptation.

Water deficit significantly affected the physiological and biochemical variables of the melon cultivars, revealing marked distinctions between the more tolerant genotype ("Dali") and the less tolerant one

("Imperial 45"). This pattern is evident in the dissimilarity dendrogram (Fig. 1). This analysis is important because it integrates, in a multivariate manner, the morphological, physiological, and biochemical responses of cultivars to water deficit, revealing patterns of similarity that are not evident in isolated analyses.

This approach allows the grouping of cultivars with similar behaviors, facilitating the identification of different levels of tolerance to water stress and common adaptive strategies, such as maintenance of germination, root growth, and solute accumulation.

The osmotic potential of −0.15 MPa was not selected with the aim of causing a severe reduction in the performance of the tolerant cultivar, but rather to represent a mild to moderate level of water stress, which is frequently used to investigate early physiological responses and adaptive mechanisms, rather than only visible deleterious effects. In tolerant genotypes, it is expected that this level of stress does not result in marked losses in morphological variables, which in itself already constitutes an indication of tolerance.

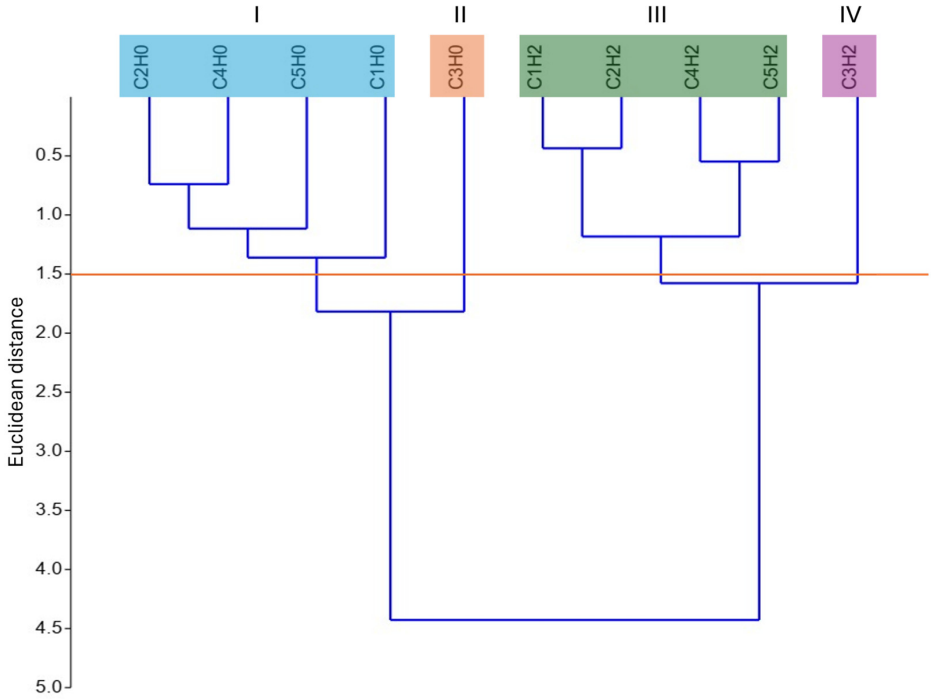

Figure 1: Dissimilarity dendrogram of groups formed by the combination of water deficit levels (H) and melon cultivars (C) (*Cucumis melo* L.). H0 = 0.0 MPa; H2 = −0.3 MPa; C1 = "Dali"; C2 = "Supreme"; C3 = "Imperial 45"; C4 = "Asturia"; C5 = "Premier".

Data were subjected to cluster analysis using Euclidean distance as the measure of dissimilarity. A cutoff value of 1.5 was adopted to define the formation of four groups resulting from the combinations of cultivars (C) and water restriction levels (H). The cut at 1.5 is statistically appropriate and biologically interpretable, allowing a robust classification of treatments according to their responses to water stress.

Groups I and II consisted of the five cultivars sown under no water deficit (0.0 MPa), considered the control groups (Fig. 1). Groups III and IV included the cultivars evaluated under water restriction (−03.0 MPa). Within this context, the cultivar Dali exhibited the most favorable responses under water deficit, positioning closer to the control Group I. Based on these results, Dali and Imperial 45 were selected for Stage II as the tolerant and sensitive cultivars, respectively.

3.2 Stage II

The analysis of variance revealed a significant interaction ($p < 0.01$) for all variables except catalase activity, for which isolated effects of cultivar and treatment were observed.

Reduced water availability markedly affected the germination of Imperial 45, causing an 86% reduction in normal seedlings (Fig. 2A). In contrast, seed pre-treatments with gibberellic acid (GA), ascorbic acid (ASC), and hydrogen peroxide (HP) mitigated the effects of water deficit, with no significant differences among these attenuators. Hydropriming was not beneficial for Dali seeds during germination.

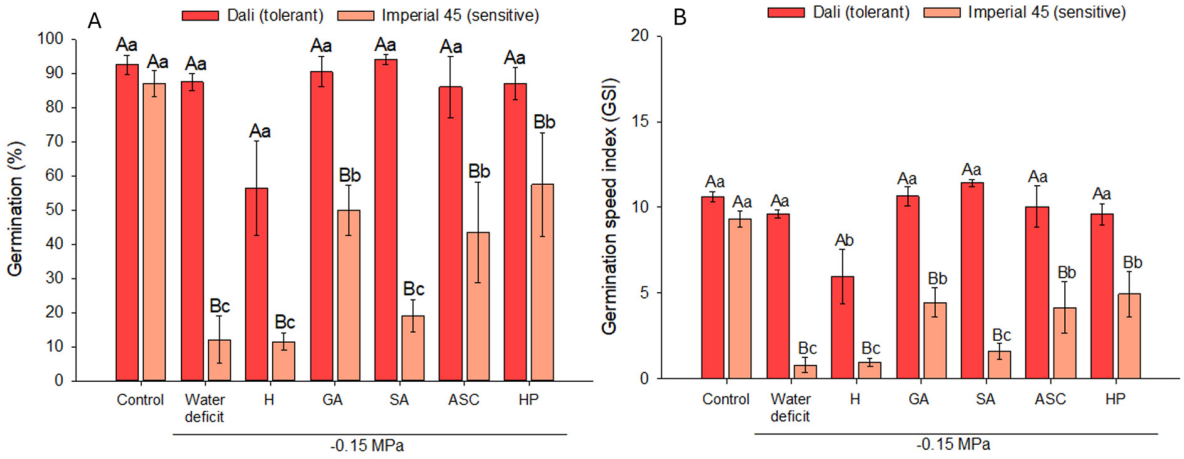

Figure 2: Germination (**A**) and germination speed index (**B**) of melon cultivars (*Cucumis melo* L.) in response to seed pre-treatments and water deficit. Control (0.0 MPa); water deficit without attenuator (−0.15 MPa); water deficit + hydropriming (H); water deficit + gibberellic acid (GA); water deficit + salicylic acid (SA); water deficit + ascorbic acid (ASC); water deficit + hydrogen peroxide (HP). Means followed by the same uppercase (cultivars) and lowercase (treatments) letter do not differ from each other by the Scott-Knott test at the 5% probability level.

For the germination speed index, results followed a pattern similar to germination, with a 91% reduction in Imperial 45 under stress relative to the control (Fig. 2B). In this cultivar, attenuator treatments increased germination speed compared with the water-deficit condition, with gibberellic acid (344%), ascorbic acid (314%), and hydrogen peroxide (393%) being the most effective, with no differences among them.

An increase in root length was observed under water deficit for both cultivars (Supplementary Fig. S2). Hydroprimed seeds of Imperial 45, considered sensitive to water stress, showed reductions in both root and shoot length, coinciding with reduced germination and germination speed index.

Shoot length was reduced by water deficit (Supplementary Fig. S2), with the tolerant cultivar Dali showing a 54% decrease compared with the control. However, the application of salicylic acid promoted a 30% increase relative to the treatment subjected only to water deficit. In contrast, in the cultivar Imperial 45, hydro-priming and the application of gibberellic and salicylic acids further impaired shoot development compared with the water-deficit treatment without seed preconditioning.

Root dry mass of the cultivar Imperial 45 was not significantly affected, except when subjected to hydropriming, which resulted in a 42% reduction in root growth (Fig. 3A). Conversely, seedlings of the cultivar Dali accumulated 58% more root biomass under water deficit compared with the control.

Shoot dry mass of Dali seedlings was reduced by 40% when exposed to the osmotic potential of −0.15 MPa relative to the control (Fig. 3B). However, pre-treatment with salicylic acid increased this variable by 29% compared with the water-deficit condition. In Imperial 45, water deficit did not reduce shoot

dry mass; however, seeds subjected to hydropriming and gibberellic acid pre-treatment showed reductions of 47.2% and 25%, respectively, compared with the water-deficit treatment.

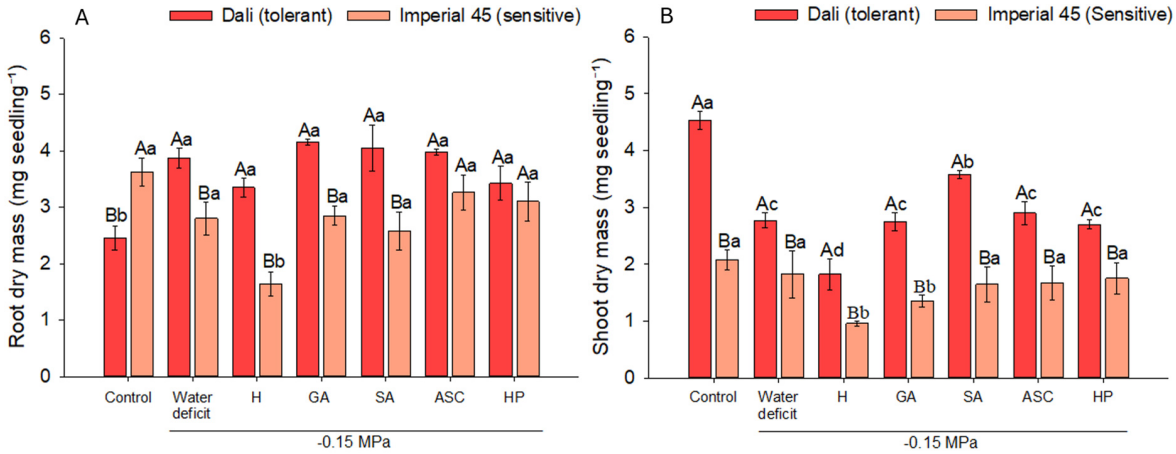

Figure 3: Root (**A**) and shoot (**B**) dry mass of melon cultivars (*Cucumis melo* L.) in response to pre-treatments and water deficit. Control (0.0 MPa); water deficit without attenuator (−0.15 MPa); water deficit + hydropriming (H); water deficit + gibberellic acid (GA); water deficit + salicylic acid (SA); water deficit + ascorbic acid (ASC); water deficit + hydrogen peroxide (HP). Means followed by the same uppercase (cultivars) and lowercase (treatments) letter do not differ from each other by the Scott-Knott test at the 5% probability level.

Soluble sugar content increased under water deficit in both Dali and Imperial 45, with increments of 166% and 64%, respectively, compared with the control (Fig. 4A). For Dali, hydropriming resulted in 21% higher soluble sugar accumulation compared with the water-deficit treatment. In Imperial 45, the use of gibberellic (22.9%), salicylic (22.5%), and ascorbic acids (12.6%), as well as hydropriming (13.4%), promoted greater sugar accumulation relative to seedlings exposed only to water deficit.

The amount of free amino acids (Fig. 4B) increased due to reduced water availability in both cultivars, particularly in the more tolerant one, which showed a 171% increment. Hydropriming stood out as an inducer of amino-acid accumulation in Dali, although its performance was 11% lower than that observed under water deficit.

Under optimal conditions, the cultivars exhibited similar levels of proline (Fig. 4C) and citrulline (Fig. 4D). However, under water deficit, proline production increased by 124% in both cultivars compared with the control group. In addition, the sensitive cultivar accumulated more citrulline (156%) when subjected to water deficit. Hydropriming also led to substantial increases in proline (157%) and citrulline (326%) in Dali relative to the water-deficit treatment. In Imperial 45, pre-treatment with gibberellic acid promoted a 65% increase in proline accumulation, whereas hydropriming increased citrulline content by 48% compared with the water-deficit treatment, although without differing from the other treatments (Fig. 4D).

Under water deficit, hydrogen peroxide levels increased, reaching values 272% higher than those observed in the control treatment of the cultivar Dali (Fig. 5A). However, all attenuators produced satisfactory results in the tolerant cultivar compared with the water-deficit treatment. In the sensitive cultivar, hydrogen peroxide was the most efficient attenuator, reducing the production of this oxidizing agent by 78%.

Water deficit resulted in malondialdehyde accumulation in both cultivars (Fig. 5B). Gibberellic acid in Dali and salicylic acid in Imperial 45 significantly reduced malondialdehyde production, with decreases of 40% and 45%, respectively, compared with the water-deficit condition (Fig. 5B).

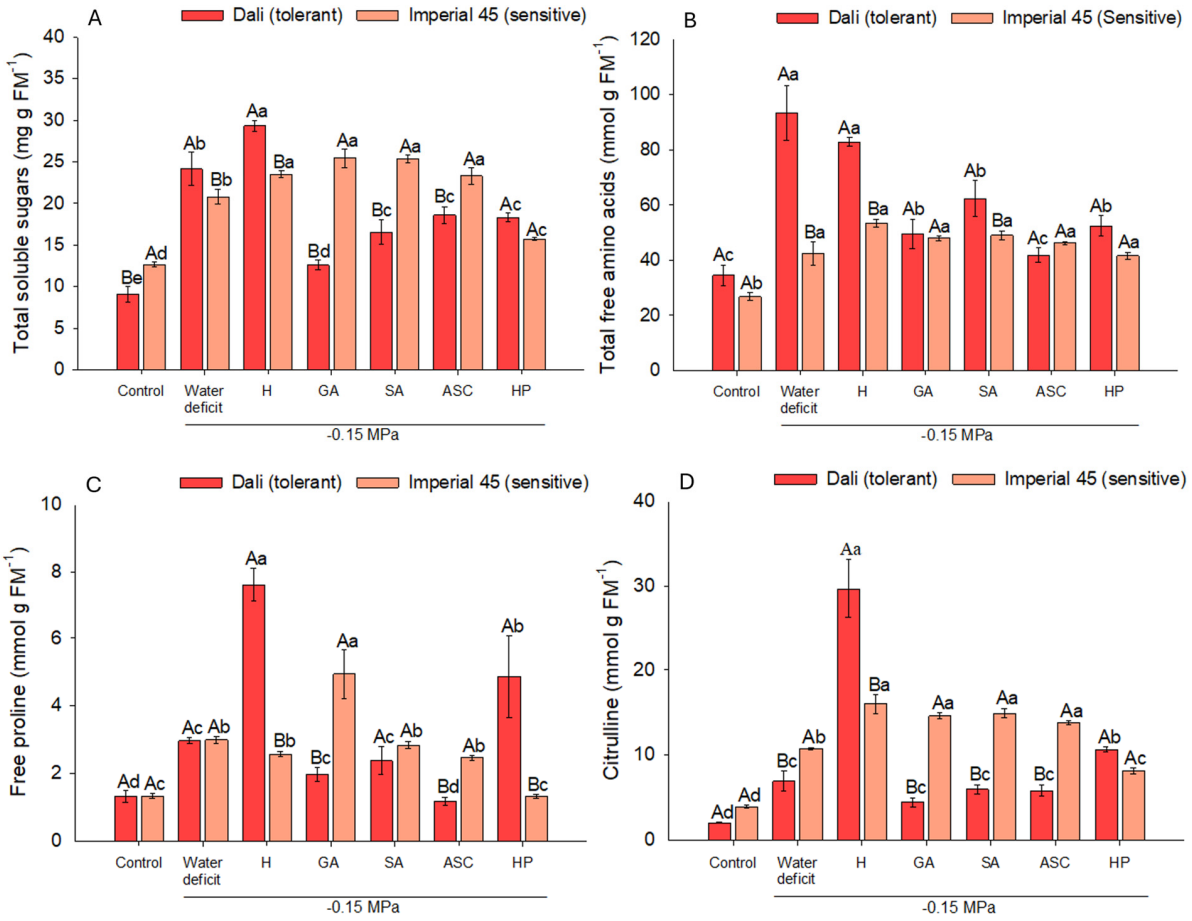

Figure 4: Total soluble sugars (**A**), total free amino acids (**B**), free proline (**C**), and citrulline (**D**) in melon cultivars (*Cucumis melo* L.) in response to pre-treatments and water deficit. Control (0.0 MPa); water deficit without attenuator (−0.15 MPa); water deficit + hydropriming (H); water deficit + gibberellic acid (GA); water deficit + salicylic acid (SA); water deficit + ascorbic acid (ASC); water deficit + hydrogen peroxide (HP). Means followed by the same uppercase (cultivars) and lowercase (treatments) letter do not differ from each other by the Scott-Knott test at the 5% probability level.

Overall, the activity of the main antioxidant enzymes was significantly affected by water restriction and by the action of the attenuator treatments in both cultivars. The exception was catalase, for which no interaction occurred between factors, only an isolated effect of cultivars (Fig. 5).

The treatments maintained superoxide dismutase activity at basal levels, with no differences among them for the cultivar Imperial 45 (Fig. 5C). In Dali, SOD activity was strongly influenced by hydrogen peroxide, showing a 231% increase compared with the water-deficit treatment.

Differences in catalase activity were observed between cultivars, with levels 19% lower in the sensitive cultivar (Fig. 5D). Conversely, a strong isolated effect was observed for hydropriming (55%) and gibberellic acid (54%) in both cultivars, compared with water deficit.

Ascorbate peroxidase activity increased by 348% under stress conditions after 12 h of hydropriming in Imperial 45 (Fig. 5E). A similar trend was observed in seeds pre-treated with gibberellic acid (264%) and hydrogen peroxide (227%). The cultivar Dali exhibited an average increase of 137% in ascorbate peroxidase activity with seeds pre-treated with gibberellic, ascorbic, and salicylic acids, as well as hydrogen peroxide, compared with untreated seeds.

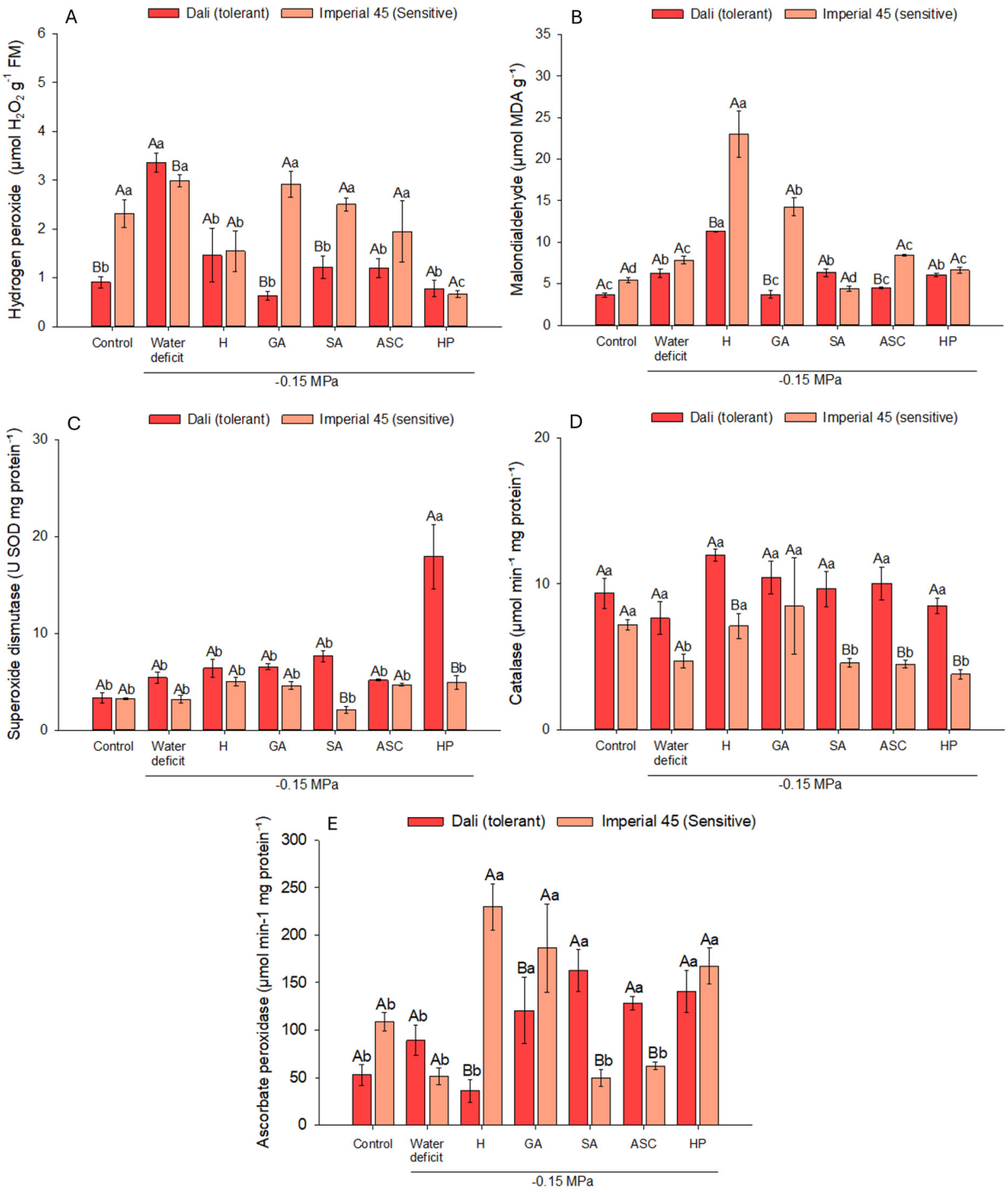

Figure 5: Hydrogen peroxide (**A**), malondialdehyde content (**B**), Activity of the enzymes superoxide dismutase—SOD (**C**), catalase—CAT (**D**) and ascorbate peroxidase—APX (**E**) in melon cultivars (*Cucumis melo* L.) in response to pre-treatments and water deficit. Control (0.0 MPa); water deficit without attenuator (−0.15 MPa); water deficit + hydropriming (H); water deficit + gibberellic acid (GA); water deficit + salicylic acid (SA); water deficit + ascorbic acid (ASC); water deficit + hydrogen peroxide (HP). Means followed by the same uppercase (cultivars) and lowercase (treatments) letter do not differ from each other by the Scott-Knott test at the 5% probability level.

4 Discussion

Water deficit reduced germination and the germination speed index due to delays in the resumption of metabolic activity during the germination process [29]. Moreover, the cultivars that performed better under moderate stress exhibited increases in soluble sugars and proline contents, indicating the activation of osmotic adjustment mechanisms that contribute to cellular homeostasis and protect structures under water-deficit conditions [30].

The use of pre-germination techniques enhances germination capacity and resilience to environmental stresses by promoting metabolic recovery, DNA repair, increased mitochondrial activity, and mobilization of nutrient reserves [31]. Thus, these techniques emerge as a viable alternative to mitigate the effects of abiotic stresses, particularly during the early stages of development.

The reduction in germination speed caused by limited water availability is primarily attributed to difficulties in tissue rehydration and consequent delays in the processes that trigger germination [32]. Therefore, the use of stress-attenuating agents that improve germinative performance is highly relevant. Among these, ascorbic acid and hydrogen peroxide, at appropriate concentrations, exert protective effects under stress [33,34].

Increased root length under water-deficit conditions occurs due to the translocation of solutes from the shoot to the root system, promoting its growth [35]. In the present study, root length was less affected than shoot length. As observed in most cases, a greater investment in root development occurs at the expense of aerial growth [36].

Plants under stress may accumulate sugars as a means of osmoregulation, a process often accompanied by growth reduction [37]. This may explain the decline in shoot growth and dry mass in the cultivar Imperial 45 when exposed to water deficit after hydropriming. The effects of exogenous gibberellic acid on shoot dry mass vary markedly depending on dosage, species, and cultivar. In sensitive cultivars, GA_3 application has been shown to reduce shoot dry biomass under saline stress, indicating that responses to GA_3 may differ according to cultivar tolerance to water deficit as well [38].

The accumulation of osmoprotective molecules is essential for maintaining osmotic adjustment and sustaining water uptake and retention in plants under water deficit [11,39]. In this regard, Chaudhry and Sidhu [40] reported that increased cellular levels of inert organic solutes such as sucrose and proline exert protective effects on macromolecules and cellular structures, including membranes.

Citrulline is a non-protein amino acid that rapidly accumulates in cucurbits such as melon in response to osmotic stress, being preferentially synthesized over other osmoprotectants such as proline and glycine betaine [7]. In watermelon under water deficit, approximately 21% of total amino acids corresponded to citrulline. These findings are associated with enhanced protection of green tissues against oxidative damage induced by water-deficit stress [41]. Taken together, the results demonstrate that proline and citrulline play complementary roles in the response to water deficit, with variations depending on genotype and treatment. Proline stands out as the main osmolyte associated with osmotic adjustment, while citrulline appears to act predominantly in mitigating oxidative stress, reinforcing its relevance as a biochemical marker of response to water stress in cucurbits.

The antioxidant system is a defense mechanism that maintains the balance between the production and detoxification of reactive oxygen species (ROS) under favorable conditions. However, under water deficit, ROS production becomes excessive, and their accumulation damages membranes, proteins, and other molecules, ultimately leading to cellular destruction [42]. In this study, both cultivars exhibited increased hydrogen peroxide production under water deficit. Similar findings have been reported, highlighting H_2O_2 as an important signal molecule in oxidative balance [43–45].

Hydrogen peroxide acts as a stress messenger and signal molecule due to its greater stability (relative to other ROS) and ease of diffusion, performing multiple functions at various organizational levels in plants [46]. Together with plant hormones, H_2O_2 activates enzymatic and non-enzymatic antioxidant systems, facilitating adaptation and survival under adverse conditions [47].

Among the stress attenuators tested, hydrogen peroxide and gibberellic acid induced the lowest H_2O_2 accumulation in the cultivar Dali, although without differing from the other treatments. Additionally, treatments with gibberellic and ascorbic acids reduced malondialdehyde accumulation in this cultivar. Seed treatment with hydrogen peroxide and salicylic acid proved effective in reducing peroxide and malondialdehyde levels, respectively, in the sensitive cultivar, consistent with findings from other studies in cucumber [12,48].

The reduction in malondialdehyde induced by gibberellic and ascorbic acids in the cultivar Dali, and by salicylic acid in Imperial 45, indicates improvements in the agronomic performance of both cultivars. These treatments favored increased root dry mass in Dali and shoot dry mass in Imperial 45. Similar results were reported by Silva et al. [49] in watermelon seedlings subjected to water deficit, where a 9% increase in shoot dry mass was observed following the application of salicylic acid (1.0 μmol L^{-1}).

Among antioxidant components, superoxide dismutase is one of the first enzymes to respond to water shortage, converting superoxide (O_2^-) into the less reactive hydrogen peroxide. Exogenous application of hydrogen peroxide also enhanced superoxide dismutase activity in barley and oat under drought conditions [50,51]. Consequently, superoxide dismutase acts upstream in hydrogen peroxide metabolism, without substantially increasing its levels under continuous irrigation. However, under water deficit and elevated O_2^- concentrations, superoxide dismutase activity increases [52].

Hydropriming and gibberellic acid application modulate antioxidant enzymatic systems and are effective in detoxifying ROS under water scarcity [53]. This action may be induced both by oxidative stress—promoting greater activation of enzymes such as CAT, directly responsible for hydrogen peroxide elimination—and by enhanced osmotic adjustment capacity. Ascorbate peroxidase is considered a key enzyme in hydrogen peroxide scavenging across different species. It acts directly in this process or as part of the ascorbate–glutathione cycle, a major non-enzymatic mechanism involved in ROS metabolism [54,55].

The coordinated action of enzymatic and non-enzymatic defense mechanisms maintains the balance of ROS production in plants, although their effectiveness is multifactorial [56]. In this context, the differences observed in this study regarding the expression of drought tolerance in melon cultivars may be associated with the greater capacity for enzymatic modulation and accumulation of compatible solutes in the cultivar Dali. This cultivar showed an effective response, mainly through the synchronized activation of osmotic and antioxidant mechanisms, especially when exposed to attenuators. Imperial 45 reacts to stress by accumulating larger amounts of antioxidant metabolites (such as citrulline) as stress sets in, resulting in greater physiological damage.

In addition to demonstrating that distinct levels and capacities for coping with water deficit are linked to the specific strategies adopted by each cultivar, this study also shows that these mechanisms can be optimized through the pregerminative treatments evaluated. Therefore, seed treatments with potential stress-attenuating agents are recommended for melon crops, offering agronomic advantages in regions prone to water scarcity.

5 Conclusions

Germination and early seedling growth of melon are impaired under a water deficit of −0.15 MPa, with the cultivars Dali and Imperial 45 identified as tolerant and sensitive to this stress, respectively.

The use of salicylic acid mitigates the effects of water deficit on physiological germination variables, seedling length, and dry mass in the cultivar Dali.

Pre-treatment of melon seeds with hydrogen peroxide reduces the production and accumulation of hydrogen peroxide, primarily through the action of the enzymatic antioxidant system, enhancing germination performance under water deficit in the sensitive cultivar (Imperial 45).

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