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ARTICLE

Elicitors of Salt Stress Tolerance during Germination and Early Growth of Basil (*Ocimum basilicum* L.)

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ABSTRACT: Salt stress attenuators assist seed germination by reducing the effects of osmotic and ionic stress, y promoting water uptake, they stabilize enzymes and enhance metabolic tolerance, resulting in higher germination rates and more uniform early development. This study evaluates the effects of applying salt stress attenuators on the germination and seedling formation of *O. basilicum* as a pre-germination treatment. Two cultivars, Limoncino (tolerant) and Genaro de Menta (sensitive), were subjected to pre-germination treatments combining salt stress with a stress-attenuating agent: no pre-germination treatment and no salt stress; salt stress (6.98 dS m^{-1} NaCl); hydropriming + salt stress; gibberellic acid (50 mg L^{-1}) + salt stress; salicylic acid (50 mg L^{-1}) + salt stress; ascorbic acid (50 mg L^{-1}) + salt stress. Basil cultivars responded differently to salinity simulated by NaCl (6.98 dS m^{-1}). Limoncino maintained high germination percentage, germination speed index, shoot and root growth, and biomass accumulation under saline conditions, regardless of pre-treatment. In contrast, Genaro de Menta showed reductions in germination speed, root length, and total seedling growth under salinity. Seed pre-treatment with ascorbic acid and salicylic acid attenuated the effects of salinity when applied at 6.98 dSm^{-1}, improving germination speed, root development, and osmotic adjustment in the sensitive cultivar, indicating physiological performance under salt stress. Thus, Limoncino exhibits inherent tolerance to salinity during germination, whereas seed pre-treatment with ascorbic acid (AsA) or salicylic acid (SA) is required to partially restore early growth in the salt-sensitive cultivar Genaro de Menta.

KEYWORDS: Seed germination; salinity stress; seed priming; osmotic adjustment

1 Introduction

The cultivation of medicinal plants has gained importance in global trade due to growing demand from the pharmaceutical, food, and cosmetic industries [1]. Basil (*Ocimum basilicum* L.), an aromatic plant rich in essential oils and widely used for culinary and pharmacological purposes, has shown reduced biomass production compared to other countries, with approximately 2432 t recorded in the latest Agricultural Census conducted in 2017 [2,3]. Environmental factors such as salinity may be among the causes of this reduced productivity [4].

The Brazilian semi-arid region presents a dynamic interaction of physical-geographic factors that shape the region's typical edaphoclimatic conditions [5]. With an average annual rainfall of 749 ± 140 mm

(over the past 35 years) and evapotranspiration rates ranging from 1200 to 2200 mm/year, the region faces an increasing risk of water scarcity and expansion of arid zones [6,7], a scenario that exacerbates soil salinization processes.

Globally, salinization is considered the second leading cause of soil degradation, second only to erosion processes [8]. The most recent estimates indicate that 73% of the world's terrestrial area is affected by salinized soil, with two-thirds located in arid and semi-arid regions [9]. The expansion of salinized areas and the high concentration of toxic ions in the soil can severely impair plant growth, both in native species (compromising natural ecosystem regeneration) and in agricultural crops, with direct impacts on productivity and, consequently, regional food security [10].

High concentrations of salt in soil and irrigation water directly compromise seed germination and early plant establishment, triggering a series of physiological stress responses [11]. This process leads to significant metabolic changes as plants attempt to acclimate to saline conditions. Specifically, excessive accumulation of ions such as Na^+ and Cl^- triggers ionic stress, which disrupts the plant's nutritional balance, primarily by inhibiting potassium (K^+) uptake [12].

When cultivated under water electrical conductivity of 4.90 dS m^{-1}, *O. basilicum* shows a considerable reduction in productivity, with physiological responses varying depending on the cultivar used [13]. At an osmotic potential of −0.3 MPa (8.3 dS m^{-1}), germination of the species is reduced by approximately 80% relative to the control, whereas doubling salinity to −0.6 MPa (16.6 dS m^{-1}) results in a further decline, with germination reaching only 20% [14].

Thus, understanding and developing strategies to mitigate salinity-induced damage in O. basilicum plants may offer viable alternatives for crop cultivation. Pre-germination treatments, such as seed soaking in stress-attenuating agents or stress elicitors, have shown effectiveness in mitigating the effects of abiotic stresses like drought and salinity [15–17].

Plant growth regulators and signaling molecules, such as gibberellic acid (GA_3) and salicylic acid (SA), stand out as effective elicitors in alleviating abiotic stress, acting through mechanisms such as osmotic regulation, ion detoxification, and energy balance [15]. When applied exogenously (via foliar spray) or through seed soaking, they enhance adaptive responses such as antioxidant synthesis and metabolic adjustments, with efficiency varying according to dosage, plant species, and stress intensity [16,17].

Different mechanisms of action can be observed among the stress-attenuating agents used during seed germination under saline conditions. GA_3 promotes germination under salinity by reactivating gibberellin biosynthesis through the induction of GA_{20}-oxidase and GA_3-oxidase, which a commonly inhibited by excess salts accumulation, thereby supporting embryo growth even under osmotic constraint [18]. Additionally, GA counteracts the inhibitory effects of abscisic acid (ABA), stimulating α-amylase production and reserve mobilization, which ensures metabolic and enzymatic activity essential for successful germination under saline stress [19].

The SA, in turn, plays a key role in maintaining ionic homeostasis and reinforcing antioxidant defenses systems by enhancing the activity of enzymes such as superoxide dismutase and catalase, thereby reducing the accumulation of reactive oxygen species under saline conditions. Furthermore, SA limits Na^+ and Cl^- uptake and transport, preserving cellular ionic balance during the early stages of germination [20,21].

Ascorbic acid (AsA) contributes to enzyme stabilization and membrane integrity by acting as a cofactor in antioxidant systems responsible for reactive oxygen species (ROS) scavenging. Its exogenous application reduces lipid peroxidation and preserves metabolic functionality in seeds exposed to salinity [22]. Additionally, AA enhances the activity of violaxanthin de-epoxidase (VDE), promoting the conversion of

violaxanthin to zeaxanthin and facilitating excess energy dissipation, thereby minimizing oxidative damage associated with saline stress [23].

Techniques such as hydropriming further potentiate these effects by inducing prior metabolic activation, promoting osmotic adjustment and increasing the expression of antioxidants and protective proteins, which collectively enhance enzyme stability during germination [24]. In rice seeds, for example, pre-hydration minimizes damage caused by water deficit and salinity, ensuring more uniform germination and improved initial seedling vigor [24,25].

Investigating the application of salt stress attenuators in basil cultivars may provide effective tools to overcome salinity-induced damage and ensure species productivity. Therefore, this study aimed to evaluate the effects of applying salt stress attenuators on the germination and seedling formation of *O. basilicum*.

The hypothesis is that the application of salt stress attenuators, such as GA_3, SA, and AsA, as well as hydropriming, mitigates the effects of salt stress in *O. basilicum* seeds, promoting greater uniformity in germination, early growth, and osmolyte accumulation, especially in sensitive cultivars.

2 Materials and Methods

2.1 Experimental Site and Seed Acquisition

The experiment was conducted at the Plant Physiology and Biochemistry Laboratory of the State University of Rio Grande do Norte (UERN) and at the Federal Rural University of the Semi-Arid Region (UFERSA), Mossoró-RN, Brazil. Seeds of the basil cultivars Limoncino and Genaro de Menta were obtained from Isla Sementes and stored in a cold chamber (16–18°C and 40% relative humidity) throughout the experimental phase.

2.2 Experimental Design

The salinity level of 6.98 dS m^{-1} was defined based on preliminary screening conducted with six basil cultivars under 5.57 dS m^{-1}, which allowed the selection of contrasting genotypes (Limoncino and Genaro de Menta), and was subsequently increased to impose a more restrictive yet non-lethal stress condition. This electrical conductivity falls within the range previously reported to cause significant reductions in germination and early growth of Ocimum basilicum under saline conditions [13,14].

2.3 Analyzed Variables

Initially, seed moisture content was determined using two replicates of 4.5 ± 0.5 g. The samples were dried in an oven at 105 ± 3°C for 24 h [26]. Moisture content was calculated on a wet basis and expressed as a percentage, using the formula proposed by Cromarty, Ellis, and Roberts [27], see Eq. (1):

$$\text{Moisture content (\%)} = \left(\frac{\text{FW} - \text{DW}}{\text{FW}}\right) * 100 \quad (1)$$

where FW is the fresh weight of the seeds before oven drying and DW is the dry weight obtained after drying.

Germination percentage was determined on the fourteenth day after showing and calculated according to Eq. (2) [26].

$$\text{Germination (\%)} = \left(\frac{N_g}{N_t}\right) * 100 \quad (2)$$

where, N_g is number of germinated seeds and N_t is the total number of seeds sown.

The germination speed index (GSI) was calculated using Maguire's formula, Eq. (3) [28].

$$\mathrm{GSI} = \sum\left(\frac{G_i}{T_i}\right) \tag{3}$$

in which G_i corresponds to the number of seeds germinated on the i-th count, and T_i represents the time (days) elapsed from sowing to each count.

The mean germination time (MGT) according to Labouriau [29], as expressed in Eq. (4).

$$\mathrm{MGT} = \frac{\sum(n_i * t_i)}{\sum n_i} \tag{4}$$

where n_i is the number of seeds germinated at time t_i, and t_i is the time (days) from sowing to each germination count.

Shoot, root, and total lengths were measured at the end of the germination test, from the seedling apex to the root tip using a ruler graduated in centimeters. Seedlings were then separated, and their parts placed in kraft paper bags and dried in a forced-air oven at 65°C for 72 h. Dry mass (DM) of shoot, root, and total was determined using a precision scale (0.0001 g). The root-to-shoot ratio (R/S) was determined as the ratio between the dry mass of the root system and the dry mass of the shoot of the seedlings.

For compatible osmolyte quantification, extracts were obtained from fresh tissue previously frozen in an ultra-freezer at −20°C. Samples were ground with liquid nitrogen, and 0.2 g of each sample (in triplicate) were placed in sealed Eppendorf tubes. One milliliter of 80% ethanol was added and homogenized in a shaker. Samples were incubated in a water bath at 60°C for 20 min, followed by centrifugation at 10,000 RPM at 4°C for 10 min. The supernatant was collected and stored in Falcon tubes. The ethanol extraction was repeated twice, and the final supernatant was stored in a freezer [16].

Total soluble sugars were quantified by absorbance at 620 nm using the Anthrone method [30]. A standard curve was generated using glucose, followed by the addition of 2 mL of Anthrone reagent under cooling. Tubes containing 100 µL of each sample were vortexed and placed in a boiling water bath at 100°C for 3 min. After cooling, absorbance was read using a UV spectrophotometer, and results expressed in µMol GLU g^{-1} fresh mass.

For total free amino acid determination, the ethanol-extracted supernatant was used. The acidic ninhydrin method was applied, with absorbance measured at 570 nm [31]. Glycine was used as the standard. For each sample (40 µL), 0.5 mL sodium citrate buffer (0.2 M, pH 5.0), 0.2 mL 5% ninhydrin in methyl cellosolve, and 1 mL 2% potassium cyanide (KCN) in methyl cellosolve were added. After vortexing, samples were incubated in a boiling water bath at 100°C for 20 min to develop the purple color. Then, 1.3 mL of 60% ethanol was added and vortexed again. After cooling, absorbance was read in a spectrophotometer, and results expressed in µMol GLY g^{-1} fresh mass.

Proline content was determined following the method described by Bates, Waldren & Teare [32]. Concentrations were calculated using a standard curve based on L-proline, with absorbance measured at 520 nm. In screw-cap test tubes, 400 µL of crude extract were added, followed by 1 mL of acidic ninhydrin and 1 mL of glacial acetic acid. Tubes were sealed, vortexed, and incubated in a boiling water bath for 1 h at 100°C. The reaction was stopped by cooling in an ice bath. Then, 2 mL of toluene were added, tubes were sealed and vigorously shaken. The supernatant was collected using a Pasteur pipette, and absorbance was read in a spectrophotometer. Results were expressed in µMol PRO g^{-1} fresh mass.

2.4 Statistical Analysis

Data were subjected to analysis of variance (ANOVA), and when significant effects were detected by the F-test ($p < 0.05$), means were compared using the Scott-Knott test ($p < 0.05$). Statistical analyses were performed using the Sisvar 5.7 software [33], and graphs were generated using Excel.

3 Results

3.1 Germination and Early Growth

According to the analysis of variance, the interaction between cultivars and salt stress attenuators (C × A) was significant for germination speed index (GSI), shoot length (SL), root length (RL), root-to-shoot ratio (R/S) at the 1% probability level, and for total length (TL) at the 5% probability level (Table 1). For germination percentage, the effect was isolated for cultivars, with 99% significance (Table 1).

Table 1: Analysis of variance for germination percentage (G), germination speed index (GSI), mean germination time (MGT), shoot length (SL), root length (RL) and total length (TL) of basil cultivars (*Ocimum basilicum* L.) subjected to salt stress attenuators.

SV	DF	RMS					
		G (%)	GSI	MGT	SL	RL	TL
Cultivars (C)	1	772.0**	84.3**	0.08ns	5.67**	45.2**	80.3**
Att + sal (A)	5	27.6ns	21.1**	0.42**	0.11**	2.25**	2.12**
C × A	5	25.8ns	4.6**	0.007ns	0.27**	2.14**	1.73*
Error	36	701.6	1.19	0.03	0.03	0.42	0.50
CV (%)		4.7	6.71	7.3	7.9	19.68	12.95

**; * = significant at 1% and 5% probability levels; ns = not significant. SV = source of variation; DF = degrees of freedom; RMS = residual mean square; CV = coefficient of variation.

Although the cultivars showed differences, both achieved high germination percentages: 96% for Limoncino and 94% for Genaro de Menta (Fig. 1a). Regardless of treatment, germination remained above 80%.

Germination percentage alone cannot determine whether a species is tolerant to salt stress. Under ideal conditions (control), both cultivars showed high GSI values (18.1 and 17.7), with no significant difference. However, under 6.98 dS m^{-1} salinity, GSI decreased by 20.8% and 31%, respectively, demonstrating the impact of salinity.

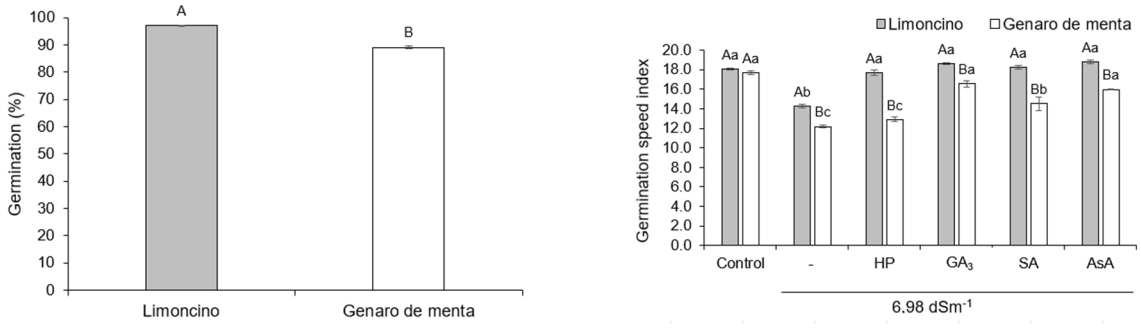
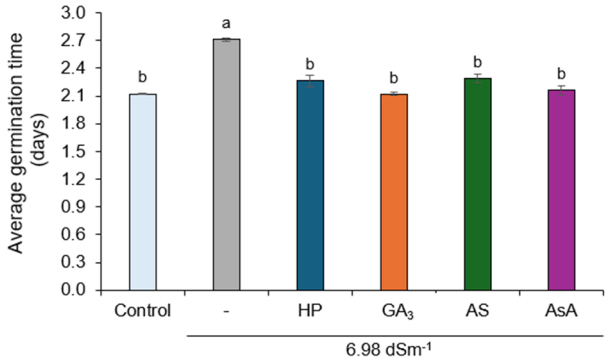


Figure 1: *Cont.*



(**c**)

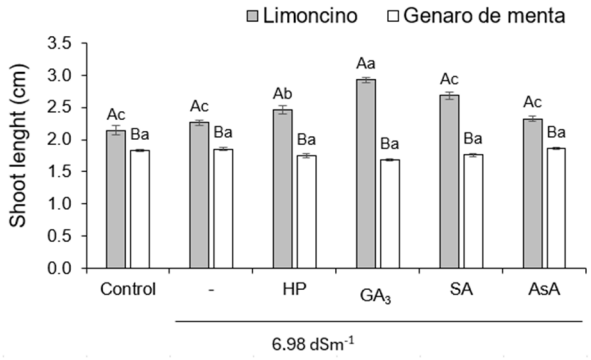
Figure 1: Mean values for germination percentage (**a**), germination speed index (**b**), and mean germination time (**c**) of basil seedlings (*Ocimum basilicum* L.) subjected to salt stress attenuators: hydropriming (HP), gibberellic acid (GA_3), salicylic acid (SA), and ascorbic acid (AA). Means followed by the same uppercase letter compare cultivars (A and B); lowercase letters compare attenuator and salinity combinations (a, b, and c). No statistical difference according to Scott-Knott test at 5% probability.

In Limoncino, all pre-treatments mitigated salinity effects, yielding results like the control (Fig. 1b). In Genaro de Menta, only GA_3 and AsA restored GSI to control levels (Fig. 1b). Salinity increased the time required for germination (MGT), but all stress attenuators produced MGT values like the control (Fig. 1c).

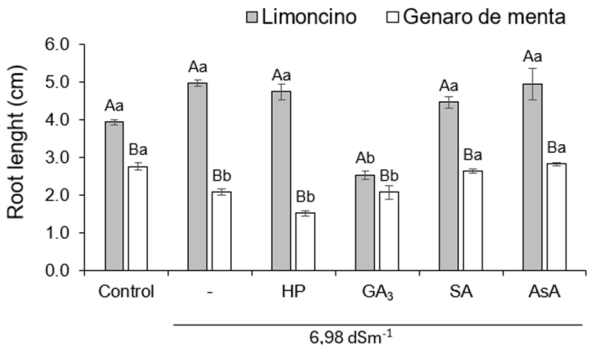
For SL, salinity (6.98 dS m^{-1}) did not differ from the control in either cultivar. However, Limoncino seeds pre-soaked in water (hydropriming) and GA_3 showed gains of 14% and 36%, respectively, compared to the control. Genaro de Menta maintained an average SL of 1.8 cm (Fig. 2a).

In Limoncino, salt presence increased RL by 26.6% compared to the control, with similar results for hydropriming, SA, and AsA treatments (Fig. 2b). In Genaro de Menta, salinity reduced RL by 24.8%, but pre-treatment with SA and AsA restored RL to 2.6 cm, statistically like the control (Fig. 2b).

Similar behavior was observed for TL. In Limoncino, seedling growth increased under salinity, with no differences among stress attenuators, except for GA_3, which further reduced growth (Fig. 2c). In Genaro de Menta, TL decreased by 14.5% under salinity, but Sa and AsA increased TL by 11.9% and 25.5%, respectively, compared to the saline treatment (Fig. 2c).

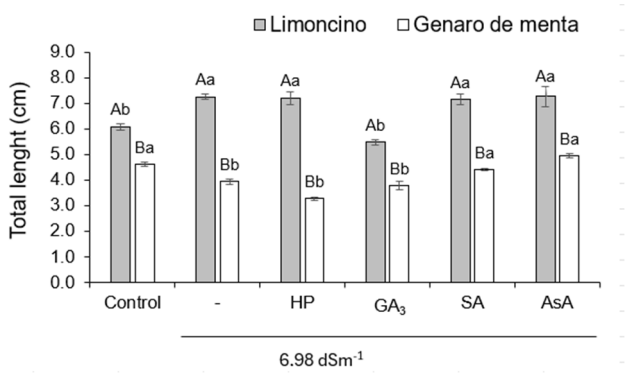


(**a**)



(**b**)

Figure 2: *Cont.*



(**c**)

Figure 2: Mean values for shoot length (**a**), root length (**b**) and total length (**c**) of basil seedlings (*Ocimum basilicum* L.) subjected to salt stress attenuators: hydropriming (HP), gibberellic acid (GA_3), salicylic acid (SA), and ascorbic acid (AA). Means followed by the same uppercase letter compare cultivars (A and B); lowercase letters compare attenuator and salinity combinations (a, b and c). No statistical difference according to Scott-Knott test at 5% probability.

3.2 Dry Mass and Osmolyte Quantification

Analysis of variance showed no significant interaction between cultivars and stress attenuators (C × A) for dry mass, only for the root-shoot ratio ($p < 0.05$). However, shoot dry mass (SDM) showed isolated effects for cultivars ($p < 0.05$), and both SDM and root dry mass (RDM) showed isolated effects for stress attenuators ($p < 0.01$) (Table 2). Significant effects ($p < 0.01$) were also observed for total soluble sugars (TSS), total amino acids (AA), and free proline (PRO) (Table 2).

Table 2: Analysis of variance for shoot dry mass (SDM), root dry mass (RDM), total dry mass (TDM), root-to-shoot ratio (R/S), total soluble sugars (TSS), total amino acids (AA), and free proline (PRO) of basil cultivars (Ocimum basilicum L.) subjected to salt stress attenuators.

SV	DF	RMS						
		SDM	RDM	TDM	R/S	TSS	AA	PRO
Cultivares (C)	1	0.03*	0.00005^{ns}	0.03ns	0.003ns	733.5**	314.7**	9.42**
Att + sal (A)	5	0.04**	0.004**	0.03*	0.024**	72.6**	223.2**	0.23**
C × A	5	0.007^{ns}	0.003^{ns}	0.009^{ns}	0.007*	53.1**	350.4**	0.48**
ErroR	36	0.006	0.0008	0.008	0.002	4.38	11.6	0.03
CV (%)		10.8	14.3	9.63	15.58	25.9	13.7	16.0

**; * = significant at 1% and 5% probability levels; ns = not significant. SV = source of variation; DF = degrees of freedom; RMS = residual mean square; CV = coefficient of variation.

Although Genaro de Menta had slightly lower RDM than Limoncino (7.6% less), SDM remained high across treatments (Fig. 3a), with a 23.7% average increase from control to saline treatments (Fig. 3b). RDM was stable under 6.98 dS m^{-1} salinity, except when GA_3 was applied, which further reduced RDM (Fig. 3c). Consequently, TDM was not reduced under any saline treatment (Fig. 3d).

Although Genaro de Menta had lower germination than Limoncino, it still reached 89%, which is considered high. For commercial purposes, most crop species require germination rates above 80–85% and maintaining this rate under water deficit indicates high seed viability. However, Genaro de Menta is considered sensitive to 6.98 dS m^{-1} salinity due to reductions in GSI, RL, and TL. Imposing salinity stress without a buffer reduced this ratio, mainly in the Genaro de Menta, demonstrating greater sensitivity of this

cultivar to salinity in terms of relative root development. The Limoncino, on the other hand, maintained relatively higher values, providing a greater capacity to sustain root system growth even under salinity (Fig. 3e).

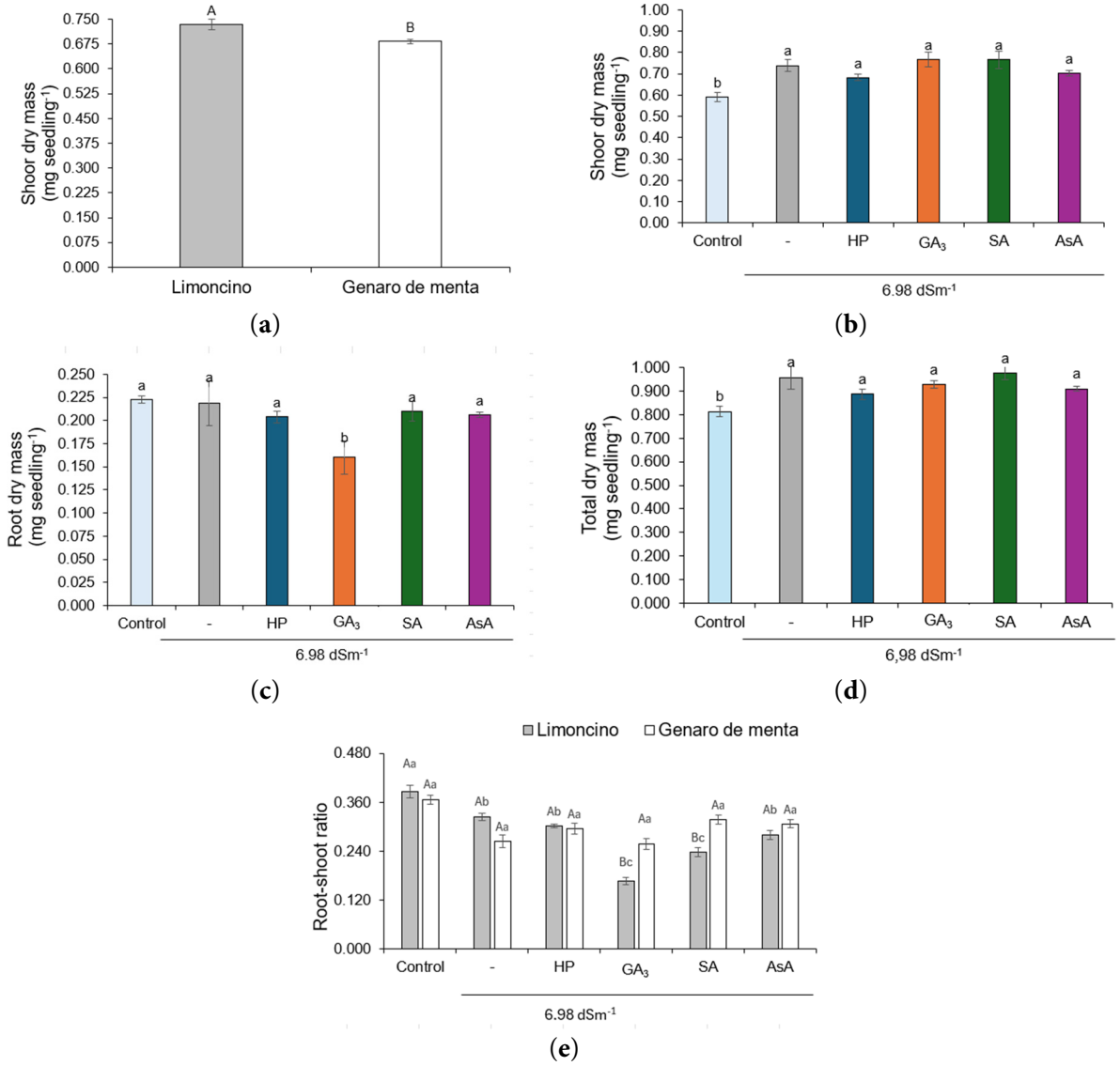


Figure 3: Mean values for shoot dry mass (**a**,**b**), root dry mass (**c**), total dry mass (**d**) and root-shoot ratio (**e**) of basil seedlings (*Ocimum basilicum* L.) subjected to salt stress attenuators: hydropriming (HP), gibberellic acid (GA_3), salicylic acid (SA), and ascorbic acid (AsA). Means followed by the same uppercase letter compare cultivars (A and B); lowercase letters compare attenuator and salinity combinations (a, b and c). No statistical difference according to Scott-Knott test at 5% probability.

In Genaro de Menta, GSI recovery under salinity occurred with GA_3 and AsA pre-treatment, like the control. RL recovery was only achieved with AsA and SA soaking. However, TL remained unchanged compared to the saline treatment. In contrast, treatments with SA and AsA promoted a significant increase in the root/shoot ratio when compared to saline solution without attenuator, standing out as the most efficient attenuators in mitigating the effects of salinity.

3.3 Biochemical Parameters

In the Limoncino cultivar, no significant increase in TSS was observed compared to Genaro de Menta (Fig. 4a). Among stress attenuators, pre-treatment with GA_3 led to an 181.9% increase in TSS accumulation relative to the control, with no statistical difference from AsA. In Genaro de Menta, GA_3 resulted in a 200.8% increase compared to the control, also statistically similar to hydropriming (Fig. 4a).

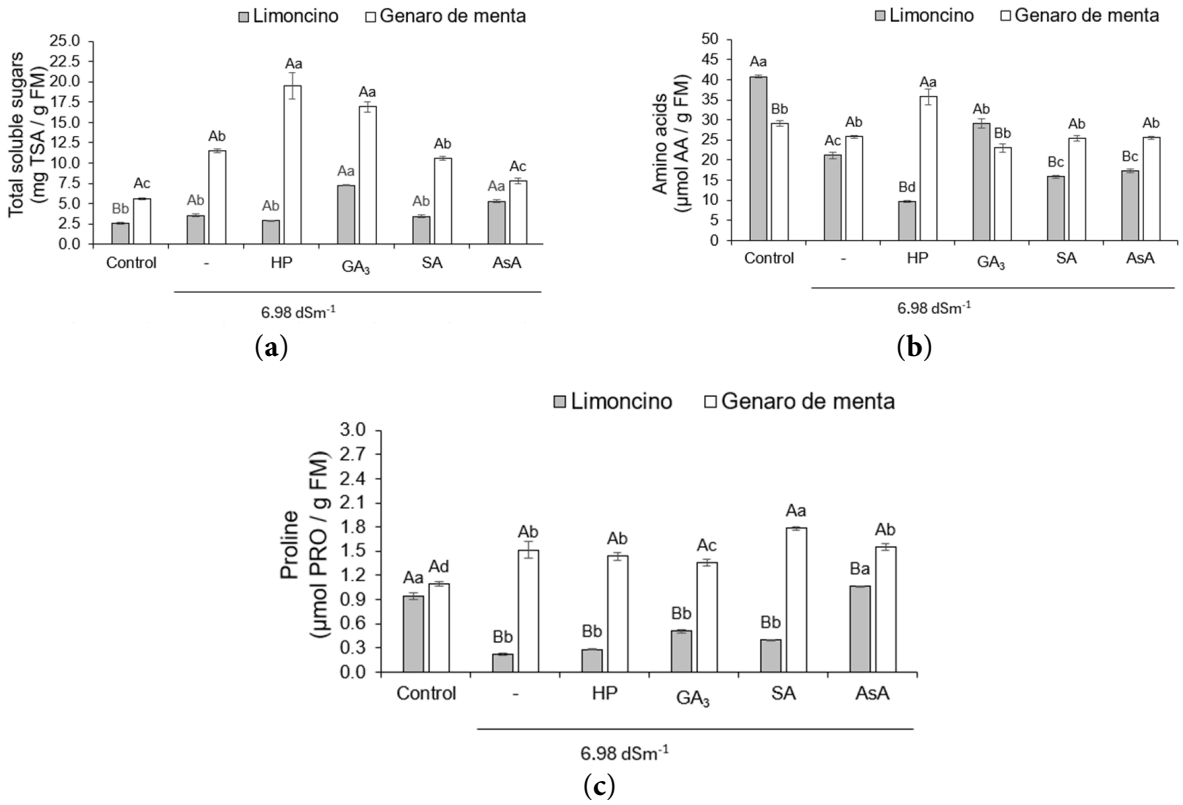


Figure 4: Mean values of total soluble sugars (**a**), total amino acids (**b**), and free proline (**c**) in basil seedlings (*Ocimum basilicum* L.) subjected to salt stress attenuators: hydropriming (HP), gibberellic acid (GA_3), salicylic acid (SA), and ascorbic acid (AsA). Means followed by the same uppercase letter compare cultivars (A and B); lowercase letters compare attenuator and salinity combinations (a, b, c and d). No statistical difference according to Scott-Knott test at 5% probability.

Under salt stress, the Limoncino cultivar accumulated fewer AA, even when seeds were pre-treated with stress attenuators. For this cultivar, only GA_3 produced results 37.7% higher than the saline treatment (Fig. 4b). In contrast, Genaro de Menta showed increased amino acid production following hydropriming, with a 38.9% gain compared to the saline treatment (Fig. 4b).

Regarding proline, both cultivars exhibited similar levels under ideal conditions. However, in Limoncino, all treatments under 6.98 dS m^{-1} salinity led to reduced proline content, except for AsA, which maintained levels equal to the control (Fig. 4c). In Genaro de Menta, salinity induced proline accumulation, and SA resulted in a 63.3% increase compared to the control (Fig. 4c).

4 Discussion

4.1 Germination Perfomance and Growth under Salinity and Attenuators

Seed germination under saline conditions is primarily constrained by osmotic stress and ion toxicity, which delay water uptake and impair metabolic activation during imbibition. In the present study, the reduction in germination speed index (GSI) in both basil cultivars under 6.98 dS m^{-1} confirms that salinity slowed germination, likely due to restricted water absorption and reduced cellular turgor caused by Na^+ and Cl^- ions, as described by Taiz et al. [34]. However, the maintenance of high germination percentages (>80%) indicates that seed viability was not compromised, emphasizing that germination percentage alone is insufficient to characterize salt tolerance.

The contrasting responses observed between cultivars during early seedling growth highlight differences in biomass allocation strategies under salinity. The Limoncino cultivar-maintained root length and root-to-shoot ratio under saline conditions, suggesting a preferential allocation of assimilates to the root system. The maintenance of a higher root-to-shoot ratio under stress is commonly interpreted as an adaptive response that enhances water and nutrient acquisition, thereby supporting seedling establishment in saline environments [35]. This response likely explains why Limoncino seedlings exhibited normal growth and total length even under salinity, indicating intrinsic tolerance at the early developmental stage.

Seed quality and vigor are key factors influencing plant establishment under saline conditions. High-vigor seeds tend to maintain higher germination rates and produce more robust seedlings under salinity stress [36]. In the present study, the reduction in GSI, both cultivars maintained high germination percentages, suggesting that salinity primarily affected germination dynamics rather than seed viability. Nevertheless, the superior early growth performance of Limoncino indicates that vigor-related traits may contribute to enhance tolerance during seedling establishment.

The reduction in GSI observed in both cultivars is consistent with the osmotic effects of salinity on seed imbibition. According to Taiz et al. [34], limited water penetration into seed tissues delays embryo hydration, resulting in reduced cell turgor and impairing both cell division and expansion. Despite this constraint, Limoncino seedling maintained normal growth, as reflected by shoot and root development and a stable root-shoot ratio under 6.98 dS m^{-1} salinity.

In contrast, the recovery of GSI in Genaro de Menta following GA_3 and AsA pre-treatments highlights the importance of seed pre-conditioning strategies to improve germination performance under saline conditions. GA_3 likely promoted faster germination by enhancing the hormonal balance during imbibition, stimulating hydrolytic enzyme activity and reserve mobilization, which are critical processes for embryo growth under osmotic constraint [19,37]. AsA, in turn, may have contributed by strengthening the antioxidant system during early germination, reducing oxidative damage caused by salt-induced reactive oxygen species and preserving membrane integrity, thereby facilitating water uptake and metabolic reactivation [38]. Additionally, root system development is particularly critical under saline and water limited conditions, as root length directly influences the plant's capacity for water and nutrient uptake. Well-developed root systems can enhance shoot growth and overall plant performance by improving resource acquisition during early growth stages [39]. In this study, Limoncino maintained or increased root growth under salinity, while Genaro de Menta showed root length reduction, reinforcing the greater sensitivity of this cultivar to salinity stress.

Although all stress attenuators mitigated the effects of salinity on GSI in Limoncino, growth parameters did not exceed those observers under salinity alone, indicating that this cultivar already exhibits a high inherent tolerance to electrical conductivity up to 6.98 dS m^{-1}. It is likely that higher salinity levels would

be required to induce growth limitations in this cultivar Nevertheless, tolerance mechanisms in basil may involve not only osmotic adjustment but also ionic homeostasis and antioxidant defense systems, which warrant further investigation [22,40].

4.2 Biochemical Responses of Seedlings to Salt Stress

Biochemical analysis revealed that pre-treatment with GA_3 under salt stress increased the accumulation of soluble sugars and amino acids in Limoncino seedlings. This response coincided with increased shoot length and shoot dry mass under 6.98 dS m^{-1} salinity. However, despite stimulating shoot growth, GA did not promote proportional root development, resulting in a reduced root-to-shoot ratio and limiting its effectiveness in improving overall seedlings performance.

Gibberellins are phytohormones that regulate diverse aspects of plant growth and development through complex biosynthetic and signaling pathways [19,41]. However, GA_3 is known to induce excessive elongation of aerial organs due to its high biological activity [42], which can compromise biomass balance under stress conditions.

Salinity is widely recognized as a major abiotic stress that negatively affects plant growth, physiology, and biochemical properties [12,43,44]. Understanding salt tolerance mechanisms is particularly relevant in regions where irrigation water exhibits high electrical conductivity and total dissolved solids, increasing the risk of soil salinization and sodification [10,45]. Even at the seedling stage, plants may display adaptive responses that enable survival under saline conditions, with osmotic adjustment being a key mechanism [16,46].

In this study, both basil cultivars exhibited relatively high amino acid levels under non-stress conditions, particularly Limoncino. Amino acids serve as precursors for a wide range of metabolites involved s in growth, stress protection and cellular stricture, and can also function as readily available energy sources under adverse conditions [47].

The reduction in amino acids accumulation observed in Limoncino under salinity may indicate efficient metabolic utilization, possibly through enhanced catabolism via the tricarboxylic acid (TCA) cycle to meet the increase in energy demands imposed by stress [48]. This efficient energy use may explain the maintenance of normal seedling growth in this cultivar despite salinity exposure. In contrast, Genaro de Menta showed pronounced accumulation of TSS and proline under saline conditions, with and without attenuators. Such accumulation is often associated with osmotic adjustment and cellular protection in salt sensitive genotypes. This may provide an energy reserve for later growth stages. Although hydropriming increased sugar and amino acid concentrations, it did not yield the highest growth results.

The contrasting responses observed between the Limoncino and Genaro de Menta cultivars indicate the involvement of distinct physiological and biochemical strategies underlying salt tolerance. The capacity of Limoncino to maintain normal seedling development under salinity, despite reduced amino acid accumulation, suggests a more efficient metabolic adjustment, possibly associated with improved energy use efficiency and ionic homeostasis rather than reliance on osmolyte buildup. According to Munns et al. [46], salt-tolerant genotypes tend to allocate metabolic energy toward growth maintenance instead of excessive osmoprotectant accumulation.

In contrast, the marked accumulation of soluble sugars and proline in Genaro de Menta appears to reflect a stress-induced response typical of salt-sensitive cultivars, where osmotic adjustment functions primarily as a protective mechanism. The partial recovery of growth observed in Genaro de Menta following SA and AsA pre-treatments suggests that these compounds act mainly through the modulation of redox homeostasis and stress signaling pathways, enhancing cellular protection and allowing limited growth

recovery under saline conditions. Together, these results indicate that salt tolerance in basil involves a coordinated interplay between metabolic efficiency, osmotic regulation, and redox balance, rather than a single dominant mechanism.

5 Conclusions

The results of this study demonstrate that the basil cultivars (*Ocimum basilicum* L.) Limoncino and Genaro de Menta respond differently to salinity stress induced by NaCl at 6.98 dS m^{-1}. The Limoncino cultivar-maintained germination and seedling formation under salinity, regardless of pre-treatment, indicating greater tolerance. In contrast, Genaro de Menta was negatively affected in terms of germination speed and seedling growth, confirming its sensitivity to this salinity level.

However, pre-treatment of Genaro de Menta seeds with GA_3, SA and AsA promoted partial recovery of physiological and biochemical parameters. These treatments improved germination speed, root development, and osmotic adjustment through increased accumulation of soluble sugars, amino acids, and proline.

Therefore, the application of salinity stress attenuators as pre-germination treatments can be an effective strategy to mitigate the effects of salinity stress in basil cultivation, especially for sensitive cultivars. These findings contribute to the development of low-cost, accessible techniques to improve seedling establishment and productivity in saline environments.

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