ORIGINAL ARTICLE

Bioestimulant with *Ascophyllum nodosum* and fulvic acids as mitigating factors of salinity damage in soybean[1]

Bioestimulante com *Ascophyllum nodosum* e ácidos fúlvicos como fatores mitigadores dos danos causados pela salinidade na soja

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HIGHLIGHTS:

Biostimulant with algae extract and humic substances enhances soybean performance grown under saline conditions.
The biostimulant promoted stomatal opening, increasing photosynthesis and reducing leaf temperature in soybean plants.
These findings suggest a promising strategy for addressing soil salinity and increasing agricultural productivity.

ABSTRACT: Soil salinization negatively affects the growth and yield of economically valuable crops such as soybeans. New technologies are being developed to minimize the damage caused by saline stress and improve crop performance. Therefore, the use of biostimulants in agriculture may offer an alternative method to mitigate the deleterious effects of salinity. Extracts of the seaweed, *Ascophyllum nodosum* and humic substances (fulvic acids) are commonly used. Therefore, this study aimed to evaluate the action of a biostimulant composed of *A. nodosum* seaweed extract and fulvic acid applied at different phenological stages on the biometric, biochemical, and physiological parameters of soybeans (*Glycine max*) subjected to irrigation with saline water. The experimental design used was completely randomized in a $3 \times 2 + 2$ factorial scheme, referring to three applications of the biostimulant at different soybean phenological stages: V3, V3R1, and V3R1R4, two levels of electrical conductivity of irrigation water (ECw): 0.5 (S0) and 5.0 dS m-1 (S1), two additional controls, one with a positive control (PC) and the other with a negative control (NC). The experiment had five replicates with one plant per plot. The results of this study suggest that the application of the biostimulant can mitigate the harmful effects of salt stress in soybeans. The results obtained with V3R1 application in the parameters stem diameter, leaf area, shoot dry mass, electrolyte leakage, and chlorophyll b represent greater efficiency in mitigating the harmful effects of salinity in soybean cultivation.

Key words: *Glycine max* L., salt stress, growth stimulator, photosynthesis

RESUMO: Processo de salinização do solo afeta negativamente o crescimento e o rendimento de culturas economicamente valiosas, como a soja. Com o objetivo de minimizar os danos causados pelo estresse salino e melhorar o desempenho das culturas, novas tecnologias estão sendo desenvolvidas. Neste contexto, o uso de bioestimulantes na agricultura pode oferecer uma alternativa para mitigar os efeitos deletérios da salinidade. O extrato da alga *Ascophyllum nodosum* e substâncias húmicas (ácidos fúlvicos) são comumente empregados. Portanto, o estudo teve como objetivo avaliar a ação de um bioestimulante composto pelo extrato da alga marinha A. *nodosum* e ácidos fúlvicos aplicados em diferentes estádios fenológicos sobre parâmetros biométricos, bioquímicos e fisiológicos da soja (*Glycine max*) submetida à irrigação com água salina. O delineamento experimental utilizado foi inteiramente casualizado em esquema fatorial 3 x 2 + 2, referente à três aplicações do bioestimulante em diferentes estádios fenológicos da soja: V3, V3R1 e V3R1R4, dois níveis de condutividade elétrica da água de irrigação (CEa): 0,5 (S0) e 5,0 dS m-1 (S1), dois controles adicionais, um com controle positivo (PC) e outro com controle negativo (NC). O experimento contou com cinco repetições e uma planta por parcela. Os resultados deste estudo sugerem que a aplicação do bioestimulante pode ser uma alternativa na mitigação dos efeitos nocivos do estresse salino em soja. Os resultados foram obtidos com a aplicação V3R1 nos parâmetros diâmetro do caule, área foliar, massa seca da parte aérea, extravasamento de eletrólitos e clorofila b representam maior eficiência na mitigação dos efeitos nocivos da salinidade no cultivo da soja.

Palavras-chave: *Glycine max* L., estresse salino, estimulador de crescimento, fotossíntese

INTRODUCTION

Plant growth and development can be significantly affected by abiotic stressors, such as drought and salinity (Taiz et al., 2017). Among these stressors, salinity is particularly detrimental to food production, and irrigated areas are highly vulnerable to its effects (Hussain et al., 2019). Salinity can be categorized into primary and secondary types. Primary salinity arises from natural processes, such as rising water tables, whereas secondary salinity is a consequence of human activities, including the use of brackish water and improper soil management practices (Liu et al., 2022). The osmotic and ionic aspects of salt stress result in reduced water absorption, alterations in stomatal conductance, and disturbances in ionic equilibrium, leading to ionic toxicity (Acosta-Motos et al., 2017). Furthermore, these factors stimulate the excessive production of reactive oxygen species (ROS), which contribute to the peroxidation of cell membranes and the denaturation of proteins (Zuo et al., 2023).

Soybean (*Glycine max* L.) is a primary leguminous crop cultivated worldwide that serves as a crucial source of oil and protein (Sun et al., 2018). In Brazil, the expansion of soybean cultivation in the northeastern region, where many soils are affected by salinity, has led to reduced soybean production (Pessoa et al., 2022). Therefore, biostimulants can be considered as alternatives to mitigate the detrimental effects of salinity in plants. Biostimulants contain organic and inorganic compounds that stimulate chlorophyll synthesis, photosynthesis, root growth, water absorption, and nutrient uptake (Bulgari et al., 2019).

The extract of the seaweed *Ascophyllum nodosum* is widely used commercially because of its influence on growth, biosynthesis of plant hormones, and its role in the defense mechanisms of the plant's antioxidative system in soybeans under water restriction (Martynenko et al., 2016; Shukla et al., 2019). Yıldıztekin et al. (2018) reported an increase in biomass and efficiency of the antioxidant defense system in pepper (*Capsicum annuum* L.); however, little is known about the efficiency of biostimulant applications in soybean under salt stress.

Therefore, this study aimed to evaluate the action of a biostimulant composed of *A. nodosum* seaweed extract and fulvic acid applied at different phenological stages on the biometric, biochemical, and physiological variables of soybeans (*Glycine max*) subjected to irrigation with saline water.

MATERIAL AND METHODS

The experiment was conducted in a greenhouse at the Department of Agronomy, Universidade Federal do Ceará (UFC), Campus do Pici, in Fortaleza, Ceará, Brazil (3° 43' 02" S and 38° 32' 35" W, altitude of 12 m), with an average temperature of 32.2 ºC and relative air humidity of 71%, between November 2020 and January 2021.

Soil was collected from the Experimental Field of the Brazilian Agricultural Research Corporation (EMBRAPA) located in Pacajus, Ceará, Brazil. The soil is classified as Ultisol (USDA, 1999). The soil characteristics are presented in Table 1. Water-holding capacity was determined following the method described by Souza et al. (2000).

Soybean seeds ('Monsoy 8349 IPRO') were sown in polyethylene pots containing 6 kg of soil, and thinning was performed seven days after sowing (DAS), maintaining one plant per pot. Salinity stress was gradually imposed by increasing the electrical conductivity of the irrigation water (ECw) from 2.5 dS m^{-1} over the course of two days. Subsequently, it was maintained at 5.0 dS m^{-1}.

The saline solution was composed of NaCl, CaCl$_2$.2H$_2$O, and MgCl$_2$.6H$_2$O (5.0 dS m^{-1}) in the equivalent proportion 7:2:1 (Silva Júnior et al., 1999), following the relationship between ECw and the respective concentration (mmol$_c$ L^{-1} = EC × 10) according to Richards (1954). To avoid excessive accumulation of salts, excess water was applied every six days in amounts above those required to saturate the soil, constituting a leaching fraction of 0.15 (Ayers & Westcot, 1999).

The biostimulant FH Attivus consists of a composition that includes extracts from the seaweed *A. nodosum*, fulvic acids, and nutrients. A dosage of 2.5 g pot^{-1} was applied in accordance with the manufacturer's recommendations for soybean cultivation. The detailed composition of the biostimulant, as provided by the manufacturer (Fertilizantes Heringer), was as follows: N = 2.0%, Mg = 1.0%, S = 3.6%, B = 0.1%, Fe = 0.05%, Mn = 0.1%, Mo = 0.1%, Cu = 0.04%, Zn = 0.2%, *Ascophyllum nodosum* = 64%, and fulvic acid = 6.5%.

The experimental design was completely randomized using a 3 × 2 + 2 factorial design. The first factor corresponded to the biostimulant with *A. nodosum* and fulvic acid (FA) applied at different soybean phenological stages: V3, V3R1, and V3R1R4. The second factor corresponds to the electrical conductivity of the irrigation water (ECw), with two levels: 0.5 (S0) and 5.0 dS m^{-1} (S1). There were also two additional controls: a positive control (S0 without a biostimulant) and a negative control (S1 without a biostimulant). The experiment included five replications, totaling 40 units.

Nondestructive analyses were performed for all treatments at 52 days after sowing (DAS). Plant height was measured using a graduated ruler, and stem diameter was measured using a digital caliper.

The net photosynthetic rate (*A* - µmol CO$_2$ m^{-2} s^{-1}), transpiration (*E* - mmol H2O m^{-2} s^{-1}), stomatal conductance (*gs* – mol m^{-2} s^{-1}), and internal CO$_2$ concentration (Ci - µmol CO$_2$ mol^{-1} air^{-1}) were measured at 49 DAS, between 9:00 a.m. and 12:00 a.m., on the third fully expanded leaf, using a portable infrared gas analyzer (IRGA; portable model LI-6400XT, Li-Cor Biosciences Inc., Lincon, Nebraska, USA).

Destructive analyses were performed for all treatments at 65 DAS, and the following variables were evaluated. Root

Table 1. Characteristic of the soil of the experimental

Layer (cm)	P (mg dm^{-3})	OM (g kg^{-1})	pH H$_2$O	K$^+$	Ca^{2+}	Mg^{2+} (mmol$_c$ dm^{-3})	Na$^+$	H$^+$+Al^{3+}	Al^{3-}	SB (mmol$_c$ dm^{-3})	CEC	m
0-20	3	3	6	0.4	5	3.6	0.1	8	0	9.1	17.1	0

SB- Sum of exchangeable bases; OM – Organic matter; CEC – Cation exchange capacity; m – Aluminium saturation percentage

length was measured using a graduated ruler. Leaf area was determined using a surface integrator (LI-3100; Li-COR, Inc., Lincoln, NE, USA). Shoot dry mass and total dry mass were obtained by drying the samples in an oven at 65 °C until a constant dry mass was reached, and then weighing them on a precision scale.

Leaf water potential (Ψwl) was assessed at 50 DAS in fully expanded leaves starting from the apex, using a Scholander-type pressure chamber (Soil Moisture Equipment Corporation, 3005, Santa Barbara, CA, USA). Electrolyte leakage was determined using the methodology described by Singh et al. (2007) and photosynthetic pigments (chlorophyll a, b, total chlorophyll, and carotenoids) were quantified as described by Wellburn (1994).

The results were subjected to analysis of variance (ANOVA), and normality was assessed using the Shapiro-Wilk test. When significant differences were found by the F-test, means were compared using the Duncan's test (p≤0.05). The positive and negative controls were compared to the other treatments using Dunnett's test (p≤0.05). Statistical analyses were performed using R software (R Core Team, 2017) and graphs were generated using Sigma Plot (2007) (version 11.0).

Results and Discussion

Analysis of plant height revealed a significant interaction between biostimulant application and salinity. At the V3 phenological stage, in the absence of salinity (S0), there was a significant increase in plant height, which was 11.1 to 13.1% higher than that of the other treatments (Figure 1A). In contrast, in the presence of salinity (S1), the application at the V3 stage resulted in a poorer performance, with an increase of only 3.9 to 7.0% compared with the other applications. Under non-saline conditions (S0), plant height was 3.5% higher than that of the positive control, whereas under saline conditions (S1), height was 1.9% lower than that of the negative control. It is worth noting that application at the V3 stage under S0 conditions showed a significant difference compared to the positive control.

Biostimulants often include components such as amino acids and plant hormones, with auxin being a notable example (Battacharyya et al., 2015), because it plays a pivotal role in the longitudinal growth of plants (Taiz et al., 2017). In biostimulants containing seaweed extract, it has been observed that soybean plants achieved greater heights even when subjected to osmotic stress and ion toxicity (Ali et al., 2022).
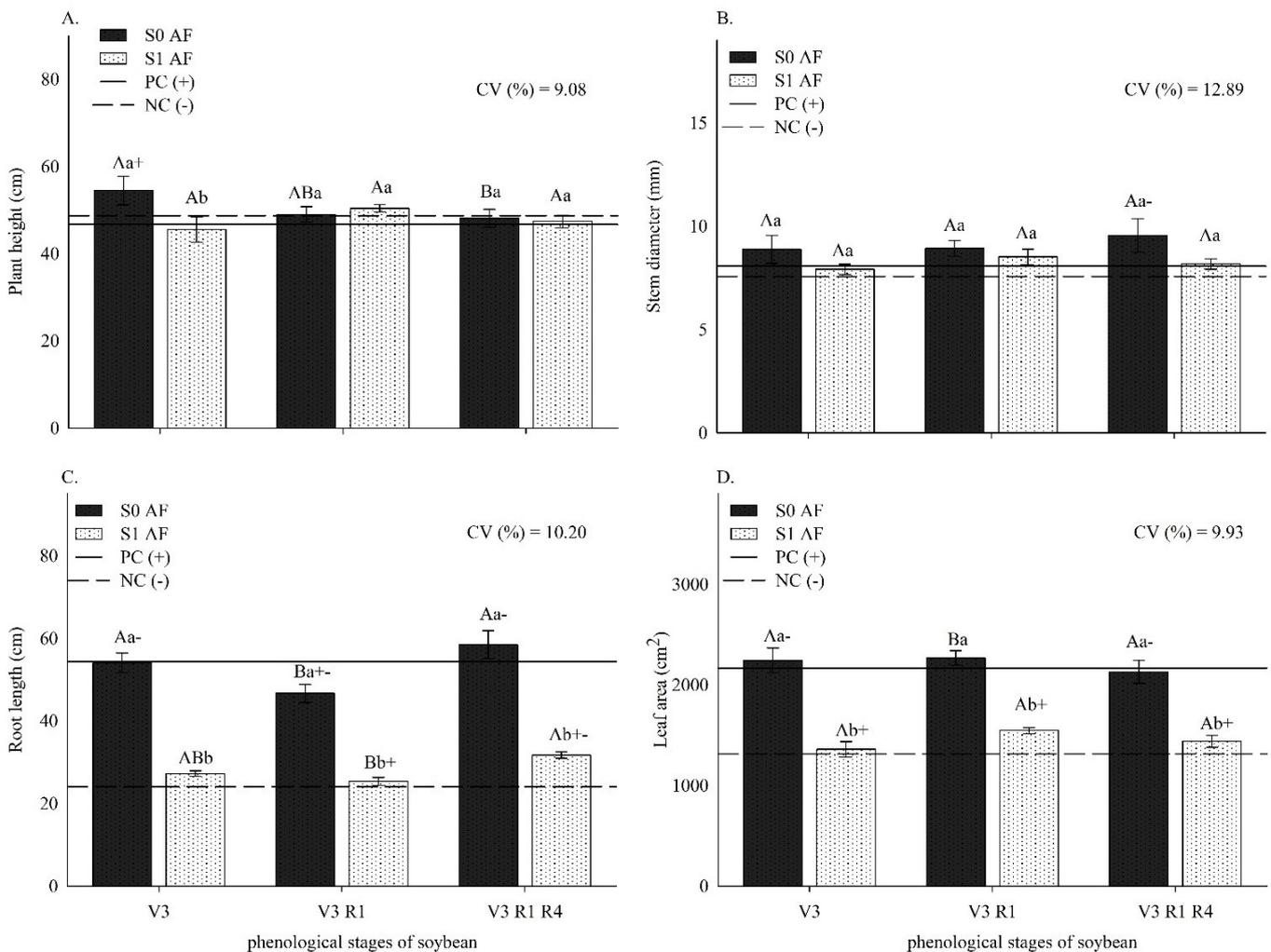
Means followed by the same uppercase letter do not differ for developmental stage, and means followed by the same lowercase letter do not differ for salt stress according to Duncan's test (p≤0.05). The symbols (+) and (-) indicate differences with the positive control and negative control treatments, respectively, based on Dunnett's test (p≤0.05)

Figure 1. Plant height (A), stem diameter (B), root length (C), and leaf area (D) at different phenological stages of soybean (*Glycine max*) grown under saline stress and application of *Ascophyllum nodosum* and fulvic acids

Stem diameter did not exhibit distinct behavior among the different biostimulant applications and salinities (Figure 1B). However, plants under saline stress conditions with biostimulants had a stem diameter that was 7.6% larger than that of the negative control. As excess salts influence the water potential of plants, the flow of water inside affects the resulting thin and slender stems (Acosta-Motos et al., 2017; Wang et al., 2017). Therefore, biostimulants may reduce water flow impairment in plants.

Root length revealed an interaction between the factors analyzed. In the absence of salinity (S0), applications at the V3 and V3R1R4 stages resulted in superior root lengths, whereas the V3R1 application showed a significant reduction, ranging from 15.7 to 25.2% (Figure 1C). Under saline conditions (S1), the greatest root lengths were observed in applications at stages V3 and V3R1R4, whereas the V3R1 application showed the lowest results, with reductions ranging from 7.6 to 25.2%. It is important to highlight that all applications under saline (S1) and the V3R1 stage under non-saline conditions (S0) showed differences compared to the positive control. In contrast, all applications under non-saline conditions (S0) at the V3R1R4 stage differed from those of the negative control. Notably, the root length of the negative control plants was 16.5% shorter than that observed under saline conditions (S1). Plants in the negative control group had shorter roots, which compromised water absorption and replenishment (Acosta-Motos et al., 2017). Indeed, biostimulants containing humic substances stimulate root growth by activating H^+-ATPase (Canellas et al., 2015).

Salinity had a significant effect on leaf area (Figure 1D). In the absence of salinity (S0), the largest leaf area was recorded at the V3R1 stage, whereas the smallest leaf area was observed at the V3R1R4 stage. Under saline conditions (S1), the largest leaf area was observed in the V3R1 stage, with a reduction of 5.0 to 6.0% in the V3R1R4 stage compared to this condition. The V3 and V3R1R4 applications in S0 showed distinct results compared to the negative control. Furthermore, all

the applications in S1 differed significantly from those in the positive control. It is important to highlight that, as an adaptation mechanism to salinity, glycophytic plants such as soybeans tend to reduce their leaf area as a strategy to minimize water loss through transpiration (Sharif et al., 2019). However, in response to biostimulant application, the soybean plants exhibited a larger leaf area than the negative control group, resulting in higher photosynthetic rates.

In plants grown under non-saline conditions (S0), the highest shoot dry mass was observed after the V3 application (Figure 2A). However, there was a reduction in shoot dry mass ranging from 3.8 to 11.5% in plants subjected to application at the V3R1R4 stage. Under saline conditions (S1), the lowest shoot dry mass was recorded in the V3 treatment, whereas the highest was observed in the V3R1 treatment. Remarkably, soybean plants under salt stress (S1) exhibited higher shoot dry mass than those in the negative control.

In plants grown under non-saline conditions (S0), the highest total dry mass accumulation was obtained with V3 application, with a reduction ranging from 0.9 to 7.7% at the V3R1R4 stage (Figure 2B). In contrast, under saline conditions (S1), the V3R1 stage had the highest total dry mass, whereas the lowest was observed in the V3 treatment. Remarkably, the V3R1 stage under the S1 conditions was 3.2% higher than that of the negative control. All applications in S0 demonstrated significant differences compared with the negative control, as did all applications in S1. It is important to emphasize that the inhibition of cell division and elongation induced by salt stress is recognized as the primary cause of reduced growth and biomass production (Nigam et al., 2022).

The biostimulant had a significant effect on leaf water potential (Ψwl) (Figure 3A). Under non-saline conditions (S0), the Ψwl approached zero in the V3R1 stage. The same application in plants subjected to salinity (S1) showed a less negative result, while the V3R1R4 stage resulted in an even more negative Ψwl. All applications in S0 demonstrated significant differences compared with the negative control, as
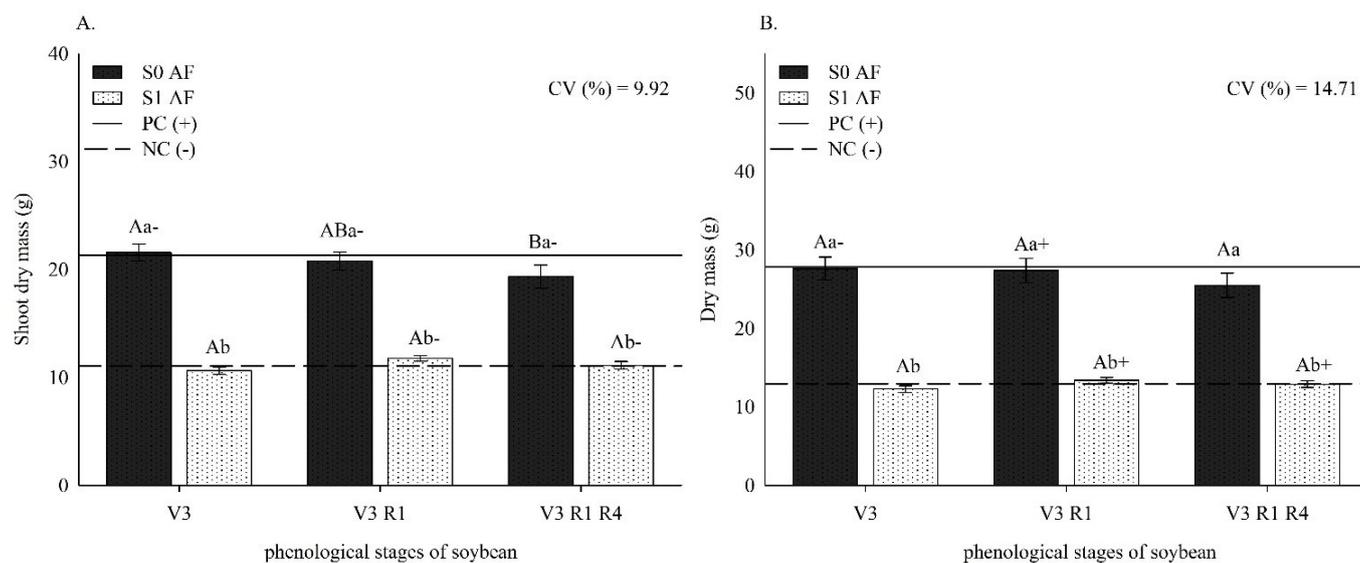
Means followed by the same uppercase letter do not differ for developmental stage, and means followed by the same lowercase letter do not differ for salt stress according to Duncan's test (p≤0.05). The symbols (+) and (-) indicate differences with the positive control and negative control treatments, respectively, based on Dunnett's test (p≤0.05)

Figure 2. Shoot dry mass (A) and total dry mass (B) at different phenological stages of soybean (*Glycine max*) grown under saline stress and application of *Ascophyllum nodosum* and fulvic acids
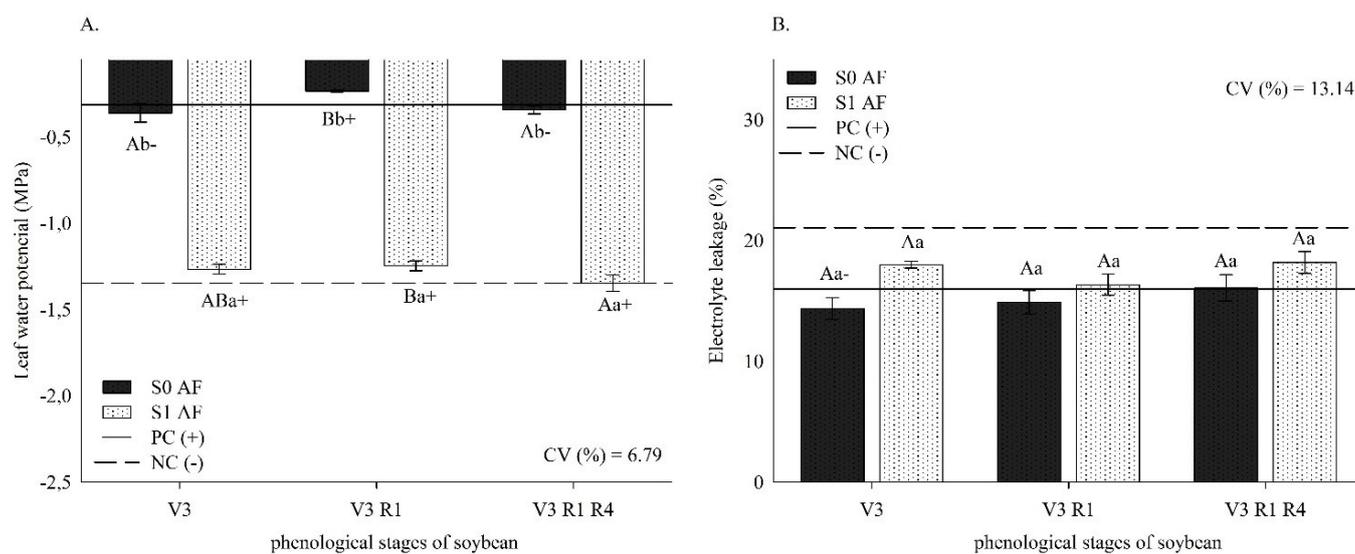
Means followed by the same uppercase letter do not differ for developmental stage, and means followed by the same lowercase letter do not differ for salt stress according to Duncan's test (p≤0.05). The symbols (+) and (-) indicate differences with the positive control and negative control treatments, respectively, based on Dunnett's test (p≤0.05)

Figure 3. Leaf water potential (A) and electrolyte leakage (B) at different phenological stages of soybean (*Glycine max*) grown under saline stress and application of *Ascophyllum nodosum* and fulvic acids

did all applications in S1 compared with the positive control. Notably, the leaf water potential of the negative control group was 4.5% lower than that of the plants under salt stress (S1). This result indicates an adaptation of plants to salt stress with a less negative leaf water potential, which can be partly attributed to biostimulant application.

Excessive salt accumulation in the root zone leads to a decrease in osmotic potential, resulting in a subsequent reduction in water potential, which, in turn, limits water availability to plants (Moura et al., 2019). In the present study, we observed that the leaf water potential of soybean plants became more negative owing to salinity. However, despite this adverse effect, the results were superior to those observed in the negative control group.

Biostimulant application in combination with salinity had no significant effect on electrolyte leakage (Figure 3B). However, it is worth noting that under saline conditions (S1), the result was lower than that observed in plants in the negative control group, with the V3R1 stage showing a reduction of 10.1 to 11.2%. Under non-saline conditions (S0), the V3 stage showed a distinct result compared with the negative control group. Notably, an increase in Na^+ and Cl^- ions in the root environment can lead to increased formation of ROS, resulting in peroxidation and oxidation of the cell membrane (Taiz et al., 2017; Xavier et al., 2022). Although the results did not reach statistical significance, it was observed that under saline conditions (S1), electrolyte leakage was lower than that observed in the negative control group, suggesting the effectiveness of the biostimulant in maintaining cell membrane integrity.

In plants grown under non-saline conditions (S0), the chlorophyll a content did not show significant differences. This content continued to increase throughout the different applications, ranging from 9.4 to 11.3% (Figure 4A). In contrast, in plants under saline conditions (S1), the highest chlorophyll a content was observed at the V3 stage, whereas the V3R1R4 stage had the lowest content, with a reduction ranging from 4.7

to 36.6%. It was observed that the V3 application in S0 differed significantly from the positive control group. It is noteworthy that the negative control group (S1 without biostimulant) presented a lower chlorophyll a content than that observed in plants subjected to salinity stress (with biostimulant), whereas plants in S0 (with biostimulant) presented a chlorophyll a content of 19.6%, higher than that of the positive control group (S0 without biostimulant).

No significant differences were observed in the chlorophyll b content of plants grown under non-saline conditions (S0) in relation to biostimulant application or salinity. However, an increase of 6.5 to 15.2% was observed in the V3R1R4 stage (Figure 4B). In contrast, in plants subjected to salinity (S1), the highest chlorophyll b value was obtained at the V3R1 stage, with a significant reduction ranging from 45.2 to 46.7%, at the V3R1R4 stage. An increase in the total chlorophyll content was observed across different biostimulant applications in both S0 and S1 (Figure 4C). The highest values were recorded in plants that received only the V3 stage, with a significant reduction ranging from 39.1 to 41.4% with the V3R1R4 application. Notably, the negative control group had a higher total chlorophyll content than that observed in plants subjected to salinity (S1), whereas plants in S0 had a total chlorophyll content 19.8% higher than that of the positive control group.

Salt stress can trigger changes in the concentration of photosynthetic pigments and enzyme activities (Shahverdi et al., 2019). The activity of chlorophyllase, which is responsible for chlorophyll degradation, tends to increase under saline conditions (Soares et al., 2021). Despite the detrimental effects caused by salts, there was a progressive increase in the contents of chlorophyll a, b, and total chlorophyll throughout the biostimulant applications. Therefore, it is plausible that a higher content of these pigments reflects a greater capacity for light capture, and consequently, a higher photosynthetic rate (Shahverdi et al., 2019).

In the biostimulant applications under non-saline conditions (S0), no significant differences were observed in the
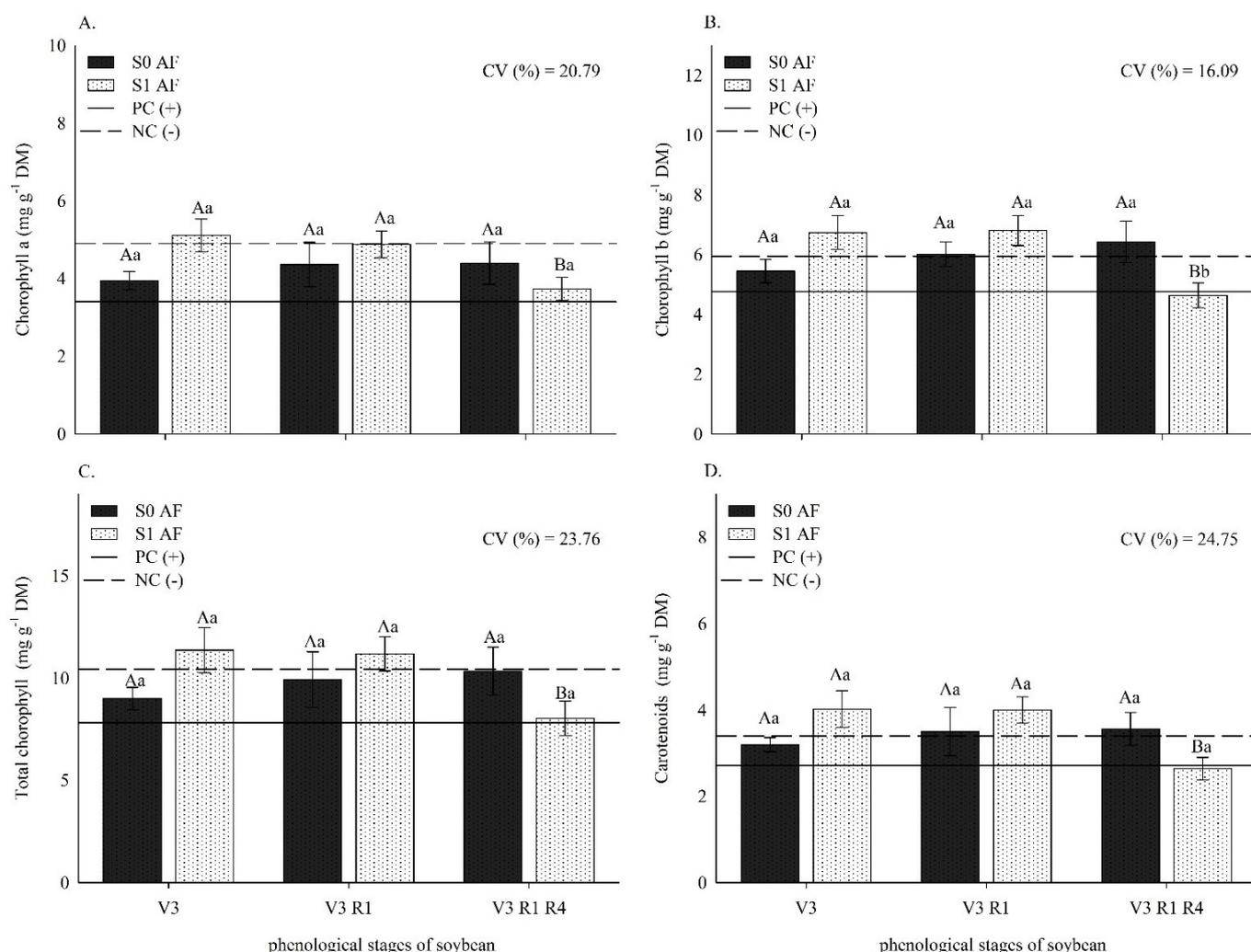
Means followed by the same uppercase letter do not differ for developmental stage, and means followed by the same lowercase letter do not differ for salt stress according to Duncan's test (p≤0.05). The symbols (+) and (-) indicate differences with the positive control and negative control treatments, respectively, based on Dunnett's test (p≤0.05)

Figure 4. Chlorophyll a (A), chlorophyll b (B), total chlorophyll (C), and carotenoids (D) at different phenological stages of soybean (*Glycine max*) grown under saline stress and application of *Ascophyllum nodosum* and fulvic acids

carotenoid content (Figure 4D). However, in plants subjected to salinity (S1), the highest content was observed in the V3 and V3R1 stages, with a significant reduction ranging from 34.0 to 34.3% at the V3R1R4 stage. The carotenoid content of the S1 plants was 4.2% higher than that of the negative control group. Additionally, S0 plants showed a carotenoid content 20.4% higher than that of the positive control group.

Carotenoids play a crucial role as photoprotective pigments, aiding in the thermal dissipation of energy. Under conditions of excess salt, a reduction in carotenoid content is commonly observed, as evidenced by previous studies, such as those by Sharif et al. (2019) with cotton. Despite the detrimental effects of salt stress and the associated oxidative stress, soybean plants treated with the biostimulant showed an increase in carotenoid concentration compared with the negative control group. This suggests that soybean plants that received the product became more efficient at neutralizing ROS and protecting the photosynthetic apparatus.

The photosynthetic rates in the V3 and V3R1 stages of plants grown under non-saline conditions (S0) did not differ, and these applications resulted in higher photosynthetic rates (Figure 5A). However, there was a reduction in photosynthetic rates ranging from 17.2 to 17.8%, in plants at the V3R1R4 stage.

In contrast, in plants subjected to salinity (S1), the V3 and V3R1 stages did not show significant differences, and unlike S0, the V3R1R4 stage resulted in an increase in photosynthetic rates, ranging from 26.8 to 30.5%. Notably, both the S0 and S1 plants exhibited photosynthetic rates that were 29.2 and 39.6% higher than those of the positive and negative control groups, respectively. At S0, the V3 and V3R1 stages differed significantly from those in the additional control groups. V3R1 application in S1 yielded different results compared to the positive control. Furthermore, the V3R1R4 stages in both S1 and S0 showed significant differences compared with the negative control group.

Salinity can affect photosynthesis owing to both stomatal and non-stomatal limitations, resulting in reduced carbon assimilation. To reduce water loss through transpiration, plants reduce stomatal conductance, leading to a decrease in the influx of CO_2 into the RuBisCO enzyme sites (Ali et al., 2022). The application of the biostimulant showed that the plants exhibited greater stomatal opening, which promoted higher photosynthetic rates than the plants in the control group.

The transpiration rate showed a significant interaction between the biostimulants and salinity. No significant differences were observed after biostimulant application in
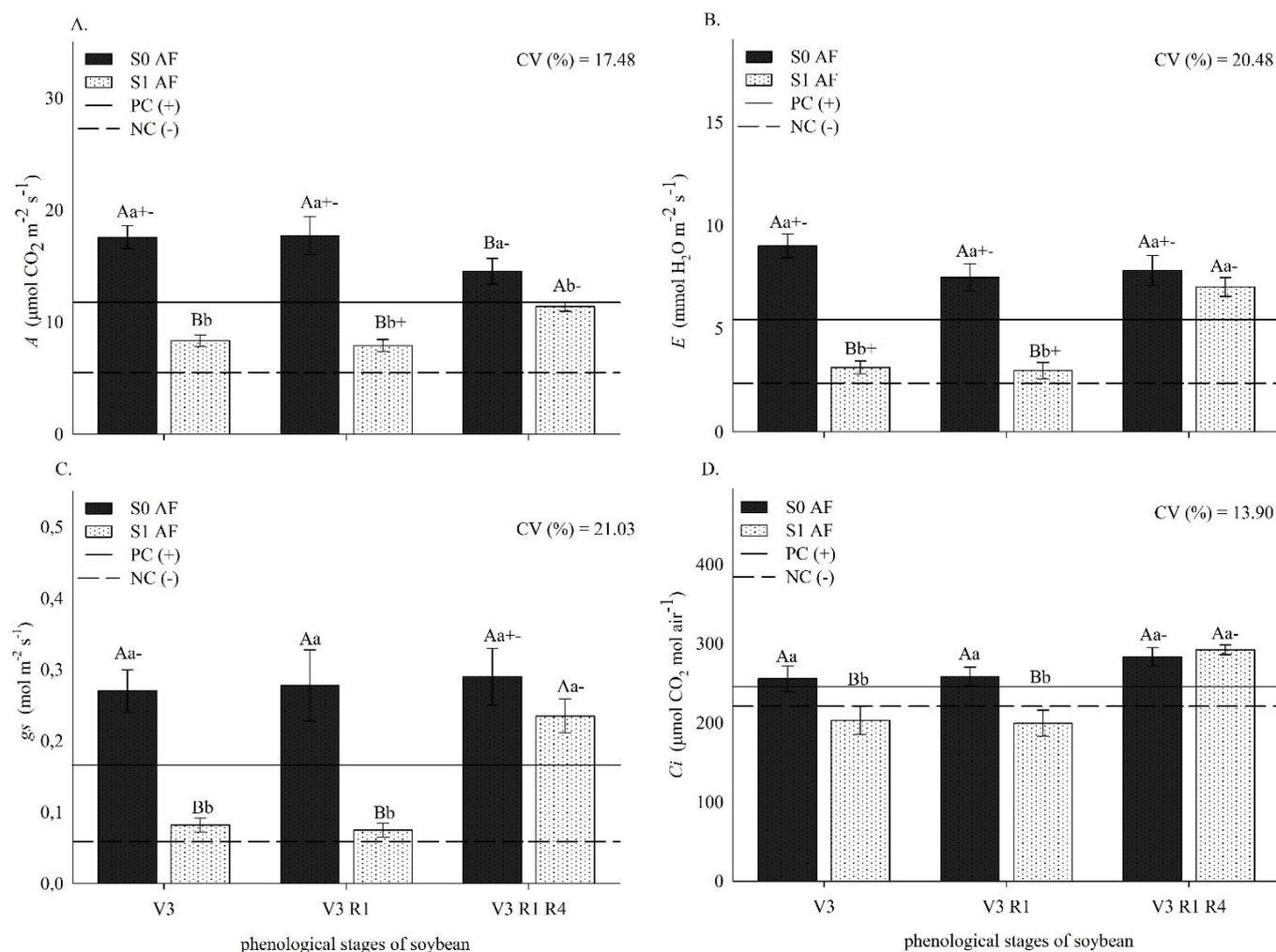
Means followed by the same uppercase letter do not differ for developmental stage, and means followed by the same lowercase letter do not differ for salt stress according to Duncan's test (p≤0.05). The symbols (+) and (-) indicate differences with the positive control and negative control treatments, respectively, based on Dunnett's test (p≤0.05)

Figure 5. Net photosynthesis - *A* (A), transpiration - *E* (B), stomatal conductance - *gs* (C), and internal CO_2 concentration - *Ci* (D) at different phenological stages of soybean (*Glycine max*) grown under saline stress and application of *Ascophyllum nodosum* and fulvic acids

plants grown under salinity-free conditions (S0) (Figure 5B). Notably, the V3 and V3R1 stages in plants subjected to salinity (S1) did not differ, and these applications showed the lowest transpiration rates. However, there was a significant increase of 65.0 to 68.0% in the transpiration rate in S1 plants that received the V3R1R4 stage. Plants from S0 and S1 showed transpiration rates that were 29.9 and 46.1% higher than those observed in the positive and negative control groups, respectively. All applications in S0 showed significant differences compared to the additional control groups. The V3 and V3R1 stages differed from the positive control group, and the V3R1R4 stage differed from the negative control group.

Under salt stress, plants tend to reduce their stomatal openings to decrease water loss through transpiration. However, the consequent reduction in stomatal opening owing to salinity leads to an increase in leaf temperature (Acosta-Motos et al., 2017). Indeed, the application of the biostimulant to soybean plants subjected to salt stress resulted in higher transpiration rates compared with the control groups, indicating that these plants were more efficient in cooling the leaf surface.

Stomatal conductance showed a significant interaction between the biostimulant and salinity (Figure 5C). Under saline conditions (S1), applications in the V3 and V3R1 stages resulted in low stomatal conductance, whereas the application at V3R1R4 showed a significant increase. The negative control group exhibited a stomatal conductance 55.2% lower than that observed in the S1 plants. Application of V3R1 differed significantly from the negative control, whereas application of V3R1R4 in both S0 and S1 differed from the control groups.

The internal CO_2 concentration progressively increased in plants grown under non-saline conditions (S0) across the biostimulant applications (V3R1R4 stage), resulting in an increase of 8.8 to 9.7% (Figure 5D). In contrast, in plants subjected to salinity (S1), the lowest internal carbon content was recorded in the V3R1 stage, while the highest was observed in the V3R1R4 stage, representing an increase of 30.5 to 31.7%. Compared with the negative control group, which exhibited an internal CO_2 concentration 4.8% lower than that of S1 plants, and the positive control group, which showed an 8.2% reduction in internal CO_2, the V3R1R4 stage in both S0 and S1 plants differed significantly from that of the negative control group.

The biostimulants applied to soybean plants under salt stress significantly improved stomatal control, resulting in

higher stomatal conductance (gs) values. This allowed for greater uptake of CO_2 and transpiration, which, in turn, helped reduce leaf temperature. Furthermore, plants treated with the biostimulants exhibited higher photosynthetic rates than those in the negative control group. Previous studies have highlighted the positive effects of seaweed extract-based biostimulants, such as increased stomatal conductance (Shukla et al., 2019) and reduced leaf temperature (Martynenko et al., 2016).

Conclusions

1. The biostimulant application improved the performance of soybean plants grown under salt stress conditions. This was reflected in the increased biomass production, plant height, and leaf area compared to plants in the negative control group.

2. This biostimulant demonstrated the ability to regulate stomatal control, thereby allowing greater stomatal opening. This facilitates the entry of CO_2, promoting higher photosynthetic rates and consequently, greater plant growth.

3. The results of this study highlight the potential use of biostimulants based on seaweed extracts and humic substances. The application carried out in the V3R1 stage of the biostimulant provided effectiveness in mitigating the adverse effects of salinity on soybeans.

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