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Proceeding Paper

A Biostimulant Based on Algae Extract and Fulvic Acids Is Able to Improve Photosynthetic Performance and Mitigate the Effects of Salinity in Soybean †

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† Presented at the 2nd International Electronic Conference on Plant Sciences—10th Anniversary of Journal Plants, 1–15 December 2021; Available online: https://iecps2021.sciforum.net/.

Abstract: The expansion of the salinization of agricultural areas limits the production of crops of high economic importance, such as soybeans. To attenuate the effects of salts on the plants and improve photosynthetic performance under salinity conditions, the application of a biostimulant based on seaweed extract (*Ascophyllum nodosum* (L.)) and fulvic acids as a physiological enhancer was adopted. In the present study, we evaluated whether applications of the biostimulant at different phenological stages can reduce damage to the photosynthetic apparatus in soybean plants under salt stress. The experiment was conducted in a greenhouse, and the design adopted was completely randomized in a double factorial scheme consisting of three applications (V3, V3/R1, and V3/R1/R4) and two levels of salinity of the irrigation water (S0—absence of salt and S1—saline solution at 5.0 dS m^{-1} prepared with the salts NaCl, $CaCl_2 \cdot 2H_2O$, and $MgCl_2 \cdot 6H_2O$ in the ratio 7:2:1). There were two additional controls without the application of the biostimulant (with and without stress) and five repetitions. Soybean plants were irrigated daily with the solutions, and a weekly depth of 25% higher than the demand of the culture was applied. The evaluations were carried out 49 days after sowing, evaluating the potential quantum efficiency of photosystem II (Fv/Fm), the effective quantum yield of PSII (ΦFSII), the photochemical (qP) and non-photochemical (qN) quenching, and the rate of electron transport (ETR). Plants subjected to irrigation with saline water showed reductions in the evaluated parameters, suggesting that the salts caused damage to the photosynthetic apparatus in the photochemical stage. The application of the biostimulant was effective in reducing damage to the photosynthetic apparatus, providing greater efficiency in dissipating excess energy and less reduction in ETR. The application that provided the best results was V3/R1.

Keywords: *Glycine max* (L.) Merril; abiotic stress; photosynthesis

Citation: Silva, B.A.d.; Castro, C.S.d.; Silva, J.d.S.; Costa, R.S.d.; Cardoso, F.B.; Mesquita, R.O. A Biostimulant Based on Algae Extract and Fulvic Acids Is Able to Improve Photosynthetic Performance and Mitigate the Effects of Salinity in Soybean. *Biol. Life Sci. Forum* **2022**, *11*, 92. https://doi.org/10.3390/IECPS2021-12041

Academic Editor: Feibo Wu

Published: 3 December 2021

1. Introduction

The salinization of soils around the world is one of the main causes of the reduced productivity of soybeans and other crops sensitive to high salt content [1]. The effects caused by salinity in plants are triggered by both osmotic and ionic components [2]; they are responsible for promoting changes in plant metabolism, reduced nutrient absorption, the excess accumulation of toxic ions in tissues, hormonal imbalances, and the loss of photosynthetic efficiency [3–5].

Biostimulants act to improve nutritional efficiency; with their attenuating effect, they improve the plant's defense mechanisms, increase growth, and improve photosynthetic rates. Among the main components of biostimulants are bioregulators, other substances such as seaweed extract (*Ascophyllum nodosum* (L.)), humic and fulvic acids, growth regulators, and amino acids [6].

Therefore, given the economic importance of soybean cultivation and soil salinization, this article aims to evaluate whether the use of a kelp extract (*Ascophyllum nodosum* (L.)) and fulvic acids applied at different phenological stages can reduce damage to the photosynthetic apparatus in soybean plants subjected to salt stress.

2. Materials and Methods

The experiment was carried out in a greenhouse covered with plastic of 200 UV microns from the Department of Crop Science, between the months of November 2020 and January 2021, Campus Pici, at the Federal University of Ceará, Fortaleza, Ceará, Brasil.

The biostimulant used includes natural compounds in its composition (sea algae and fulvic acids). A dose of 0.25 kg ha^{-1} of the biostimulant was applied as recommended for soybean by the manufacturer. For the imposition of salt stress, the electrical conductivity (EC) was gradually increased from 2.5 dS m^{-1} until reaching an EC of 5.0 dS m^{-1}; the plants were irrigated for 2 days in this EC. For the characterization of salt stress, a solution composed of three salts was prepared: NaCl, $CaCl_2 \cdot 2H_2O$, and $MgCl_2 \cdot 6H_2O$ in the proportion of 7:2:1 [7].

The seeds used were Monsoy 8349 IPRO, sanitized and inoculated with Bradyrhizobium japonicum. Field capacity (FC) was determined according to Souza et al. (2000), considering the difference between the weight of wet soil after saturation and free drainage and the weight of air-dried soil.

The first application was performed when the plants were at stage V3 (15 DAS) and before the imposition of salt stress. Salt stress was imposed at phenological stage V4 (20 DAS). Irrigation was carried out daily, keeping the soil at 80% of the CRA. The saline solution was applied to the soil for six days, and on the seventh day, a leaching layer was applied, 25% higher than the crop demand, in order to reduce the accumulation of salts in the soil. The controls were sprayed with distilled water only.

The experimental design used was completely randomized in a factorial scheme (3×2) + 2. The first factor concerns the applications of the biostimulant in the phenological phases of the crop: V3, V3/R1, and V3/R1/R4. The second factor corresponds to the electrical conductivity of the irrigation water: S0 (absence of salt) and S1 (saline solution), plus two controls, one positive (S0; without biostimulant) and the other negative (S1; without biostimulant), with five replications, totaling 40 experimental units.

The evaluations were carried out 49 days after sowing; we evaluated the potential quantum efficiency of photosystem II, the potential quantum efficiency of photosystem II (Fv/Fm), the effective quantum yield of PSII (ΦFSII), the photochemical (qP) and non-photochemical (qN) quenching, and the rate of electron transport (ETR). The analysis was carried out from 09:00 to 12:00 in the third completely expanded trefoil using an infrared gas analyzer (IRGA; model Li6400XT, LI-COR Biosciences Inc., Lincoln, NE, USA) with CO_2 concentration in the chamber at 400 µmol mol^{-1} and 1500 µmol photons m^{-2} s^{-1}.

The results were submitted to an analysis of variance (ANOVA), the Shapiro-Wilk normality and homogeneity test and, when found to be significant by the F test, they were also submitted to mean comparison analysis by the Tukey test. The additional controls (positive and negative controls) were compared to the other treatments using the Dunnett means test considering a 5% probability ($p < 0.05$). For statistical analysis, the computer program RStudio was used, and for making the graphics, SigmaPlot version 11.0 was used.

3. Results

The application of the biostimulant to plants with salt stress provided a 4.38% increase in photochemical efficiency (Figure 1A). The effective quantum yield of PSII (Figure 1B) showed a result of 33.78% in the V3/R1/R4 application in plants with stress compared to the negative control (S1; without biostimulant).

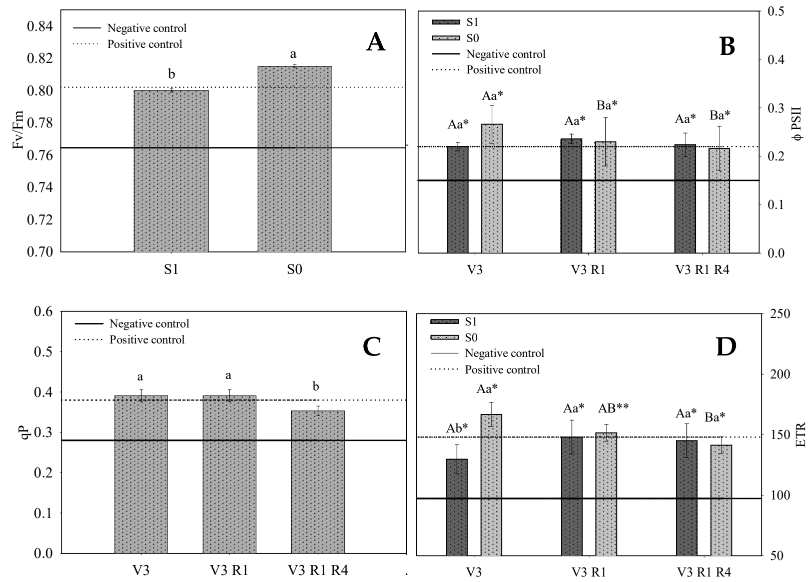

Figure 1. Effects of biostimulant applications based on algae extract and fulvic acids in soybean under saline stress. Potential quantum efficiency of photosystem II (**A**), Effective quantum yield of PSII (**B**), quenching photochemical (**C**), and the rate of electron transport (**D**). Means followed by the same letters do not differ statistically using the Student's *t*-test at 5% probability. The absence of capital letters indicates that there were no significant differences.

For the quenching photochemical (Figure 1C), among applications with and without salt stress, those performed in V3 and V3/R1 were superior to the others. In general, plants subjected to stress were 26.32% higher than the negative control (S1; without biostimulant), and the positive control (S0; without biostimulant) was 1.55% higher than the treatment without stress. The electron transport rate (Figure 1D) had the highest average when the biostimulant was applied only in V3 in plants without stress. In plants with stress, the best result was obtained in applications V3/R1/R4: the result of 145.86 was 33.94% higher than the negative control (S1; without biostimulant).

In the analysis of the non-photochemical quenching (Figure 2), the application carried out in V3/R1 in plants presented a result superior to the other applications and was 8.04% superior to the negative control (S1; without biostimulant). Later, in the application V3/R1/R4, the mean of the variable in the same treatment reduced 47.44%. In the treatment without stress, it was observed that only in V3 did the application present a value lower than the other applications; it was 18.60% lower than the positive control (S0; without biostimulant).

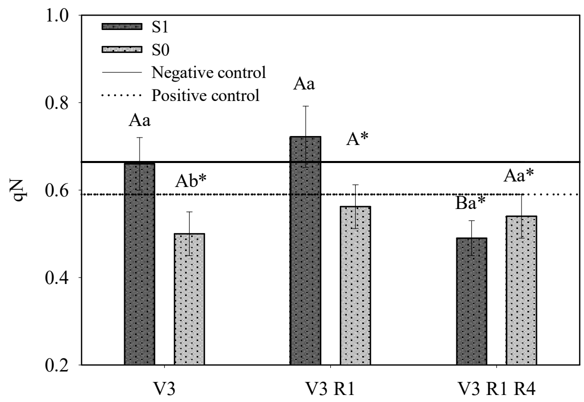

Figure 2. Non-photochemical quenching in soybean under saline stress and the application of the biostimulant based on algae extract and fulvic acids. Means followed by the same letters do not differ statistically using the Student's *t*-test at 5% probability. The absence of capital letters indicates that there were no significant differences.

4. Discussion

A high salt content causes changes in the composition and function of the photosynthetic apparatus of plants and in the functional state of thylakoid membranes in chloroplasts, which cause changes in the characteristics of the fluorescence signals and the potential quantum yield, especially of photosystem II [8], which is an indicator of the efficiency in the use of photochemical radiation and, consequently, in the assimilation of carbon by plants [9].

The potential photochemical efficiency (Figure 1A) was lower in plants with salt stress. This is mainly due to the damage that may be related to the reduction in the intercellular concentration of CO_2 due to stomatal closure, the increase in lipid peroxidation due to the deviation of the electron flow from CO_2 assimilation to the reduction of O_2, and a drop in photosynthetic activity [10]. The PSII quantum yield (Figure 1B), despite having reduced values compared to the treatment without stress, was superior to the negative control. The photochemical quenching (Figure 1C) showed better results when the biostimulant was applied in V3 and V3/R1.

The electron transport rate (Figure 1D) showed a reduction only in the V3 application, probably due to the increase in the salt content in the soil and, therefore, a reduction in the availability of water for the plants. The photosynthetic electron transporters are inactivated with the increase in the osmotic potential, which can reach an irreversible level [11]. As seen in the V3/R1/R4 application, the results probably showed an increase in the use of the biostimulant.

The parameter non-photochemical quenching is induced by changes in the trans-thylakoid pH-gradient, photoinhibitory processes [12], the application of the biostimulant performed in V3/R1/R4 showed a decline in non-photochemical extinction (Figure 2).

Author Contributions: Conceptualization, B.A.d.S.; methodology, C.S.d.C.; software, J.d.S.S.; validation, R.O.M. and J.d.S.S.; formal analysis, B.A.d.S.; investigation, B.A.d.S. writing—review and editing, R.S.d.C.; visualization, B.A.d.S. and C.S.d.C.; supervision, R.O.M.; supervision, F.B.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Council for Scientific and Technological Development (CNPq).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Acknowledgments: The authors wish to thank the Federal University of Ceara for laboratory support.

Conflicts of Interest: The authors declare no conflict of interest.

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