

Proceeding Paper

# Biostimulant Based on Algae Extract and Fulvic Acids Is Able to Improve Photosynthetic Performance and Mitigate the Effects of Salinity in Soybean <sup>†</sup>

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**Abstract:** The expansion of salinization of agricultural areas limits the production of crops of high economic importance such as soybeans. To attenuate the effects of salts on the plant 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, consisting of three applications (V3, V3/R1 and V3/R1/R4), two levels of salinity of the irrigation water (S0—absence of salt and S1—saline solution at 5.0 dS m<sup>-1</sup> prepared with the salts NaCl, CaCl<sub>2</sub>·2H<sub>2</sub>O and MgCl<sub>2</sub>·6H<sub>2</sub>O in the ratio 7:2:1) with two additional controls without 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 in V3/R1.

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

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## 1. Introduction

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

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

Therefore, given the economic importance of soybean cultivation and soil salinization, the 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, Brazil.

The Biostimulant used has natural compounds in its composition (sea algae and fulvic acids). The dose of 0.25 kg ha<sup>-1</sup> of the biostimulant was applied as recommended by the manufacturer for soybean. For the imposition of salt stress, the electrical conductivity (EC) was gradually increased from 2.5 dS m<sup>-1</sup> until reaching an EC of 5.0 dS m<sup>-1</sup>, being irrigated for 2 days in this EC. For the characterization of salt stress, a solution composed of three salts was carried out: NaCl, CaCl<sub>2</sub>·2H<sub>2</sub>O and MgCl<sub>2</sub>·6H<sub>2</sub>O, in the proportion of 7:2:1, according to Rhoades et al. (1992).

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), 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 (Macêdo et al., 2007). Controls were sprayed with distilled water only.

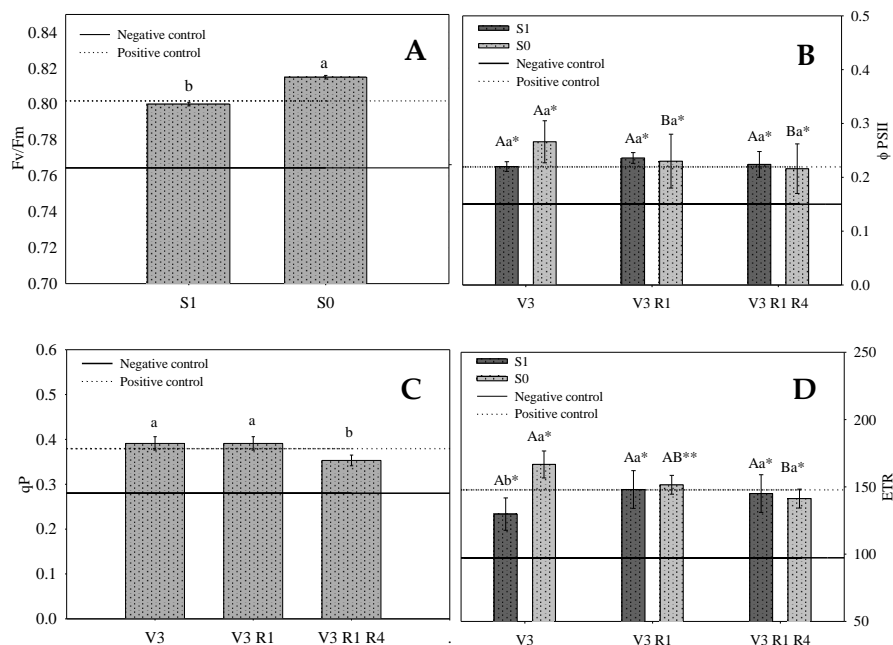
The experimental design used was completely randomized, in a factorial scheme (3 × 2) + 2; the first factor concerns the applications of the biostimulant in the phenological phases of the crop: V3, V3/R1 and V3/R1/R4, the second factor corresponds to the electrical conductivity of the irrigation water, 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 evaluating 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 of the 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, Nebraska, USA) with CO<sub>2</sub> concentration in the chamber at 400 μmol mol<sup>-1</sup> and 1500 μmol photons m<sup>-2</sup> s<sup>-1</sup>.

The results were submitted to analysis of variance (ANOVA), the Shapiro-Wilk normality and homogeneity test and when significant by the F test, they were 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

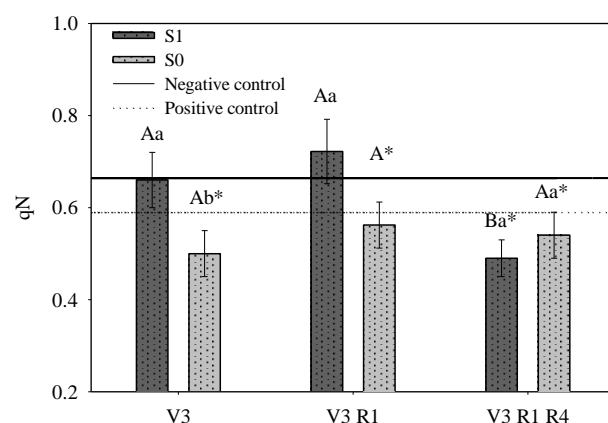
The application of the biostimulant in plants with salt stress provided a 4.38% increase in photochemical efficiency (Figure 1A). The effective quantum yield of PSII (Figure 1B) showed a result of 33.78% in the V3/R1/R4 application in plants with stress compared to the negative control (S1; without biostimulant).



**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 t test at 5% probability. The absence of capital letters indicates that there were no significant differences.

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; and 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, 145.86, it was 33.94% higher than the negative control (S1; without biostimulant).

The non-photochemical quenching (Figure 2), the application carried out in V3/R1 in plants with this one, 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, in the application only in V3 it presented a value lower than the other applications and 18.60% lower than the positive control (S0; without biostimulant).



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

#### 4. Discussion

The 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, in addition to the potential quantum yield, especially of photosystem II [7] which is an indicator of the efficiency in the use of photochemical radiation and, consequently, in the assimilation of carbon by plants [8].

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<sub>2</sub> due to stomatal closure, the increase in lipid peroxidation due to the deviation of the electron flow from CO<sub>2</sub> assimilation to the reduction of O<sub>2</sub> and a drop in activity photosynthetic [9]. The PSII quantum yield (Figure 1B), despite having reduced values compared to the treatment without stress, it 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) in the application only in V3 showed a reduction, 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 of the osmotic potential, which can reach an irreversible level [10]. As seen in the V3/R1/R4 application, the results probably showed an increase in the use of the biostimulant.

The non-photochemical quenching (Figure 2) among the application of the biostimulant performed in V3/R1/R4, there was a decline in non-photochemical extinction.

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

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