

## Article

# Saccorhiza polyschides Extract as Biostimulant for Reducing Salt Stress Effect in Common Bean Crops

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**Abstract:** Salinity stress emerges as an important menace to global food security, particularly affecting susceptible crops such as common bean plants (*Phaseolus vulgaris* L.). The disruption of ion balances caused by salt stress within plant cells reduces water and nutrient absorption and hampers growth and productivity. *Saccorhiza polyschides*, a brown macroalga rich in essential elements and macrominerals, has drawn attention as a natural biostimulant, being a potential remedy to bolster crop resilience against salt stress. This study delves into the effect of water extracts of *Saccorhiza polyschides* (SPE) across varying concentrations (1%, 2%, and 5%) in common bean plants under salt stress (68.4 mM NaCl) conditions. Two different methods of application were used: by spraying the aerial part of the plant or by irrigation together with the watering solution. The effects of both systems of application were studied on the aerial parts of the plants. SPE exhibited significant enhancements in morphological parameters, chlorophyll content, carbon–nitrogen activities, and antioxidant enzymatic system activities, alongside increasing osmolyte accumulation, compared to salt-stressed plants without biostimulation. Moreover, SPE reduced reactive oxygen species (ROS) levels caused by salt stress, mitigating lipid peroxidation. The concentrations of 2% and 5% SPE applied by spraying showed the most significant results in most of the parameters analyzed. By shedding light on the underlying science behind this biostimulant effect, this research underscores the potential of SPE to enhance crop resilience and augment food production in salt-challenged environments.

**Keywords:** abiotic stress; algal extract; enzyme activities; osmolytes; *Phaseolus vulgaris* L.; reactive oxygen species



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## 1. Introduction

Salinity stress is an environmental constraint that produces substantial limitations on crop productivity and quality worldwide [1], especially in those crops that are sensitive to this stress such as common bean (*Phaseolus vulgaris* L.) [2]. It was estimated that up to 20% of irrigated lands in the world are affected by different levels of salinity and sodium content [1]. Common beans are a vital source of proteins and essential nutrients for millions of people worldwide. However, plant growth and productivity can be significantly

affected by environmental stressors, with salt stress being a major concern [3]. Excessive salt concentration produces damage in common bean plants by disrupting water uptake, producing ion toxicity and interfering with nutrient uptake [4]. For instance, excessive salts in the soil can replace essential nutrients like potassium ( $K^+$ ) and calcium ( $Ca^{2+}$ ), affecting vital cellular processes [5]. Ion toxicity results from the buildup of toxic amounts of  $Na^+$  in plant tissues and cells, which negatively impacts the ability of the plant to grow and develop.  $Na^+$  is not necessary for plants, in contrast to animals (except in some C4 plants) [6,7], and its overabundance will be extremely harmful to common bean plants, causing cytosolic  $K^+$  efflux, which will disrupt cellular homeostasis and oxidative stress; interfere with  $K^+$  and  $Ca^{2+}$  functions; and cause nutrient shortage, stunted growth, and even cell death [8,9].

Salt stress significantly affects common bean plants by impairing their growth and physiological processes, leading to reduced photosynthetic efficiency, ion imbalance, and oxidative stress [10]. The oxidative stress caused by salinity in common beans increases the formation of reactive oxygen species (ROS), such as superoxide anion ( $O_2^{\cdot-}$ ) and hydrogen peroxide ( $H_2O_2$ ), which can perturbate the structure of cells, leading to DNA mutation, protein denaturation, and lipid peroxidation [11,12]. Maintaining vital processes in these conditions of high salinity therefore requires a resistance of the plant to dehydration by an adaptation of its osmotic potential to restore water relations and ensure a suitable water supply, also by means of an effective control of intracellular ion fluxes [13]. To prevent ROS-induced damage and reduce its negative effects, plant cells are outfitted with highly developed antioxidant defense systems, both enzymatic and non-enzymatic. Within each organelle of cells, there are one or more antioxidants that work to detoxify a specific ROS [14]; therefore, plants exposed to salinity and water stressors have adopted the important strategy of enhanced synthesis and accumulation of low molecular weight compounds. To survive under stressful circumstances, plants often store free amino acids, non-structural carbohydrates, and quaternary ammonium compounds [15].

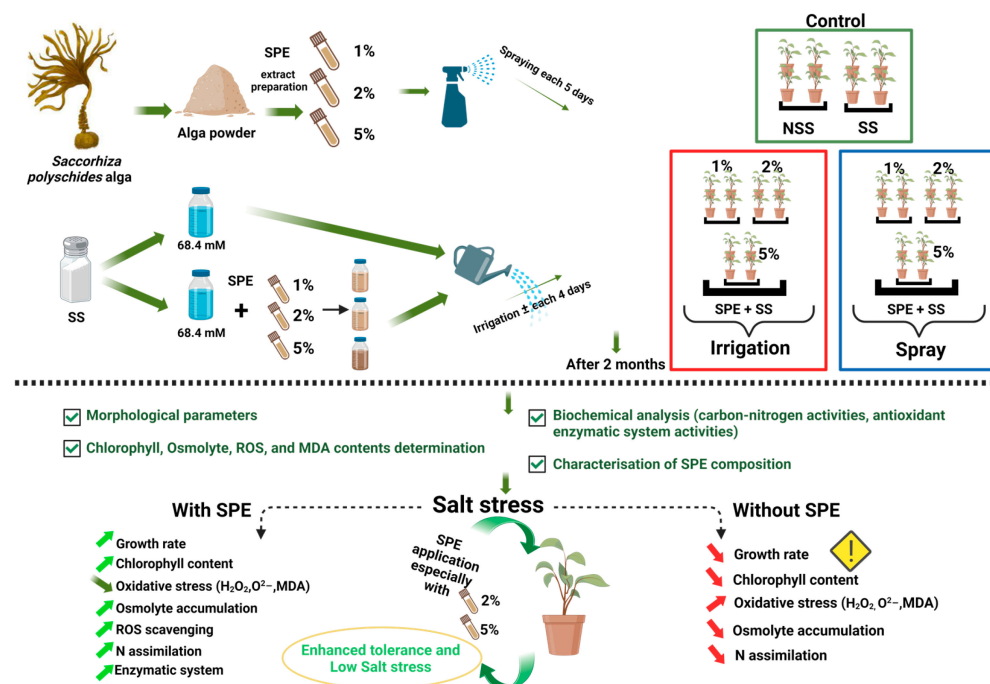
In recent years, natural biostimulant exploration has emerged as a promising approach for improving crop resilience against salt stress [16]. *Saccorhiza polyschides* (SP) is a large and bulky brown macroalgae species belonging to the Phyllariaceae family, whose size varies from 1.5 to more than 5 m in length with up to 3.5 m in width. Usually found on the lowest shore part, it is widely distributed on West European, Mediterranean, and North-West African coasts. Samples of SP were taken from natural algae fields located on Sidi Rahal marine coast, in the south of Casablanca, Morocco. These algae possess a unique bioactive composition with significant amounts of relevant macrominerals and trace elements essential for plant growth [17]. Apart from this, this edible seaweed has the potential to ensure the production of large amounts of biomass for feed and food [18]. The appeal of such a natural solution lies in its potential to mitigate the environmental and ecological impacts of traditional agricultural interventions [19]. As a potential biostimulant source, SP could provide a natural and sustainable solution to mitigate the adverse effects of salt stress on crops like common beans and other salt-sensitive plants, enhancing their resilience in saline environments. Mamede et al. [20] reported that the application of the *S. polyschides* extract (SPE) increased soil fertility and hence, turnip green plants enhanced their root system, photosynthetic activity, and essential nutrient uptake.

The current research focuses on the exploration of the role of SPE at different concentrations (1%, 2%, and 5%) in the mitigation of salt stress (68.4 mM NaCl) in common bean plants. The biostimulant effect is analyzed by testing physiological and biochemical processes on the plant such as growth parameters, indole acetic acid, sugars and amino acids, chlorophyll content, and antioxidant and carbon-nitrogen enzyme activities. The objective was not only to scientifically understand the biostimulant phenomenon but also to provide practical insights into applications in agriculture to alleviate salt-induced stress in common bean crops.

## 2. Materials and Methods

### 2.1. Graphical Experimental Design

All the chemicals use for this work were obtained from Sigma-Aldrich, Steinheim, Germany. In this study, we investigated the use of brown seaweed extract derived from *Saccorhiza polyschides* (SPE) to improve the ability of common bean (*Phaseolus vulgaris* L.) plants, a major global legume, to withstand salt stress. We evaluated the impact of foliar application and irrigation with SPE in water at different concentrations (0, 1, 2, 5% (v/v)) on common bean plants under salt stress. The experimental design and the main results of this study are illustrated in Figure 1.



**Figure 1.** Overview of experimental design used to test the effect of different SPE applications (1%, 2%, and 5%) in common bean plants after 2 months of growing under non-salinity stress (NSS; water) and salinity stress (SS; 68.4 mM of NaCl) culture conditions. Two different SPE applications were used: spraying or mixing in the irrigation water. A summary of the main affected parameters under these different conditions is also shown in the lower part (illustration made using Biorender).

### 2.2. Preparation of Seaweed Extract

*Saccorhiza polyschides* (SP) specimens were carefully selected and identified and picked with complete thalli from natural algae fields located on the Sidi Rahal marine coast, in the south of Casablanca, Morocco. Samples were thoroughly rinsed with tap water to eliminate salt and any potentially unwanted substances or dirt. The SP was dried using an oven-drying method at a temperature of 50 to 55 °C, which took approximately 96 h to achieve full dryness. After that, the SP was finely ground. Crude extract stock solutions were obtained by mixing 100 g of dried algae powder with 1 L of distilled water. For two hours, these solutions were heated at 100 °C and then filtered to eliminate any solid debris by gravity filtration using Whatman No. 1 filter paper. The resulting filtrate served as 100% crude extract stock, from which experimental biostimulant solutions were later prepared. Working biostimulant solutions were made by diluting the crude extract with additional distilled water to obtain experimental solutions containing 1%, 2%, and 5% of *Saccorhiza polyschides* extracts (SPE). The SPE was stored at 4 °C, never for more than 1 month, in a dark container to protect it from light that could degrade sensitive compounds.

We conducted an initial preliminary experiment to ascertain the suitable range of concentrations for SPE to be utilized. On common bean plants, concentrations of SPE at 1%, 2%, 5%, 10%, and 20% in combination with salt stress (68.4 mM NaCl) were tested.

For four weeks, this initial study was carried out in starter trays. After the plants were harvested, the diameters of the shoots, their fresh weight, and their shoot length were measured (results not included). Following careful thought, we determined that 1%, 2%, and 5% would be the best concentrations to use in a larger-scale investigation.

### 2.3. Plant Material and Growth Conditions

Seeds of a variety of common bean (*Phaseolus vulgaris* L. var. *nanus* (L.)) were selected based on size and color to be as similar as possible. After two to three minutes of disinfection with a 5% sodium hypochlorite solution, they were rinsed with distilled water. Sterilized seeds were sown in commercial substrate (Projar, Madrid, Spain), and, after germination, five plants were selected and cultivated per pot. They were kept at 22 °C with a 15 h lighting photoperiod and an irradiance of 250  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . A total of 32 pots were used for the present experiment (4 repetitions for each condition were made) (Figure 1).

Plants were sprayed every 5 days with different SPE concentrations (1%, 2%, and 5%) and were watered every 4 days with fresh water (NSS), with NaCl solution (68.4 mM for SS), or with NaCl solution mixed with the SPE in the irrigation method. The salt stress concentration was selected based on preliminary and previous results of our group. After 2 months, all plants were harvested and morphologically characterized, measuring growth parameters, mainly length, weight, and leaf diameter. Chemical and enzymatic analyses were carried out using freshly harvested plant leaves (Figure 1).

For shoot length, we utilized a standardized ruler to measure the distance from the base of the plant at soil level to the tip of the main shoot. Each plant was measured individually to ensure accuracy and consistency. To measure the leaf diameter, we first determine the center point of each leaf. From this central point, we measured the diameter in centimeters using a caliper. Lastly, for weight measurement, we carefully harvested the plants (without the roots) and weighed them using a digital scale. The weight was recorded in grams, and each plant was weighed individually.

### 2.4. Chlorophyll Assay

The leaves' chlorophyll content was measured using the methodology described by Arnon [21]. During the process, 800  $\mu\text{L}$  of 80% acetone was mixed with 200  $\mu\text{L}$  of soluble plant homogenate pellets in water. Three independent replicates of the amount of chlorophyll were measured following a 72 h incubation period at 4 °C. The levels of Chlorophyll-a and Chlorophyll-b were evaluated by measuring the absorbance at two different optical densities: at 663 (OD<sub>663</sub>) and 645 (OD<sub>645</sub>) nm. The following formulae were applied:

$$\text{Chlorophyll-a (mg/mL)} = (0.0127 \times \text{OD}_{663}) - (0.0269 \times \text{OD}_{645})$$

$$\text{Chlorophyll-b (mg/mL)} = (0.0229 \times \text{OD}_{645}) - (0.00468 \times \text{OD}_{663})$$

The total chlorophyll content was expressed as the sum of Chlorophyll-a + Chlorophyll-b.

### 2.5. Plant Extract Preparation for Measurement of Soluble Sugar, Amino Acid, and Indole Acetic Acid Contents

After drying the leaves of common bean at 37 °C and using 80% ethanol, 200 mg of the dried material was extracted at 4 °C. The amount of soluble sugar, amino acids, and indole acetic acid in the supernatant was measured after centrifugation at 5000  $\times g$  for 15 min at 4 °C.

The amount of indole acetic acid (IAA) generated in common bean leaves was determined according to Ennoury et al. [22] using Salkowski's reagent method and commercial IAA as standard. Thus, 500  $\mu\text{L}$  of this reagent (2% 1.5 M ferric chloride ( $\text{FeCl}_3$ ) in 36% perchloric acid ( $\text{HClO}_4$ )) was combined with 1.5 mL of common bean plant extract, and the mixture was incubated for 30 min at 25 °C. Subsequently, absorbance

was determined at 530 nm using a blank containing 500  $\mu$ L of Salkowski's reagent and 1.5 mL of distilled water.

Amino acid content was evaluated by applying the methodology described in Sun et al. [23]. Thus, 1.5 mL of 2% ninhydrin solution (solubilized in 0.2 M citrate buffer, pH 5, and ethylene glycol) was mixed with an aliquot of 500  $\mu$ L plant extract. The mixture was boiled for 15 min after being stirred with a Vortex. After allowing this mixture to cool at room temperature, the absorbance was measured at 546 nm. A calibration curve made with various glycine concentrations was used to calculate the number of amino acids.

Soluble sugar concentration was determined using the method reported by Yemm and Willis [24]. Thus, 2 mL of anthrone reagent (1 g/L of anthrone dissolved in 95% sulfuric acid  $\text{H}_2\text{SO}_4$ ) and 1 mL of common bean leaf extract were mixed. After being vortexed and heated to 100  $^{\circ}\text{C}$  in a water bath for 10 min, the mixture was left to cool on ice. The absorbance of the samples was measured at 630 nm, and a calibration curve with various concentrations of anhydrous glucose was used to calculate the sugar content of the samples.

## 2.6. Oxidative Stress Estimation

The malondialdehyde (MDA) content was measured using the method described by Bouchmaa et al. [25] with a few minor modifications to ascertain the extent of lipid peroxidation. To create a cell homogenate, trichloroacetic acid (20%) and tetrabutylammonium hydroxide (TBA) (0.67%) were added to plant cell homogenate and mixed with a vortex. Following an hour of boiling, ice was used to cool the mixture, and 1 mL of n-butanol was added before centrifuging for 12 min at  $12,000\times g$ . Afterward, the supernatant was collected and analyzed with a spectrophotometer at 532 and 600 nm.

The superoxide ( $\text{O}_2^{\cdot-}$ ) content in common bean plant leaves was measured according to Kubis [26].

The content of peroxide ( $\text{H}_2\text{O}_2$ ) was ascertained in accordance with Mrid et al. [27]. After pulverizing 0.2 g of fresh leaves with 1 mL of 0.1% TCA, the mixture was centrifuged at  $12,000\times g$  for 15 min. The obtained supernatant was combined with 2 mL of 1 M iodide potassium and 1 mL of 10 mM phosphate buffer pH 7. Following a 1 h incubation period in a dark environment, the optical density (OD) was measured at 390 nm. A standard curve was used to calculate the concentrations of  $\text{H}_2\text{O}_2$ .

## 2.7. Extraction and Assay of Antioxidant Activities

For the enzyme extraction process, fresh common bean leaves (0.2 g) were combined with a buffer that contained 10 mM magnesium chloride, 100 mM HEPES-KOH, 20  $\mu$ M FAD, 1 mM phenylmethylsulfonyl fluoride, and 14 mM  $\beta$ -mercaptoethanol. The homogenate was centrifuged for 20 min at 4  $^{\circ}\text{C}$  and  $20,000\times g$ . The supernatant was used to determine the subsequent enzyme activities. Thereby, Beauchamp and Fridovich [28] methodology was used to measure the superoxide dismutase (SOD) activity (EC 1.15.1.1).

Glutathione reductase (GR) activity was determined using NADPH oxidation at 340 nm (extinction coefficient  $6.2\text{ mM}^{-1}\text{ cm}^{-1}$ ) in accordance with Mrid et al. [27]. In this regard, 0.2 mM GSSG and 0.2 mM NADPH were combined with 0.1 M phosphate buffer, pH 7.8. Glutathione peroxidase (GPx) activity was assessed at 360 nm after NADPH oxidation. The Bouchmaa et al. [29] protocol was used to measure the enzyme activity.

Glutathione S-Transferase (GST) activity was measured using the protocol outlined by Latique et al. [30]. The reaction mixture was supplemented with 0.1 M sodium buffer pH 6.5, 2 mM 1-chloro-2,4-dinitrobenzene, 5 mM glutathione, and 0.1% triton x100. Then, a molar extinction coefficient of  $9.6\text{ mM}^{-1}\text{ cm}^{-1}$  was used to measure the activity of GST at 340 nm.

## 2.8. Extraction and Assay of Nitrogen and Carbon Activities

Using a 100 mM Tris-HCl buffer (pH 8) mixture containing 10 mM  $\text{MgCl}_2$ , 1.4 mM glycerol, 1.4 mM  $\beta$ -mercaptoethanol, 1 mM EGTA, 1 mM phenylmethylsulfonyl fluoride (PMSF), 16.5  $\mu$ M chymostatin, 1 mM EDTA, and 9.4  $\mu$ M leupeptin, common bean leaves were ground in a cooled



mortar. The homogenate was centrifuged for 15 min at 4 °C at 12,000× g. Solid ammonium sulfate was added to the supernatant after 30 min until it reached 60% saturation. The pellet was resuspended in 300 µL of extraction buffer and utilized for enzyme activities after the saturated supernatant underwent another centrifugation.

The method described by El Omari et al. [31] was used to measure the activity of phosphoenolpyruvate carboxylase (PEPC). Glutamine synthetase (GS) and Aspartate aminotransferase (AAT) activities were measured using methods outlined by Kchikich et al. [32], and Glutamate dehydrogenase (GDH) activity was measured spectrophotometrically for 30 min at 340 nm, as described by Ben Mrid et al. [33].

The total soluble protein content of the enzyme extracts was determined using the Bradford method [34], with the protein standard for the calibration curve being bovine serum albumin (BSA).

## 2.9. Statistical Analysis

For statistical analysis, all datasets were treated by one-way ANOVA using a general linear model in SAS, incorporating fixed salt stress level and SPE treatment. A comparison was achieved using Tukey's test. Data analysis was performed using SAS 9.4 software. A *p*-value below 0.05 was considered significant.

To create a biplot of the studied parameters, principal component analysis (PCA) was carried out under IBM SPSS Statistics version 25.0, using KMO and Bartlett's test as well as Oblimin with the Kaiser Normalization in Rotation Method.

## 3. Results

### 3.1. *Saccorhiza polyschides* Extract Enhanced the Growth Parameters of Common Bean Plants under Salt Stress

Results of the salt stress (68.4 mM NaCl) effect on growth parameters of common bean plants, mainly shoot length (cm), plant fresh weight (g), and leaf diameter (cm), are shown in Figure 2. These results clearly indicated that salt stress (SS) has a detrimental effect on the growth of common bean plants. As a matter of fact, plant length, plant weight, and leaf diameter were significantly reduced by salt stress compared to control (NSS).

Results shown in Figure 2 indicated also that SPE applied by the spraying method had a strong biostimulant effect. Overall, a significant improvement was observed in all plant growth parameters analyzed in SPE treatments combined with salt stress conditions (SPE + SS), compared to those irrigated only with SS. The best results in all parameters (plant length, plant weight, and leaf diameter) were observed using 5% of SPE under SS, followed by 2% SPE + SS. In the irrigation method, SPE application did not influence the reduction in SS symptoms, except for leaf diameter when plants were irrigated with 2% or 5% SPE.

According to these results, it clearly seems that, under SS conditions, 5% SPE treatment with the spraying method is more efficient than the irrigation method in terms of the performance of growth parameters. However, compared to control results (NSS), all treatments showed equal or less effect except in the two cases of plant length parameter using 5% and 2% SPE in the spraying method.

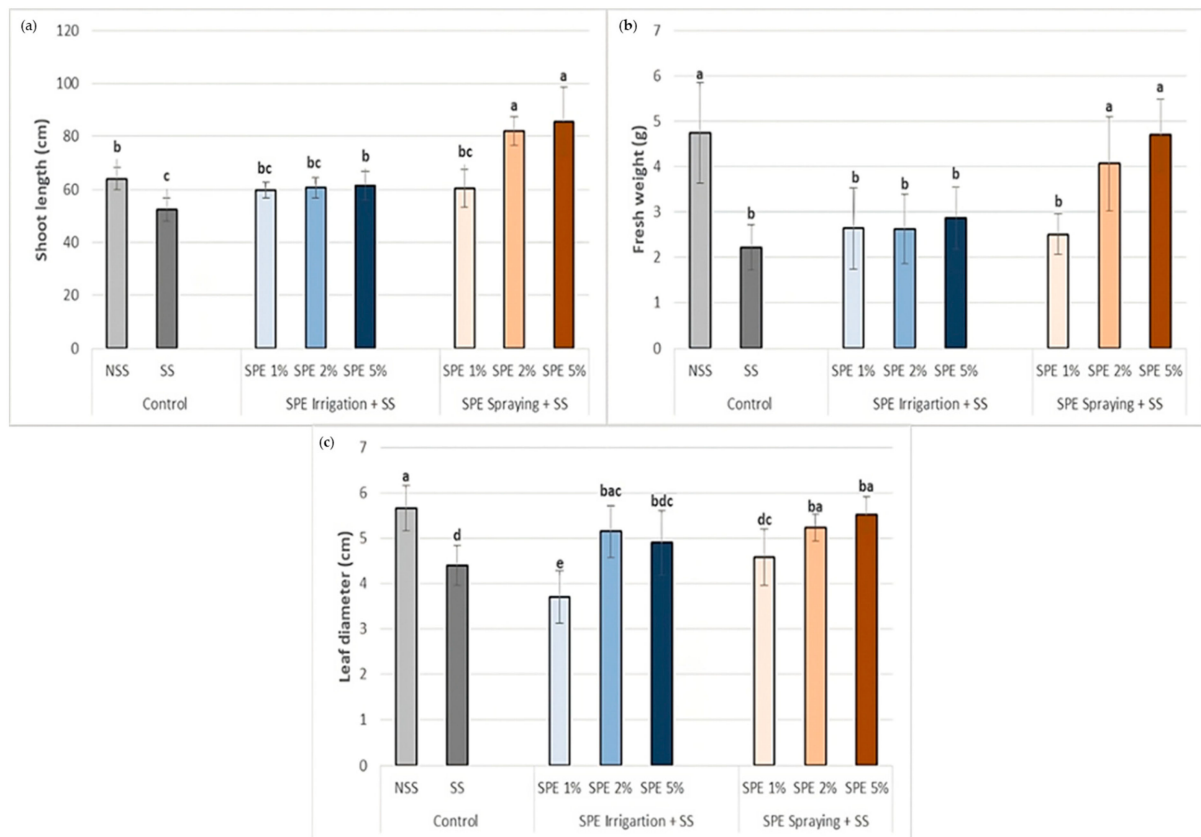
### 3.2. *Saccorhiza polyschides* Extract Enhanced Chlorophyll and Osmolytes in Common Bean Plants under Salt Stress

#### 3.2.1. Chlorophyll Content Increase in Common Bean Leaves after Biostimulation

As shown in Figure 3, chlorophyll content in common bean plants was negatively impacted by salt stress (SS) when compared to control (NSS), showing a reduction of 40%.

When compared to salt stress (SS control), chlorophyll content in stressed plants significantly increased after SPE application, both with irrigation and spraying conditions and at all biostimulant concentrations. All treatments showed relevant improvements compared to SS control, but SPE being sprayed produced better results than when it was irrigated. The best result was observed using 5% SPE in both application methods

compared to SS, but only the application of SPE 5% by spraying increased the chlorophyll content by 11% with respect to the content of the NSS plants.



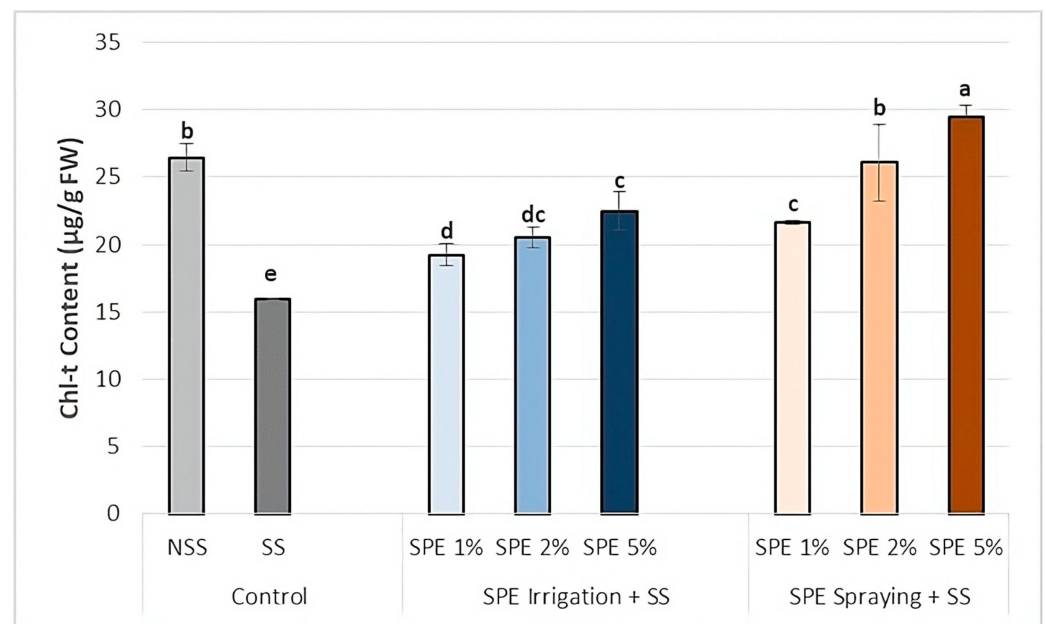
**Figure 2.** Biostimulant effect of 1%, 2%, and 5% SPE applied by irrigation method (SPE irrigation + SS), or spraying method (SPE spraying + SS) on common bean plants' growth parameters: shoot length (a), fresh weight (b), leaf diameter (c), under salt stress (68.4 mM of NaCl). Results of control plants neither with biostimulation nor salt stress (NSS) and under salt stress without biostimulation (SS) were also included for comparison. The value is the average  $\pm$  standard deviation (SD) of three or four independent observations. Different letters indicate significant differences by Tukey's test ( $p < 0.05$ ).

### 3.2.2. Indole Acetic Acid and Osmolytes Content (Soluble Sugars and Amino Acids) Increase in Common Bean Leaves after Biostimulation

Within the present investigation, salt stress induced a significant reduction in IAA and osmolyte content in common bean plants but with variable magnitudes depending on osmolyte type (Figure 4).

In this regard, and compared to NSS control, IAA, soluble sugar, and AA contents have been decreased under salt stress conditions. SPE application helped re-establish and increase hormone content in common bean plants under salt-stressed conditions. This positive effect was important, particularly at 5% SPE being sprayed. So, in terms of IAA, the best result was recorded using 5% SPE being sprayed which generated an important increase compared to SS control (Figure 4a). However, the content of AIA leaves under all SPE treatments was always lower than those found in NSS plants.

SPE treatments significantly increased the soluble sugar content of common bean plants under SS control (Figure 4b). This increase was proportional to biostimulant concentrations. The application of SPE by spraying showed better results than by irrigation. As a matter of fact, the highest value recorded with 5% of SPE treatment in the spraying method showed a great increase of soluble sugars compared to SS plants, with the content being higher than those found in NSS plants.



**Figure 3.** Biostimulant effect of 1%, 2%, and 5% SPE applied by irrigation method (SPE irrigation + SS) or spraying method (SPE spraying + SS) on total chlorophyll content of common bean plants under salt stress (68.4 mM of NaCl). Results of control plants neither with biostimulation nor salt stress (NSS) and under salt stress without biostimulation (SS) were also included for comparison. The value is the average  $\pm$  standard deviation (SD) of three or four independent observations. Different letters indicate significant differences by Tukey's test ( $p < 0.05$ ).

In the case of amino acid (AA) content in common bean plants under salinity conditions (Figure 4c), all SPE treatments were higher than SS control with almost the same improvement levels in both irrigation and spraying methods. The best results were recorded with 5% SPE in both methods providing enhanced AA content in stressed common bean plants in comparison to the control (SS). However, compared to NSS control, all SPE treatment results were lower except 5% SPE, which were almost equal in both irrigation and spraying methods.

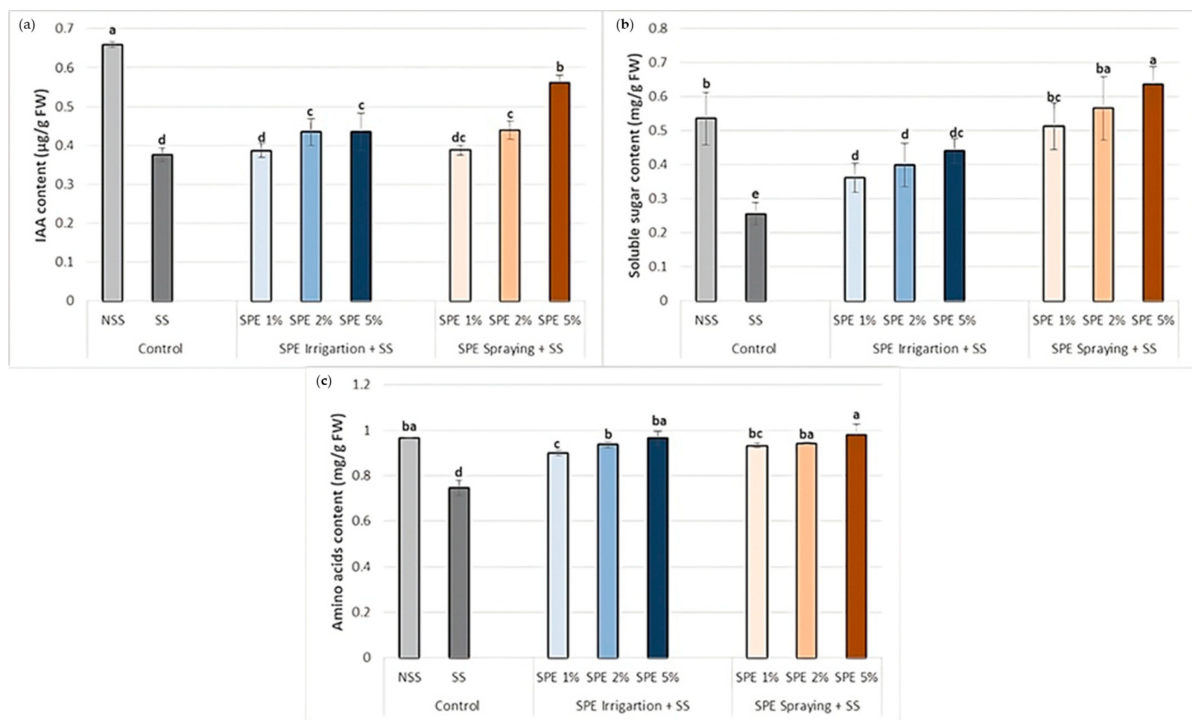
### 3.3. *Saccorhiza polyschides* Extract Limits Oxidative Stress Markers in Common Bean Plants under Salt Stress

The presence of NaCl in soil produced oxidative stress, which in the end produced lipid peroxidation in the cells and could be measured as MDA content. In fact, the MDA content was 2.5 times higher in SS plants than in NSS plants (Figure 5a). In addition,  $O_2^{\cdot-}$  and  $H_2O_2$  contents, which are the oxidant agents that produce lipid peroxidation, were also significantly increased under SS conditions (Figure 5b,c). However, the application of SPE, by both methods, reduced the lipid peroxidation levels in all concentrations assayed (Figure 5a) although the best treatment was SPE 5%. In line with this, the SPE application reduced the levels of  $O_2^{\cdot-}$  (Figure 5b) and  $H_2O_2$  (Figure 5c) compared to SS plants, with the  $O_2^{\cdot-}$  and  $H_2O_2$  concentrations being almost lower than in NSS when SPE 5% was sprayed on the leaves.

### 3.4. *Saccorhiza polyschides* Extract Boosts Antioxidant Enzymatic Activities in Common Bean Plants under Salt Stress

Salt stress increased all antioxidant activities assayed (SOD, GPx, GST, and GR) in common bean plants (Figure 6), with 5% being the best concentration for all activities except for GPx in which SPE 1% was the best application. The irrigation methods also increased the activities assayed but to a minor extent and normally, at the highest concentration (SPE 5%).





**Figure 4.** Biostimulant effect of 1%, 2%, and 5% SPE applied by irrigation method (SPE irrigation + SS), or spraying method (SPE spraying + SS) on common bean content of IAA (a), soluble sugars (b), amino acid (AA) (c), under salt stress (68.4 mM of NaCl). Results of control plants neither with biostimulation nor salt stress (NSS), and under salt stress without biostimulation (SS) were also included for comparison. The value is the average  $\pm$  standard deviation (SD) of three or four independent observations. Different letters indicate significant differences by Tukey's test ( $p < 0.05$ ).

### 3.5. *Saccorhiza polyschides* Extract Boosts the Carbon-Nitrogen Enzymatic System of Common Bean Plants under Salt Stress

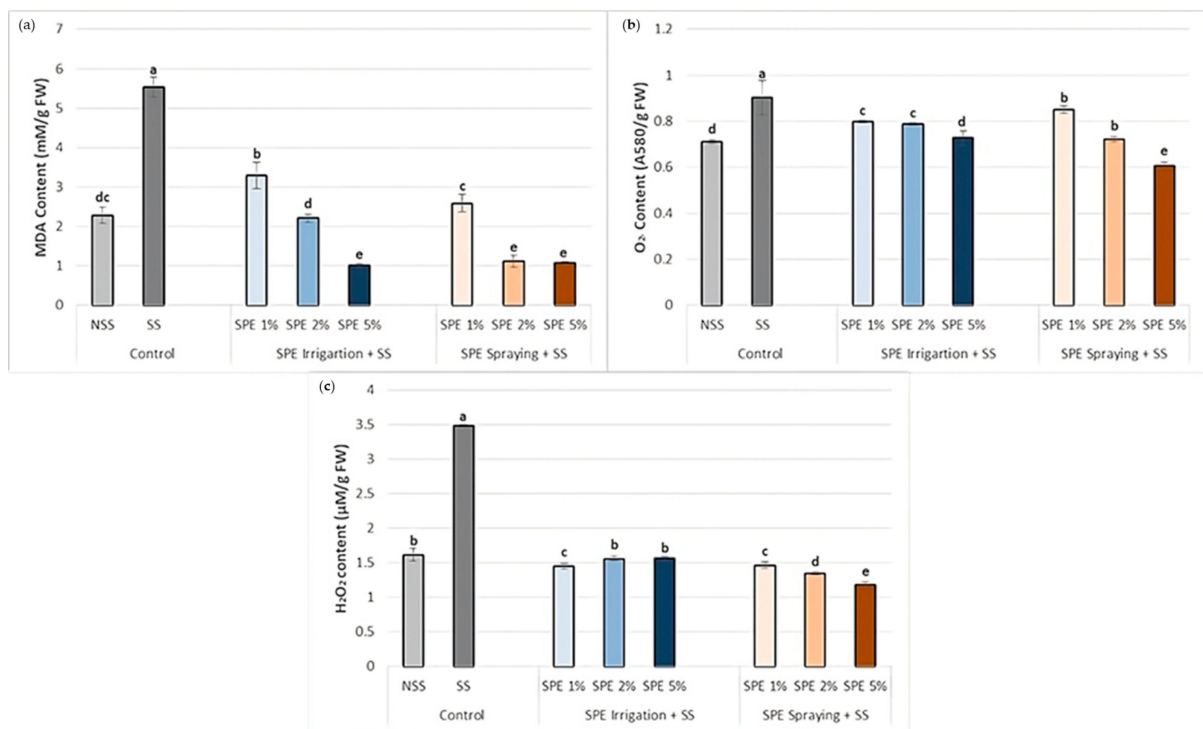
The effects of salt stress in common bean plants, in terms of the carbon–nitrogen enzymatic system, compared to NSS conditions showed variable results (Figure 7). In control plants, SS led to a marked increase in GDH and AAT activities compared to NSS (Figure 7b,d). However, PEPC and GS activities decreased under SS conditions compared to NSS (Figure 7a,c).

Moreover, the enzymatic system in stressed common bean plants treated with SPE using the three concentrations was significantly increased, especially when it was sprayed using 2% and 5%, for PEPC, GDH, and GS compared to SS (Figure 7a–c).

SPE treatment for plants under SS helped restore AAT activity, especially when this extract was sprayed compared to SS (Figure 7d) but were slightly higher in all three SPE treatments (1%, 2%, and 5%) with almost the same level compared to SS (Figure 7d). However, although the SPE applied by irrigation also increased AAT activity compared to NSS, the levels never reached those measured in SS plants.

### 3.6. Principal Component Analysis (PCA)

In our research, we performed two principal component analyses (PCA) to investigate the relationships between the treatments applied and the morpho-physiological (Figure 8) and biochemical traits (Figure 9) observed in common bean plants. As depicted in Figure 8A, the first PCA component (PC1) explained approximately 52.38% of the total variation and was related to the treatment, being the SS plants located at the negative part of this component. The second component (PC2) accounted only for 27.64% of the variance observed in the dataset.

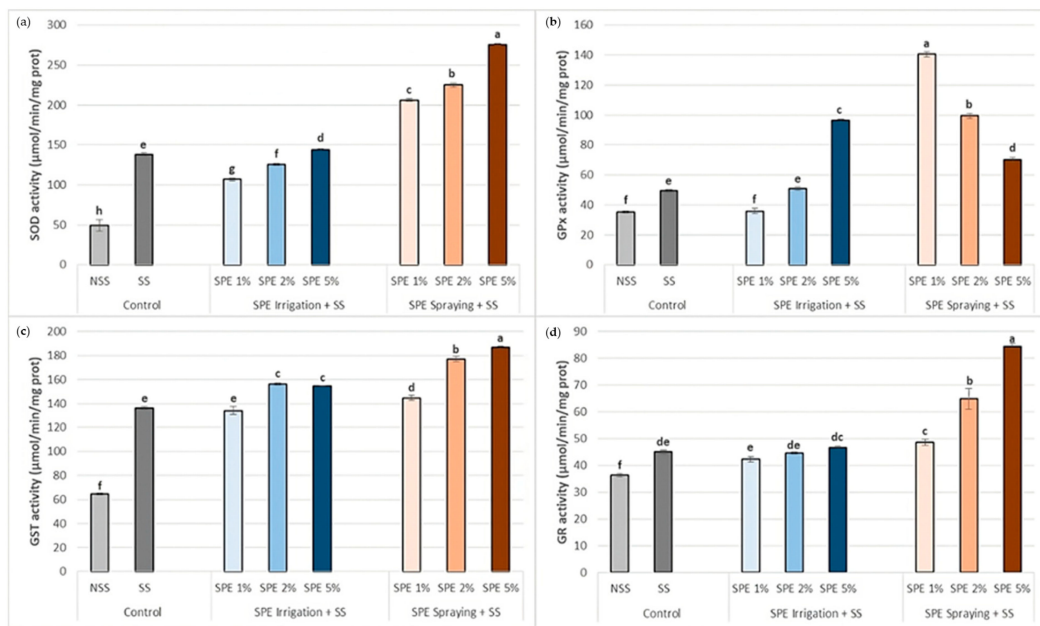


**Figure 5.** Biostimulant effect of 1%, 2%, and 5% SPE applied by irrigation method (SPE irrigation + SS), or spraying method (SPE spraying + SS) on common bean lipid peroxidation (MDA) (a), superoxide  $O_2^-$  (b), and hydrogen peroxide  $H_2O_2$  (c), under salt stress (68.4 mM of NaCl). Results of control plants neither with biostimulation nor salt stress (NSS), and under salt stress without biostimulation (SS) were also included for comparison. The value is the average  $\pm$  standard deviation (SD) of three or four independent observations. Different letters indicate significant differences by Tukey's test ( $p < 0.05$ ).

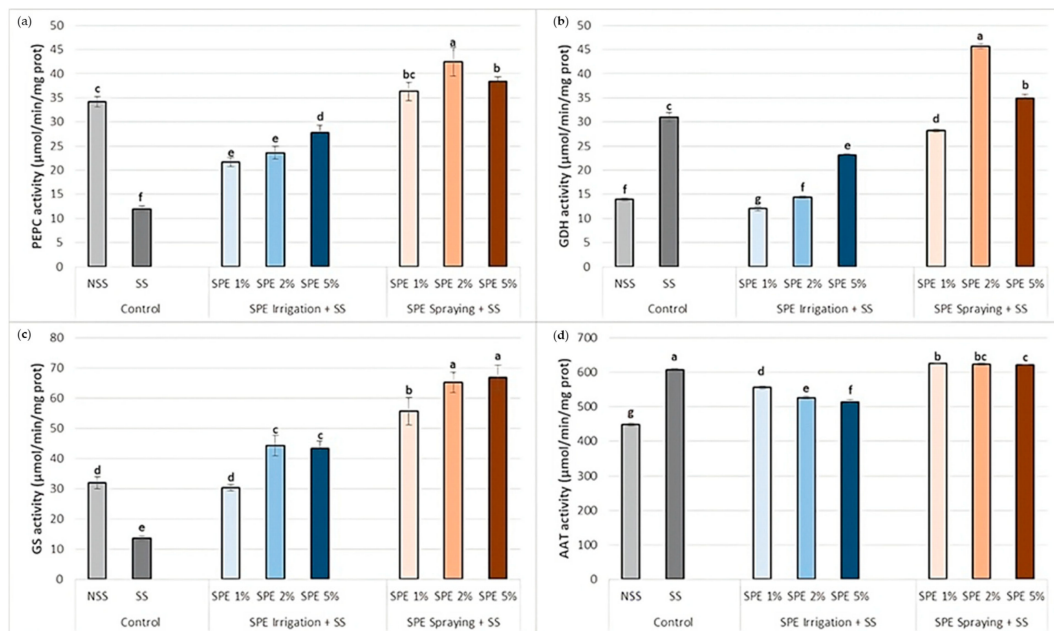
The scatter plot of treatments (Figure 8B) revealed that under salt stress, the effects of SPE at the concentration of 2% and 5% (SSPE2% and SSPE5%) were positively associated with the measured common bean growth parameters. However, these associations were almost completely lost when irrigation was used at 1% SPE (ISPE1%). SSPE application at 2% and 5% was negatively related to SS.

The PCA shown in Figure 9 revealed the relationship between the treatments, the enzymatic activities, and oxidative stress markers analyzed in common bean plants under the different treatments. In this case, PC1 and PC2 represented 51.44% and 26.50% of the total variation, respectively, and collectively accounted for 77.94%. The pattern matrix with the rotation method showed that all enzymes were correlated with the positive part of the PC1, whereas the oxidative stress markers such as lipid peroxidation (MDA),  $H_2O_2$ , and  $O_2^-$  content were correlated with the negative part of PC1. However, PC2 seems to be related to the treatment, with NSS and SPE-biostimulated (SPC) plants in the positive part, and SS plants in the negative part (Figure 9A). The IAA content was a parameter not related to these treatments.

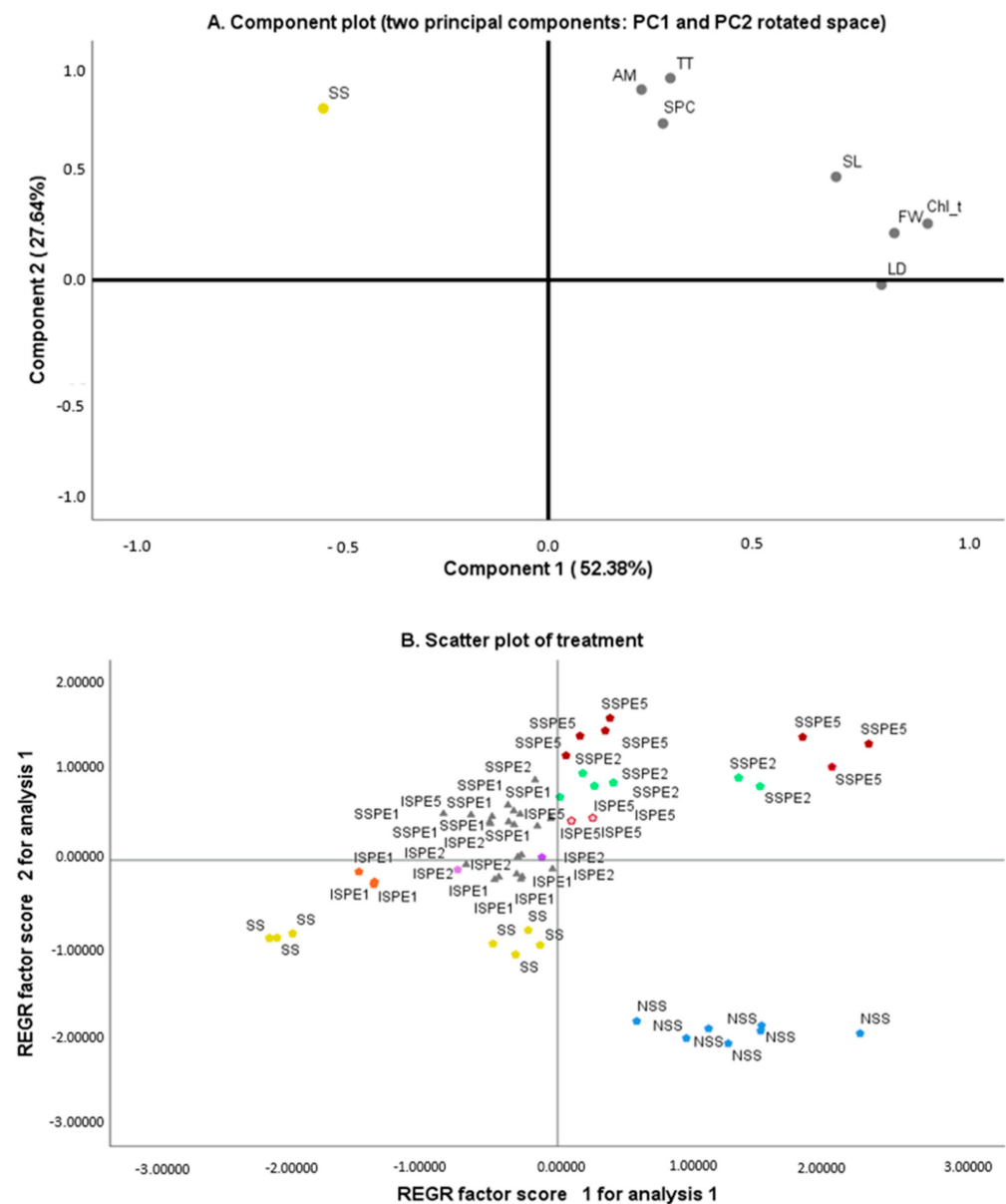
The scatter plot of treatments (Figure 9B) illustrated that SPE at 2 and 5% concentrations by means of spraying (SSPE) had a positive correlation with NSS and a negative correlation with SS plants. However, the rest of the applications were between NSS and SS plants.



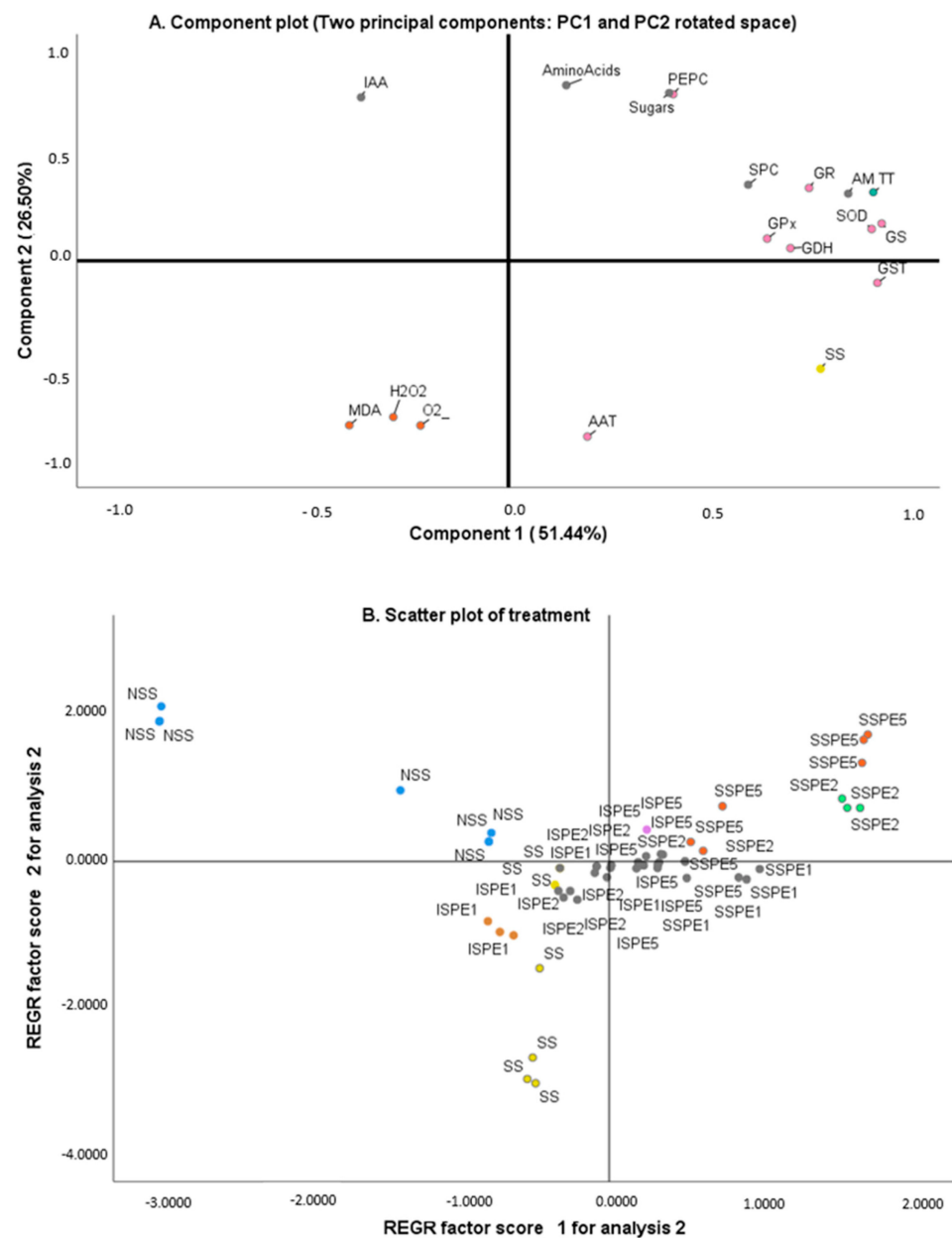
**Figure 6.** Biostimulant effect of 1%, 2%, and 5% SPE applied by irrigation method (SPE Irrigation + SS), or spraying method (SPE Spraying + SS) on common bean antioxidant enzyme activities; superoxide dismutase (SOD) (a), glutathione peroxidase (GPx) (b), glutathione-S-transferase (GST) (c), and glutathione reductase (GR) (d) under salt stress (68.4 mM of NaCl). Results of control plants neither with biostimulation nor salt stress (NSS), and under salt stress without biostimulation (SS) were also included for comparison. The value is the average  $\pm$  standard deviation (SD) of three or four independent observations. Different letters indicate significant differences by Tukey's test ( $p < 0.05$ ).



**Figure 7.** Biostimulant effect of 1%, 2%, and 5% SPE applied by irrigation method (SPE irrigation + SS), or spraying method (SPE spraying + SS) on common bean carbon–nitrogen enzyme activities: phosphoenolpyruvate carboxylase (PEPC) (a), glutamate dehydrogenase (GDH) (b), Glutamine synthase (GS) (c), and Aspartate Aminotransferase (AAT) (d) under salt stress (68.4 mM of NaCl). Results of control plants neither with biostimulation nor salt stress (NSS), and under salt stress without biostimulation (SS) were also included for comparison. The value is the average  $\pm$  standard deviation (SD) of three or four independent observations. Different letters indicate significant differences by Tukey's test ( $p < 0.05$ ).



**Figure 8.** Principal component analysis (PCA) of morpho-physiological and biochemical traits observed in common bean plants (**A**) and scatter plot of treatment (**B**). The variables included were shoot length (SL), fresh weight (FW), leaf diameter (LD), and total chlorophyll content. The total treatments (TT) included were control (NSS: irrigation only with water), NaCl application (SS: irrigation only with 68.4 mM of NaCl), *Saccorhiza polyschides* extract (SPE) at different concentrations ((SPC) SPE 1%, SPE 2% or SPE 5%) and 68.4 mM of NaCl applied through two application methods (AM): irrigation (I) and spraying (S).



**Figure 9.** Principal component analysis (PCA) of enzyme activities and some biochemical traits observed in common bean plants (**A**) and scatter plot of treatment (**B**). The enzymatic activities included were superoxide dismutase (SOD), glutathione-s-transferase (GST), glutathione-peroxidase (GPx), phosphoenolpyruvate carboxylase (PEPC), aspartate aminotransferase (AAT), glutamate dehydrogenase (GDH), and glutamine synthetase (GS). The content of indoleacetic acid (IAA), amino acids, soluble sugars, malonaldehyde acid (MDA), H<sub>2</sub>O<sub>2</sub>, and O<sub>2</sub><sup>-</sup> was also included. The treatments (TT) included were control (NSS: irrigation only with water), NaCl (SS: irrigation only with 68.4 mM of NaCl), *Saccorhiza polyschides* extract (SPE) at different concentration ((SPC) SPE 1%, SPE 2% or SPE 5%) and 68.4 mM of NaCl applied through two methods (AM): irrigation (I) and spraying (S).

#### 4. Discussion

The main objective of this work is to evaluate the putative biostimulant effect of *Saccorhiza polyschides* extracts (SPE) on common bean plants under salt stress conditions (68.4 mM NaCl), testing two types of SPE application: by spraying the aerial part of the plant or by irrigation. Salt stress has one of the most inhibitory impacts on several glycophyte crops such as *Solanum*, *Capsicum*, or *Triticum* plants, causing reduced growth, development, and productivity [35,36]. The application of algae biostimulants has been



described as a useful tool for improving crop protection against several stresses [37,38], and brown seaweeds have been largely employed mostly due to their year-round availability and large size. Most of the experiments in this sense have been developed using *Ascophyllum nodosum* extracts, which are used as fertilizers to enhance growth, quality, and resilience under salt stress conditions in *Lactuca sativa*, *Arabidopsis thaliana*, and *Solanum lycopersicum*, among others [39–41]. However, as far as we know, no experiments were made using SPE to improve the resilience and productivity of common bean plants under salt stress conditions.

The biostimulant action of algae extracts was attributed to high amounts of macro- and micronutrients, hormones, phenolics, amino acids, carotenoids, vitamins, and polyamines [42,43]. SP alga is rich in valuable macro and trace elements that aim for its potential application as a biofertilizer [16], which could provide insight into why SPE application positively impacts the growth of plants and their ability to withstand salt stress conditions. Our preliminary analysis showed that SPE used in this study was composed of flavonoid ( $4.5 \text{ mg.g}^{-1} \text{ DW}$ ) and polyphenol ( $1.3 \text{ mg.g}^{-1} \text{ DW}$ ) as well as amino acids ( $0.4 \text{ mg.g}^{-1} \text{ DW}$ ) and IAA ( $0.3 \text{ mg.g}^{-1} \text{ DW}$ ), which could participate in salt stress protection.

The increment of salt in the substrate modifies the electrical conductivity of the soil, limiting the uptake of water by most of the glycophyte plants [44] such as the common bean. The reduction in water content impairs the transpiration and stomatal conductance, affecting the expansion of old leaves and the emergence of new ones [45]. This effect reduces plant growth, as we observed in common bean plants under salt stress, which reduced their shoot length, fresh weight, and leaf diameter (Figure 2). Salt stress also affected photosynthetic parameters negatively, producing pigment degradation (Figure 3) and reducing the carbon fixation capacity of the plant, which led to the accumulation of less soluble sugars and amino acids (Figure 4). However, the application of algae extracts increased growth parameters, chlorophyll content, and photosynthesis in *Cucurbita pepo* [46] and *Triticum aestivum* [47] under salt stress. In accordance with this, 5% SPE application by spraying significantly increased growth parameters (Figure 2) and chlorophyll content (Figure 3) in common bean plants under salt stress compared to plants without biostimulation. The irrigation method only had a significant effect at 2% and 5% SPE on leaf diameter and chlorophyll content (Figures 2 and 3), suggesting an effect of SPE on cytokinin synthesis and/or mobilization from the root to the leaves [48].

The accumulation of osmolytes in the cells is a well-known strategy of halophyte plants to facilitate water entrance and protect cells and subcellular structures upon salt stress [45]. However, common bean plants reduced soluble sugar and amino acid content under salt stress (Figure 4) probably because it is a glycophyte plant. Similarly, El-Samad et al. [49] reported a progressive decrease in amino acid content in maize and broad bean plants with increasing salt stress, as well as a decrease in soluble sugar content. However, the SPE application increased their content to the levels of those found in non-stressed plants (Figure 4). This result has also been noted in tomato plants treated with *A. nodosum* extract [39], and the exogenous application of other algal extracts significantly increased osmolytes accumulation in plants exposed to various types of stress [50]. In this regard, a variety of organic compounds found in algal extracts have been described, including amino acids (such as proline, glycine, and glutamine) and soluble sugars (such as glucose, sucrose, and mannitol) that regulate osmotic balance and mitigate stress in plants [19].

When subject to salt stress, plants suffer an accumulation of reactive oxidizing species (ROS), such as superoxide anion ( $\text{O}_2^{\cdot-}$ ) radicals and hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) [51], which could cause adverse effects such as increased lipid peroxidation as evidenced by elevated malondialdehyde (MDA) levels [51].

Recorded results of the current investigation revealed a noteworthy rise in lipid peroxidation damage, and a high  $\text{O}_2^{\cdot-}$  and  $\text{H}_2\text{O}_2$  content in common bean plants under salt stress conditions (Figure 5). However, the application of 2% or 5% SPE significantly reduced the ROS content in salt-stressed plants to that level found in control plants or even less,

resulting in a drastic reduction in the lipid peroxidation levels. This could be attributed to SPE boosting non-enzymatic antioxidant activity pathways as well as enzymatic ones [37]. The content of natural antioxidants in SPE [18], including polyphenols, plays an important role in reducing lipid peroxidation. Compounds recognized as excellent anti-oxidants are found in algae comprising certain pigments such as carotenoids, astaxanthin, fucoxanthin, and polyphenols such as phenolic acids, flavonoids, and tannins [52].

Apart from antioxidant compounds, plants use enzymatic systems to deal with oxidative stress. Superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione-S-transferase (GST), and glutathione reductase (GR) create an intricate network to reduce, absorb, and scavenge ROS detoxification, allowing cellular survival [53]. It is well known that SOD is the first line of defense against damage caused by ROS because it catalyzes the dismutation of  $O_2^{\cdot-}$  into oxygen and  $H_2O_2$  [54]. A noteworthy rise in SOD activity was observed in the present research in reaction to salt stress. Nevertheless, it was overcome by plants treated with sprayed SPE mainly at 5%, which, in addition to neutralizing the harmful salt stress effect, further increased SOD activity. As expected, in SPE-treated plants under stress, the increase in SOD activity was accompanied by a decrease in  $O_2^{\cdot-}$  content (Figures 5 and 6). Hence, the  $H_2O_2$  generated by SOD could be used for other types of procedures such as cell wall reinforcement [55] as well as a signaling molecule that activates plant defense systems and gets detoxified by other antioxidant enzymes such as peroxidases and catalases. These results suggest that SPE could help common bean plants defend themselves against salinity-induced oxidative stress.

In the present study, common bean leaves exposed to salt stress presented an increase in GPx and GR activities, especially when plants were sprayed with SPE rather than irrigated (Figure 4). GPx catalyzes the reduction in the generated  $H_2O_2$  by SOD to water and oxygen, using GSH directly as a reducing agent [53]. GSSG reduction through glutathione reductase (GR) facilitates the regeneration of GSH. By employing NADPH as a reducing agent, the GR enzyme maintains a balanced ratio of GSSG/GSH, which is essential for preserving the oxidative balance in plant cells [56].

GST activity also plays an essential role in ROS detoxification [57]. Plants under salinity have higher GSH content which is linked to higher GST and GPx activities (Figure 6; [57]). Additionally, SPE treatments have been shown to reduce the oxidative stress response in common bean plants, especially at 5% SPE (Figure 4c), suggesting it provides tolerance to salt toxicity. In sum, SPE reduced the production of ROS and minimized oxidative damage caused by salinity by modulating the antioxidant defense system, allowing common beans to develop normally even in salt-stressed environments. In line with these results, Latique et al. [58] examined liquid seaweed extract's effects on mitigating salt stress on wheat plants, resulting in increased growth, antioxidant enzymes, and boosting the overall health of crops subject to salt stress.

The present investigation highlighted how salt stress influences carbon and nitrogen metabolism in common bean plant leaves with and without SPE treatment. Salt stress caused a striking drop in PEPC activity (Figure 7), which is known as a key enzyme in carbon metabolism. Considering that there is plenty of evidence showing adverse salt toxicity effects on PEPC performance [59], this enzyme plays diverse functions in plant cells by exchanging phosphoenolpyruvate (PEP) into oxaloacetate and inorganic phosphate (Pi) in the presence of  $HCO_3^-$  [60]. This enzyme has the potential to support the replenishment of intermediates necessary for nitrogen assimilation and biosynthesis in the tricarboxylic acid cycle (TCA). This is necessary not only for amino acid synthesis but also for carbohydrates and nucleic acid production [30,61]. Furthermore, PEPC activity showed a significant increase in SPE treatment, particularly by spraying application (Figure 7a), suggesting a potential alleviating effect on demand for carbon skeletons required for nitrogen assimilation in common bean plants under salt stress conditions.

GDH activity rose while GS activity was significantly suppressed under salt stress (Figure 7b,c). As an essential enzyme for the metabolism of nitrogen, GS produces glutamine by condensing glutamate and ammonia [61]. Moreover, GDH primarily functions

as a bidirectional enzyme reaction, catalyzing the assimilation of ammonium to glutamate and the deamination of glutamate to 2-oxoglutarate and ammonium [32]. The increasing GDH activity and GS/GOGAT cycle decreasing capacity under salt stress have been previously reported by various studies [62–64]. The results presented here may indicate that ammonium assimilation under salinity stress might largely depend on the GDH pathway. As reported, SPE increased GDH and GS activities, much more by spraying than by irrigation, highlighting the ability of SPE to stimulate ammonium assimilation in common bean plants. AAT activity plays a crucial role in the synthesis of amino acids from glutamate, making it a necessary enzyme for nitrogen metabolism in plants [64]. Salt stress markedly increased AAT activity, compared to control plants (Figure 7d); however, when stressed plants were treated with SPE by irrigation, AAT activity was reduced compared to SPE-sprayed plants, which showed a significant increase. AAT is an aminotransferase, oxaloacetate and glutamate are produced when AAT facilitates the reversible transfer of the amino group of aspartate to  $\alpha$ -ketoglutarate [64]. AATs have been shown to be crucial in several physiological functions in plants, involving the carbon skeleton recycling that occurs when roots absorb ammonia. The current observations of elevated AAT activity in SPE-treated plants are consistent with the rise in the amino acid pool. The ammonia that tends to build up in salty environments is detoxified in large part by the keto acids and certain amino acids [65]. Considering all these results SPE treatment could be assessed as an excellent potential to induce resistance in common bean plants against salt stress toxicity due to its potential for modulating carbon and nitrogen metabolism, antioxidant system, morphological parameters, and content in osmolytes.

Overall, the PCA results reveal that the treatments applied have a significant effect on the morpho-physiological biochemical and enzymatical traits in common bean plants. The positive association between the SSPE2% and SSPE5% treatments and the measured parameters under salt stress suggests that these treatments are effective in mitigating the negative effects of salt stress. In contrast, the relationship between SSPE1% and the measured parameters under salt stress indicates that this treatment may not be as effective as the SSPE2% and SSPE5% in these conditions. The spray application method has proven to be more effective than the irrigation method. One possible explanation could be that a stimulation of the metabolism (produced by SPE irrigated in roots) in the same organ that is being directly stressed (by NaCl applied in watering solution) may lead to an overstimulation, which may reduce the positive effects of SPE. However, further research is needed to test this hypothesis.

Therefore, our findings highlight the need for a precise selection of SPE concentrations and an effective application method for agricultural use.

## 5. Conclusions

This study highlights the effectiveness of *Saccharhiza polyschides* extract (1%, 2%, and 5%) in alleviating salt stress in common bean plants (68.4 mM NaCl). Salt stress impaired growth and physiological functions, reducing chlorophyll content, photosynthetic activity, and essential metabolic processes. SPE treatment significantly countered these effects, enhancing osmolyte accumulation and plant growth, chlorophyll content as well as antioxidant defenses. Key findings include increased activity of nitrogen metabolism enzymes and antioxidant enzymes and decreased ROS and lipid peroxidation levels with SPE application.

The SPE application by spraying in leaves, especially at 2% and 5%, seems to be a potent biostimulant able to enhance common bean resilience to salt stress, making SPE a sustainable solution for improving crop productivity in saline environments.

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