Article

Use of a Biostimulant to Mitigate Salt Stress in Maize Plants

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Abstract: Salinity is considered among the abiotic stresses most impacting agriculture for its ability to interfere with crop development and quality. For this reason, practices and innovations that could contain the deleterious effects of such stress are of pivotal importance for maintaining acceptable crop yields. In this context, this work has concerned the study of severe salt stress (100 mM NaCl) on maize seedlings and the effects of a plant biostimulant (Megafol–Meg) in helping plants to cope with this adversity. Biomass production, pigments, the content Na⁺ and K⁺, the accumulation of hydrogen peroxide (H₂O₂) and lipid peroxidation products (MDA), total phenolic compounds (TPC), 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), and 2,2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS) were investigated in control samples, in samples treated with NaCl alone, and in samples treated with NaCl in combination with the biostimulant. The results showed that the biostimulant significantly mitigated the impact of the salt stress on shoot length and fresh weight, on chlorophyll and carotenoid contents, and reduced the amount of Na⁺ taken up by the species. Regarding the oxidative status, the biostimulated samples revealed lower amounts of H₂O₂ and MDA, while maize seedlings grown with NaCl alone exhibited the highest increases in the TPC, ABTS, and FRAP. The explanation for these effects is provided by highlighting the effectiveness of the biostimulant in avoiding Na⁺ accumulation, which resulted in a lower content of H₂O₂, MDA, TPC, and antioxidant activity.

Keywords: maize; salt stress; biostimulant; Na acquisition; oxidative impact

1. Introduction

Salinity is considered among the abiotic stresses that have the highest detrimental effects on plant growth and development [1]. Furthermore, the increase in water and soil salinity is closely related to climate change [2]; specifically, climate change has led to rises in temperatures and decreases in precipitations, thus degrading water quality and elevating the salt content of groundwater [3]. As for as agriculture is concerned, the salinisation of soil and irrigation water has a heavy impact on crop yield. In particular, soil salinity regards about 20% and 33% of the total cultivated and irrigated agricultural lands, respectively [4]. As a result, salinity is taking vast areas away from cultivation [1]; to date, it has been estimated that 800 million arable hectares are affected by this problem [5]. These issues mainly affect arid and semi-arid regions and coastal areas where climate change is causing sea-level rises, coastal flooding, and salt intrusion into the soil, severely degrading its quality [4,5]. To date, this problem affects about 600 million people, and by 2050, it is expected to cover areas populated by about 1 billion people [2].

Salinity impacts crops by negatively affecting plant establishment and causing stunted growth [6]. High salt concentrations exert direct toxicity on plants or lead to nutritional disorders [7]. The adverse effects of salt stress can lead to the destruction of water and ion homeostasis in plants, decreased photosynthetic activity, degradation of biomolecules, or alteration of cellular redox potential [8]. In addition, salinity may hamper the crop’s ability to acquire and distribute nutrients among tissues or even interfere with the activity of some critical enzymes for plant life [9]. Excessive salt uptake can also reduce or even
interrupt the production of pigment and specific metabolites involved in plant growth or result in the leaves’ death for the excessive amount of salt in the cytoplasm or cell wall [10]. Further effects prompted by excessive salt concentrations can regard alterations in root morphology, root to shoot ratio, or, more in general, substantial reductions in biomass production [11]. Another critical pathway in which salinity demonstrates its detrimental action on plants relates to the onset of oxidative stress, namely the overproduction of reactive oxygen species (ROS). ROS are highly harmful to plant survival for their oxidising capacity, leading to disturbing essential plant cell functions [7]. In particular, ROS can degrade, among other things, pigments, proteins, nucleic acids, and membranes, thus determining in the most severe cases plant death [12,13].

Different approaches have been studied, developed, and adopted to tackle salinity, and they are based on the use of salt-tolerant genotypes, the application of amendments, the implementation of different irrigation techniques [4,14]. However, there is recent and increasing attention in using biostimulants to improve plant resistance to salt stress, even though these substances have been initially employed to increase the production and quality of horticultural species [15]. Plant biostimulants can be defined as substances that promote beneficial effects in crops by stimulating physiological, morphological, and biochemical processes, mainly related to nutrient assimilation, enhanced plant growth, and biomass production [16]. In addition, the beneficial effects of biostimulants can often relate to improving the quality of end-products and increasing the plant’s ability to cope with biotic and abiotic stresses [16]. Given the diverse and varied nature of the starting materials from which biostimulants can be obtained, they have been grouped into seaweed extracts, plant extracts, vegetal and animal protein hydrolysate, microorganisms, and humic and fulvic substances [16,17]. Some recent studies have also pointed out as biostimulants of various origins can improve the resistance of diverse plants to salt stress thanks to the upregulation of genes involved in stress tolerance, the improvement of nutrient uptake and pigments production, or decreasing oxidative stress [18,19].

Maize represents one of the most important crops globally, and it is a cereal characterized by high nutritional and economic values [20]. This crop is generally considered sensitive to salt stress because if grown in saline soils or irrigated with inappropriate water, it can show alterations in some physiological and biochemical parameters, germination, and biomass production [20]. Several studies focused on the effect of biostimulants on maize grown under normal conditions, highlighting the effectiveness of the various materials tested on the crop productivity, quality, and yield [21–24]. Differently, less attention has been paid to the use of biostimulants to increase maize resistance to salinity, even though it has been recently demonstrated that biostimulants can help this crop in contrasting the deleterious effects of salt stress [25,26]. In light of the above, this work aimed to investigate the effect of Megafol (Meg), a commercial biostimulant, on the maize’s ability to resist a high dosage of salt (100 mM). In order to collect evidence on the possible beneficial effects of this biostimulant, some physiological and biochemical parameters were investigated in samples subjected to salt stress, treated or not with Meg, compared to control samples. In particular, biomass production, pigment and sodium, and potassium content, oxidative stress, and antioxidant activities were investigated in young maize seedlings biostimulated or not and compared to samples grown in the absence of salt stress.

2. Materials and Methods
2.1. Plant Material and Growth Conditions

Maize seeds (cv Belgrano) were disinfected in a solution containing hypochlorite (0.25% w/v) for two minutes. Then, the seeds were washed with copious amounts of distilled water and placed into Petri dishes (diameter 14 cm: 10 seeds/plate in triplicate for each treatment) with water (10 mL), on filter papers. Seeds were allowed to germinate in the dark. After 4 days, the young seedlings were transferred in hydroponic solutions containing Half Hoagland solution. Afterwards, three treatments were applied: control samples (0 NaCl), salt-stressed samples (100 mM NaCl), and biostimulated salt-stressed samples...
(100 mM + Meg). The biostimulant was applied at the concentrations corresponding to the field dosage suggested by the manufacturer (2.5 L per hectare). For each treatment, three replicates were performed. At the third leaf stage (21 days after sowing, DAS), maize samples were harvested and submitted to the following determinations.

2.2. Pigments Determination

The concentration of chlorophylls (Chlorophyll a, ChlA; Chlorophyll b, ChlB) and carotenoids (Car) were determined in maize shoots according to the method of Lichtenthaler and Wellburn [27]. Results were then expressed as milligrams per gram of fresh weight (mg g\(^{-1}\) FW). The sum of ChlA and ChlB was reported as the total concentration of chlorophylls (TotChl).

2.3. Na\(^+\) and K\(^+\) Concentrations in Shoot Maize

The Na\(^+\) and K\(^+\) contents were determined on maize samples subjected to the different treatments (control and treated with salt with or without Meg) at 21 days after sowing. For this scope, the aerial parts of plants were collected. First, the dry mass was determined after drying the samples at 60 °C for 24. Then, dry samples were ground, added with HNO\(_3\) 65% (v/v) and H\(_2\)O\(_2\) 30% (v/v), and digested at 90 °C for 60 min. Finally, the concentrations of Na\(^+\) and K\(^+\) in the acid digested samples were determined using a flame photometer, and the results were expressed in mg of cation for gram of dry weight (DW).

2.4. Hydrogen Peroxide (H\(_2\)O\(_2\)) and Malondialdehyde (MDA) Contents

The H\(_2\)O\(_2\) and MDA contents were determined in maize shoots collected at 21 days after the treatments. To this end, maize leaves (0.5 g fresh weight–FW) were collected and homogenized in trichloroacetic acid (TCA) 0.1% (w/v). The resulting suspension was centrifuged at 7000 \(\times\) g for 25 min. Then, 0.5 mL of the supernatant was employed to estimate H\(_2\)O\(_2\) concentration spectrophotometrically at 390 nm, according to Velikova et al. [28]. The MDA content was determined in maize shoots (0.5 g FW) which were extracted in a solution containing 10% (w/v) trichloroacetic acid and 0.25% (w/v) thiobarbituric. The suspension was then centrifuged for 20 min at 10,000 \(\times\) g [29]. After that, the MDA content was determined spectrophotometrically at 532 and 600 nm and referred to the samples’ FW.

2.5. Samples Methanolic Extract

One gram of fresh shoots samples was cut and then extracted twice in 80% aqueous methanol (volume for each extraction) by shaking it at room temperature for 90 min. Supernatants were then centrifuged (6000 rpm for 5 min), filtered on filter papers. Moreover, the volume of each of these filtrates was made up to 30 mL with the methanolic solution. The extracts were stored at −20 °C for further analysis.

2.6. Determination of Total Phenolic Content (TPC)

The total phenolic content of the methanolic extracts, obtained as described above, was determined using the Folin-Ciocalteu method (Singleton and Rossi, 1965). Briefly, two millilitres of Folin–Ciocalteau reagent were added to 0.4 mL of extracts and 1.6 mL of sodium carbonate solution (7.5%). Then the absorbance of these solutions was determined spectrophotometrically at 760 nm after incubation for 120 min in the dark. Standard solutions of gallic acid (GA) were used to realize a calibration curve (50–500 µg GA). Then, the TPC was expressed as mg gallic acid equivalents (GAE) g\(^{-1}\) FW.

2.7. Antioxidant Activity Measurement

The measurement of the antioxidant capacity of the extracts was performed with assays based on electron transfer (Et) assays. These methods included DPPH (2,2-diphenyl-1-picrylhydrazyl), FRAP (ferric reducing antioxidant power), and ABTS (2,2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid)) assays. An aliquot of the methanolic extract (150 µL) was mixed with 2850 µL of a DPPH solution (54 mg L\(^{-1}\)), and the absorbance was
read at 515 nm after 30 min in the dark, following the same procedure used by Falcinelli et al. [30].

For ABTS, an aliquot of methanolic extract (150 µL) was mixed with the ABTS\textsuperscript{••} solution (1 mL), and the absorbance was read at 734 nm after 2 h in the dark following modified procedure used by Arnao et al. [31].

For the FRAP assay, an aliquot of methanolic extract (300 µL) was mixed with the FRAP working solution and maintained at 37 °C, in the dark, for 30 min. The FRAP reaction mixture of the samples was read at 593 nm, following the modified procedure used by Benzie et al. [32].

A standard curve was obtained with Trolox (10 and 800 µM Trolox), and the results of DPPH, ABTS, and FRAP were expressed as µM Trolox Equivalent (TE) g\textsuperscript{−1} FW (µM TE g\textsuperscript{−1} FW).

2.8. Statistical Analysis

Data were analysed by one-way ANOVA according to a randomised block design with three replicates. The mean values of the determinations in triplicate ± standard errors are shown. In addition, the means were compared with Fisher’s least significant difference (LSD) at \( p < 0.05 \). The statistical environment R was used to perform the analysis [33].

3. Results

3.1. Effect of Salinity on Maize Growth and Pigment Content in Control Samples and Treated with \( \text{NaCl} \) Alone or in Combination with Meg

Salt stress severely impacted the shoot length at 21 DAS (Table 1), determining significant decreases in this parameter compared to control samples. However, when the samples subjected to salinity were biostimulated with Meg, decreases in shoot length were still recorded but were of a more modest entity compared to the sample grown in \( \text{NaCl} \) without the biostimulant (Table 1). On the other hand, root length was unaffected by salinity and the samples treated with \( \text{NaCl} \)—regardless of whether they biostimulated or not—did not show statistically significant differences compared to control samples.

As far as shoot and root fresh weights are concerned, salinity significantly reduced shoot fresh weight, while root fresh weight was unaffected (Table 1). Furthermore, no significant differences were recorded between samples grown in salinity, whether biostimulated or not. Regarding pigments, salinity reduced the content of chlorophyll a (ChlA) (Table 2); on the contrary, when samples were treated with the biostimulant, the content of this pigment was not significantly different from those shown by control samples (Table 2). Regarding chlorophyll b (ChlB), samples grown in salinity without the biostimulant showed a significant decrease in this pigment. As a consequence of the abovementioned effects, the total chlorophyll content of ChlA + ChlB (TotChl) was significantly decreased in samples subjected to salinity alone (Table 2). Regarding the ratio ChlA/ChlB, increases were recorded in samples grown in salinity, regardless of whether they were biostimulated or not (Table 2).
Table 2. Chlorophyll a (ChlA), chlorophyll b (ChlB), total chlorophyll (TotChl), ChlA/ChlB ratio, carotenoids (Car) and ChlA/Car ratio recorded in maize seedling controls, and grown with NaCl alone, and NaCl with the biostimulant (i.e., Control, Salt, Salt + Meg, respectively).

|          | ChlA         | ChlB         | TotChl       | ChlA/ChlB | Car          | ChlA/Car  |
|----------|--------------|--------------|--------------|-----------|--------------|-----------|
|          | mg g⁻¹ FW    | mg g⁻¹ FW    | mg g⁻¹ FW    |           | mg g⁻¹ FW    |           |
| Control  | 0.70 ± 0.05 c| 0.22 ± 0.02 a| 0.92 ± 0.06 a| 3.18      | 0.67 ± 0.02 a| 1.04      |
| Salt     | 0.59 ± 0.01 a| 0.15 ± 0.04 b| 0.74 ± 0.05 b| 3.93      | 0.37 ± 0.05 b| 1.59      |
| Salt + Meg| 0.79 ± 0.04 b| 0.20 ± 0.02 ab| 0.99 ± 0.05 a| 3.65      | 0.74 ± 0.05 a| 1.07      |

Values are the mean of three replicates ± standard deviation. Different letters within each column indicate statistically significant differences at \( p \leq 0.05 \).

Carotenoids (Car) were significantly decreased by salinity in samples treated with only NaCl (Table 2). However, samples subjected to salinity biostimulated with Meg did not evidence any significant difference from the untreated control samples. Finally, the ChlA/Car ratio was increased in the samples treated with only NaCl, while biostimulated samples did not show changes in this parameter (Table 2).

3.2. \( \text{Na}^+ \) and \( \text{K}^+ \) Content, and Relative Ratio, in Control Samples and in Maize Treated with NaCl Alone or in Combination with Meg

Salinity strongly increased the content of \( \text{Na}^+ \) in leaves of maize at 21 DAS compared to control samples (Figure 1). On the other hand, when samples were biostimulated with Meg, a significant reduction in the amount of \( \text{Na}^+ \) contained by leaves was recorded, even though it was significantly higher than those shown by the control samples (Figure 1). As for \( \text{K}^+ \), a significant increase in the content of this cation was observed in samples grown in salinity. However, biostimulated samples did not show significant differences in the \( \text{K}^+ \) content compared to control samples (Figure 1). In addition, the highest \( \text{Na}^+ / \text{K}^+ \) ratio was recorded in samples grown in salinity, regardless of the biostimulant application (Figure 1).

Figure 1. Contents of sodium (\( \text{Na}^+ \)), potassium (\( \text{K}^+ \)) and their ratio in maize seedling controls, and grown with NaCl alone, and NaCl with the biostimulant (i.e., Control, Salt, Salt + Meg, respectively). Different letters within each column indicate statistically significant differences at \( p \leq 0.05 \).
3.3. Hydrogen Peroxide (H$_2$O$_2$) and Malondialdehyde (MDA) Content Samples and in Maize Treated with NaCl Alone or in Combination with Meg

The hydrogen peroxide (H$_2$O$_2$) content was investigated in maize samples subjected to the various treatments collected at 21 DAS. Salinity determined the highest increase in the content of this oxidant (Figure 2A). However, when samples were biostimulated, a significant reduction in the content of H$_2$O$_2$ was recorded, even though it was significantly higher than the values found for the untreated controls. In line with the trend recorded for the hydrogen peroxide content, samples grown in salinity without the biostimulant application showed the highest content of MDA (Figure 2B). On the other hand, when maize was treated with the biostimulant, it showed significant decreases in the content of this lipid peroxidation product, albeit the amount recorded was significantly higher than those shown by untreated control samples (Figure 2B).

![Figure 2A](image1.png)  
**Hydrogen Peroxide**

![Figure 2B](image2.png)  
**MDA content**

Figure 2. Contents of hydrogen peroxide (H$_2$O$_2$) (A) and malondialdehyde (MDA) (B) determined in maize seedling controls, grown with NaCl alone and NaCl with the biostimulant (i.e., Control, Salt, Salt + Meg, respectively). Different letters within each column indicate statistically significant differences at $p \leq 0.05$.

3.4. TPC Content, DPPH, FRAP and ABTS Assays, in Control Samples and Maize Treated with NaCl Alone or in Combination with Meg

As for total phenol content (TPC), it ranged between 549 ± 28 µg GAE g$^{-1}$ FW (control samples) to 821 ± 102 µg GAE g$^{-1}$ FW (salt treated maize) (Table 3). Therefore, when plants were salt-stressed, TPC resulted significantly higher than unstressed plants (control group). However, the TPC in biostimulated samples did not show significant differences with respect to the control maize. The DPPH, FRAP, and ABTS assays were used for
determining antioxidant activity measured in methanolic extracts obtained from maize shoot control and treated with salt with or without Meg. The results showed that the DPPH activity was significantly higher in the salt and salt + Meg samples with respect to the controls (Table 3). The FRAP assay showed that the samples treated with NaCl alone exhibited a significantly higher antioxidant activity than the other treatments (Table 3). Finally, ABTS activity was highest in the salt-treated samples, followed by salt + Meg, and, finally, the control samples (Table 3).

Table 3. Total phenolic content (TPC), DPPH, FRAP, and ABTS determined in control maize samples, treated with NaCl alone, and NaCl with the biostimulant (i.e., Control, Salt, Salt + Meg, respectively).

| Sample          | TPC   | DPPH  | FRAP  | ABTS  |
|-----------------|-------|-------|-------|-------|
| control         | 549 ± 28 b | 9.2 ± 0.6 a | 14.7 ± 1.8 b | 23.1 ± 1.5 c |
| Salt            | 821 ± 102 a | 13.6 ± 1.8 b | 20.6 ± 2.6 a | 35.0 ± 2.0 a |
| Salt + Meg      | 697 ± 74 b  | 14.0 ± 2.3 b | 14.2 ± 2.6 b | 28.8 ± 1.1 b |

β: µM TE g⁻¹ sample: µM Trolox Equivalent for gram. µg GAE g⁻¹ sample: µg gallic acid equivalents for gram. Values are the mean of three replicates ± standard deviation. Different letters within each column indicate statistically significant differences at p ≤ 0.05.

4. Discussion

Salinity is among the abiotic stresses that have the highest detrimental effects on agriculture for its ability to interfere with crop development and quality [1]. Furthermore, salt stress mainly affects crop production in regions with an arid or semi-arid climate, particularly the coastal areas [6]. Therefore, implementing practices and innovations that can contain the deleterious effects of such stress is considered essential to maintaining acceptable crop yields. For this reason, this work concerned the study of the effects of a commercial biostimulant (Megafol) on maize seedlings on which severe salt stress (100 mM NaCl) was imposed. In order to provide evidence of possible positive effects of Megafol on this adversity, specific physiological and biochemical parameters, the content of oxidants and lipid peroxidation products, K⁺ and Na⁺, as well as certain antioxidant activities were investigated in control samples treated only with salt, or with salt in combination with a biostimulant. In general, salt stress causes physiological, morphological, and biochemical changes in plants, leading to impaired or stunted growth, yield losses, and decreased end-products quality [34].

The results obtained in this study evidenced that the salt concentration applied reduced the biomass production by treated maize at shoot level, but the NaCl did not affect root length and fresh weight (Table 1). Thus, although some studies documented that salinity can cause reductions in plant biomass development and specifically to plant roots [35,36], the response is often species-dependent and conditioned by the mechanisms activated in response to this stress [37]. In addition, our experiments evidenced that salinity determined a general decrease in the content of chlorophyll (Table 2–ChlA, ChlB, and TotChl). It has been documented that decreases in chlorophyll resulting from senescence can occur during salt stress, as evidenced in lettuce grown in salinity [38]. In particular, the synthesis of chlorophylls can be very sensitive to various stress conditions such as osmotic stress and then can undergo degradative pathways that are also enzymatically mediated [39]. The decreases in chlorophyll content have also been accounted for as a defensive mechanism activated to remobilize nitrogen from pigment-binding proteins [39].

When maize was grown in salinity and biostimulated with Meg, it maintained a content of ChlA and TotChl at levels similar to those shown by control samples (Table 2). Therefore, this result reveals that the biostimulant may permit maize, even though grown in a considerable NaCl concentration, to show a high chlorophyll content to sustain higher photosynthetic activity than unbiostimulated salt-stressed samples. It is in line with the increase in shoot biomass found in biostimulated samples compared to samples treated only with NaCl (Table 1). Such an effect on chlorophyll is not unusual when biostimulants
are applied to plants grown in stressful conditions, and it could depend on the presence of specific metabolites, which can protect this pigment from degradation [40] or stimulate its biosynthesis [41]. In the case of Megafol, a previous study conducted on tomato plants showed that this biostimulant induced in plants grown under stress the chlorophyll content and then biomass production [42]. This beneficial effect was attributed to the capacity of Megafol to activate the expression of specific genes involved in stress responses and tolerance, and it allowed plants to recover quicker when the stress was removed [42].

The ratio ChlA/ChlB is worth mentioning as it is usually around 3 and related to the amounts of chlorophyll associated with photosystems [43]. If this ratio increases, it indicates environmental changes and stress or external stimuli, which could also result from the lack of nitrogen nutrition [43]. Our experiments showed significant increases in this ratio in maize exposed to NaCl (Table 2), mainly when the stress was imposed without the biostimulant. This effect is worth consideration as the chlorophyll-protein complex, which has the function to harvest light, is positively related to the activity of photosystem II (PSII) [43]. Therefore, the results show that Meg can help the plant to cope with the salt stress stimulating the content of chlorophyll a and the activity of PSII. The carotenoids content strongly decreased in maize grown with NaCl alone, while biostimulated samples exhibited values of these pigments similar to those of control samples (Table 2). The importance of this effect prompted by the biostimulant lies in the biological role of this class of pigments since they operate as light-harvesting molecules and exert a protective function [44]. In particular, carotenoids remove ROS, leading to the protection of chloroplast from oxidants, whose overproduction is frequently caused by biotic and abiotic stresses [44].

Finally, the interest towards the possibility of increasing carotenoids for their nutraceutical value should be mentioned, given their health-promoting action [45]. Therefore, the effect of Meg on maize in salt stress considered in terms of maintaining a high carotenoid content has an immediate protective effect against salinity as it is well known that this adversity can give rise to the overproduction of ROS. Further, given the healthy action of these compounds, the use of biostimulants to improve or maintain a high carotenoid content deserves attention for the production of quality food. Finally, the ChlA/Car has been proposed as an index to evaluate the entity of environmental stresses in plants [43]. Our data show that this ratio remained practically the same in control and biostimulated maize grown in salinity, whereas samples treated with NaCl alone showed a considerable increase in this ratio [43]. In particular, the lower values exhibited by biostimulated samples indicated a good relationship between the light-harvesting complex and the capacity to carry out non-photochemical quenching [43]. Several hypotheses have been formulated to explain the mechanisms of tolerance/resistance of crops to salinity, but, in many cases, they are dependent on the species in question. In general, however, among the most critical pathways adopted by species to counteract the deleterious effects of salinity mechanisms, the controlling of Na\(^+\) uptake and ROS production are reputed of particular importance [2,6,46,47]. Regarding the latter aspect, a large body of literature documents that salinity causes oxidative stress, and this mainly affects chloroplasts and mitochondria with negative consequences on their functionality [6,46,47].

As far as Na\(^+\) is concerned, as already mentioned, there is some variability between different species, but in some cases, plants can cope with this stress, limiting this cation translocation into the shoots [48]. Furthermore, some plants can improve their tolerance to salinity by increasing K\(^+\) levels in leaves [49]. Indeed, increases in this ion result in osmotic adjustments, which in turn elevate the plant tolerance to stress [35]. Our results show that Meg strongly limited the translocation of Na\(^+\) to the shoots in samples grown in salinity when compared to non-biostimulated samples grown under NaCl (Figure 1). The K\(^+\) significantly increased in the samples grown under salt stress alone, while in the biostimulated samples, it remained unchanged compared to the control samples (Figure 1). Salinity tolerance is based on a complex load of ions and ion exchange systems at the roots and shoots level [50]. In the initial phases of salt stress, high amounts of Na\(^+\) are
quickly transported to the shoot, then a progressive reduction of this cation can take place to avoid excessive accumulations in shoots [50]. In addition, it has been demonstrated that an excessive ROS accumulation could activate non-selective cation channels that are permeable, among others, to Na\(^+\), thus revealing, in particular, the role of the hydrogen peroxide (H\(_2\)O\(_2\)) in regulating ion-loading in shoots [51].

Our results indicate that plants exposed to salt without the biostimulant exhibited a non-selective and deleterious increase in Na\(^+\) in shoots, accompanied by an increase in K\(^+\). On the contrary, the biostimulant strongly limited the amount of Na\(^+\) accumulated in the shoots. This effect has been accounted for in several biostimulants, although the mechanisms of such an effect still remain unclear [25,36,52,53]. However, limiting the concentration of Na\(^+\) in the shoot is a crucial mechanism for plant survival in salinity, and it was prompted by the biostimulant investigated in this study. Salt stress can give rise to the overproduction of ROS, thus affecting the redox status of cells and generating oxidative stress. Excessive ROS accumulation has a negative impact mainly for its ability to degrade proteins, membranes, and DNA [54]. H\(_2\)O\(_2\) is one of the main oxidizing species and can seriously affect cell functionality if accumulated to excessively high levels [55]. Furthermore, as already stated, the accumulation of H\(_2\)O\(_2\) in salinity could lead to non-selective Na\(^+\) acquisition by plants, as appeared to happen in our experiments, especially in unbiostimulated plants. Despite this, the biostimulant significantly decreased the amount of H\(_2\)O\(_2\), as evidenced in Figure 2A compared to maize samples grown with only NaCl. This last aspect should be discussed with the other parameter investigated in this study, thus the content of malondialdehyde (MDA).

MDA is a lipid peroxidation product that accumulates in oxidative perturbations, even though it is commonly produced in mitochondria and chloroplasts, where metabolic processes characterized by high electron flow occur [6,29]. The results of our study evidenced that salinity decisively increased the amount of MDA when maize was treated with NaCl alone (Figure 2B), but it decreased when the samples were biostimulated. MDA accumulates when the oxidative stress is particularly severe, and antioxidant defences fail to contain ROS [56]. The results obtained in this study shed light on the Meg capacity to ameliorate the oxidative status of maize and are in line with other studies on such a protective effect of biostimulants [29,57]. Phenolic compounds represent one of the most abundant families of secondary metabolites in plants and are involved in metabolic and physiological processes, including adaptation in stress conditions [58]. In fact, the biosynthesis and content of phenolic compounds are particularly sensitive to abiotic stresses [58]. Our experiment showed that the salt stress caused significant increases in TPC in unbiostimulated plants, and this can be seen as a plant response to improve its free radical scavenging activity (Table 3). Therefore, it can be concluded based on TPC results that the biostimulant reduced the stress and, consequently, the TPC in maize [59].

Finally, the antioxidant activity of the plant methanolic extracts was measured using the DPPH, ABTS, and FRAP. Each method provides an estimate of antioxidant capacity which is dependent on the conditions and reagents used. Concerning the results of these parameters, the highest values were generally found in methanolic extracts from plants grown in salinity when the biostimulant was not applied (Table 3). The only exception was the DPPH, which did not vary significantly between biostimulated and non-biostimulated salt-stressed samples. This result can be explained by the fact that the DPPH assay has some disadvantages which can limit its reliability. In particular, this method is influenced by the pH, the solvent, and the steric accessibility of the radical: large antioxidants that react rapidly with peroxyl radicals might not be quantified by this test. In addition, the DPPH does not show affinity with the reactive oxygen species [60]. Consequently, the DPPH does not allow assessing the antioxidant capacity correctly in some instances. On the contrary, ABTS and FRAP indicated that the samples subjected to salt stress without the biostimulant evidenced higher antioxidative activities than the biostimulated samples (Table 3). These findings evidence that the biostimulant was able to decrease the oxidative impact of NaCl and such an effect can be linked to the ability of this substance to reduce
the Na\textsuperscript{+} taken up by the crop (Figure 1), which in turn led to reduced accumulations of hydrogen peroxide and MDA (Figures 1 and 2A,B). Furthermore, as already discussed, the reduced content of H\textsubscript{2}O\textsubscript{2} in biostimulated samples can avoid the unregulated loading of Na\textsuperscript{+} in shoots [51]. Finally, it should be noticed that the ABTS and FRA well correlate with TPC for the electron-donating capacity of polyphenolic compounds [61,62].

5. Conclusions

In conclusion, this research has shown that it is possible to significantly reduce the negative effects of salinity in maize by using a biostimulant. This beneficial action affected several physiological and biochemical parameters and was justified by the biostimulant ability to reduce the amount of sodium translocated by the treated species into the shoots. As a result, the biostimulant ameliorated some aspects concerning biomass production, pigments, and the oxidative state of the studied grown under salinity. Therefore, based on the results presented in this study, it can be indicated that biostimulants can be seen as a helpful tool for improving maize performance under severe salinity conditions.

Author Contributions: Conceptualization, R.D. and D.D.B.; methodology, R.D. and D.D.B.; investigation, R.D. and D.D.B.; data curation, R.D. and D.D.B.; writing—original draft preparation, R.D. and D.D.B.; writing—review and editing, D.D.B. Both authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: Main data are contained within the article; further data presented in this study are available on request from the corresponding author.

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

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