agronomy

MDPI

Article

Chlorophyll Fluorescence Parameters and Antioxidant Defense System Can Display Salt Tolerance of Salt Acclimated Sweet Pepper Plants Treated with Chitosan and Plant Growth Promoting Rhizobacteria

Muneera D. F. ALKahtani [1], Kotb A. Attia [2], Yaser M. Hafez [3], Naeem Khan [4], Ahmed M. Eid [5], Mohamed A. M. Ali [6] and Khaled A. A. Abdelaal [7,*]

1 Biology Department, College of Science, Princess Nourah bint Abdulrahman University, Riyadh POX 102275-11675, Saudi Arabia; mdfkahtani@gmail.com
2 Center of Excellence in Biotechnology Research, King Saud University, Riyadh POX 2455-11451, Saudi Arabia; kattia1.c@ksu.edu.sa
3 Plant Pathology and Biotechnology Lab., Faculty of Agriculture, Kafrelsheikh University, Kafr El Sheikh 33516, Egypt; hafezyasser@gmail.com
4 Department of Agronomy, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, FL 32611, USA; naeemkhan@ufl.edu
5 Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Nasr City 11884, Egypt; aeidmicrobiology@azhar.edu.eg
6 Department of Horticulture, Faculty of Agriculture, New Valley University, El-Kharga, New Valley 72511, Egypt; Mohamedahmedali77@aun.edu.eg
7 Excellence Center (EPCRS), Plant Pathology and Biotechnology Lab., Faculty of Agriculture, Kafrelsheikh University, Kafr El Sheikh 33516, Egypt
* Correspondence: khaled.elhaies@gmail.com

Received: 15 July 2020; Accepted: 1 August 2020; Published: 12 August 2020

Abstract: Salinity stress deleteriously affects the growth and yield of many plants. Plant growth promoting rhizobacteria (PGPR) and chitosan both play an important role in combating salinity stress and improving plant growth under adverse environmental conditions. The present study aimed to evaluate the impacts of PGPR and chitosan on the growth of sweet pepper plant grown under different salinity regimes. For this purpose, two pot experiments were conducted in 2019 and 2020 to evaluate the role of PGPR (*Bacillus thuringiensis* MH161336 $10^{6–8}$ CFU/cm^3) applied as seed treatment and foliar application of chitosan (30 mg dm^{-3}) on sweet pepper plants (cv. Yolo Wonder) under two salinity concentrations (34 and 68 mM). Our findings revealed that, the chlorophyll fluorescence parameter (*Fv/Fm* ratio), chlorophyll *a* and *b* concentrations, relative water content (RWC), and fruit yield characters were negatively affected and significantly reduced under salinity conditions. The higher concentration was more harmful. Nevertheless, electrolyte leakage, lipid peroxidation, hydrogen peroxide (H_2O_2), and superoxide (O_2^-) significantly increased in stressed plants. However, the application of *B. thuringiensis* and chitosan led to improved plant growth and resulted in a significant increase in RWC, chlorophyll content, chlorophyll fluorescence parameter (*Fv/Fm* ratio), and fruit yield. Conversely, lipid peroxidation, electrolyte leakage, O_2^-, and H_2O_2 were significantly reduced in stressed plants. Also, *B. thuringiensis* and chitosan application regulated the proline accumulation and enzyme activity, as well as increased the number of fruit $plant^{-1}$, fruit fresh weight $plant^{-1}$, and total fruit yield of sweet pepper grown under saline conditions.

Keywords: sweet pepper; salinity; *Bacillus*; chitosan; chlorophyll fluorescence; fruit yield

1. Introduction

Sweet pepper belongs to Solanacease family. It is an annual plant in the cultivated lands in many countries, however it is grown as a perennial plant in tropical areas. It is one of the most widespread and popular vegetables, and has a greatest economic importance worldwide [1]. It is the richest source of different antioxidants and vitamins and has several health benefits [2]. However, salinity is a very significant factor that threatens the production of economic plants such as sweet pepper [1], strawberry plants [3], and cucumber plants [4]. Salinity damages plant growth and proliferation by creating water stress and cytotoxicity due to the excess in uptake of ions, such as sodium and chloride. Furthermore, salinity is usually accompanied by oxidative stress due to the generation of reactive oxygen species [5,6]. Salinity stress adversely affects morpho-physiological characters of sweet pepper such as plant height and leaf area which are significantly reduced [7]. Likewise, chlorophyll *a* and *b* as well as RWC were reduced under salinity in cucumber [4]. Photosynthesis is harmfully affected by salinity through the reduction in stomatal conductance. Also, salinity led to increased ion toxicity and negatively affected nutrients uptake, especially potassium uptake, so the salt stressed plants showed low membrane stability [8]. The chlorophyll fluorescence parameters were adversely affected with salinity and the content of chlorophyll pigments significantly decreased in cucumber [9]. Also, the study of Misra et al. [10] pointed out that salt stress causes photoinhibition in PSII and decreases its activity. Salt stress led to decreased chlorophyll concentrations, leaf area and mungbean yield [11] and led to an increase in the accumulation of Na^+, decreasing the uptake of mineral nutrients such as nitrogen and potassium [12]. The high level of Na^+ was associated with the ROS accumulation such as H_2O_2 and O^{2-}. The excessive formation of ROS causes protein oxidation and lipid peroxidation under several stresses mainly under salinity stress [1,13]. Previous studies have shown that the adverse effects of salinity stress on leaf number, plant length, fresh and dry weights of shoots, and plant yield also increases with the increase in NaCl concentration [14–16].

According to salinity concentrations, the plants are classified to euhalophytes or glycophytes. Euhalophytes have the salinity thresholds of 250 mM NaCl, i.e., euhalophytes are able to complete their life cycle upon salinities exceeding 250 mM NaCl. Glycophytes cannot grow under high salinity concentrations and their response to salinity differs in terms of osmotic regulation, photosynthetic electron transport, chlorophyll content, and reactive oxygen species (ROS) formation as well as antioxidant defense system [1,7]. The excessive accumulation of ROS under stress, such as salinity [1,17], drought [18,19], and biotic stress factors [20–23], results in the activation of the enzymatic and non-enzymatic antioxidant system to enhance stress tolerance in plants to cope with increased accumulation of ROS [24]. The antioxidative system also consists of some of the non-enzymatic systems, such as salicylic acid and carotenoids. Nonetheless, the enzymatic defense system contains ascorbate peroxidases (APX), glutathione reductases (GR), superoxide dismutases (SOD), catalases (CAT), and peroxidases (POD), which protect the plant tissues against stress factors [25]. Also, the plants have adaptive mechanisms to salinity stress through morphological, anatomical, and biochemical changes. Euhalophytes can cope with salinity stress through different mechanisms, such as salt exclusion, salt elimination, salt succulence and salt redistribution [7]. Furthermore, EL%, lipid peroxidation, and ROS were increased significantly under salinity, as these parameters are signals to various stresses, such as salinity, drought, and heat [26–29], that enable plants to respond to a particular stress. Some plants protect themselves from salinity stress by maintaining ion homeostasis and transportation of the excess salt to the vacuole or sequestering in the older tissues which ultimately are sacrificed, thereby defending itself from salinity stress [30]. Meanwhile, other plants keep the ion concentration in the cytoplasm at a low level. Membranes along with their linked components play an essential role in retaining ion concentration within the cytosol during the period of stress by regulating ion uptake and transport [31,32]. Chlorophyll fluorescence is a fast method for photosynthetic processes measurements [33] and provides a lot of information about the plant status under abiotic and biotic stresses to understand the mechanisms of photosynthesis and how plants respond to various stresses [34]. Chlorophyll fluorescence parameters are important indicators used to measure the

quantum yield of photosystem II (PSII), display the plant response to stress and the harmful effects, particularly on photosynthesis and chlorophyll concentrations [35].

Chitosan or chitin is a natural polysaccharide consisting of two molecules of D-glucosamine and naturally present in the cell walls of many organisms such as crabs, shrimp, fungi, and the exoskeleton of insects [36]. In the agricultural field, it improves the morpho-physiological parameters and alleviates the injurious effect of abiotic stresses through stress transduction pathway [37]. Application of chitosan led to increased plant tolerance to many stresses in various plants [38,39], enhance growth characters and improve germination rate of many plants [38,40]. The fruit yield of tomato plants was improved with chitosan treatments [41]. Under drought, barley plants treated with chitosan showed a significant increase in chlorophyll, RWC, total soluble sugar, and grain yield [42]. Plant growth-promoting rhizobacteria (PGPR) can prompt plant tolerance to stress through some chemical and physical changes which are identified as induced systemic tolerance [43]. The application of PGPR led to improved growth and yield production [44]. Under stress conditions, PGPR can improve the injurious impacts and enhance the yield production under salt conditions [45], as a bio-fertilizer in sugar beet and sweet sorghum plants [20,46,47] and as a bio-control agent [48–50]. There are many PGPR strains, such as *Bacillus*, *Azotobacter*, *Azospirillum*, *Pseudomonas*, *Rhizobium,* and *Serratia,* which can be used in improving plant growth even under various stress factors [51,52] by the production of antioxidants, phytohormones and vitamins [53]. There is a lot of information about the effect of PGPR, nevertheless studies about chitosan and its effects on plants under salinity stress are still scarce and have not yet been fully understood. Hence, in this research, we focus on the effect of chitosan and *Bacillus thuringiensis* MH161336 in alleviating the harmful effect of salinity to improve chlorophyll fluorescence parameters, chlorophyll concentration, enzymes activity, and fruit yield of sweet pepper.

2. Materials and Methods

2.1. Experiments Preparation and Plant Materials

Two pot experiments were conducted at Kafrelsheikh University, Agricultural Botany Department during two summer seasons 2019 and 2020, to evaluate the effect of seed treatment with plant growth promoting rhizobacteria (*B. thuringiensis* MH161336 $10^{6–8}$ CFU/cm^3) and foliar spray with chitosan 30 mg·dm^{-3} on sweet pepper plants under salinity (sodium chloride at 34 and 68 mM). The physio-biochemical characters were done at Plant Pathology & Biotechnology Lab., and EPECRS Excellence Center, Kafrelsheikh University. The seeds of sweet pepper (*Capsicum annuum* L.) cv. Yolo Wonder (obtained from a private agricultural company) were divided into three groups (the first group was treated with *B. thuringiensis* and the others without treatments). Seed treatment was done with *B. thuringiensis*. Thereby, the seeds underwent surface sterilization by sodium hypochlorite 2.5% for 5 min, 70% ethanol for 1 min, and were then washed 5 times by sterile distilled water. *B. thuringiensis* MH161336 which was isolated from the halophytic plant *Spergularia marina* (obtained from Dr. Ahmed Eid), *B. thuringiensis* pure cultures were grown in nutrient broth at 35 ± 2 °C on a shaker at 180× *g*. Bacterial cultures were diluted in sterilized distilled water to reach a final concentration of $10^{6–8}$ CFU/cm^3 [54]. Sterilized seeds were incubated with bacterial suspensions at room temperature for 6 h and sown in the nursery in foam trays on 7th and 3rd January in the two seasons, respectively. After forty-five days from the sowing, the transplantation was done in pots 50 cm^3 in diameter, each one containing two seedlings and the pots were divided into three groups (control, *B. thuringiensis* treatment and chitosan treatment 30 mg·dm^{-3}). The plants irrigated with two concentrations (34 and 68 mM) of saline water (was prepared from NaCl) and the group of chitosan treatment was treated with chitosan 30 mg·dm^{-3} twice after 20 and 40 days from transplanting. The compound fertilizer containing nitrogen, phosphorus, and potassium (NPK) (135:40:35 kg·ha^{-1}) was used as recommended in two doses, the first dose after 12 days from transplanting and the second at the flowering stage initiation. The experiments were in a completely randomized design with 4 replicates, the physiological and

biochemical studies were done at 80 days from transplanting. The chemical and physical characters of experimental soil were determined [55] and are presented in Table 1.

Table 1. Chemical and physical characters of the experimental soil before conducting the experiments in 2019 and 2020 seasons.

Seasons	PH	* EC Ds/m	Mechanical Analysis			Soil Texture	Organic Matter (%)	Total N (%)	Total P (ppm)
			Sand%	Silt%	Clay%				
2019	8.11	0.464	21.96	23.98	47.4	Clay	1.79	0.158	8.8
2020	8.16	0.483	22.17	24.29	46.8	Clay	1.82	0.149	8.2

Seasons	Soluble Cations				Soluble Anions		
	Na^+	K^+	Ca^{++}	Mg^{++}	HCO_3^-	SO_4^{--}	Cl^-
2019	2.14	0.18	2.02	2.4	4.2	2.07	0.6
2020	2.19	0.17	2.04	2.3	4.3	1.93	0.5

* EC = Electrical conductivity.

2.2. Biochemical and Physiological Characters

The physiological and biochemical characters were recorded at 80 days from transplanting.

2.2.1. Relative Water Content (RWC%)

According to Sanchez et al. [56], twenty leaf discs were used to determine RWC, the fresh weight (FW) for the discs was determined, the same discs were saved in petri dishes containing distilled water for 1 h to determine the turgid weight (TW), after that the discs were dried for 24 h at 80 °C to determine the dry weight (DW). Relative water content (RWC%) was calculated as follows: RWC = (FW − DW)/(TW − DW) × 100.

2.2.2. Determination of Chlorophyll *a* and *b* Concentrations

The extraction was done using *N-N* Dimethyl formamide, whereby 5 cm^3 *N-N* Dimethyl formamide was added to 1 g fresh leaves and kept in the refrigerator overnight, and the absorbance was measured at 647 and 664 nm according to Moran [57].

2.2.3. Electrolyte Leakage Assay (EL%)

Ten discs (1 cm^2) of sweet pepper leaves were placed into flasks containing deionized water (25 cm^3). Flasks were shaken for 20 h, initial electrical conductivity was recorded for each vial and then flasks were immersed in a water bath at 80 °C for 1 h. The vials were shaken for 20 h at 21 °C. Final conductivity was measured for each flask. Electrolyte leakage % was calculated according to Szalai et al. [58] with the following formula: initial conductivity/final conductivity × 100.

2.2.4. Chlorophyll Fluorescence Parameter

Chlorophyll *a* fluorescence parameter (*Fv*/*Fm* ratio) was measured at 80 days from the sowing using a chlorophyll fluorometer (PEA, Hansatech Instrument Ltd., version 1.21, Norfolk, UK).

According to Schreiber [59], middle-aged sweet pepper leaves were placed in the dark for 30 min to stimulate the reaction of photosystem II. The minimum chlorophyll fluorescence (*Fo*) was measured using a measuring beam of <0.1 μmol $m^{-2}{\cdot}s^{-1}$. The maximum fluorescence (*Fm*) was determined after a 1 s saturating pulse (>3500 μmol$\cdot m^{-2}{\cdot}s^{-1}$). Variable fluorescence (*Fv*) was determined by the difference between the maximum fluorescence and the minimum fluorescence (*Fm* − *Fo*). The maximum efficiency of PSII was determined as the ratio of (*Fv*) to (*Fm*) as follows: *Fv*/*Fm* = (*Fm* − *Fo*)/*Fm*.

2.2.5. Proline Determination

According to Bates et al. [60], proline was assayed in sweet pepper plants, 0.5 g fresh leaf in 3% sulphosalicylic acid and centrifuged for 20 min at 3000× *g*. Then, 2 cm^3 of glacial acetic acid and 2 cm^3 ninhydrin reagent was boiled with 2 cm^3 supernatant for 1 h, the reaction was completed in an ice bath, and proline was separated using toluene. Proline was determined as $\mu g \cdot g^{-1}$ FW using a spectrophotometer at 520 nm.

2.2.6. Determination of Lipid Peroxidation

According to Davenport et al. [61], lipid peroxidation was measured as malondialdehyde (MDA) using 100 mg fresh leaves in 1% trichloro acetic acid and centrifuged at 10,000× *g* for 5 min. 0.5% thiobarbituric acid was then added, and mixture was boiled at 95 °C for half an hour. The samples were placed on an ice bath and centrifuged for 5 min at 5000× *g*, the measurements were done using spectrophotometer at 532 and 600 nm. MDA ($nmol \cdot g^{-1}$ FW) = [6.45 × (A532 − A600) − (0.56 × A450)] × $V^{-1}W$, where V = volume (cm^3); W = weight (g).

2.2.7. Determination of Superoxide (O_2^-) and Hydrogen Peroxide (H_2O_2)

Sweet pepper leaves were vacuum infiltrated with 10 mM potassium salicylate buffer containing 0.1 *w/v*% nitro blue tetrazolium (NBT) or 0.1 *w/v*% 3,3-diaminobenzidine (DAB). The leaves were incubated in the light for 140 min and two hours, respectively. The samples were cleared with trichloroacetic acid in ethanol: chloroform 4:1 *v/v* for 1 day, the samples were washed and placed in 50% glycerol. O_2^- and H_2O_2 were determined as $nmol \cdot g^{-1}$ FW according to Huckelhoven et al. [62] using a ChemiImager 4000 digital imaging system (Alpha Innotech Corp., San Leandro, CA, USA).

2.2.8. Assay of Enzymes Activity

For the determination of enzymes, 0.5 g fresh leaves were homogenized in 3 cm^3 of 50 mM Tris buffer at 0–4 °C, containing 1 mM EDTA-Na_2 and 7.5% polyvinyl pyrrolidone. The samples were centrifuged 12,000× *g* for 20 min at 4 °C and the total soluble enzyme activities were measured using spectrophotometer in the supernatant [63]. Catalase activity (CAT) was determined through the decomposition of H_2O_2 by catalase results in the decrease of the ultraviolet absorption of H_2O_2 at 240 nm, catalase activity can be calculated from this decrease. The reaction mixture contained 2.15 cm^3, 2 cm^3 0.1 M Na-phosphate buffer, 100 µL H_2O_2, and 50 µL leaves extract. The solution is mixed, and the absorptions were recorded at 240 nm according to Aebi [64]. Peroxidase activity (POX) was calculated according to Hammerschmidt et al. [65]. The reaction mixture consisted of 2.9 cm^3 of a 100 mM sodium phosphate buffer containing 0.25% (*v/v*) guaiacol and 100 mM H_2O_2. The reaction was done with adding 100 µL of crude enzyme extract, the changes in absorbance were recorded every 30 s intervals for 3 min at 470 nm, the activity was determined for $min^{-1} \cdot g^{-1}$ fresh weight. Activity of superoxide dismutase (SOD) was measured according to Mishra et al. [66]. Then, we add 290 µL of a mixture containing 100 mM potassium phosphate buffer, 0.1 mM EDTA, 11 mm^3 xanthine, cytochrome-c, and 0.002 units of xanthine oxidase to 20 µg of protein extracts was prepared. Xanthine oxidase regulation produced an increase in the absorbance due to the reduction of cytochrome-c (0.025 ± 0.005 min^{-1}). Activity of SOD was stated by McCord and Fridovich [67]. According to Goldberg and Spooner [68], GR activity was measured, approximately 0.05 cm^3 enzyme extract was mixed with 1 cm^3 phosphate buffer combined with EDTA, 0.1 cm^3 glutathione, and 0.1 cm^3 NADPH, the absorbance was determined at 340 nm.

2.2.9. Fruit Yield

The harvest date starts at 120 days from transplanting to determine number of fruit $plant^{-1}$, fruit fresh weight $plant^{-1}$ (g), and total fruit yield (ton $hectare^{-1}$).

2.3. Statistical Analysis

Statistical analysis was done using analysis of variance (ANOVA) procedures according to the method of Gomez and Gomez [69] using the MSTAT-C statistical software package. The means between treatments were compared by Duncan [70] when the difference was significant ($P \leq 0.05$). The correlation analysis was done using XLSTAT 2014.5.03 statistical software.

3. Results

3.1. Effect on Relative Water Content (RWC%)

The presented results in Figure 1A showed a significant decrease in RWC in sweet pepper under two salinity concentrations (57.6% at the low concentration (34 mM) (S1) and 52% at the high concentration (68 mM) (S2) comparing with control plants (74.6%) as the mean of the two seasons. Likewise, the results in Figure 1 revealed that seed treatment with *B. thuringiensis* showed a significant increase in RWC in stressed plants (65.7% compared with 57.6% at the low concentration and 60.8% compared with 52% at the high concentration). Furthermore, chitosan application at 30 mg dm^{-3} caused a significant increase in RWC (71.5% compared with 57.6% at the low concentration of salinity) and (67.1% compared with 52% at the high concentration) as a mean of both seasons in the stressed plants. The best treatment under salinity conditions was chitosan at 30 mg·dm^{-3} which achieved 71.5% when compared with control plants 74.6% without any significant difference.

3.2. Effect on Chlorophyll a and b Concentrations

It is obvious from the achieved results in Figure 1B–C that chlorophyll was significantly reduced in stressed plants; chlorophyll *a* significantly decreased at low concentration of salinity (2 mg·g^{-1} FW^{-1}) compared with control (2.85 mg·g^{-1} FW^{-1}) as the mean of both seasons. Furthermore, the high salinity concentration caused a significant reduction in chlorophyll *a* (1.25 mg·g^{-1} FW^{-1}) in stressed plants compared to control (2.85 mg·g^{-1} FW^{-1}). Similarly, salinity stress led to a significant decrease in chlorophyll *b* concentration, the two concentrations caused significant decreases (0.84 and 0.55 mg·g^{-1} FW^{-1} respectively) compared with control (2.85 mg·g^{-1} FW^{-1}). Nonetheless, seed treatment with *B. thuringiensis* and chitosan application led to significant increases in chlorophyll *a* and *b*. The greatest result was obtained with chitosan (S1 + Chitosan) treatment (2.85 mg·g^{-1} FW^{-1}) in the stressed plants with the low salinity concentration compared to the stressed plants (S1) without treatments (2 mg·g^{-1} FW^{-1}).

3.3. Effect on Electrolyte Leakage (EL%)

The presented data in Figure 1D exhibited that EL% significantly increased in the stressed plants, the low salinity concentration caused significant increase (42.3%) comparing with control (13.8%) as the mean of two seasons. Furthermore, the high salinity concentration was more harmfully effective and caused a significant increase in EL% (52.6%) compared with control (13.8%). Nevertheless, chitosan application 30 mg dm^{-3} and seed treatment with *B. thuringiensis* led to significant decrease in EL% in the stressed plants under the two concentrations. Seed treatment with *B. thuringiensis* caused a positive effect and significant decrease in EL% (30.2% and 37.6%) in the stressed plants at the two concentrations compared with untreated plants (42.3% and 52.6%), respectively. Furthermore, EL% was reduced significantly in the stressed treated plants with chitosan 30 mg dm^{-3} (21.7% and 27.2%) that compared with the stressed untreated plants (42.3% and 52.6%).

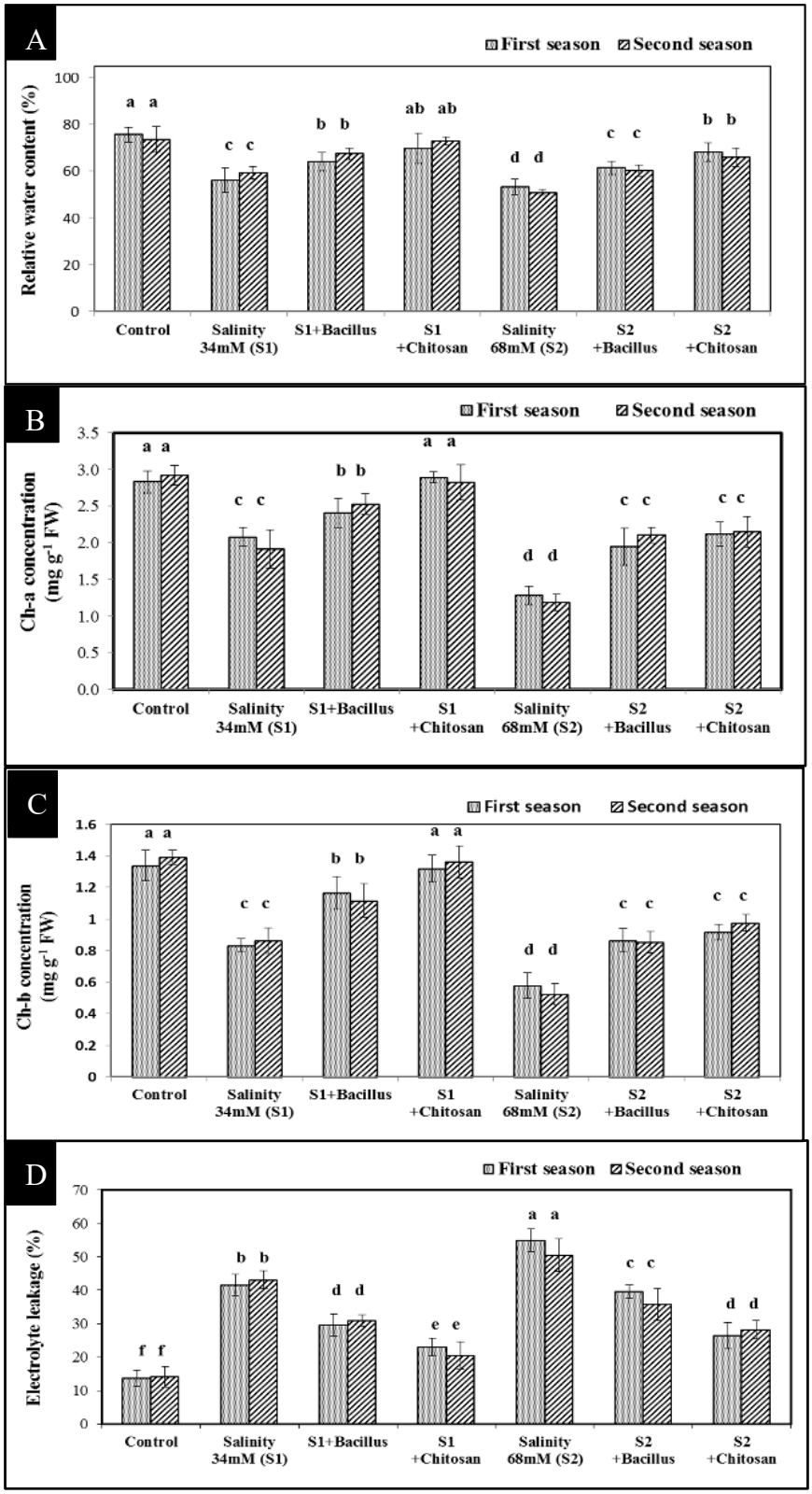

Figure 1. Effect of *B. thuringiensis* and chitosan on relative water content (**A**) chlorophyll *a*, (**B**) chlorophyll *b*, (**C**) and electrolyte leakage (**D**) under two salinity concentrations in sweet pepper plants during two seasons [first season (2019) and second season (2020)]. Data is the mean (±SE) of four replicates. Different letters above the data columns indicate significant differences between the samples determined by ANOVA, Duncan's multiple range test at 0.05 level.

3.4. Effect on Proline Concentration

It could be noted from Figure 2A that the exposed plants to salinity at (S1) and (S2) caused a significant increase in proline concentration, the high concentration of salinity (S2) achieved the high concentration of proline (24 μg·g^{-1} FW) comparing to control (9.1 μg·g^{-1} FW) as the mean of both seasons in sweet pepper. Application of seed treatment with *B. thuringiensis* and chitosan application in stressed plants led to regulate proline accumulation when compared with the control and the stressed untreated plants. *B. thuringiensis* seed treatment led to the regulation of proline accumulation in the stressed plants (12.7 μg·g^{-1} FW at the low concentration of salinity and 13.6 μg·g^{-1} FW at the high concentration comparing to the stressed untreated plants 17.4 and 24 μg·g^{-1} FW) at the two concentrations, respectively. Chitosan application had a significant effect on proline content (9.8 and 12.2 μg·g^{-1} FW) compared with stressed untreated plants (17.4 and 24 μg·g^{-1} FW) at the two concentrations, respectively. The difference was not significant between the both seasons.

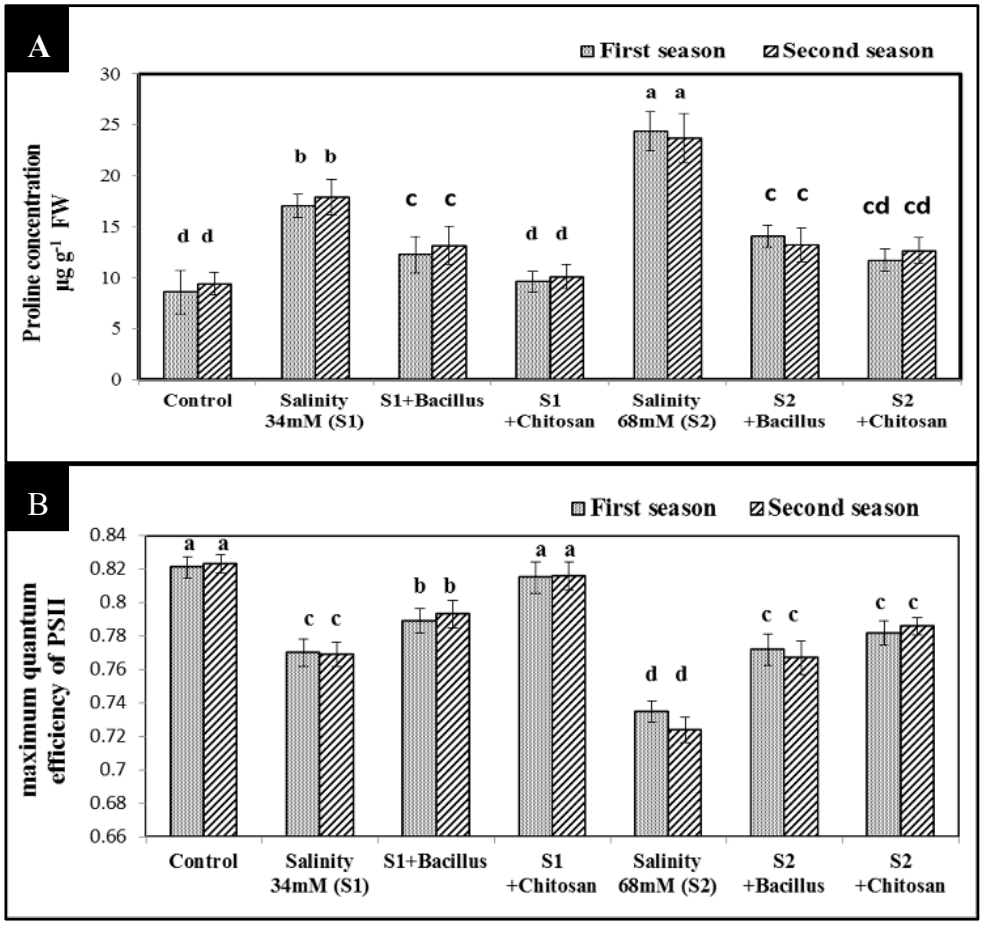

Figure 2. Effect of *B. thuringiensis* and chitosan on proline content (**A**) and maximum efficiency of PSII (*Fv*/*Fm*) (**B**) under two salinity concentrations in sweet pepper during two seasons. Data is the mean (±SE) of four replicates. Different letters above the data columns indicate significant differences between the samples determined by ANOVA, Duncan´s multiple range test at 0.05 level.

3.5. Effect on Chlorophyll Fluorescence Parameter (Fv/Fm)

Our results in Figure 2B indicated that chlorophyll fluorescence parameters were adversely affected under salinity conditions. The maximum efficiency of PSII (*Fv*/*Fm*) significantly reduced in sweet pepper (0.790) at the low salinity concentration and (0.729) at the high salinity concentration, respectively comparing to the control (0.822). However, seed treatment with *B. thuringiensis* caused significant increase in *Fv*/*Fm* ratio in the stressed plants (0.791) at the low concentration of salinity

and (0.769) at the high salinity concentration when compared with the stressed untreated plants (0.790) at the low concentration and (0.729) at the high salinity concentration. Likewise, under the two concentrations, chitosan caused a significant increase in *Fv/Fm* ratio. The best treatment was chitosan at the low salinity concentration (0.815) compared with control (0.822).

3.6. Effect on Lipid Peroxidation as Malondialdehyde

According to the findings in Figure 3, lipid peroxidation (MDA) significantly increased in sweet pepper (11.35 and 13.8 μmol·g^{-1} FW) at the two salinity concentrations, respectively as the mean of both seasons when compared with control plants (6.75 μmol·g^{-1} FW). Nevertheless, MDA significantly decreased in the stressed plants according to seed treatment with *B. thuringiensis* and chitosan treatment. *B. thuringiensis* treatment had a positive effect on MDA and led to significant reduction in the MDA content at the two salinity concentrations (8.8 and 10.5 μmol·g^{-1} FW) when compared with the stressed untreated plants (11.35 and 13.8 μmol·g^{-1} FW). The application of chitosan significantly reduced MDA content in sweet pepper under the two salinity concentrations (7 and 7.85 μmol·g^{-1} FW) when compared with stressed untreated plants (11.35 and 13.8).

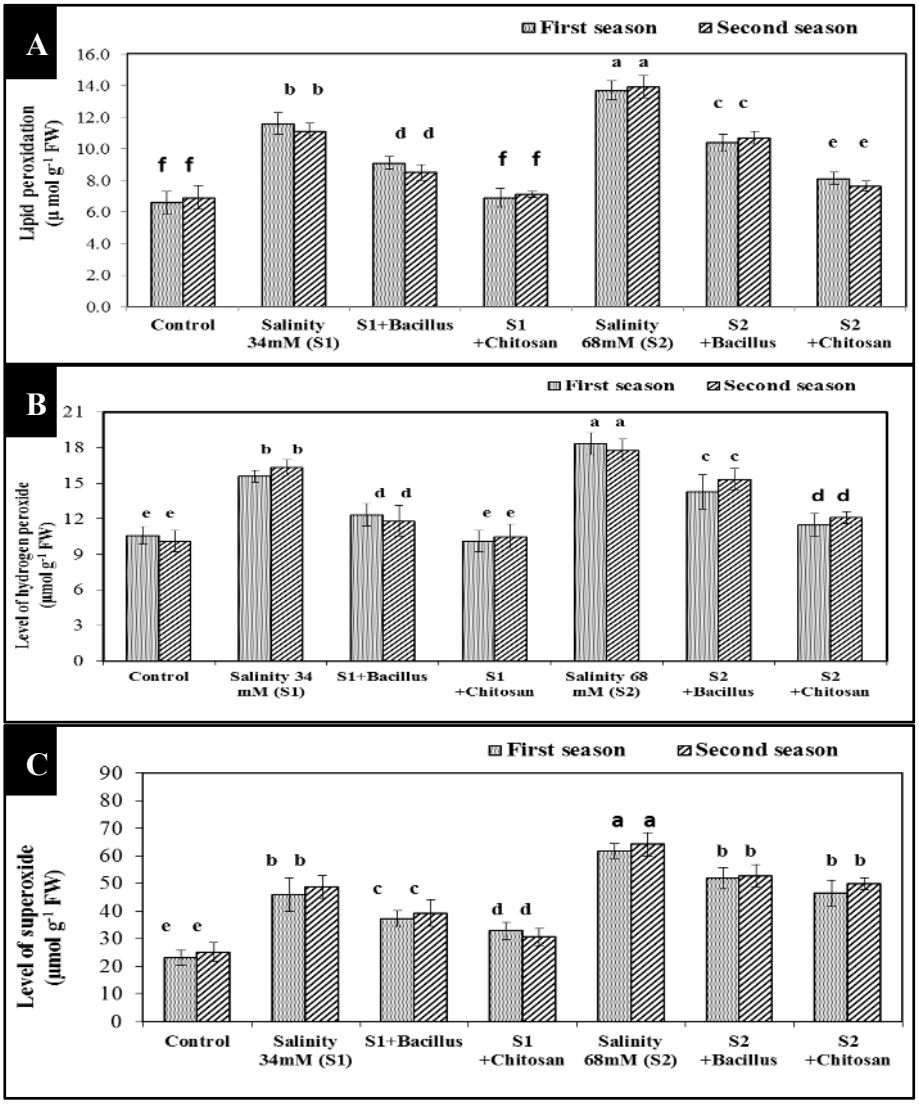

Figure 3. Effect of *B. thuringiensis* and chitosan on lipid peroxidation (**A**), H_2O_2 (**B**) and O_2^- (**C**) under two salinity concentrations in sweet pepper during two seasons. Data is the mean (±SE) of four replicates. Different letters above the data columns indicate significant differences between the samples determined by ANOVA, Duncan´s multiple range test at 0.05 level.

3.7. Effect on O_2^- and H_2O_2

ROS, mainly O_2^- and H_2O_2 significantly increased under the both salinity concentrations (Figure 3). O_2^- significantly increased (47.4 and 63 units) at the two salinity concentrations compared with control (24.16 units). Conversely, *B. thuringiensis* treatment caused a significant decrease in O_2^- in the salt stressed plants (38.3 and 52.3 units) in comparison with stressed untreated plants (47.4 and 63 units). Also, chitosan treatment caused a significant decrease in O_2^- (31.7 and 48.3 units) when compared with the stressed untreated plants (47.4 and 63 units).

Salinity stress caused a significant increase in H_2O_2 in sweet pepper (16 and 18.1 units) at the two concentrations, respectively as compared to control (10.3 units). However, the levels of H_2O_2 were decreased significantly according to *B. thuringiensis* seed treatment and chitosan application in the stressed plants at the two salinity concentrations. Chitosan application gave the best and most significant results (10.3 and 11.8 units) compared to stressed untreated plants (16 and 18.1 units) at the two salinity concentrations, respectively.

3.8. Effect on the Activity of Catalase (CAT), Peroxidase Activity (POX), Superoxide Dismutase (SOD) and Glutathione Reductase (GR) Enzymes

Salinity stress at both concentrations caused significant increases in CAT, POX, SOD and GR enzyme (Figure 4). CAT activity significantly increased in the stressed plants (124.8 and 149.3 mM H_2O_2 g^{-1} FW min^{-1}) at the two salinity concentrations, respectively, when compared with control (78.6 mM H_2O_2 g^{-1} FW min^{-1}).

However, chitosan treatment and *B. thuringiensis* seed treatment caused significant reduction in CAT activity at both salinity concentrations. Chitosan with the low salinity concentration (S1 + Chitosan) gave the best result (85.8 mM H_2O_2 g^{-1} FW min^{-1}) as compared to stressed untreated plants (124.8 mM H_2O_2 g^{-1} FW min^{-1}) and control plants (78.6 mM H_2O_2 g^{-1} FW min^{-1}). Moreover, POX, SOD and GR activities significantly increased in the stressed plants at the low salinity concentration (0.6 µmol tetra-gualacol g^{-1} FW min^{-1}, 74.5 and 0.59 unit/cm^3) as compared to control plants (0.24, 38.3 and 0.36), also, the enzymes activity significantly increased in the stressed plants at the high concentration (0.76, 98.7 unit mg^{-1} FW min^{-1} and 0.59 unit/cm^3) respectively. Nevertheless, chitosan application and seed treatment with *B. thuringiensis* caused a significant reduction in POX, SOD, and GR activity in the stressed plants at the two salinity concentrations compared to the stressed untreated plants.

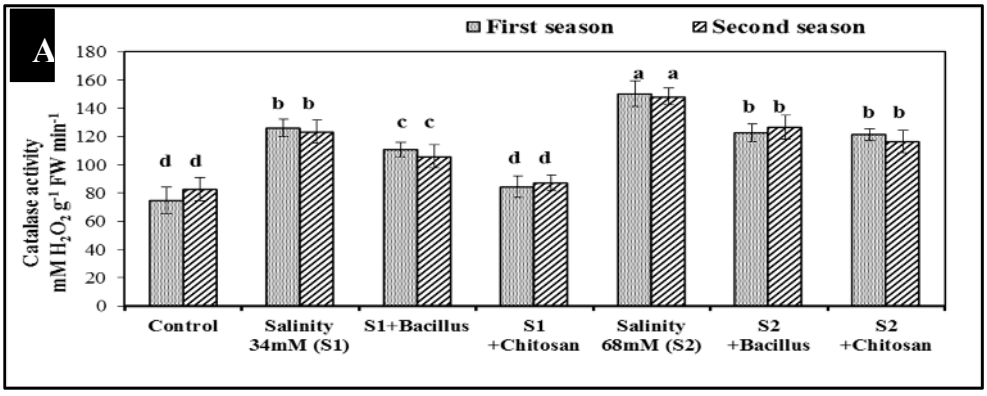

Figure 4. *Cont.*

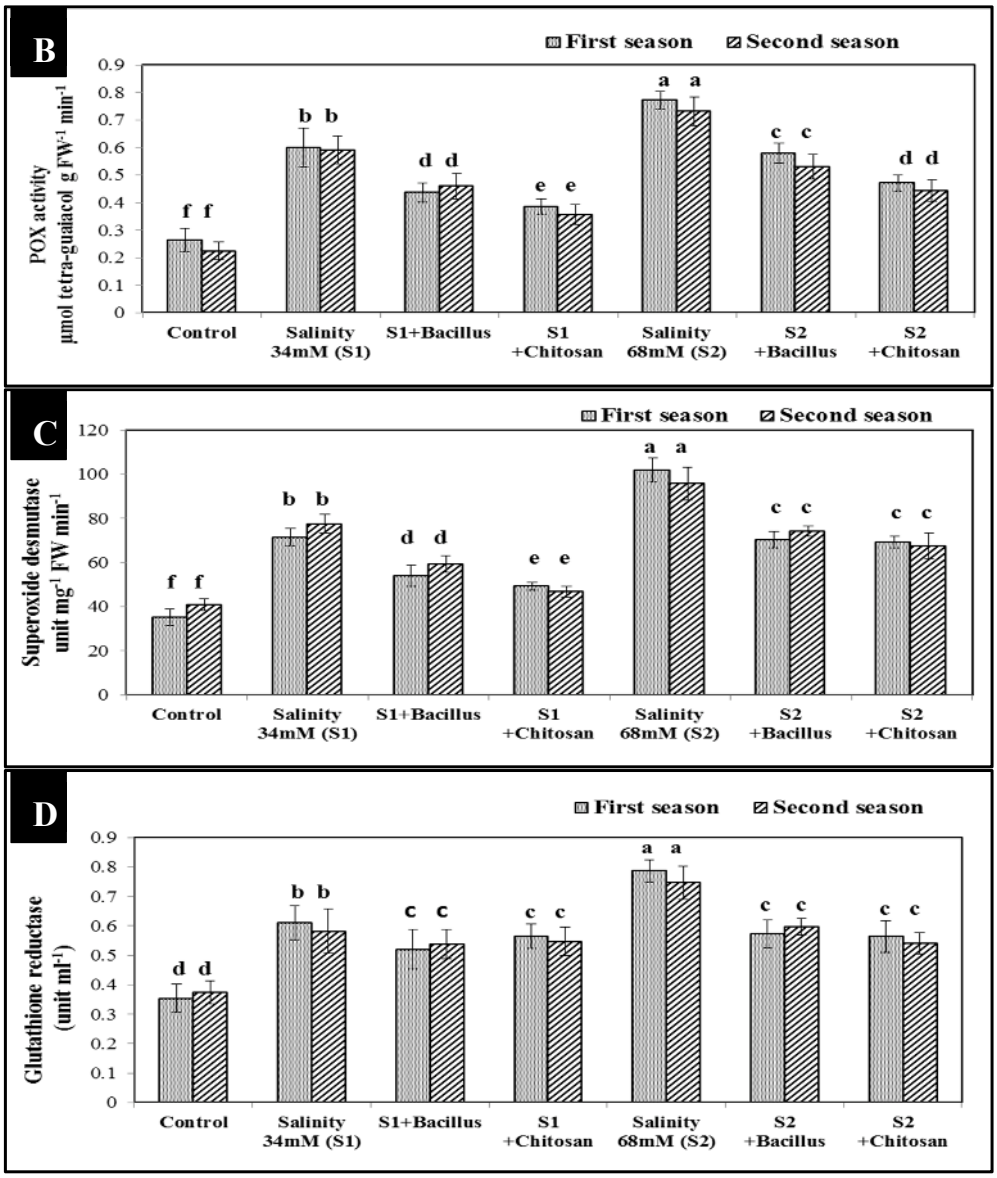

Figure 4. Effect of *B. thuringiensis* and chitosan on the activity of CAT (**A**), POX (**B**), SOD (**C**) and GR (**D**) under two salinity concentrations in sweet pepper during two seasons. Data is the mean (±SE) of four replicates. Different letters above the data columns indicate significant differences between the samples determined by ANOVA, Duncan´s multiple range test at 0.05 level.

3.9. Effect on Number of Fruit Plant^{-1}, Fruit Fresh Weight Plant^{-1} and Total Fruit Yield (Ton Hectare^{-1}).

In the present study, the results in Figure 5 point out that salinity at the both concentrations caused a significant decrease in number of fruit plant^{-1} (7.7 and 4.8 fruit), fresh weight of fruit plant^{-1} (524.5 and 356.4 g) and total fruit yield hectare^{-1} (7.05 and 5 ton) as the mean of the both seasons when compared to control plants (15.7 fruit plant^{-1}, 974 g plant^{-1} and 14.9 ton hectare^{-1}). However, *B. thuringiensis* and chitosan significantly increased the number of fruit plant^{-1}, fruit fresh weight (g plant^{-1}) and total fruit yield (ton hectare^{-1}) in the stressed plants compared with untreated plants. Interestingly enough, under the both salinity concentrations, chitosan application gave the best results and significantly increased the number of fruit plant^{-1} (14.9 and 12.7), fruit fresh weight plant^{-1} (911 and 527 g plant^{-1}), and total fruit yield (14 and 10.8 ton hectare^{-1}) as the mean of the both seasons.

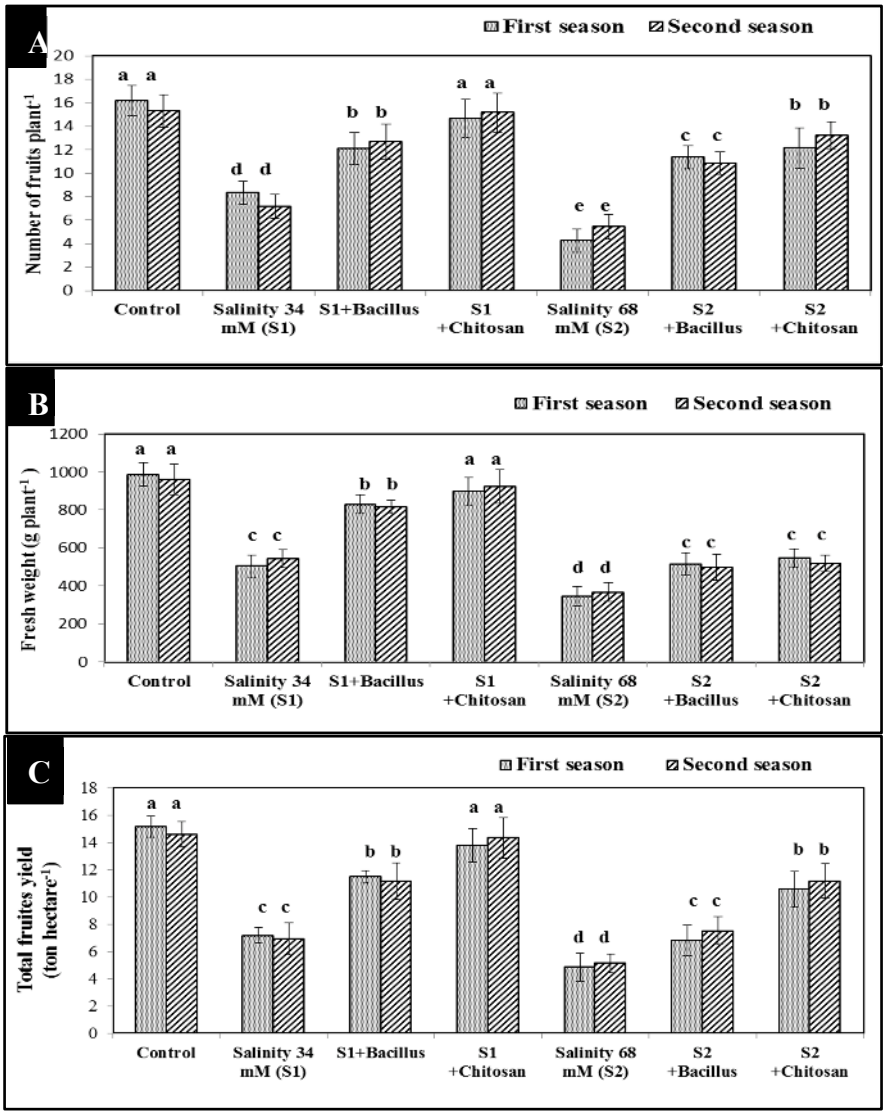

Figure 5. Effect of *B. thuringiensis* and chitosan on number of fruit plant^{-1}(**A**), fruit fresh weight plant^{-1} (**B**) and total fruit yield (ton hectare^{-1}) (**C**) under two salinity concentrations in sweet pepper during two seasons. Data is the mean (±SE) of four replicates. Different letters above the data columns indicate significant differences between the samples determined by ANOVA, Duncan's multiple range test at 0.05 level.

3.10. Correlation Studies

In the present study chlorophyll *a* was positively and significantly correlated with chlorophyll *b* (r = 0.99), number of fruits (r = 0.98), RWC (r = 0.97), GR (r = 0.80) and MDA (r = 0.75). Among the treatment it has a negative correlation with salinity stress @ 34 mM (r = −0.05), salinity stress @ 68 mM (r = −0.02), however, a positive correlation was noted among the chlorophyll *a* and treatments of Bacillus sp. and chitosan (Figure 6 and Supplementary Table S1). A similar trend of relationship was shown by chlorophyll *b*. Proline showed highly positive correlation with MDA (r = 0.98), H_2O_2 (r = 0.97), SOD (r = 0.96) and GR (r = 0.96) but was negatively correlated with the treatments Bacillus sp. (r = −0.04) and chitosan (r = −0.05). A very similar correlation was observed among all the studies. Antioxidant enzymes and H_2O_2 concentration that were highly correlated with each other also showed a negative correlation with the treatments of *Bacillus* sp. and chitosan. The number of fruits showed a highly significant correlation with chlorophyll *a* and *b* (r = 0.98) and with RWC (r = 0.95). However,

this trait was inversely related to the treatments of salinity @ 34 mM (r = − 0.15) and @ 68 mM (r = − 0.25).

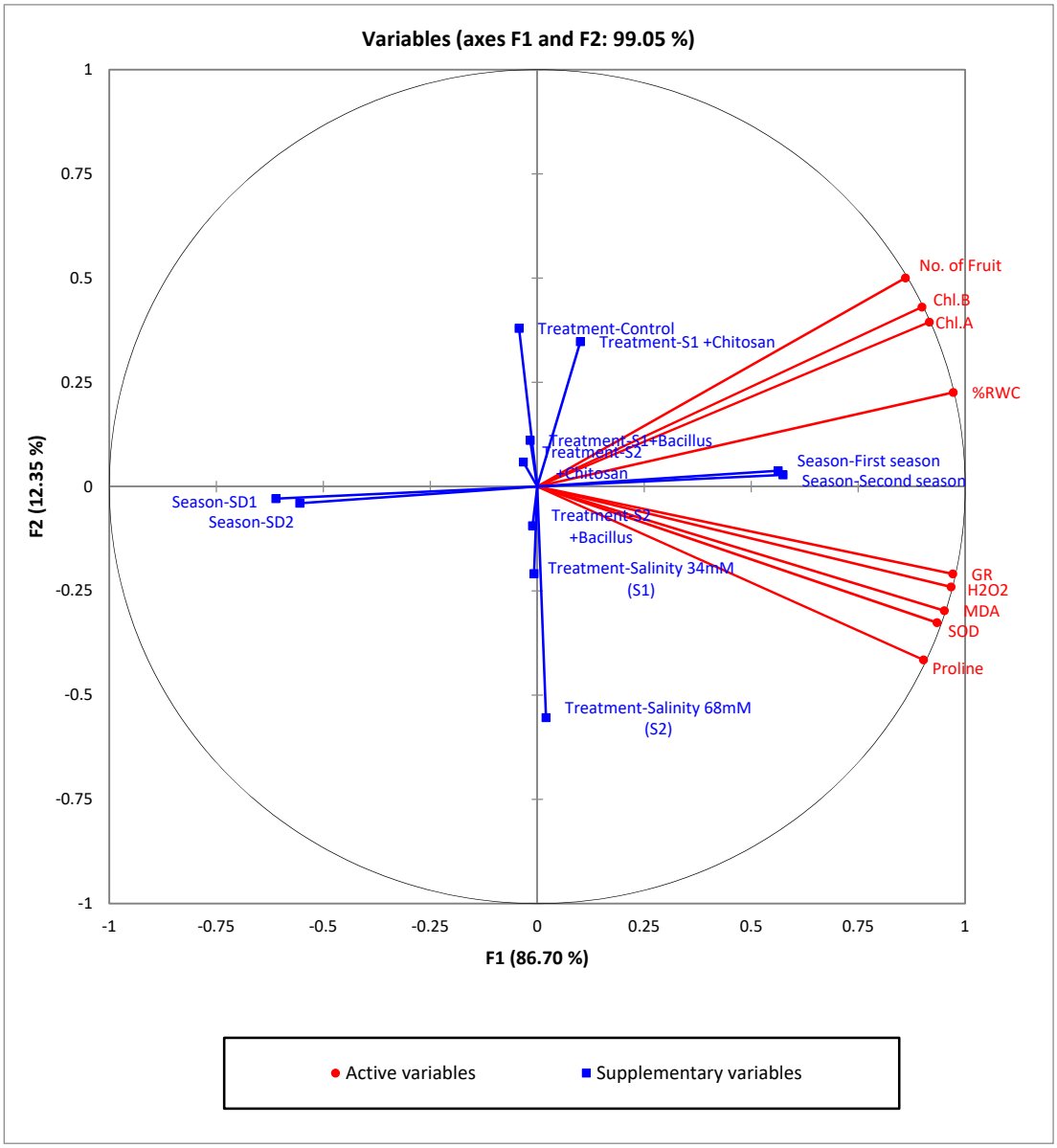

Figure 6. Circle of correlation between variables and factors for sweet pepper.

4. Discussion

Salinity stress adversely affects plant growth, inhibiting plant development and reducing fruit yield of sweet pepper. The present data revealed the deleterious effects of salinity at the two different concentrations (34 and 68 mM) on RWC. This might be due to the injurious influence of salinity on the cell wall structure [71], thereby increasing ethylene concentration, which reduces the growth of roots [44]. This effect causes changes in cell wall properties, the reduction in osmotic potential, and the decrease in water balance [72], consequently reducing RWC in sweet pepper [1]. These deleterious impacts of salinity were overcome by seed treatment with *B. thuringiensis* and treating stressed sweet pepper with chitosan. The pivotal role of *B. thuringiensis* under salinity stress could be due to the formation of Indole-acetic acid which causes enhancement of root growth and increased water uptake [73]. Likewise, PGPR can produce exopolysaccharides (EPSs) which aggregate with soil particles and improve soil structure as well as water uptake [74]. Further, the application of PGPR

causes a decay in the soil bulk density and enhances the availability of soil water. Chitosan application positively affects RWC in stressed plants, this progressive effect of chitosan could be due to the positive role of chitosan on water availability in stressed plants. These valuable effects were documented in barley under drought [19].

Chlorophyll *a* and *b* are very important pigments in the process of photosynthesis, in this process, two reactions take place. One such reaction is the light reaction, in which NADPH and ATP are produced, and the second is the dark reaction, in which carbon dioxide is fixed [75]. Demonstrated data revealed a significant decrease in chlorophyll content under the two salinity concentrations, this decrease in chlorophyll was more considerable at the high concentration (68 mM) than at lower concentration (34.mM) and this might be due to the damaging effect of salinity on the chloroplast structure [3,76], that decrease energy transport from PSII to PSI [77] and, consequently, reduce the chlorophyll formation in stressed sweet pepper plants. The harmful effect of salinity on the content of chlorophyll was also due to reduction in stomatal conductance and destruction of biochemical processes [78]. These findings are in accordance with those reported by Abdelaal et al. [1] in sweet pepper under salinity stress. Also, Asrar et al. [79] indicated that a high salinity concentration caused harmful effects on PSII and decreased chloroplast proteins as well as chlorophyll concentrations. This decrease in chlorophyll concentrations is related to the reduction in RWC under high salt concentration.

Conversely, inoculation of seeds with *B. thuringiensis* mitigates the adverse effects of salinity on the content of chlorophyll that improve the overall growth and proliferation of plants under stressful environments [80]. Beside this, the application of chitosan had also synergistic effects on the contents of chlorophyll *a* and *b*. This increase in the content of chlorophyll with the application of chitosan may be attributed to the fact that chitosan is a rich source for amino acids which increase the chloroplast number and chlorophyll formation. These results are in harmony with the findings of Possingham [81]. During the present study, a significant increase was found in EL% under two different salt concentrations mainly. The higher salt concentration was more effective and significantly increased the EL%. This negative influence of salinity on EL% may be due to its damaging impacts on the cytoplasmic membrane and permeability process. Previously, a similar result was reported by Abdelaal et al. [1] in sweet pepper. Contrariwise, EL% significantly reduced in stressed plants as a result of seed treatment with *B. thuringiensis* and chitosan, these valuable effects of *B. thuringiensis* treatment and chitosan application is attributed to the positive roles of *B. thuringiensis* and chitosan on membrane stability and an improvement in the selective permeability of cell plasma membrane.

In the present study, the chlorophyll fluorescence parameter was adversely affected under two salinity concentrations. Salinity stress causes a significant decrease to maximum efficiency of PSII (*Fv/Fm*). This adverse effect of salinity on (*Fv/Fm*) might be due to its role in the inhibition of electron transport and the reaction centers at the PSII sites as well as destroys the oxygen-evolving complex [82–84]. Also, salinity stress has a negative effect on enzymes activity and decreases the activity of water splitting enzyme complexes and electron transport chains resulting in decrease *Fv/Fm* [85]. However, seed treatment with *B. thuringiensis* and the application of chitosan caused a significant increase *Fv/Fm* ratio in the stressed plants. These results are credited to the helpful role of *B. thuringiensis* and chitosan in increasing the production of protective metabolites, increasing N and K content as well as the number of chloroplasts under stress [81,86], and consequently, improving the chlorophyll fluorescence parameter. The obtained results indicated that proline significantly increased in the stressed plants under both the salinity concentrations (34 and 68 mM). This impact of salinity may be due to its role in reducing the proline oxidation to glutamate, consequently increasing the proline content [87]. Proline is one of the most important osmoprotectants, plays a key role in osmotic regulation, and protects the plants under stress [1,8]. Chitosan application and seed treatment with *B. thuringiensis* regulated proline content under salinity conditions. Seed inoculation with *B. thuringiensis* positively regulated proline content under stress because this species regulates the osmotic balance under saline conditions. Similar results for proline production under saline conditions were also reported by Egamberdieva et al. [88].

Salinity could hamper plant growth and increase lipid peroxidation, O_2^-, and H_2O_2. A significant increase was noted in the mentioned parameters during the present study. These reactive compounds can damage lipids and proteins, essential for the process of photosynthesis and electron transport chain. Islam et al. [18] noted similar results in two wheat cultivars grown under saline conditions. However, in the present study, a significant decrease was noted in the lipid peroxidation upon treatment with chitosan. This may be due to the involvement of chitosan in cell protection from oxidative stress under salinity conditions. Similarly, O_2^- and H_2O_2 were significantly reduced with chitosan due to the presence of hydroxyl and amino groups which react with ROS, thus chitosan can scavenge superoxide radicals [89]. Chitosan derived from the pathogen is recognized by a specific cellular receptor resulting in enhancing the defense response to abiotic and biotic stresses [90]. The positive effect of chitosan in the plant cell protection was also noted in plants under drought stress [20]. Interestingly, seed treatment with *B. thuringiensis* led to improved cell membrane stability and decreased the formation of MDA in the stressed sweet pepper, this effect of *B. thuringiensis* is due to its improved phenol content and defense enzyme system [91]. Also, *B. thuringiensis* causes decreases in O_2^- and H_2O_2 by increasing reactive oxygen scavenging enzyme activity [92].

Enzymes up-regulation (CAT, POX, SOD, and GR) is involved in the mitigation of salinity stress in sweet peppers compared with control plants. The significant increase in these enzymes is a natural defense system, which helps to cope with salinity stress and reduces the osmotic and toxic effects by scavenging ROS. Our results are in agreement with those reported by Abdelaal et al. [17] and Foyer et al. [93]. Nevertheless, it was clear from our results that the application of seed treatment with *B. thuringiensis* led to improved and regulated up-regulation of CAT, POX, SOD, and GR in the stressed sweet pepper. The induction of these enzymes is involved in the mitigation of salt stress in sweet pepper treated with *Bacillus*. A similar trend of enzyme activity was recorded in the findings of Kohler et al. [94]. Likewise, chitosan application causes an increase in enzymes activity to protect the plant from oxidative damage and reduce lipid peroxidation as well as scavenge O_2^- due to its structure and protective role in sweet pepper plants subjected to salinity stress. These results are in agreement with those reported by Hafez et al. [19]. The presented study showed that two salinity concentrations caused a significant reduction in the number of fruit plant^{-1}, fruit fresh weight plant^{-1}, and total fruit yield. This harmful impact of salinity may be due to the decrease in reproductive organs, such as pollen grains in stressed plants [95], and also due to the decrease in water absorption, nutrients uptake, and chlorophyll content [1,4], resulting in a significant decrease in fruit yield [96]. The vital role of *B. thuringiensis* might be due to the formation of growth regulators such as gibberellins, auxin, and cytokinins, as well as an increase in proline content [87], up-regulation of essential enzymes and solubilization of nutrients [89], and an increase in the number of fruits and fruit yield hectar^{-1} in sweet pepper. These findings are in agreement with the previous results reported by Hafez et al. [19], Katiyar et al. [36], and Hidangmayum et al. [37].

5. Conclusions

The present research concluded that seeds treated with *B. thuringiensis* and foliar application of chitosan 30 mg dm^{-3} on sweet pepper plants under two salinity concentrations (34 and 68 mM) led to an improvement of the adverse effects of salinity and enhanced the growth and yield of sweet pepper. RWC, chlorophyll *a* and *b* concentrations, chlorophyll fluorescence parameters, and fruit yield characters significantly increased with *B. thuringiensis* and chitosan treatments in sweet pepper under two salinity concentrations. Conversely, lipid peroxidation, electrolyte leakage, and reactive oxygen species (O_2^- and H_2O_2) were decreased significantly as a result of *B. thuringiensis* and chitosan treatments. Overall, seed treatment with *B. thuringiensis* and chitosan foliar application was an effective and cheaper approach to cope with the deleterious effects of salinity on sweet pepper by improving the chlorophyll fluorescence parameters, proline accumulation, and up-regulation of enzymes activity as well as the enhancement of fruit yield characters.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/10/8/1180/s1, Table S1: Correlation matrix among different treatments and quantitative traits of sweet pepper.

Author Contributions: Conceptualization, K.A.A.A., Y.M.H., K.A.A., and M.D.F.A.; methodology, K.A.A.A., Y.M.H., K.A.A., M.D.F.A., A.M.E., and M.A.M.A.; software, K.A.A.A., Y.M.H., K.A.A., M.D.F.A., and M.A.M.A.; formal analysis, K.A.A.A., Y.M.H., K.A.A., A.M.E., M.A., and N.K.; investigation, K.A.A.A., Y.M.H., K.A.A., A.M.E., M.A.M.A., and N.K.; resources, K.A.A.A., Y.M.H., K.A.A., A.M.E., M.A.M.A., and N.K.; data curation, K.A.A.A., Y.M.H., K.A.A., A.M.E., M.A.M.A., and N.K.; writing-original draft preparation, K.A.A.A., Y.M.H., K.A.A., A.M.E., M.A.M.A., M.D.F.A., and N.K.; writing-review and editing, K.A.A.A., Y.M.H., A.M.E., M.A., N.K., and M.D.F.A.; visualization, K.A.A.A., Y.M.H., and K.A.A.; funding acquisition, M.D.F.A., K.A.A.A., M.A., A.M.E., and K.A.A. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Deanship of Scientific Research at Princess Nourah bint Abdulrahman University through the Fast-track Research Funding Program.

Acknowledgments: The authors extend their appreciation to all members of PPBL and EPCRS excellence center, Faculty of Agriculture, Kafrelsheikh University, and the Deanship of Scientific Research at Princess Nourah bint Abdulrahman University Fast-track Research Funding Program.

Conflicts of Interest: The authors declare no conflict of interest

References

1. Abdelaal, K.A.A.; EL-Maghraby, L.M.; Elansary, H.; Hafez, Y.M.; Ibrahim, E.I.; El-Banna, M.; El-Esawi, M.; Elkelish, A. Treatment of Sweet Pepper with Stress Tolerance-Inducing Compounds Alleviates Salinity Stress Oxidative Damage by Mediating the Physio-Biochemical Activities and Antioxidant Systems. *Agronomy* **2020**, *10*, 26. [CrossRef]
2. Chavez-Mendoza, C.; Sanchez, E.; Muñoz-Marquez, E.; Sida-Arreola, J.P.; Flores-Cordova, M.A. Bioactive compounds and antioxidant activity in different grafted varieties of bell pepper. *Antioxidants* **2015**, *4*, 427–446. [CrossRef] [PubMed]
3. El-Banna, M.F.; Abdelaal, K.A.A. Response of Strawberry Plants Grown in the Hydroponic System to Pretreatment with H_2O_2 before Exposure to Salinity Stress. *J. Plant Prot. Mansoura Univ.* **2018**, *9*, 989–1001. [CrossRef]
4. Helaly, M.N.; Mohammed, Z.; El-Shaeery, N.I.; Abdelaal, K.A.A.; Nofal, I.E. Cucumber grafting onto pumpkin can represent an interesting tool to minimize salinity stress. Physiological and anatomical studies. *Middle East J. Agric. Res.* **2017**, *6*, 953–975.
5. Isayenkov, S.V.; Maathuis, F.J. Plant salinity stress: Many unanswered questions remain. *Front. Plant Sci.* **2019**, *10*, 80. [CrossRef] [PubMed]
6. Hernández, J.A.; Almansa, M.S. Short-term effects of salt stress on antioxidant systems and leaf water relations of pea leaves. *Physiol. Plant.* **2002**, *115*, 251–257. [CrossRef]
7. Koyro, H.W. Effect of salinity of growth, photosynthesis, water relations and solute composition of the potential cash crop halophyte *Plantago coronopus* (L.). *Environ. Exp. Bot.* **2006**, *56*, 136–146. [CrossRef]
8. Gorhman, J.; Hardy, C.; Wyn, R.G.; Jones, L.R.; Law, C.N. Chromosomal location of a K/Na discrimination character in the D genome of wheat. *Theor. Appl. Genet.* **1987**, *74*, 584–588.
9. Harizanova, A.; Koleva-valkova, L. Effect of silicon on photosynthetic rate and the chlorophyll fluorescence parameters at hydroponically grown cucumber plants under salinity stress. *J. Cent. Eur. Agric.* **2019**, *20*, 953–960. [CrossRef]
10. Misra, N.; Ansari, M.; Gupta, A. Differential response of scavenging of reactive oxygen species in green gram genotype grown under salinity stress. *Am. J. Plant Physiol.* **2006**, *1*, 41–53. [CrossRef]
11. Hasan, M.K.; El Sabagh, A.; Sikdar, M.S.; Alam, M.J.; Ratnasekera, D.; Barutcular, C.; Abdelaal, K.A.A.; Islam, M.S. Comparative adaptable agronomic traits of blackgram and mungbean for saline lands. *Plant Arch.* **2017**, *17*, 589–593.
12. Carmen, B.; Roberto, D. Soil bacteria support and protect plants against abiotic stresses. In *Abiotic Stress in Plants Mechanisms and Adaptations*; Shanker, A., Ed.; Pub. In Tech: London, UK, 2011; pp. 143–170.
13. Islam, F.; Yasmeen, T.; Ali, S.; Ali, B.; Farooq, M.A.; Gill, R.A. Priming induced antioxidative responses in two wheat cultivars under saline stress. *Acta Physiol. Plant.* **2015**, *37*, 153. [CrossRef]
14. Memon, S.A.; Hou, X.; Wang, L.J. Morphological analysis of salt stress response of pak Choi. *Electron. J. Environ. Agric. Food Chem.* **2010**, *9*, 248–254.

15. Khan, N.; Bano, A. Role of plant growth promoting rhizobacteria and Ag-nano particle in the bioremediation of heavy metals and maize growth under municipal wastewater irrigation. *Int. J. Phytorem.* **2016**, *18*, 211–221. [CrossRef]
16. Gama, P.B.S.; Inanaga, S.; Tanaka, K.; Nakazawa, R. Physiological response of common bean (*Phaseolus vulgaris* L.) seedlings to salinity stress. *Afr. J. Biotechnol.* **2007**, *6*, 79–88.
17. Abdelaal, K.A.; Mazrou, Y.S.; Hafez, Y.M. Silicon Foliar Application Mitigates Salt Stress in Sweet Pepper Plants by Enhancing Water Status, Photosynthesis, Antioxidant Enzyme Activity and Fruit Yield. *Plants* **2020**, *9*, 733. [CrossRef]
18. Abdelaal, K.A.; Hafez, Y.M.; Sabagh, A.E.L.; Saneoka, H. Ameliorative effects of Abscisic acid and yeast on morpho-physiological and yield characteristics of maize plant (*Zea mays* L.) under drought conditions. *Fresenius Environ. Bull.* **2017**, *26*, 7372–7383.
19. Hafez, Y.; Attia, K.; Alamery, S.; Ghazy, A.; Al-Doss, A.; Ibrahim, E.; Rashwan, E.; El-Maghraby, L.; Awad, A.; Abdelaal, K.A. Beneficial Effects of Biochar and Chitosan on Antioxidative Capacity, Osmolytes Accumulation, and Anatomical Characters of Water-Stressed Barley Plants. *Agronomy* **2020**, *10*, 630. [CrossRef]
20. Abdelaal, K.A.; Hafez, Y.M.; Badr, M.M.; Youseef, W.A.; Esmaeil, S.M. Biochemical, histological and molecular changes in susceptible and resistant wheat cultivars inoculated with stripe rust fungus *Puccinia striiformis* f.sp. *tritici*. *Egypt. J. Biol. Pest Control* **2014**, *24*, 421–429.
21. Abdelaal, K.A.; Omara, I.R.; Hafez, Y.M.; Esmail, S.M.; EL Sabagh, A. Anatomical, biochemical and physiological changes in some Egyptian wheat cultivars inoculated with *Puccinia graminis* f.sp. *tritici*. *Fresenius Environ. Bull.* **2018**, *27*, 296–305.
22. Esmail, S.M.; Omara, R.I.; Abdelaal, K.A.; Hafez, M. Histological and biochemical aspects of compatible and incompatible wheat-Puccinia striiformis interactions. *Physiol. Mol. Plant Pathol.* **2019**, *106*, 120–128. [CrossRef]
23. Omara, R.I.; El-Kot, G.A.; Fadel, F.M.; Abdelaal, K.A.; Saleh, E.M. Efficacy of Certain Bioagents on Patho-Physiological Characters of Wheat Plants under Wheat Leaf Rust Stress. *Physiol. Mol. Plant Pathol.* **2019**, *106*, 102–108. [CrossRef]
24. Hafez, Y.M.; Abdelaal, K.A. Investigation of susceptibility and resistance mechanisms of some Egyptian wheat cultivars (*Triticum aestivum* L.) inoculated with *Blumeria graminis* f.sp. *tritici* using certain biochemical, molecular characterization and SEM. *J. Plant Prot. Path. Mansoura Univ.* **2015**, *6*, 431–454.
25. Khan, N.; Bano, A.; Zandi, P. Effects of exogenously applied plant growth regulators in combination with PGPR on the physiology and root growth of chickpea (*Cicer arietinum*) and their role in drought tolerance. *J. Plant Interact.* **2018**, *13*, 239–247. [CrossRef]
26. Abdelaal, K.A.; Attia, K.A.; Alamery, S.F.; El-Afry, M.M.; Ghazy, A.I.; Tantawy, D.S.; Al-Doss, A.A.; El-Shawy, E.E.; Abu-Elsaoud, A.M.; Hafez Y., M. Exogenous Application of Proline and Salicylic Acid can Mitigate the Injurious Impacts of Drought Stress on Barley Plants Associated with Physiological and Histological Characters. *Sustainability* **2020**, *12*, 1736. [CrossRef]
27. Abdelaal, K.A. Effect of Salicylic acid and Abscisic acid on morpho-physiological and anatomical characters of faba bean plants (*Vicia faba* L.) under drought stress. *J. Plant Prod. Mansoura Univ.* **2015**, *6*, 1771–1788. [CrossRef]
28. Abdelaal, K.A.; Hafez, Y.M.; El-Afry, M.; Tantawy, D.S.; Alshaal, T. Effect of some osmoregulators on photosynthesis, lipid peroxidation, antioxidative capacity, and productivity of barley (*Hordeum vulgare* L.) under water deficit stress. *Environ. Sci. Pollut. Res.* **2018**, *25*, 30199–30211. [CrossRef]
29. Elkelish, A.; Qari, S.H.; Mazrou, Y.M.; Abdelaal, K.A.; Hafez, Y.M.; Abu-Elsaoud, A.M.; Batiha, G.; El-Esawi, M.; El Nahhas, N. Exogenous Ascorbic Acid Induced Chilling Tolerance in Tomato Plants Through Modulating Metabolism, Osmolytes, Antioxidants, and Transcriptional Regulation of Catalase and Heat Shock Proteins. *Plants* **2020**, *10*, 431. [CrossRef]
30. Zhu, J.-K. Regulation of ion homeostasis under salt stress. *Curr. Opin. Plant Biol.* **2003**, *6*, 441–445. [CrossRef]
31. Sairam, R.K.; Tyagi, A. Physiology and molecular biology of salinity stress tolerance in plants. *Curr. Sci.* **2004**, *86*, 407–421.
32. Gupta, B.; Huang, B. Mechanism of salinity tolerance in plants: Physiological, biochemical, and molecular characterization. *Int. J. Genom.* **2014**, 18. [CrossRef] [PubMed]
33. Maxwell, K.; Johnson, G.N. Chlorophyll fluorescence—A practical guide. *J. Exp. Bot.* **2000**, *51*, 659–668. [CrossRef] [PubMed]

34. Murchie, E.H.; Lawson, T. Chlorophyll fluorescence analysis: A guide to good practice and understanding some new applications. *J. Exp. Bot.* **2013**, *64*, 3983–3998. [CrossRef] [PubMed]
35. Kalaji, M.H.; Goltsev, V.; Zuk-Golaszewska, B.; Zivcak, M.; Brestic, M. *Chlorophyll Fluorescence: Understanding Crop Performance-Basics and Applications*; Taylor and Francis: New York City, NY, USA, USA 2017; p. 222. ISBN 9781498764490.
36. Katiyar, D.; Hemantaranjan, A.; Singh, B. Chitosan as a promising natural compound to enhance potential physiological responses in plant: A review. *Indian J. Plant Physiol.* **2015**, *20*, 1–9. [CrossRef]
37. Hidangmayum, A.; Dwivedi, P.; Katiyar, D.; Hemantaranjan, A. Application of chitosan on plant responses with special reference to abiotic stress. *Physiol. Mol. Biol. Plants* **2019**, *25*, 313–326. [CrossRef] [PubMed]
38. Al-Tawaha, A.M.; Seguin, P.; Smith, D.L.; Beaulieu, C. Foliar application of elicitors alters isofl avone concentrations and other seed characteristics of field-grown soybean. *Can. J. Plant Sci.* **2006**, *86*, 677–684. [CrossRef]
39. Kim, H.J.; Chen, F.; Wang, X.; Rajapakse, N.C. Effect of chitosan on the biological properties of sweet basil (*Ocimum basilicum* L.). *J. Agric. Food Chem.* **2005**, *53*, 3696–3701. [CrossRef]
40. Balal, R.M.; Shahid, M.A.; Javaid, M.M.; Iqbal, Z.; Liu, G.D.; Zotarelli, L.; Khan, N. Chitosan alleviates phytotoxicity caused by boron through augmented polyamine metabolism and antioxidant activities and reduced boron concentration in *Cucumis sativus* L. *Acta Physiol. Plant.* **2017**, *39*, 31. [CrossRef]
41. Monirul, I.M.; Humayun, K.M.; Mamun, A.N.K.; Monirul, I.; Pronabananda, D. Studies on yield and yield attributes in tomato and chilli using foliar application of oligo-chitosan. *GSC Biol. Pharm. Sci.* **2018**, *3*, 020–028.
42. Yang, J.; Kloepper, J.W.; Ryu, C.M. Rhizosphere bacteria help plants tolerate abiotic stress. *Trends Plant Sci.* **2009**, *14*, 1–4. [CrossRef]
43. Noel, T.C.; Sheng, C.; Yost, C.K.; Pharis, R.P.; Hynes, M.F. *Rhizobium leguminosarum* as a plant growth-promoting rhizobacterium: Direct growth promotion of canola and lettuce. *Can. J. Microbiol.* **1996**, *42*, 279–283. [CrossRef] [PubMed]
44. Mayak, S.; Tirosh, T.; Glick, B.R. Plant growth-promoting bacteria that confer resistance in tomato plants to salt stress. *Plant Physiol. Biochem.* **2004**, *42*, 565–572. [CrossRef]
45. Abdelaal, K.A.; Tawfik, S.F. Response of Sugar Beet Plant (*Beta vulgaris* L.) to Mineral Nitrogen Fertilization and Bio-Fertilizers. *Int. J. Curr. Microbiol. App. Sci.* **2015**, *4*, 677–688.
46. Abdelaal, K.A.; Badawy, S.A.; Abdel Aziz, R.M.; Neana, S.M.M. Effect of mineral nitrogen levels and biofertilizer on morphophysiological characters of three sweet sorghum varieties (*Sorghum bicolor* L. Moench). *J. Plant Prod. Mansoura Univ.* **2015**, *6*, 189–203. [CrossRef]
47. Abdelaal, K.A. Pivotal Role of Bio and Mineral Fertilizer Combinations on Morphological, Anatomical and Yield Characters of Sugar Beet Plant (*Beta vulgaris* L.). *Middle East J. Agric. Res.* **2015**, *4*, 717–734.
48. Hafez, Y.M.; Attia, K.; Kamel, S.; Alamery, S.; El-Gendyd, S.; Al-Doss, A.; Mehiar, F.; Ghazy, A.; Abdelaal, K.A. *Bacillus subtilis* as a bio-agent combined with nano molecules can control powdery mildew disease through histochemical and physiobiochemical changes in cucumber plants. *Physiol. Mol. Plant Pathol.* **2020**, *111*, 101489. [CrossRef]
49. Khan, N.; Bano, A.; Ali, S.; Babar, M.A. Crosstalk amongst phytohormones from planta and PGPR under biotic and abiotic stresses. *Plant Growth Regul.* **2020**, *90*, 189–203. [CrossRef]
50. Kousar, B.; Bano, A.; Khan, N. PGPR Modulation of Secondary Metabolites in Tomato Infested with *Spodoptera litura*. *Agronomy* **2020**, *10*, 778. [CrossRef]
51. Abou-Attia, F.A.M.; Abdelaal, K.A. Effect of Bio and Mineral fertilization on the main insect pests and some characters of sugar beet plants. *J. Agric. Sci. Mansoura Univ.* **2007**, *32*, 1471–1485.
52. Egamberdieva, D. Pseudomonas chlororaphis: A salt-tolerant bacterial inoculant for plant growth stimulation under saline soil conditions. *Acta Physiol. Plant.* **2012**, *34*, 751–756. [CrossRef]
53. Khan, N.; Bano, A.M.; Babar, A. Impacts of plant growth promoters and plant growth regulators on rainfed agriculture. *PLoS ONE* **2020**, *15*, e0231426. [CrossRef] [PubMed]
54. Zhao, S.; Zhou, N.; Zhao, Z.-Y.; Zhang, K.; Wu, G.-H.; Tian, C.-Y. Isolation of endophytic plant growth-promoting bacteria associated with the halophyte *Salicornia europaea* and evaluation of their promoting activity under salt stress. *Curr. Microbiol.* **2016**, *73*, 574–581. [CrossRef] [PubMed]
55. Page, A.L. *Methods of Soil Analysis. Part 2, Chemical and Microbiological Properties*, 2nd ed.; Soil Science Society of America Inc.: Madison, WI, USA, 1982.

56. Sanchez, F.J.; de Andrés, E.F.; Tenorio, J.L.; Ayerbe, L. Growth of epicotyls, turgor maintenance and osmotic adjustment in pea plants (*Pisum sativum* L.) subjected to water stress. *Field Crop. Res.* **2004**, *86*, 81–90. [CrossRef]
57. Moran, R. Formulae for Determination of Chlorophyllous Pigments Extracted with N,N-Dimethylformamide 1. *Plant Physiol.* **1982**, *69*, 1376–1381. [CrossRef] [PubMed]
58. Szalai, G.; Janda, T.; Padi, E.; Szigeti, Z. Role of light in post-chilling symptoms in maize. *J. Plant Physiol.* **1996**, *148*, 378–383. [CrossRef]
59. Schreiber, U. *Pulse-Amplitude (RAM) Fluorometry and Saturation Pulse Method in Chlorophyll Fluorescence: A Signature of Photosynthesis*; Papageorgiou, G., Govindjee, Eds.; Springer: Dordrecht, The Netherlands, 2004; pp. 279–319.
60. Bates, L.S.; Waldren, R.P.; Teare, I.D. Rapid determination of free proline for water-stress studies. *Plant Soil* **1973**, *39*, 205–207. [CrossRef]
61. Davenport, S.B.; Gallego, S.M.; Benavides, M.P.; Tomaro, M.L. Behaviour of antioxidant defense system in the adaptive response to salt stress in *Helianthus annuus* L. cells. *Plant Growth Regul.* **2003**, *40*, 81–88. [CrossRef]
62. Huckelhoven, R.; Fodor, J.; Preis, C.; Kogel, K.H. Hypersensitive cell death and papilla formation in barley attacked by the powdery mildew fungus are associated with hydrogen peroxide but not with salicylic acid accumulation. *Plant Physiol.* **1999**, *1119*, 1251–1260. [CrossRef]
63. Hafez, Y.M.; Abdelaal, K.A.; Eid, M.E.; Mehiar, F.F. Morpho-physiological and biochemical responses of barley plants (*Hordeum vulgare* L.) against barley net blotch disease with application of non-traditional compounds and fungicides. *Egypt. J. Biol. Pest Cont.* **2016**, *26*, 261–268.
64. Aebi, H. Catalase in vitro. In *Methods in Enzymology; Oxygen Radicals in Biological Systems*; Academic Press: Cambridge, MA, USA, 1984; Volume 105, pp. 121–126.
65. Hammerschmidt, R.; Nuckles, E.M.; Kuć, J. Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum lagenarium*. *Physiol. Plant Pathol.* **1982**, *20*, 73–82. [CrossRef]
66. Mishra, N.P.; Mishra, R.K.; Singhal, G.S. Changes in the activities of anti-oxidant enzymes during exposure of intact what leaves to strong visible light at different temperatures in the presence of protein synthesis inhibitors. *Plant Physiol.* **1993**, *102*, 903–910. [CrossRef] [PubMed]
67. McCord, J.M.; Fridovich, I. Superoxide Dismutase: An enzymatic reaction for erythrocuprein (hemocuprein). *J. Biol. Chem.* **1969**, *244*, 6049–6055. [PubMed]
68. Goldberg, D.M.; Spooner, R.J. *Methods of Enzymatic Analysis*, 3rd ed.; Verlag Chemie: Weinheim, Germany, 1983; pp. 258–265.
69. Gomez, K.A.; Gomez, A.A. *Statistical Procedures for Agricultural Research*, 2nd ed.; Wiley Inter Science: New York City, NY, USA, 1984; pp. 1–690.
70. Duncan, B.D. Multiple ranges and multiple F-test. *Biometrics* **1955**, *11*, 1–42. [CrossRef]
71. Wang, Y.; Nil, N. Changes in chlorophyll, ribulose biphosphate carboxylase-oxygenase, glycine betaine content, photosynthesis and transpiration in *Amaranthus tricolor* leaves during salt stress. *J. Hortic. Sci. Biotechnol.* **2000**, *75*, 623–627. [CrossRef]
72. Parvin, K.; Hasanuzzaman, M.; Bhuyan, M.H.M.B.; Nahar, K.; Mohsin, S.M.; Fujita, M. Comparative Physiological and Biochemical Changes in Tomato (*Solanum lycopersicum* L.) under Salt Stress and Recovery: Role of Antioxidant Defense and Glyoxalase Systems. *Antioxidants* **2019**, *8*, 350. [CrossRef]
73. Wang, Y.; Li, K.; Li, X. Auxin redistribution modulates plastic development of root system architecture under salt stress in Arabidopsis thaliana. *J. Plant Physiol.* **2009**, *166*, 1637–1645. [CrossRef]
74. Naseem, H.; Ahsan, M.; Shahid, M.A.; Khan, N. Exopolysaccharides producing rhizobacteria and their role in plant growth and drought tolerance. *J. Basic. Microbiol.* **2018**, *58*, 1009–1022. [CrossRef]
75. Allakhverdiev, S.I.; Nishiyama, Y.; Miyairi, S.; Yamamoto, H.; Inagaki, N.; Kanesaki, Y.; Murata, N. Salt stress inhibits the repair of photodamaged photosystem II by suppressing the transcription and translation of psbA genes in Synechocystis. *Plant Physiol.* **2002**, *130*, 1443–1453. [CrossRef]
76. Khan, N.; Bano, A.; Curá, J.A. Role of Beneficial Microorganisms and Salicylic Acid in Improving Rainfed Agriculture and Future Food Safety. *Microorganisms* **2020**, *8*, 1018. [CrossRef]
77. Wang, W.; Wang, C.; Pan, D.; Zhang, Y.; Luo, B.; Ji, J. Effects of drought stress on photosynthesis and chlorophyll fluorescence images of soybean (*Glycine max*) seedlings. *Int. J. Agric. Biol. Eng.* **2018**, *11*, 196–201. [CrossRef]

78. Tavakkoli, E.; Rengasamy, P.; McDonald, G. High concentrations of Na^+ and Cl^- ions in soil solution have simultaneous detrimental effects on growth of faba bean under salinity stress. *J. Exp. Bot.* **2010**, *61*, 4449–4459. [CrossRef] [PubMed]
79. Asrar, H.; Hussain, T.; Midhat, S.; Hadi, S.; Gul, B.; Nielsen, B.L.; Khan, M.A. Salinity induced changes in light harvesting and carbon assimilating complexes of *Desmostachya bipinnata* (L.) staph. *Environ. Exp. Bot.* **2017**, *135*, 86–95. [CrossRef]
80. Shah, G.; Jan, M.; Afreen, M.; Anees, M.; Rehman, S.; Daud, M.K.; Malook, I.; Jamil, M. Halophilic bacteria mediated phytoremediation of salt-affected soils cultivated with rice. *J. Geochem. Explor.* **2017**, *174*, 59–65. [CrossRef]
81. Possingham, J.V. Plastid replication and development in the life cycle of higher plants. *Annu. Rev. Plant Physiol.* **1980**, *31*, 113–129. [CrossRef]
82. Plazek, A.; Rapacz, M.; Hura, K. Relationship between quantum efficiency of PSII and cold-induced plant resistance to fungal pathogens. *Acta Physiol. Plant.* **2004**, *26*, 141–148.
83. Mehta, P.; Jajoo, A.; Mathur, M.; Bharti, S. Chlorophyll a fluorescence study revealing effects of high salt stress on photosystem II in wheat leaves. *Plant Physiol. Biochem.* **2010**, *48*, 16–20. [CrossRef]
84. Kalaji, H.; Rastogi, A.; Živčgk, M.; Brestic, M. Prompt chlorophyll fluorescence as a tool for crop phenotyping: An example of barley landraces exposed to various abiotic stress factors. *Photosynthetica* **2018**. [CrossRef]
85. Yang, D.L.; Lian, J.R.; Ping, C.X.; Wei, L. Quantitative Trait Loci Mapping for Chlorophyll Fluorescence and Associated Traits in Wheat (*Triticum aestivum*). *J. Integr. Plant Biol.* **2007**, *49*, 646–654. [CrossRef]
86. Li, Z.; Zhang, Y.; Zhang, X.; Merewitz, E.; Peng, Y.; Ma, X.; Yan, Y. Metabolic pathways regulated by chitosan contributing to drought resistance in white clover. *J. Proteome Res.* **2017**, *16*, 3039–3052. [CrossRef]
87. Khan, N.; Bano, A.; Rahman, M.A.; Rathinasabapathi, B.; Babar, M.A. UPLC-HRMS-based untargeted metabolic profiling reveals changes in chickpea (*Cicer arietinum*) metabolome following long-term drought stress. *Plant Cell Environ.* **2019**, *42*, 115–132. [CrossRef]
88. Egamberdieva, D.; Davranov, K.; Wirth, S.; Hashem, A.; Abd_Allah, E.F. Impact of soil salinity on the plant-growth–promoting and biological control abilities of root associated bacteria. *Saudi J. Biol. Sci.* **2017**, *24*, 1601–1608. [CrossRef] [PubMed]
89. Prashanth, H.K.V.; Dharmesh, S.M.; Rao, K.S.; Tharanathan, R.N. Free radical-induced chitosan depolymerized products protect calf thymus DNA from oxidative damage. *Carbohydr. Res.* **2007**, *342*, 190–195. [CrossRef] [PubMed]
90. Malerba, M.; Cerana, R. Chitosan Effects on Plant Systems. *Int. J. Mol. Sci.* **2016**, *23*, 996. [CrossRef] [PubMed]
91. Sharma, I.P.; Sharma, A.K. Physiological and biochemical changes in tomato cultivar PT-3 with dual inoculation of mycorrhiza and PGPR against root-knot nematode. *Symbiosis* **2017**, *71*, 175–183. [CrossRef]
92. Bianco, C.; Defez, R. Medicago truncatula improves salt tolerance when nodulated by an indole-3-acetic acid-overproducing *Sinorhizobium meliloti* strain. *J. Exp. Bot.* **2009**, *60*, 3097–3107. [CrossRef]
93. Foyer, C.H.; Descourvieres, P.; Kunert, K.J. Protection against oxygen radicals: An important defense mechanism studied in transgenic plants. *Plant Cell Environ.* **1994**, *17*, 507–523. [CrossRef]
94. Kohler, J.; Caravaca, F.; Roldàn, A. An AM fungus and a PGPR intensify the adverse effects of salinity on the stability of rhizosphere soil aggregates of *Lactuca sativa*. *Soil Biol. Biochem.* **2010**, *42*, 429–434. [CrossRef]
95. Hu, Y.; Yu, W.; Liu, T.; Shafi, M.; Song, L.; Du, X.; Huang, X.; Yue, Y.; Wu, J. Effects of paclobutrazol on cultivars of Chinese bayberry (*Myrica rubra*) under salinity stress. *Photosynthetica* **2017**, *55*, 443–453. [CrossRef]
96. Hayat, R.; Ali, S.; Amara, U.; Khalid, R.; Ahmed, I. Soil beneficial bacteria and their role in plant growth promotion: A review. *Ann. Microbiol.* **2010**, *60*, 579–598. [CrossRef]