Article

Synergistic Effect of Plant-Growth-Promoting Rhizobacteria Improves Strawberry Growth and Flowering with Soil Salinization and Increased Atmospheric CO_2 Levels and Temperature Conditions

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Abstract: Biofertilization with plant-growth-promoting rhizobacteria (PGPR) can positively affect the growth and health of host plants and reinforce their tolerance of stressors. Here, we investigate the use of isolated PGPR consortia from halophytes to improve strawberry growth and flowering performance under saline and elevated CO_2 and temperature conditions. Growth, flower bud production, and the photosynthetic apparatus response were determined in strawberry plants grown at 0 and 85 mmol L^{-1} NaCl and in two atmospheric CO_2-temperature combinations (400/700 ppm and 25/+4 °C, respectively). Biofertilization improved strawberry plant growth and flower bud production, independently of salinity conditions, at ambient CO_2 and 25 °C, while bacterial inoculation only had a positive effect on plant growth in the presence of salt in high CO_2 and at +4 °C. Biofertilizers 1 and 3 generated the largest biomass of strawberries at 400 ppm CO_2 and 0 and 85 mmol L^{-1} NaCl, respectively, while biofertilizer 1 did so in the presence of salt and in an atmosphere enriched with CO_2 and at +4 °C. The effect of the consortia was mediated by bacterial strain PGP properties, rather than by an improvement in the photosynthetic rate of the plants. Furthermore, biofertilizers 1 and 2 increased the number of flower buds in the absence of salt, while biofertilizers 3 and 4 did so for salt-inoculated plants at 400 ppm CO_2 and at 25 °C. There was no effect of inoculation on flower bud production of plants grown at high CO_2 and at +4 °C. Finally, we concluded that the effect of bacterial inoculation on strawberry growth and flowering depended on the type of bacterial strain and growth conditions. This highlights the importance of developing studies considering stress interaction to assess the real potential of biofertilizers.

Keywords: biofertilizer; chlorophyll fluorescence; elevated atmospheric CO_2; flowering; gas exchange; inoculation; PGPR; temperature

Citation: Redondo-Gómez, S.; García-López, J.V.; Mesa-Marín, J.; Pajuelo, E.; Rodriguez-Llorente, I.D.; Mateos-Naranjo, E. Synergistic Effect of Plant-Growth-Promoting Rhizobacteria Improves Strawberry Growth and Flowering with Soil Salinization and Increased Atmospheric CO_2 Levels and Temperature Conditions. *Agronomy* **2022**, *12*, 2082. https://doi.org/10.3390/agronomy12092082

Academic Editor: Sung-Cheol Koh

Received: 28 July 2022
Accepted: 23 August 2022
Published: 31 August 2022

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1. Introduction

The strawberry is a popular berry fruit that is rich in various antioxidants, including polyphenols and vitamins. This crop is widely cultivated around the world in open fields via commercial cultivation systems and is farmed intensively in protected environments [1]. Temperatures of between 15 and 20 °C are ideal for strawberry fruitification in Europe. Consequently, the expected environmental increase in mean temperature in the future is predicted to shorten the duration of the crop cycle, thus reducing the total fruit yield [2]; up to a 32% decrease in strawberry production has been recorded due to an increase in average temperature by 4 °C [1–3]. Similarly, fruit yield was reduced at high CO_2 levels and high temperatures [1].

The atmospheric CO_2 content is increasing and is predicted to increase to 760 ppm, and with it a global mean temperature of between 2.4 and 4.8°C, along with increased salinity by the end of 2100 [4]. Soil salinity can be a consequence of poor soil management practices, through inadequate fertilization or the indiscriminate use of high salt concentration in water, and can negatively influence growth and flowering, causing decreasing yield and quality in crops [5,6]. Strawberry plants are considered a salt-sensitive crop, and salt-stress conditions have been reported to negatively affect plant growth and yield [7]. In this context, plant growth-promoting rhizobacteria (PGPR) have been suggested to mitigate the deleterious effect of salt stress on strawberry plants [8]. Under stress conditions, PGPR can stimulate plant growth through several mechanisms, including antioxidant alleviation, the regulation of stress-responsive genes, and phytohormones [9]. These microbes have been reported to offer one of the best alternatives, to alleviate the effect of salinity on plants [10].

PGPR are defined as soil bacteria that colonize plant roots and the surrounding rhizosphere and that have a positive effect on plant growth, yield enhancement, and resistance to pathogens and environmental stress, while these bacteria benefit from plant-secreted root exudates [11,12]. PGPR interact with plants through both direct and indirect mechanisms. Some direct mechanisms by PGPR are known to promote plant growth: atmospheric nitrogen fixation, the synthesis of amino acids and other bioactive substances, the production of phytohormones, fighting other diseases that restrict plant growth, and improving mineral intake from the soil [13,14]. Furthermore, PGPR promote indirect mechanisms such as biofilm production, which improves bacterial adhesion to the surface of the root tissue, intercellular communication, the facilitation of nutrient uptake, adaptation to changing environmental conditions, and the compartmentalization of toxic elements [13,15,16].

Finally, it is not often that a single microorganism elicits all the mechanisms to promote plant growth, so the use of PGPR consortia, rather than a single strain, is of current research interest for the formulation of biofertilizers [13,17]. A biofertilizer is any microbial biostimulant (defined, in turn, as materials that, in small amounts, promote plant growth) applied to plants to improve plant nutrition, tolerance to abiotic stress, and/or crop quality [17].

Specifically, we focus on the five biofertilizers used in a study by Redondo-Gómez et al. [18], which have been shown to be especially effective in improving crop growth in salinized soils. These biofertilizers are formulated with bacterial strains that show plant growth-promoting properties, such as: (1) nitrogen fixation; (2) siderophore production, which are iron-binding protein molecules that chelate Fe^{3+} from soil—when needed, Fe is used by plants, resulting in improved plant development and growth [19,20]; (3) P solubilization; (4) synthesis of the phytohormone indole-3-acetic acid (IAA), the main signal for the shoot that controls all visible vascular differentiation characteristics in plants [21]; (5) biofilm production; (6) the release of 1-aminocyclopropane-1-carboxylate deaminase (ACC deaminase). This enzyme hydrolyzes 1-aminocyclopropane-1-carboxylate (ACC), which is an immediate raw material for ethylene in higher plants, into α-ketobutyrate and ammonia [22]. Saline-induced stress in plants is partially the result of the plant's production of stress ethylene; therefore, lowering ethylene levels using PGPR containing ACC deaminase might provide some protection against this stress [23].

However, the effects of these biofertilizers on the physiological responses of plants or on flowering are unknown. Therefore, our goal was to test the effect of these five biofertilizers on strawberry growth, flowering, and the response of its photosynthetic apparatus under a complex environmental matrix characterized by salinity (0 and 85 mM NaCl) and variations in atmospheric CO_2 concentration and air temperature (400 ppm and 25/14 °C and 700 ppm at +4 °C). Studies evaluating the effect of PGPR inoculants on crops where the salinity, temperature, and CO_2 concentration have been changed are very scarce, but such studies are key to determining the feasibility of using PGPR in the context of climate change [18].

We hypothesize that these biofertilizers, isolated from halophytes, could enhance strawberry growth, flowering, and its physiological response to soil salinization and climate change. Therefore, the interactions of the root microbiota of halophytes may be

a sustainable solution to improve alternative crop production while combating abiotic stress [24].

2. Materials and Methods

2.1. Plant Materials, Growth Conditions, and Treatments

Bare-rooted cold-stored strawberry plants (*Fragaria vesca* var. Fortuna) with a well-developed crown, with a diameter of 8 to 10 mm, were planted in 1.5 L plastic pots filled with sand and an organic commercial substrate mixture (Gramoflor GmbH und Co., KG.) (4:1). The mixture was previously sterilized at 100 °C for 18 h. The sterility of the substrate was confirmed by mixing some samples, randomly selected from different bags, with sterile physiological saline solution and plating onto tryptone soya agar (TSA). No growth was observed after 3 days of incubation at 28 °C.

The plants in pots (n = 240) were kept at 400 ppm CO_2 with a diurnal regime of 16 h of light at 25 °C and 8 h of darkness at 14 °C, 50% relative humidity (80% for rice) and 300 $\mu molm^{-2}\ s^{-1}$ light flux in controlled environment chambers (Aralab/Fitoclima 18.000 EH, Lisbon, Portugal). After 15 days of growth, the different treatments were established. Before and after the establishment of the treatment, the pots were watered daily with 100 mL of 20% Hoagland's solution, since the strawberry plants were grown on a highly sandy substrate with a low cation exchange capacity (CEC); therefore, they were characterized by a low retention capacity of nutrients [25].

Twenty-four different treatments were established (n = 10 per treatment): six biofertilization treatments (5 rhizobacteria consortia + non-inoculated control), two salinity concentrations (0 and 85 mmol L^{-1} NaCl), and two combinations of CO_2- temperature: 400 ppm CO_2 at 25/14 °C (16/8 h) and 700 ppm CO_2 at 29/18 °C (16/8 h).

The salinity treatment was imposed by immersing the pots in tanks with 6 L of solution, at 0 or 85 mmol L^{-1} NaCl, for 25 min. The atmospheric CO_2 concentrations in the chambers were continuously recorded by CO_2 sensors (Aralab, Lisbon, Portugal) and maintained by supplying pure CO_2 from a compressed gas cylinder (Air Liquide, B50 35 K). Rhizobacterial inoculation was carried out the day after environmental treatments (salinity, CO_2, and temperature). After the establishment of each treatment, the pots were watered daily with 100 mL of 20% Hoagland's solution.

The substrate conductivity was monitored weekly with a conductivity meter (Probe GS3, Decagon, Pullman, WA, USA) and immersion of the pots in tanks was repeated, when necessary, at 0 or 85 mmol L^{-1} NaCl.

2.2. Rhizobacteria Selection

In this experiment, five bacterial biofertilizers that had been tested with eight crops, including strawberry [18], were used. They were made up of rhizobacteria that were originally isolated from the rhizospheres of five different halophytes that commonly inhabit salt marshes in southwestern Spain (Tinto, 37°13′ N 6°53′ W; Odiel, 37°10′35.2″ N 6°55′59.2″ W; and Piedras, 37°16′09.1″ N 7°09′36.4″ W (rivers and estuaries)). Three plant samples of each halophyte were harvested with rhizospheric soil (20 cm depth) for the subsequent isolation of cultivable bacteria. The rhizobacteria that make up Biofertilizer 1 (strains: *Pseudomonas composti* SDT3, *Aeromonas aquariorum* SDT13, and *Bacillus thuringiensis* SDT14) were isolated from *Sporobolus montevidensis* (Arechav.) P.M. Peterson and Saarela. Rhizobacteria in Biofertilizer 2 (strains: *Vibrio kanaloae* RA1, *Pseudoalteromonas prydzensis* RA15, and *Staphylococcus warneri* RA18) were isolated from the rhizosphere of *Allenrolfea occidentalis*. The rhizobacteria in Biofertilizer 3 (strains: *Bacillus methylotrophicus* SMT38, *Bacillus aryabhattai* SMT48, and *Bacillus licheniformis* SMT51) came from *Sporobolus maritimus* (Curtis) P.M. Peterson and Saarela, while those in Biofertilizer 4 (strains: *Vibrio spartinae* HPJ2, *Marinobacter sediminum* HPJ15 and *Vibrio parahaemolyticus* HPJ50) were from *Atriplex portulacoides*. Finally, rhizobacteria from Biofertilizer 5 (strains: *Vibrio neocaledonicus* SRT1, *Thalassospira australica* SRT8, and *Pseudarthrobacter oxydans* SRT15) were isolated from the *Salicornia europaea* rhizosphere.

These five biofertilizers were selected because they exhibited interesting properties in terms of promoting plant growth, as well as a salt tolerance of up to 2 M NaCl (see Table 1).

Table 1. Plant growth promoting rhizobacterial (PGPR) traits for the strains of biofertilizers used in this study (information obtained from Redondo-Gómez et al. [18]).

	Biofertilizers														
	1			**2**			**3**			**4**			**5**		
PGPR Properties	**SDT3**	**SDT13**	**SDT14**	**RA1**	**RA15**	**RA18**	**SMT383**	**SMT483**	**SMT513**	**HPJ2**	**HPJ15**	**HPJ50**	**SRT1**	**SRT8**	**SRT15**
N fixation			×	×	×		×	×	×	×		×	×		×
P solubilization	×	×						×	×	×		×	×		×
Siderophores production	×	×	×	×	×		×	×	×	×	×		×		
IAA production		×		×	×	×		×	×	×	×	×	×		×
Biofilm production							×		×	×		×	×	×	
ACC deaminase										×				×	

To prepare the bacterial suspension for inoculation, strains were grown separately in 250 mL Erlenmeyer flasks containing 50 mL of TSB (tryptone soya broth) medium and incubated on a rotary shaker for 18–24 h at 28 °C. The cultures were then centrifuged in 50 mL Falcon tubes at 7000 rpm (6300× *g*) for 5 min and the supernatant was discarded. The pellets were washed twice with sterile tap water (by resuspension and centrifugation) and were finally resuspended in tap water to reach an OD_{600} of approximately 1.0, in order to produce a uniform bacterial concentration of all strains. The bacterial suspensions were mixed to produce the five final inoculant suspensions, as follows: strains SDT3, SDT13, and SDT14 were mixed to obtain Biofertilizer 1; strains RA1, RA15, and RA18 were mixed to obtain Biofertilizer 2; strains SMT38, SMT48, and SMT51 were mixed to obtain Biofertilizer 3; strains HPJ2, HPJ15, and HPJ50 were mixed to obtain Biofertilizer 4; strains SRT1, SRT8, and SRT15 were mixed to obtain Biofertilizer 5. For plant inoculation, every 1.5 L pot was watered with 20 mL of the inoculant suspensions to achieve a final bacteria concentration of 10^5 CFU/mL (estimating that a suspension of OD_{600} 1 corresponds to a concentration of approximately 10^8 CFU/mL).

2.3. Growth Measurements

After 30 d of growth in the different treatments, the number of flower buds (n = 10) was determined. Finally, the plants were harvested and divided into roots, shoots, and flower buds. The dry mass was determined after drying the samples at 80 °C for 48 h.

2.4. Gas Exchange

One day before the plants were harvested, the gas exchange parameters were measured in random leaves (n = 8) using an infrared gas analyzer (LI-6400, LI-COR Inc., Lincoln, NE, USA, equipped with a light leaf chamber, LI-6400-02B) in an open system. Net photosynthetic rate (A), intercellular CO_2 concentration (Ci), stomatal conductance (Gs) and instantaneous water use efficiency (iWUE—the ratio between A and Gs) were determined at a photon flux density (PPFD) of 1000 mmol photons m^{-2} s^{-1} (with 15% blue light to maximize stomatal aperture), a CO_2 concentration surrounding the leaf of 400 mmol mol^{-1} air, an air temperature of 24 ± 1 °C, relative humidity of 45 ± 5%, and a vapor pressure deficit of 2.0–3.0 kPa [26].

2.5. Chlorophyll Fluorescence

Chlorophyll fluorescence was measured in fully expanded random leaves ($n = 10$) using a portable modulated fluorimeter (FMS-2; Hansatech Instruments Ltd., Kings Lynn, UK) after 30 d of treatment. Thus, the maximum quantum efficiency of PSII photochemistry (Fv/Fm) was measured from 30 min of dark-adapted leaves. Fv/Fm reflects the potential maximum efficiency of PSII (i.e., the quantum efficiency if all PSII centers were open) [27].

2.6. Statistical Analysis

Statistical analysis was performed using the SPSS 26.0 statistical program (SPSS Inc., Chicago, IL, USA). Data were analyzed using generalized linear models (GLMs). The Duncan test was applied to establish the significance between treatments ($p < 0.05$). Before statistical analysis, the Kolmogorov–Smirnov and Levene tests were used to verify the assumptions of normality and homogeneity of the variances, respectively. The data were transformed using an arc-tangent function when the homogeneity of variance was not reached.

3. Results

3.1. Growth Measurements

Biofertilization had a significant effect on strawberry plants under different salinity conditions (0 and 85 mmol L^{-1} NaCl) at 400 ppm CO_2 and 25 °C (GLM, $p < 0.01$; Figure 1). Plants treated with biofertilizer 1 showed the highest total biomass in the absence of salt, while biofertilizer 3 showed the highest total biomass at 85 mmol L^{-1} NaCl with respect to non-inoculated control plants (57 and 71%, respectively; Figure 1A). Furthermore, biofertilizer 3 increased the biomass to values similar to those with the non-saline treatment. This increase recorded in the total dry weight for plants treated with inoculum 1 was mainly due to significant differences in aboveground biomass production, while plants inoculated with consortium 3 showed significant differences for both above- and below-ground biomasses at 85 mmol L^{-1} NaCl (Figure 1B,C).

When strawberry plants were grown under high CO_2 and +4 °C, biofertilization only had a positive effect in the presence of salt. Biofertilizer 1 enhanced strawberry growth at 85 mmol L^{-1} NaCl, compared to the control ($p < 0.05$; Figure 1D). This effect was due to changes in below-ground biomass production, which increased by 127% (Figure 1E,F).

Inoculation also stimulated flower bud production at 400 ppm CO_2 and 25 °C (GLM, $p < 0.05$; Figure 2A). Biofertilizers 1 and 2 increased the number of flower buds in the absence of salt to the same extent, while biofertilizers 3 and 4 did the same for salt-inoculated plants. However, the mean dry weight of the flower bud did not change in plants grown at 0 mmol L^{-1} NaCl ($p > 0.05$); biofertilizer 2 showed the highest value of this parameter in the presence of salt (Figure 2B).

Finally, no beneficial effect from biofertilization on the flower bud production of plants grown at high CO_2 and +4 °C was recorded; indeed, inoculum 4 significantly reduced the number of flower buds and the mean dry weight of the flower buds of strawberry plants treated with 85 mmol L^{-1} NaCl ($p > 0.05$ for both; see Figure 2C,D).

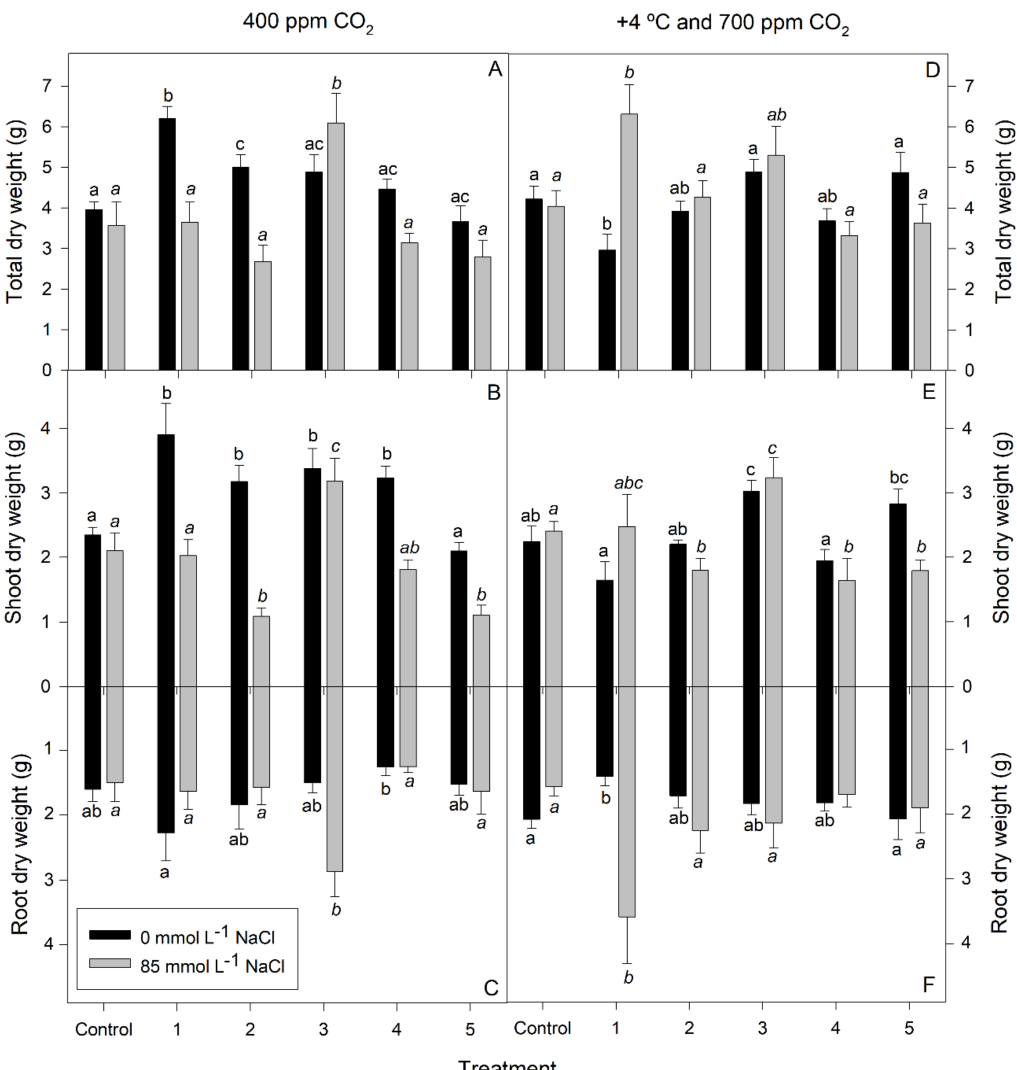

Figure 1. Total (**A**,**D**), shoot (**B**,**E**) and root (**C**,**F**) dry weights of strawberry plants inoculated with rhizobacteria consortia, numbered 1 to 5 (control = non-inoculated plants), after 30 d of treatment at 400 ppm CO_2 (**A**–**C**) and at +4 °C and 700 ppm CO_2 (**D**–**F**), with 0 and 85 mmol L^{-1} NaCl. Each value represents the mean of ten replicates ± SE. Different letters for each saline treatment (capital and italic letters for 0 and 85 mmol L^{-1} NaCl, respectively) indicate those means that are significantly different from each other (GLM; Duncan test, $p < 0.05$).

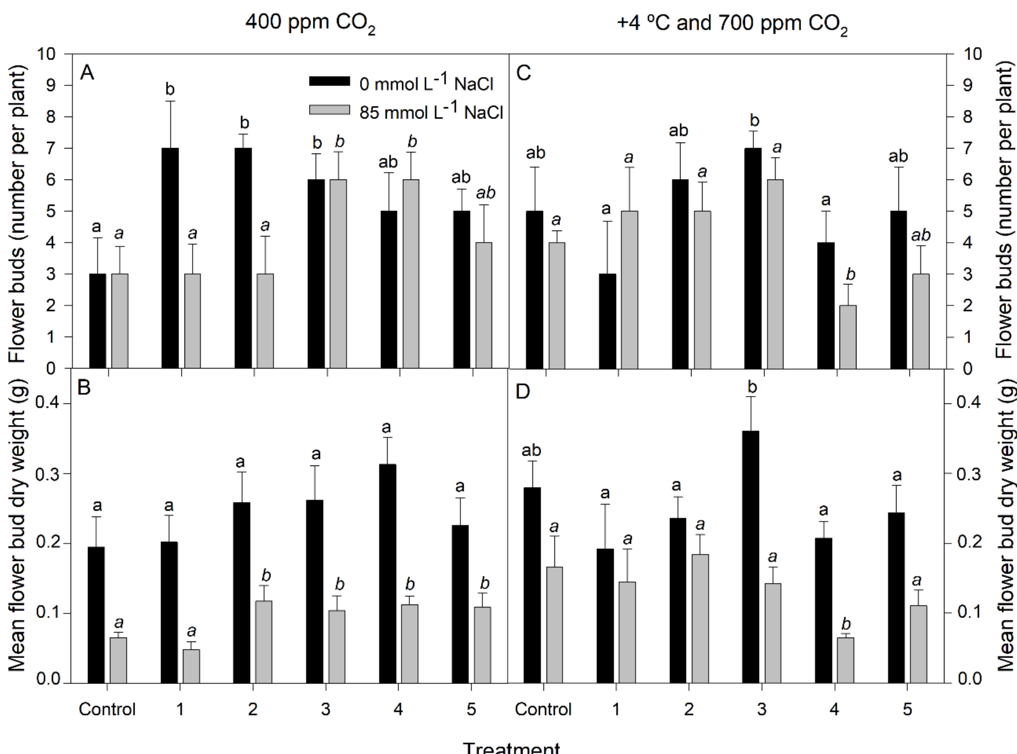

Figure 2. Number of flower buds per plant (**A**,**C**) and the mean dry weight of flower buds (**B**,**D**) of strawberry plants inoculated with rhizobacteria consortia, numbered 1 to 5 (control = non-inoculated plants), after 30 d of treatment at 400 ppm CO_2 (**A**,**B**) and at +4 °C and 700 ppm CO_2 (**C**,**D**) with 0 and 85 mmol L^{-1} NaCl. Each value represents the mean of ten replicates ± SE. Different letters for each saline treatment (capital and italic letters for 0 and 85 mmol L^{-1} NaCl, respectively) indicate those means that are significantly different from each other (GLM; Duncan test, $p < 0.05$).

3.2. Gas Exchange

There was no significant improvement in the net photosynthetic rate (A) with biofertilization (Figure 3A) in the absence of salt at 400 ppm CO_2 and 25 °C. However, inoculum 2 significantly increased the A values compared to the control treatment at 85 mmol L^{-1} NaCl (GLM, $p < 0.05$; Figure 3A). This response corresponded to a higher rate of stomatal conductance (Gs; $p < 0.05$), although the values of intercellular CO_2 concentration (Ci) were similar to the rest of the treatments (Figure 3B,C). Furthermore, biofertilization had no effect on instantaneous water use efficiency (iWUE) values, regardless of the saline treatment (Figure 3D).

In general, all the biofertilizers increased the values of the net photosynthetic rate (A) at 700 ppm CO_2 and an air temperature of +4 °C (GLM, $p < 0.01$) in plants grown without salt, while inoculation had no effect on the A values of plants treated with 85 mmol L^{-1} NaCl (Figure 3E). A similar trend to Gs was observed since inoculated plants treated with 0 mmol L^{-1} NaCl showed higher values than the control, although significant differences were only recorded for biofertilizer 5 (GLM, $p < 0.05$; Figure 3G). Finally, inoculation did not improve iWUE, regardless of saline treatment (Figure 3H).

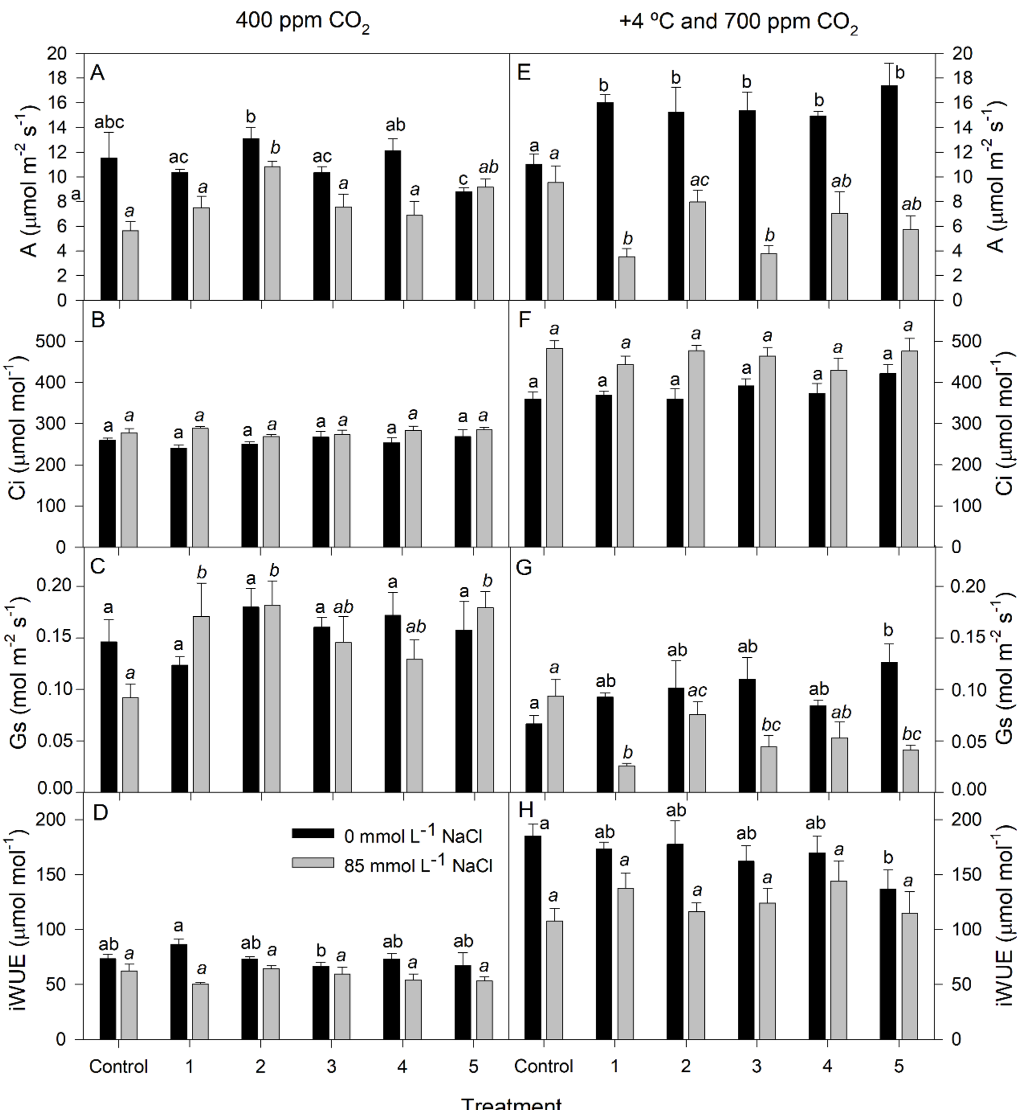

Figure 3. A, net photosynthetic rate (**A**,**E**); Ci, intercellular CO_2 concentration (**B**,**F**); Gs, stomatal conductance (**C**,**G**); and iWUE, instantaneous water use efficiency (**D**,**H**) of strawberry plants inoculated with rhizobacteria consortia, numbered 1 to 5 (control = non-inoculated plants), after 30 d of treatment at 400 ppm CO_2 (**A**–**D**) and at +4 °C and 700 ppm CO_2 (**E**–**H**), with 0 and 85 mmol L^{-1} NaCl. Each value represents the mean of eight replicates ± SE. Different letters for each saline treatment (capital and italic letters for 0 and 85 mmol L^{-1} NaCl, respectively) indicate those means that are significantly different from each other (GLM; Duncan test, $p < 0.05$).

3.3. Chlorophyll Fluorescence

Inoculation either had no effect or reduced the maximum quantum efficiency values of PSII photochemistry (Fv/Fm; Figure 4). Biofertilizer 5 reduced the Fv/Fm values of plants grown without salt at 400 ppm CO_2 and 25 °C ($p < 0.01$; Figure 4A), while inoculum 3 showed the lowest Fv/Fm values for plants treated with elevated CO_2 and 85 mmol L^{-1} NaCl (Figure 4B).

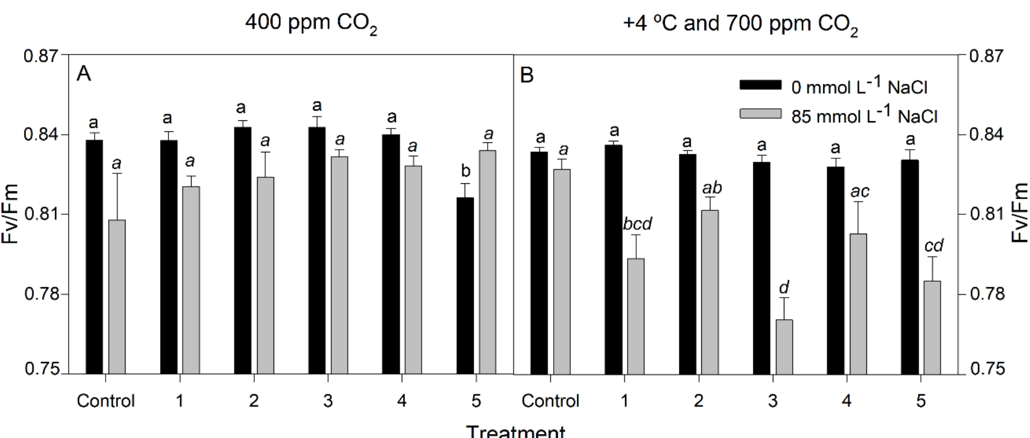

Figure 4. Maximum quantum efficiency of PSII photochemistry (Fv/Fm) of strawberry plants inoculated with rhizobacteria consortia, numbered 1 to 5 (control = non-inoculated plants), after 30 d of treatment at 400 ppm CO_2 (**A**) and at +4 °C and 700 ppm CO_2 (**B**) with 0 and 85 mmol L^{-1} NaCl. Each value represents the mean of eight replicates ± SE. Different letters for each saline treatment (capital and italic letters for 0 and 85 mmol L^{-1} NaCl, respectively) indicate those means that are significantly different from each other (GLM; Duncan test, $p < 0.05$).

4. Discussion

The effect of biofertilization with plant growth-promoting rhizobacteria (PGPR) that were isolated from halophytes on strawberry growth varied according to atmospheric CO_2 concentration and salinity. We recorded a positive effect of inoculation on plants grown at 400 ppm and 25 °C, regardless of saline treatment (0 and 85 mmol L^{-1} NaCl); however, only those plants treated with salt showed this positive effect of inoculation under an atmosphere enriched with CO_2 (700 ppm) and at +4 °C. A similar pattern was observed by Redondo-Gómez et al. [18] for alfalfa plants grown under the same CO_2 concentration and temperature and salinity conditions as those used in our experiment. They concluded that the way PGPR interacts with crops depends on the type of bacterial strain (plant growth-promoting properties) and crop growth conditions, as has been corroborated in our studies. Therefore, biofertilizers 1 and 3 generated the largest biomass of strawberries at 400 ppm CO_2 and 0 and 85 mmol L^{-1} NaCl, respectively, while bacterial consortium 1 did the same thing in the presence of salt, in an atmosphere enriched with CO_2 and +4 °C. Neither of these two biofertilizers (1 and 3) showed ACC activity (bacterial consortia 4 and 5 were the only ones showing this activity (see Table 1)) and only biofertilizer 3 demonstrated biofilm formation capability, so that saline stress relief in strawberry plants can be attributed to bacterial IAA synthesis. The primary function of this microbial phytohormone is to promote plant growth by promoting cell division, tissue expansion, elongation, and other potential advantageous impacts on plant growth and yield [28]. Etesami and Beattie [29] explained that managing the production of IAA in plants by bacteria may be an important tool by which to improve salt tolerance. Under stressed conditions, plants can release root exudates, which improve the growth and chemotaxis activities of soil microbes and ultimately enhance plant growth [20,30].

The improvement in strawberry growth with inoculation at 400 ppm CO_2 mainly corresponded to higher above-ground biomass, for plants grown without salt, and by higher above- and below-ground biomasses in the presence of salt, while improvement in growth was due to higher below-ground biomass at elevated CO_2. Conversely, in another study, both green and red leaf lettuce showed an increase in above-ground biomass production with elevated CO_2 and salinity (700 ppm CO_2 and 200 mmol L^{-1} NaCl) [31]. However, in agreement with our results, Marcondes de Andrade et al. [32] recorded that bacteria inoculation increased by 35% the aerial part length of strawberry plants compared to the control without inoculation, while Karlidag et al. [8] found that inoculation increased the shoot and root weights of plants under natural field salinity conditions (0.25 dS m^{-1});

both studies were developed under current climate conditions. In our experiment, the increase in biomass experienced by plants treated without salt and inoculum 2 at 400 ppm CO_2 was mediated by a higher net photosynthetic rate (A), which, in turn, corresponded to a higher stomatal conductance (although there were no significant differences from the control). However, the A values of the plants inoculated with biofertilizer 1 were not higher than those of the control (they were even lower at high CO_2 and 85 mmol L^{-1} of NaCl). In this case, the positive effect of inoculation on strawberry plant growth could be explained by the PGP properties of the bacterial consortium. Both biofertilizers 1 and 3 demonstrated phosphate solubilization capacity, atmospheric nitrogen fixation, siderophore production for iron uptake, and IAA synthesis. Rhizobacteria can acquire insoluble nutrients for plants via their extensive flagella or hyphae [33]. In addition, IAA stimulates root development and, as a result, those roots with a larger surface area absorb more water and nutrients from soils and translocate them to the aerial parts of plants, resulting in increased above-ground biomass [34]. Although biofertilizers 1 and 3 share the same properties mentioned above, interestingly, biofertilizer 1 did not show the best performance at 400 ppm CO_2 and 85 mmol L^{-1} NaCl (biofertilizer 3 showed the best performance), but this was at an elevated and ambient CO_2 with and without salt, respectively. We hypothesize that this may be related to the greater N fixation capacity of biofertilizer 3 (see Table 1). Nitrogen is a component of amino acids, proteins, nucleic acids, and chlorophyll, along with many other metabolites that are essential for plants, highlighting the fact that substantial amounts of N are invested in the RuBisCO protein [35,36]. Furthermore, it has been described that the concentration of N in plant leaves decreases in the presence of salt [37,38]. Therefore, the higher N fixation capacity of consortium 3 would counteract the nitrogen deficiency in the leaves caused by salinity. Finally, the leaf N imbalance in strawberry plants would be improved by CO_2 enrichment.

The stimulation of photosynthesis in C_3 species is a general response to the enrichment of atmospheric CO_2 concentration, varying between species and environmental conditions [39]. According to Keutgen et al. [40], non-inoculated strawberries did not experience this general trend in the absence of salt but did so at 85 mmol L^{-1} NaCl, since the A values remained the same as those of non-salinized plants. Our results for inoculated plants were also in agreement with that trend in the absence of salt, although the response of A did not closely correspond to that of total biomass. In the same way, Balasooriya et al. [1] reported a lower number of strawberry leaves at 30 °C than at 25°C in plants grown in 650 ppm CO_2, despite the fact that A values increased compared to plants in 400 ppm CO_2. This disparity could be explained by changes in dark respiration [41] and/or the inhibition of end products associated with a limited sink capacity [1,42]. Furthermore, the additive effect between biofertilization and the high concentration of CO_2 in A disappeared in the presence of salt. Thus, salinity has been described as affecting photosynthetic metabolism, primarily in terms of the diffusion of CO_2 in leaves through a decrease in stomatal and mesophyll conductance [43]. Therefore, we recorded a decrease in Gs with biofertilizers 1, 3, and 5, although the Ci values did not change, which was contrary to what was expected. This may be interpreted as a direct effect of NaCl on the photosynthetic apparatus [44]. Salinity may lead to biochemical limitations that affect the carboxylase activity of RuBisCO [45] and may alter metabolic functions such as protein synthesis that determine the photoinhibition levels [46]. In this regard, we recorded lower values of Fv/Fm for salt-inoculated plants grown at 700 ppm and +4 °C, indicating that there was greater photoinhibition. This photoinhibition would have been caused by a lower proportion of open reaction centers, resulting from a saturation of photosynthesis by light [47].

However, the previously described decline in Gs did not correspond to a decrease in the instantaneous water use efficiency (iWUE) values, which were similar to those in the non-inoculated control; therefore, we concluded that strawberry inoculation had no effect on this parameter. On the contrary, Redondo-Gómez et al. [48] found lower iWUE values for inoculated Swiss chard plants, which was due, in turn, to higher Gs values. In

general, as expected, iWUE values were higher at elevated CO_2 levels and temperatures than in ambient CO_2, regardless of inoculation and saline treatments. Thiagarajan et al. [49] explained that temperature and CO_2 concentration, individually and interactively, can affect WUE values in plants. A higher concentration of CO_2 in the external environment suppresses the stomatal opening and, consequently, limits the loss of water from the leaves. These water savings in plants improve WUE at elevated CO_2 levels [1,50].

Finally, we found an overall positive effect of bacterial inoculation on strawberry flowering at 400 ppm CO_2 and 25 °C, although this effect depended on growth conditions, specifically the presence or absence of salt. Kumar et al. [51] observed that carnations treated with IAA showed earlier flowering and higher total yield and number of flowers per plant; biofertilizers 1 and 3 contained at least one strain with the ability to synthesize IAA. Kurokura et al. [52] studied the response of 'Hoko-wase' and 'Tochi-otome' strawberries to the commercially available *Bacillus* PGPR application and found that it resulted in earlier flowering, higher total yield, and a higher number of fruits from the first cultivar but not from the second cultivar. Interestingly, consortia 3, 4, and 5 were the ones that showed higher flower bud production than the non-inoculated control in the presence of salt. These biofertilizers were the only ones that demonstrated the ability to form biofilms (3) and ACC activity (4 and 5), while both properties provide plants with some protection against salt stress [18]. Biofilm formation reduces Na^+ uptake in the plant by binding Na^+, and ACC activity reduces ethylene production, a plant hormone that regulates growth and plant development, as well as the responses to biotic and abiotic stresses [23,53,54]. The role of ethylene in the flowering transition is unclear, as ethylene can both promote and delay flowering [55,56].

Regarding flowering at elevated CO_2 and temperature levels, Sun et al. [57] reported that strawberry yield was reduced at 25/20 °C day/night temperature levels and 720 ppm CO_2; this reduction was further enhanced by elevated CO_2 levels at high temperatures during flowering and fruit development. However, Balasooriya et al. [1] recorded that the number of flower buds per plant was slightly higher for strawberries grown at 650 ppm CO_2 and 30 °C than at 400 ppm CO_2 and 25 °C. We did not evaluate the differences in our experiment, nor did we identify them for the inoculation¸ except in the case of inoculum 4 in the presence of salt, which had a negative effect on flowering.

5. Conclusions

Our study is the first to assess the effect of consortia isolated from halophytes on strawberry growth, flowering, and the response of strawberry plants' photosynthetic apparatus under the interaction of different salinity, temperature, and atmospheric CO_2 concentration conditions, which is key to determining the feasibility of using PGPR in the context of climate change. Thus, we find that the effect of bacterial inoculation on strawberry growth and flowering depended on the types of bacterial strain and growth conditions.

Biofertilizers 1 and 3 were the most effective, as they produced the highest plant growth and flowering. Plants treated with biofertilizer 1 showed the highest total biomass and flower bud production in the absence of salt, while biofertilizer 3 did this at 85 mmol L^{-1} NaCl. The salt-inoculated plants receiving consortium 3 showed biomass values similar to those shown by the most effective inoculum (1) in the absence of salt (although both were more than 50% above the non-inoculated control). When strawberry plants were grown with salt in high CO_2 and +4 °C, biofertilizer 1 was again the most effective.

The increased growth demonstrated by Consortia 1 and 3 was mainly due to the PGP properties of the bacterial strain, especially the synthesis of IAA, rather than to an improvement in the photosynthetic rate of the plants. Therefore, the negative additive effect of inoculation, salinity, and the high concentration of CO_2 and +4 °C recorded in the photosynthetic apparatus of strawberry plants, which experienced greater photoinhibition, did not translate into a reduction in growth.

Author Contributions: S.R.-G. and E.M.-N. conceived the study, supervised, and acquired funding for the project. S.R.-G. performed data analysis and wrote the manuscript, with input from E.M.-N., J.M.-M., with assistance from E.P., J.V.G.-L. and I.D.R.-L., provided the bacterial inocula. All authors performed the experimental development. All authors have read and agreed to the published version of the manuscript.

Funding: This work has been supported by MCIN/AEI/10.13039/501100011033 and UE "NextGenerationEU/PRTR" (PDC2021-120951-I00).

Acknowledgments: The authors thank the University of Seville Greenhouse General Services (CITIUS) for its collaboration. J.M.-M. acknowledges the European Social Fund and Junta de Andalucía for personal funding (Talento Doctores DOC_00725).

Conflicts of Interest: The authors declare that they have no conflict of interest.

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