

## PGPR-based biofertilizer modulates strawberry photosynthetic apparatus tolerance responses by severe drought, soil salinization and short extreme heat event

Jesús V. García-López<sup>a</sup>, Susana Redondo-Gómez<sup>b</sup>, Noris J. Flores-Duarte<sup>c</sup>, Ignacio D. Rodríguez-Llorente<sup>c</sup>, Eloísa Pajuelo<sup>c</sup>, Enrique Mateos-Naranjo<sup>a,b,\*</sup>

<sup>a</sup> Servicio General de Invernadero, Centro de Investigación, Tecnología e Innovación (CITUS), Universidad de Sevilla 41012 Seville, Spain

<sup>b</sup> Departamento de Biología Vegetal y Ecología, Facultad de Biología, Universidad de Sevilla 41012 Seville, Spain

<sup>c</sup> Departamento de Microbiología y Parasitología, Facultad de Farmacia, Universidad de Sevilla 41012 Seville, Spain

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### ABSTRACT

Drought, soil salinization and the extreme heat events increments associate to climate change will notably impact sensitive crop species, such as strawberry. A greenhouse experiment was arranged to evaluate the potential of a PGPR-based biofertilizer, with multiple PGP properties, including ACC deaminase production highly related to the limitation of ethylene levels under abiotic stress, in modulation of photosynthetic apparatus tolerance responses by severe drought (complete water withholding), salinity in irrigation water (340 mM NaCl) and short extreme heat event (37/28 °C maximum and minimum temperature range). Our results show that all stress factors triggered acute injury effects on strawberry carboxylation capacity and photosystem II energy assimilation efficiency ability; whose intensity varied depending on factor nature. However, bacterial inoculation diminished ~67 %, 20 % and 18 % the deleterious impact imposed by drought, heat and salinity stress on the net photosynthetic rate ( $A_N$ ). This effect was primarily mediated by counterbalancing the diffusion of CO<sub>2</sub> in the stomata and biochemical limitations in response to heat and salinity stress, while the reduction of biochemical damage was more notable in response to drought. Complementarily, inoculation was able to highly buffer the photochemical limitations imposed by all abiotic stress factors tested. Despite these positive effects, the application of PGPR-based biofertilizer was unable to completely reverse the impact of stress factors on strawberry photosynthesis metabolism. However, the signal of these ameliorative effects was significant enough to consider the implementation of PGPR-based biofertilizer application as a complementary tool in the management of strawberry cultivation in increasingly stressful agronomic contexts.

### Introduction

In the future, agriculture is expected to face problems derived from climate change; variations in rainfall regimes, temperature rise, soil desertification and associated salinization appear to be more common based on more reliable predictions (IPCC, 2022). The accumulation of abiotic stresses will reduce the production of crops, and this will push the need to increase the inputs associated with the crop, especially the water supply will become critical in a future scenario of scarcer and more irregular rains (IPCC, 2022). Additionally, the pressure to produce more food and resources for a growing world population will translate into the need to improve crop production in an agronomic context of

more extreme abiotic stress (FAO, 2023).

Strawberry cultivation stands out for its geographic location, biological characteristics and agronomic management characteristics, as one of the agricultural sectors most seriously affected by future climate conditions (Neri et al, 2012; Redondo-Gómez et al, 2022). Thus, although in the last 20 years there have been innovations to extend the harvest season regardless of climate conditions, strawberry production still depends largely on abiotic factors such as water availability and soil quality (Neri et al, 2012; Saidimoradi et al, 2019). In this sense, drought, salinity, and temperature have been underlined as the main limiting abiotic factors for strawberry production worldwide, as they can influence anatomical and physiological characteristics in plants by affecting

\* Corresponding author.

E-mail address: [emana@us.es](mailto:emana@us.es) (E. Mateos-Naranjo).

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certain molecular pathways and mechanisms (Ghaderi et al, 2018), which ultimately affects crop production. These limitations emphasize the need to promote research that facilitates the availability of abiotic stress-tolerant varieties and/or the development of tools that allow cushioning the undesired effects under environmental stress conditions, making it possible to maintain or even increase the cultivated surface covering semi-arid areas and salinized soils.

Many studies have focused on improving crop yield through classical interbreeding programs or genetic engineering (Qin et al, 2008; Mezetti et al, 2016). However, classical breeding programmes have proved inefficient, arduous and slow (Faedi et al., 2002; Arias et al, 2004). Regarding genetic engineering, several studies have identified genes involved in plant response to environmental stress and pathogens (Wei et al, 2016). However, although this is a most promising technology, it is still in an experimental stage due to its potential risks to human health and the environment, which need to be assessed (Qin et al, 2008). Considering these limitations, it should be noted that the use of PGPR-based biofertilizers is gaining momentum in recent years to apply to different types of crops (Glick, 2012; Zaidi et al, 2015) with the aim of promoting plant growth in an actual agronomic context and strengthening plant tolerance to a number of environmental stresses through different direct and/or indirect positive bacterial properties (Ahmad and Khan, 2011; Bhattacharyya and Jha, 2012; Chookietwattana and Maneewan, 2012; Nadeem et al, 2014; Paul and Lade, 2014; Etesami and Maheshwari, 2018).

Regarding strawberry crops, the use of microorganisms has focused mainly on pathogen biocontrol in the soil, with very few recent studies analyzing its potential to improve strawberry tolerance to some environmental stress (water deficit: Erdogan et al, 2016; salt stress: Karlidag et al, 2010; Dönmez and Turan, 2010; Karlidag et al., 2013). And none of them has focused on the use of high stress resistance PGP bacteria isolated from the halophyte rhizosphere and have explored their role in the tolerance response of plants to multiple stress factors including extreme weather events, such as heat waves, which are increasing in intensity and frequency especially in the Mediterranean area (ICC, 2022). Taking into account these aspects, our working hypothesis is that the use of PGPR-based biofertilizer, previously used under nutrient limitation conditions (García-López et al, 2023; Valle-Romero et al, 2023), integrated by self-compatible strains with high tolerance to environmental stress with multiple PGP properties, include 1-aminocyclopropane-1-carboxylate (ACC) deaminase (that cleaves the precursor of ethylene, which is crucial in response of plants to stress) will improve strawberry physiological performance under several abiotic stress factors. To assess this aspect, a complete factorial greenhouse study approximation was arranged to evaluate the effect of PGP bacterial consortium inoculation on strawberry plants (*Fragaria ananassa* var. *rociera*) photosynthetic apparatus performance as a proxy of the degree of plant tolerance to stress induced by high salinity concentration in irrigation water (340 mM NaCl), severe drought (complete water withholding) and short extreme heat event (three days of 37/28 °C maximum and minimum temperature range).

## Material and methods

### Plant material, growth conditions and bacteria inoculum characteristics

Seedlings of strawberry plants (*Fragaria ananassa* var. *rociera*) were provided by the company Agro GM SL and were transplanted in 3.5 L plastic pots filled with soil from an agricultural farm located in Palma del Condado (Huelva, Spain 37°36'1" N, 6°56' W; Southwest Spain). Soil physicochemical characteristics: Texture: 81 % sand, 8 % silt, and 11 % clay; pH = 7.81 and electrical conductivity (CE) = 272.05  $\mu\text{S}/\text{cm}$ . Before planting, the substrate was subjected to a sterilization treatment by adding 5 ml of  $\text{H}_2\text{O}_2$  to each pot and subsequent cover with plastic for 48 h.

After sowing, the plants were kept in a greenhouse (University of

Sevilla Research, Technology and Innovation of the University of Sevilla, CITIUS II; 37°24'0" N, 6°00' W; Southwest Spain) with  $25 \pm 2$  °C,  $50 \pm 10$  % RH, and subjected to a day/night regime of 14 h of light (maximum photosynthetic photon flux density (PPFD) incident on leaves of  $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) and 10 h of darkness and with adequate irrigation and fertilization. After 30 days plants growth the inoculation procedure began, this consisted of applying 100 mL of a previously designed bacterial inoculum to half of the plants every 15 days. These plants were kept under previously described conditions and constituted the base of plant material for the development of the different experimental setups described in the next Section.

The bacterial inoculum used in this study was integrated by five self-compatible bacteria strains with high multi-stress resistance and a variety of complementary plant growth-promoting (PGP) isolated, characterized and used in several of our previous studies (Mesa et al, 2015; Mesa-Marín et al, 2020; Flores-Duarte et al., 2022; Redondo-Gómez et al, 2022; García-López et al, 2023; see Fig. 1 for details of the PGP inoculum). For technical details on inoculum preparation, see Navarro-Torre et al (2016) and Flores-Duarte et al. (2022).

### Experimental design

Three different stress experiments were arranged in parallel to determine the potential of the designed multifunctional PGP-bacterial inoculum to improve strawberry tolerance against high salinity concentration in irrigation water, severe drought, and short extreme heat event. For that, different subgroups of inoculated and non-inoculated plants from the initial set, with a similar degree of development (mean total biomass of  $65 \pm 4$  g of fresh weight), were randomly selected and divided into different groups and arranged in randomized experimental specific stress blocks as follows:

#### Experimental block 1: salinity stress

Several non-inoculated and inoculated plants from the initial subset were randomly selected and subjected to an irrigation treatment with 340 mM NaCl for 5 days (Ss, salinity stress) obtaining two different treatments, Ss N-In (salinity stress and non-inoculated plants) and Ss In (salinity stress and inoculated plants). For the establishment of salinity stress treatment and to avoid changes in NaCl concentration caused by water evaporation during the experimental period or a high salinity concentration heterogeneity in the soil, plants were immersed every day for 2 min at ground level in a container with the appropriate salinity solution. Furthermore, CE of the soil was recorded daily in each pot using an ML3 ThetaProbe soil moisture sensor (Delta-T Devices Ltd) to verify that all plants had similar CE values. During the salinity stress period, gas exchange and chlorophyll fluorescence measurements were recorded every day.

#### Experimental block 2: water stress

Specific plant subsets (i.e. non-inoculated and inoculated) were subjected to severe drought treatment (WS, water-stressed) by completely withholding water until severe water stress was reached, obtaining two experimental treatment combinations WS N-In (water-stressed and non-inoculated plants) and WS In (water-stressed and inoculated plants). Severe water stress was considered to have occurred when the stomatal conductance to  $\text{CO}_2$  ( $g_s$ ) decreased below  $50 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ , because photosynthetic activity becomes predominantly inhibited by metabolic processes below this level (Medrano et al, 2002), which happened on the fifth day. During the stress period, gas exchange and chlorophyll fluorescence measurements were recorded every day.

#### Experimental block 3: heat stress

For this, specific plant subsets (i.e. non-inoculated and inoculated)

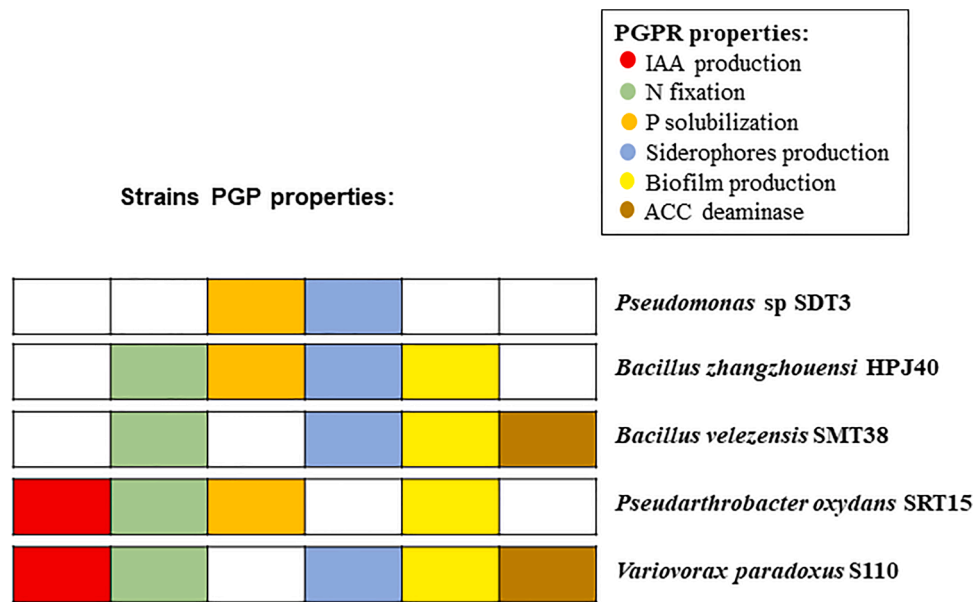


Fig. 1. Plant growth-promoting rhizobacterial (PGPR) traits for the strains of inoculum used in this study. Information adapted from García-López et al (2023).

were randomly selected and placed in a controlled environment chamber (Aralab/Fitoclima 18.000EH, Lisbon, Portugal), which was programmed with alternating diurnal regime of 16 h of light and 8 h of darkness, temperature range of 16/25 °C, 50±5 % relative humidity and light intensity of 300  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . After one week of plant acclimation to these environmental conditions, each subset of plants was subjected for three days to a short extreme temperature event of 37/28 °C maximum and minimum temperature range with an alternating diurnal regime of 16/8 h of light/darkness and 50±5 % relative humidity. The soil water content values (VWC) were monitored daily during both experiments in each pot using a soil moisture sensor (Delta-T Devices Ltd) to verify that all plants presented similar VWC values. Leave gas exchange and chlorophyll fluorescence measurements were recorded at the end of the stress period.

In parallel to each specific experimental block, another set of plants (both non-inoculated and inoculated) was maintained under non-stressful conditions (well-watered conditions by adding the amount of tap water they lost during the day and 25/16 °C maximum and minimum temperature range with alternating diurnal regime of 16/8 h of light/darkness) which were used as a control treatment to obtain reference values of gas exchange and fluorescence of non-stressed plants. These results are presented in the supplementary material (Figs. S1–2, respectively).

#### Gas exchange and chlorophyll fluorescence analysis

Measurements of leaf gas exchange and chlorophyll fluorescence were taken every day after the onset of the drought and salinity treatments, while these measurements were done at the end of the heat stress experiment.

Gas exchange measurements consisted of the development of  $A_N/C_i$  curves in fully developed leaves randomly selected ( $n = 5$  per treatment) using an infrared gas analyser (LI-6800–01, LICOR Inc., Lincoln, NE, USA). These  $A_N/C_i$  curves were developed using twelve different ambient  $\text{CO}_2$  concentration ( $C_a$ ) (400, 300, 200, 150, 100, 50, 400, 750, 10,000, 1250, 1500, 1750, 2000 ppm), flux light density 1000  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ , 2.0–3.0 kPa vapor pressure, 50±0.5 % relative humidity and leaf temperature of 25±0.5 °C for salinity and water stress experiments, and 37±0.5 °C for the heat stress experiment. After the curve development, the net photosynthetic rate ( $A_N$ ), stomatal conductance ( $g_s$ ), instantaneous water use efficiency (iWUE) and intercellular

$\text{CO}_2$  concentration ( $C_i$ ) were directly obtained. And using the curve fitting method, the mesophyll conductance ( $g_m$ ) and the maximum carboxylation rate allowed by ribulose-1,5-biphosphate (RuBP) carboxylase/oxygenase ( $V_{c,\text{max}}$ ) were obtained following the recommendations of Flexas et al (2009, 2013) and Pons et al (2009). Finally, coinciding with the gas exchange measurements, several energy efficiency traits of the plant photosystems were recorded. Thus, the actual efficiency of photosystem II ( $\Phi_{\text{PSII}}$ ), the electron transport rate (ETR) and the apparent quantum efficiency of photosynthesis ( $\Phi_{\text{CO}_2}$ ) were measured by multiphase flash fluorometer (LI-6800–01A, LICOR Inc., Lincoln, NE, USA) ( $n = 5$ ).

#### Statistical analysis

Statistica software v. 10.0 was used for statistical analyses. Thus, the effect of different stress treatment and bacterial inoculation on strawberry plant physiological traits was determined using analyses of variance (ANOVA) followed by post hoc LSD test for multiple comparisons. Although this analysis was focused on the percentage of variation of each specific physiological trait in non-inoculated and inoculated plants regarding to control under the similar stress scenario, the data corresponding to the absolute values and each sampling period are presented in the supplementary material (Figs. S3–S8).

## Results

#### Gas exchange responses and photosynthesis limitation analysis of strawberry plants

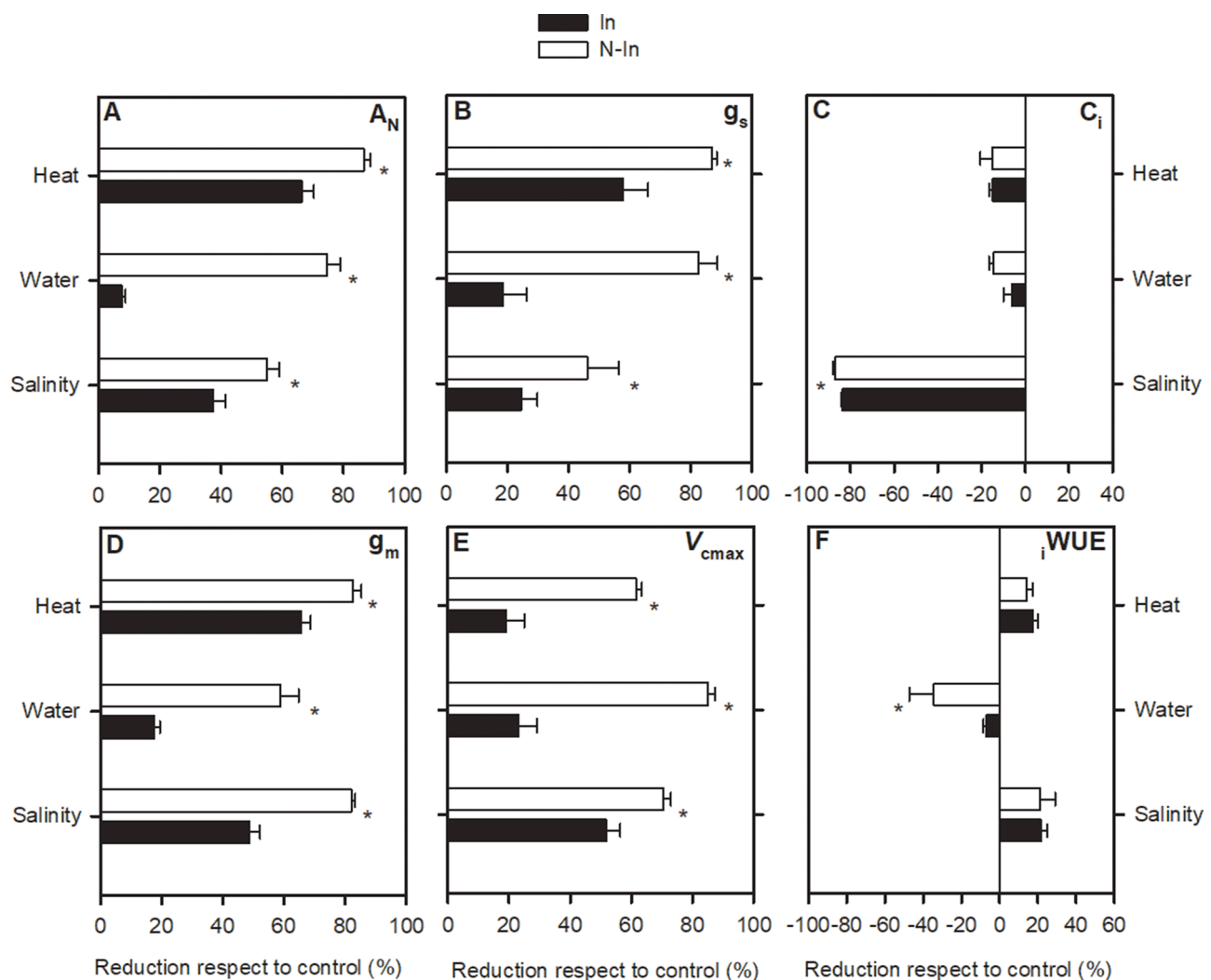
Our results revealed that there were significant effects of the different stress factors tested and bacterial inoculation on the performance of the strawberry plants photosynthetic apparatus in terms of carboxylation capacity. Consequently, we found that saline irrigation, water deficit, and short extreme high temperature events had a negative effect on the gas exchange characteristics of strawberries leaves, with respect to control plants, and this degree of injury was stress specific, and each specific trait showed a different level of sensitivity to the imposed stress factor (Anova,  $P < 0.05$ ; Supplementary material Figs. S3, S5, S7).

Focussing at detail level on the responses of gas exchange traits in terms of the percentage of variation of their absolute values with respect

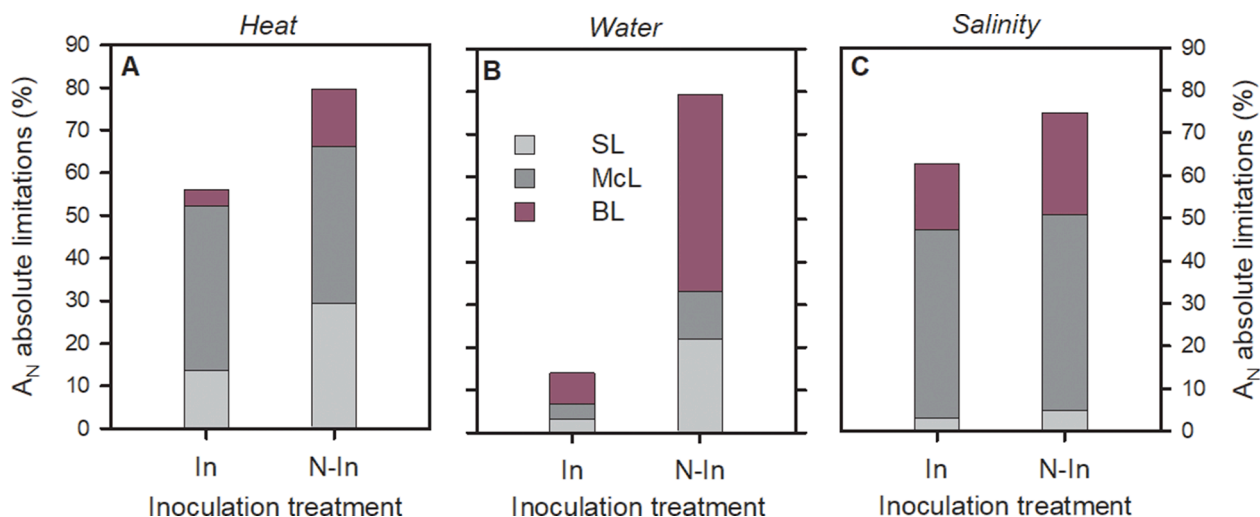
to control treatment in the maximum stress period, we found that in non-inoculated plants  $A_N$  decreased 87 %, 74 %, and 55 % for heat, water and salinity stress, respectively, compared to control plants (Anova,  $P < 0.05$ ; Fig. 2A). A very similar trend was recorded for  $g_s$  in relation to each specific stress factor for these plants (Anova,  $P < 0.05$ ; Fig. 2B), while the intercellular concentration of CO<sub>2</sub> ( $C_i$ ) tended to increase in all stress situations tested, but especially in plants subjected to saline irrigation with a percentage of variation relative to the control c. 85 % (Anova,  $P < 0.05$ ; Fig. 2C). Also, we found that  $g_m$  was affected to a greater extent by heat and salinity stress decreasing 82 % regarding to control for both, followed by water stress, which imposed a reduction of 52 % in non-inoculated plants (Fig. 2D). Similarly, it was recorded that the greatest influence on  $V_{c,max}$  occurs due to irrigation limitation with a reduction c. 85 % in non-inoculated plants, followed by a reduction of 70 % and 61 % for salinity and heat stress, respectively (Anova,  $P < 0.05$ ; Fig. 2E). Furthermore, it should be noted that these deleterious effects were, to some extent, mitigated by bacterial inoculation. Thus, we were able to identify an overall positive common pattern in the response of most of the gas exchange parameters analysed against each specific stress factor in combination with bacterial inoculation compared to non-inoculated plants (Anova,  $P < 0.05$ ; Fig. 2A-E). However, it is also to

highlight that this specific positive ameliorative effect overall was more acute in plants subjected to severe water stress conditions, followed by those grown under salinity and heat stress, respectively, for all parameters (Fig. 2A, B, D), except for  $V_{c,max}$ , where inoculation was more efficient both in response to drought and heat stress (Fig. 2E). Finally,  $iWUE$  values showed a decrease with heat and salinity stress to a similar degree in both inoculation treatments, but a contrary increase with water withholding, making this effect more acute in non-inoculated plants (Anova,  $P < 0.05$ ; Fig. 2F).

On the other hand, our photosynthetic absolute limitation analysis corroborated the different levels of sensitivity of photosynthesis to specific stress factor and bacterial ameliorative effect. According, we found that the drastic decrease in  $A_N$  due to heat, water, and salinity stress was explained by the variation in the relative importance of the diffusional and biochemical components in the carboxylation process in each specific stress factor studied, and this variation was to same extent modulated by bacterial inoculation (Fig 3A-C). Thus, in non-inoculated plants subjected to heat and salinity stress,  $A_N$  reduction was mainly attributed to diffusional component with limitations of 37 % and 29 % for mesophyll conductance (MCL) and stomatal (SL), respectively, followed by 13 % limitation in biochemical (BL) component (Fig. 3A) in



**Fig. 2.** Percentage of variation in net photosynthetic rate,  $A_N$  (A), stomatal conductance,  $g_s$  (B), intercellular CO<sub>2</sub> concentration,  $C_i$  (C), mesophyll conductance,  $g_m$  (D), maximum carboxylation rate,  $V_{c,max}$  (E) and intrinsic water use efficiency ( $iWUE$ ) (F) measured in randomly selected leaves of strawberry plants subjected to a heat stress (37/28 °C, maximum and minimum temperature range for 3 days), water stress (full water withholding during 5 days) and salinity stress (irrigation with 340 mM NaCl during 5 days) with and without bacterial inoculation respect to control plants grown under non-stressful conditions (well-watered conditions, 0 mM NaCl and 25/16 °C maximum and minimum temperature range). Values represent mean  $\pm$  standard error of five replicates. Asterisks indicate significant differences among inoculation treatments within each stress treatment (analysis of variance, LSD test:  $p < 0.05$ ).



**Fig. 3.** Quantitative analysis of absolute limitations in photosynthesis ( $A_N$ ) respect to the control treatment of strawberry plants subjected to heat, water and salinity stress with and without bacterial inoculation for more details see legend of Fig. 2). Absolute limitations of photosynthesis is represented by the sum of stomatal (SL), mesophyll (MCL), and biochemical (BL) limitations.

response to heat stress. While diffusional restrictions (McL + SL) and BL were 48 % and 23 %, respectively, in response to salinity (Fig. 3C). However, in non-inoculated plants grown under water stress, biochemical limitation was the most important explaining 46 % of  $A_N$  reduction, followed by limitations in SL and McL components with 20 % and 8 % reduction, respectively (Fig. 3B). However, these percentages of  $A_N$  limitation were substantially reduced in inoculated plants, with a similar joint reduction of the three components (McL+SL+BL) in response to water shortage, and only of stomatal and biochemical limitations under heat and salinity stress, respectively (Fig 3A-C).

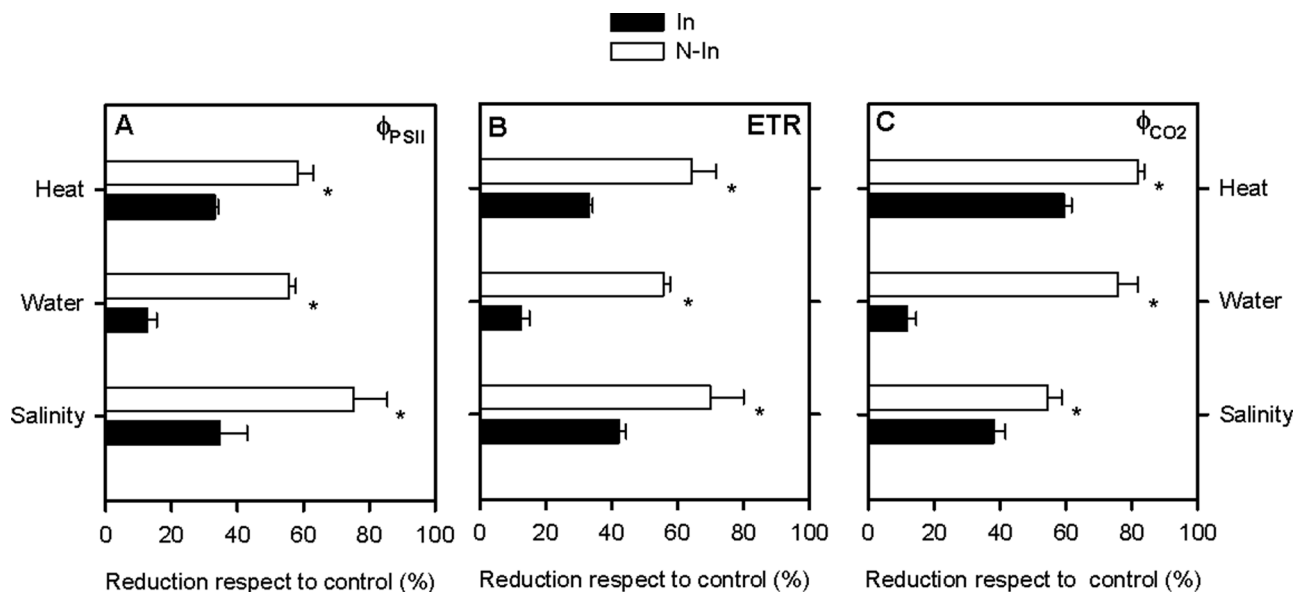
*Chlorophyll fluorescence responses in strawberry plants*

Our results revealed that there were significant effects of the different stress factors tested and bacterial inoculation on the efficiency of PSII energy use of strawberry plants (Anova,  $P < 0.05$ ; Supplementary

material Figs. S4, S6, S8). Thus, in non-inoculated plants,  $\Phi_{PSII}$  and ETR values decreased 75 % and 70 %, 58 % and 64 % and 56 % and 55 % respectively for the treatment of salinity, heat and water stress, compared to control plants (Anova,  $P < 0.05$ ; Fig. 4A, B), while the highest percentage of reduction in  $\Phi_{CO2}$  was recorded in response to heat stress, followed by water and salinity stress, respectively (Fig. 4C). However, bacterial inoculation was able to counterbalance in a certain degree these stress injury-imposed effects, this positive response being more acute under water stress for all fluorescence parameters tested regarding non-inoculated plants (Anova,  $P < 0.05$ ; Fig. 4A-C).

**Discussion**

Agricultural sectors highly dependent on quality soils and large amounts of water resources, such as the strawberry industry, will be especially impacted by climate change, due to the difficulty in



**Fig. 4.** Percentage of variation in actual efficiency of photosystem II,  $\Phi_{PSII}$  (A), electron transport rate, ETR (B) and apparent quantum efficiency of photosynthesis  $\Phi_{CO2}$  (C) measured in randomly selected leaves of strawberry plants subjected to heat, water and salinity stress with and without bacterial inoculation (for more details see legend of Fig. 2). Values represent mean  $\pm$  standard error of five replicates. Asterisks indicate significant differences among inoculation treatments within each stress treatment (analysis of variance, LSD test:  $p < 0.05$ ).



maintaining its production, yield, and fruit quality under more adverse environmental conditions (Neri et al, 2012; Redondo-Gómez et al, 2022; Schattman et al, 2022). In this study we have treated to understand the role of PGPR-based biofertilizer, designed from halophytes microbiome and highly resistant to environmental stress, could play as a sustainable technique to facilitate more resilience of strawberry cultivation against several stress factors linked with climate change, using a physiological approach based on the study of the tolerance photosynthetic apparatus responses of strawberries under different extreme stress scenarios with and without bacterial inoculation.

Our results indicate that all stress factors tested (i.e. severe drought, high salinity concentration in irrigation water, and short extreme heat event) exerted a clear injury effect on the performance of the strawberry plants photosynthetic apparatus in terms of carboxylation and PSII energy assimilation efficiency capacity, which was more pronounced depending on the nature and intensity of the stress factor involved (Suzuki et al, 2014). This response pattern is consistent with many results obtained in stress tolerance research, where it has been recognized that photosynthetic metabolism is especially sensitive to drought, salinity and air temperature variations in several plant species (Munns, 2002; Chaves et al, 2003; Flexas et al, 2012a), and particularly in previous studies developed in the abiotic stress sensitive crop specie strawberry. In this sense, several authors have indicated that water deficits between  $-0.03$  and  $-0.07$  MPa can reduce both mean strawberry weight and the number of strawberries (Serrano et al, 1992; Neri et al, 2012). A further change elicited even by moderate water stress is a fast decrease in photosynthetic rate (Martínez-Ferri et al, 2016), with a resulting reduction in plant biomass and fruit production (Tworkoski, 2001; Martínez-Ferri et al, 2016). Likewise, there are records of a reduction in plant physiological performance in response to saline irrigation (Saied et al, 2005; Wu et al, 2019; Zahedi et al, 2019) and heat stress (Kadir et al, 2006; Ledesma et al, 2008).

Focussing in detail on the results obtained in this study, it is worth highlighting that the short extreme temperature event of the maximum and minimum temperature range of  $37/28$  °C after 3 days of exposure produced the greatest limitation in the carbon assimilation metabolism functionality of strawberry plants, followed by extreme drought and saline irrigation for 5 days, as indicated by the pattern of responses of  $A_N$ . However, despite this different level of sensitivity to these stress factors, it should be noted that bacterial inoculation had an overall ameliorative effect on plant photosynthetic apparatus performance, being also stress-specific. Therefore, bacterial inoculation diminished  $\sim 67$  %,  $20$  % and  $18$  % the deleterious impact mediated by drought, heat and salinity stress on plant carbon assimilation capacity, with the potential consequence effect for plant growth and development under more stressful conditions due to the increased availability of photo-assimilates (Flexas et al, 2013). Although the reason for this variable positive ameliorative response with respect to the type of stressor imposed is not clear, it is thought that could be associated with the effect that the specific stress factor would exert on the plant-microbiome complex used in this study (Mateos-Naranjo et al, 2024). In this sense, note that compared to plants, soil microbes could be more sensitive to factors related with climatic change, as tested in this study, due to their simple life history (Jansson and Hofmockel, 2020). Consequently, these stress factors in addition to generating direct effects on plants photosynthetic performance as we previously indicated; they could have led to shifts within the microbiological component of this experiment, despite its demonstrated tolerance to abiotic stress, and consequently in its functional characteristics that modulate its effect, due to its PGP properties, on plant tolerance responses (Marín and Kohout, 2021; Yang et al, 2021). Therefore, it would be reasonable to expect a positive variable effect depending on the stress factor imposed, as revealed our results. However, despite this variability, our results agree with previous results found for strawberry, where the presence of microorganisms with PGP properties counterbalanced to some extent the detrimental effects of stressful environmental factors, such as salinity, on plant physiological

performance (Redondo-Gómez et al, 2021; 2022). And, in particular, this PGPR-based biofertilizer integrated by strains SDT3, HPJ40, SMT38, SRT15 and S110, which previously has demonstrated the potential to counterbalance to some extent the detrimental effects triggered by phosphorus and nitrogen deficits in strawberry plants (García-López et al, 2023; Valle-Romero et al, 2023) would also improve strawberry photosynthetic performance in response to other abiotic stress factors of different nature, according to our working hypothesis.

The positive ameliorative effect of PGPR-based biofertilizer on the performance of the strawberry photosynthetic apparatus under stress induced by drought, salinity and heat, regardless of its intensity level, could be partially explained by a differential inoculation modulation effect on the down-regulation in key steps of the plant photosynthetic apparatus triggered by each stress factor in terms of apparatus integrity and functioning; i.e.,  $CO_2$  diffusion limitation due to variations in stomatal and mesophyll responses, Rubisco activity, and light harvesting antenna efficiency (Flexas et al, 2012b; Pérez-Romero et al, 2018; 2019). Therefore, we found that in non-inoculated plants, photosynthetic carbon assimilation capacity injury from both heat and salinity stress treatments was mainly due to  $CO_2$  diffusion limitation, although with a different degree of relative importance of the stomatal and mesophyll component for these specific stresses. So, in these plants, the short extreme heat event imposed an acute decrease in both  $g_s$  and  $g_m$ , while in salinity irrigation treatment, the diffusion limitation was accounted to a greater extent by  $g_m$  reduction, followed by biochemical limitation according to  $V_{c,max}$  values recorded. However, bacterial inoculation was able to counterbalance in greater degree the percentage of reduction of those stress mainly at stomatal and biochemical limitations, being the improvement at the level of mesophyll conductance compared to the control treatment more limited. Furthermore, it should be noted that the positive effect of bacterial inoculation in photosynthetic response to water stress was mediated by both the reduction of diffusional and biochemical limitations, although it should be noted that its effect was more considerable at the biochemical level, as indicated by the  $V_{c,max}$  values. These observed trends in  $g_s$ ,  $g_m$ , and  $V_{c,max}$  resulted in an increase in  $C_i$  in plants grown irrigated with  $340$  mM NaCl regarding inoculation treatment, where  $CO_2$  was transported to the intercellular space, however, it was not transferred to chloroplast due to a reduction in  $g_m$  and  $V_{c,max}$ , with and without bacterial inoculation, respectively, with the consequent accumulation of  $CO_2$  and variations in the efficiency of intrinsic water use (Galle et al, 2009; Resco et al, 2009). Additionally, our results indicated that the application of biofertilizer led to a reduction in the photochemical limitations of strawberries imposed by all abiotic stress factors tested, as shown by the overall higher values of the  $\Phi_{PSII}$ ,  $\Phi_{CO2}$  and ETR values recorded in inoculated plants compared to their non-inoculated counterparts, because this protective effect also caused a specific stress as occurred with the gas exchange parameters. These results indicated that the ameliorative stress effect of bacterial inoculation on strawberry photosynthetic carbon assimilation capacity would also be mediated for the preservation of a greater photosystem efficiency, in terms of absorption, transport, and transformation of captured energy, providing enough energy substrates for carbon fixation, as reflected in the highest  $A_N$  values recorded in these plants.

The positive effect of the PGPR-based biofertilizer modulate on the tolerance of the strawberry carbon fixation capacity and photochemical efficiency under the extreme stress factor tested could be explained by several factors linked with the PGP properties of the biofertilizer. Thus, we highlight that above the possible direct effects of some PGP properties, such as bacterial indol-3-acetic acid production (IAA) on plant growth and development (Zaidi et al, 2015; Redondo-Gómez et al, 2021) or biofilm formation in the limitation of sodium plant uptake under saline stress (Nadeem et al, 2014; Navarro-Torre et al, 2017), it is possible that much of this inoculation effect on improving the tolerance of the strawberry photosynthetic metabolism performance has been mediated by particular PGP properties. In this sense, it must be indicated that the SMT38 and S110 strains (i.e. biofertilizer strains members) have

the ability to produce the enzyme 1-aminocyclopropane-1-carboxylate deaminase (ACC), which can modulate the level of ethylene hormone produced by plants in response to stress (Glick, 2014). In this sense several authors have identified an effect of ethylene hormone on different key plant functional processes, including its growth and photosynthetic performance (Acharya and Assmann, 2009; Iqbal et al., 2012; Iqbal et al., 2022) through a down-regulation of its photosynthesis rate and stomatal functioning (Pallas and Kays, 1982; Madhavan et al., 1983; Merritt et al., 2001), as we have observed in this experiment in non-inoculated plants in response to stress. In addition, Khan (2004) found a strong positive correlation between ACC and the photosynthesis of mustard cultivars. Taking into account this evidence, it is possible that the potential regulation of ethylene levels by the production of ACC deaminase due to PGPR-based biofertilizer application would allow strawberry plants to partially overcome ethylene level from becoming inhibitory for the photosynthesis process through the reduction of stomatal functionality, as indicated by the greater  $g_s$  values while  $g_m$  did not highly vary compared to non-inoculated plants regarding nature impose stress factor. Consistent with our results, Brunetti et al (2021) demonstrated a positive effect of ACC producer strains PGPR on the photosynthesis rate of the limbing species *Mucuna pruriens*, stomatal conductance, and ethylene formation under water deficit.

Finally, other PGP properties present in the biofertilizer used, such as nitrogen fixation of bacterial strains, siderophores production, and P solubilisation, could help plants acquire sufficient nitrogen, iron, and phosphorous under extreme stress factors tested, which would have a negative effect on plant uptake capacity, and consequently in its primary metabolism (Thereby-Vale et al, 2022). In this sense, several authors have highlighted the importance of having an adequate nutritional balance of essential elements such as nitrogen, phosphorus, and iron due to their role as structural elements of primary metabolites and enzymes involved in plant photosynthetic metabolism, in terms of improving plant carboxylation, photochemical, and CO<sub>2</sub> diffusion capacity (phosphorus: Warren, 2011; nitrogen: Evan and Clarke, 2019; iron: Barhoumi et al, 2015). Therefore, these biofertilizer PGP properties would have potentially contributed to a better nutritional balance in different stress situations, as has already been previously demonstrated in strawberries grown with nutritional deficits (García-López et al, 2023; Valle-Romero et al, 2023), which would help explain the amelioration effect in strawberry carbon fixation capacity and photochemical efficiency in extreme stress scenarios.

## Conclusions

Our findings demonstrated that the PGPR-based biofertilizer designed from halophytes microbiome integrated by high multi-stress resistance bacterial strains and with several PGP properties, which previously had demonstrated its efficacy to improve strawberry nutritional deficit tolerance, would also be useful to strengthen plant capacity to cope with extreme drought, salinity, and short high temperature events, which would make it a broad spectrum multifunctional management tool for strawberry crops adaptation to abiotic stress and to reinforce the resilience of this crop to climate change. Thus, our results revealed that all stress factors evaluated imposed an acute injury effect on strawberry carboxylation capacity and photochemical apparatus efficiency, whose magnitude and damage in key steps of the photosynthetic process was specific-stress. However, bacterial inoculation ameliorates these adverse effects in highly notable percentages, representing improvements of ~67 %, 20 % and 18 % of the deleterious impact mediated by drought, heat, and salinity stress on the carbon assimilation capacity; through up-regulation of the diffusional limitation of CO<sub>2</sub> due to variations in stomatal functionality, Rubisco activity and light-harvesting photosystem efficiency. Finally, from an agronomic approach these results revealed that although PGPR-based biofertilizer application was unable to completely reverse abiotic stress factor on strawberry photosynthesis metabolism, the signal of the positive effect

of bacterial inoculation was notable enough to consider the implementation of this biotool in the management of strawberry cultivation that would contribute to facilitate together with other potential techniques a more efficient management of water resources in order for production to require a smaller water supply and/or even to rely on brackish water and poor soil quality.

## CRedit authorship contribution statement

**Jesús V. García-López:** Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Susana Redondo-Gómez:** Conceptualization, Funding acquisition, Investigation, Project administration, Resources, Supervision, Validation, Writing – review & editing. **Noris J. Flores-Duarte:** Investigation, Methodology, Writing – review & editing. **Ignacio D. Rodríguez-Llorte:** Conceptualization, Investigation, Resources, Supervision, Writing – review & editing. **Eloísa Pajuelo:** Investigation, Resources, Supervision, Writing – review & editing. **Enrique Mateos-Naranjo:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data Availability

Data will be made available on request.

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.stress.2024.100448.

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