

# Growth-promoting rhizobacteria improve physiological variables in lemon balm, *Melissa officinalis* L., subjected to water stress

Rizobacterias-promotoras de crecimiento mejoran variables fisiológicas en toronjil, *Melissa officinalis* L., sometido a estrés hídrico

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## ABSTRACT

Climate change has caused droughts in regions that previously did not have water issues, affecting the production of medicinal plants grown in small-scale agricultural units. Plants such as lemon balm, *Melissa officinalis* L., are sensitive to water stress, which reduces their yield. One alternative to mitigate water stress is the use of plant growth-promoting rhizobacteria (PGPR). However, in the high tropics of Colombia, the use of these microorganisms is not common due to a lack of knowledge about how they can improve water absorption and increase the yield of medicinal plants. This study aimed to determine the effect of native PGPR on lemon balm plants subjected to water stress conditions using a completely randomized design with six treatments and four replications. Applications of *Bacillus cereus* and *Bacillus amyloliquefaciens* were made, and the plants were subjected to two water levels (field capacity and 50% field capacity). Physiological variables of stomatal

conductance, chlorophyll content, and fluorescence were measured at the end of the experiment. *Bacillus cereus* significantly improved growth parameters such as number of leaves ( $115.00 \pm 34.71$ ), fresh weight of root ( $5.51 \pm 3.07$  g) and shoot ( $8.32 \pm 4.27$ ), *Bacillus amyloliquefaciens* increased stomatal conductance ( $401.3 \mu\text{mol H}_2\text{O m}^2 \text{s}^{-1}$ ) in water-stressed plants. These results suggest that the use of native PGPR considerably improves the growth and development parameters of lemon balm plants and provides a viable alternative for farmers to enhance yield and resistance to water stress conditions.

**Key words:** *Bacillus*, bacterial strains, climate change, PGPR, plant hormones, yield.

## RESUMEN

El cambio climático ha causado sequías en regiones que anteriormente no tenían problemas hídricos, afectando la producción de plantas medicinales cultivadas en unidades agrícolas pequeñas. Plantas como *Melissa officinalis* L. (toronjil) son sensibles al estrés hídrico, lo que reduce su rendimiento. Una alternativa para reducir el impacto hídrico es el uso bacterias promotoras del crecimiento vegetal (PGPR). Sin embargo, en los trópicos altos de Colombia, el uso de estos microorganismos no es común debido a la falta de conocimiento sobre cómo pueden mejorar la absorción de agua y aumentar el rendimiento de las plantas medicinales. Este estudio tuvo como objetivo determinar el efecto de PGPR nativas en plantas de toronjil sometidas a condiciones de estrés hídrico, se utilizó un diseño completamente aleatorizado DCA con seis tratamientos y cuatro repeticiones, se realizaron aplicaciones de *Bacillus cereus* y *Bacillus amyloliquefaciens* y las plantas se sometieron a dos niveles de hídricos (capacidad de campo y 50% de capacidad de campo), se midieron las variables fisiológicas de conductancia estomática, contenido de clorofila y fluorescencia al finalizar el experimento. *Bacillus cereus* mejoró número de hojas ( $115,00 \pm 34,71$ ), peso fresco de raíces ( $5,51 \pm 3,07$  g) y brotes ( $8,32 \pm 4,27$ ), *Bacillus amyloliquefaciens* aumentó la conductancia estomática ( $401,3 \mu\text{mol H}_2\text{O m}^2 \text{s}^{-1}$ ) en plantas sometidas a estrés hídrico. Estos resultados sugieren que el uso de PGPR nativas mejora considerablemente los parámetros de crecimiento y desarrollo de las plantas de toronjil, proporcionando una alternativa viable para los agricultores con el fin de mejorar el rendimiento y la resistencia a condiciones de estrés hídrico.

**Palabras clave:** *Bacillus*; cepas bacterianas; cambio climático; fitohormonas; PGPR, rendimiento

## INTRODUCTION

Lemon balm (*Melissa officinalis* L) is a medicinal plant belonging to the Lamiaceae family and one of the main aromatic species traded worldwide. It contains secondary metabolites such as unsaturated sterols, triterpenes, phenylpropanoids, catechins, tannins, polyphenols, flavonoids, glycosides, and alkaloids in its essential oil (Moradkhani *et al.*, 2010; Vélez *et al.*, 2018). Recent studies have demonstrated that lemon balm extract exhibits antitumor properties (Jahanban-Esfahlan *et al.*, 2017) and antimicrobial activity against human disease-causing microorganisms such as

*Escherichia coli* and *Listeria monocytogenes* (Vélez *et al.*, 2018; Carvalho *et al.*, 2023). Due to these properties, it is considered a promising crop globally, especially in developing countries. However, it is highly affected by water stress, which leads to reduced yield and a decrease in essential oil production (Ahmadi *et al.*, 2019; Mohasseli *et al.*, 2020).

The use of plant growth-promoting rhizobacteria (PGPR) offers an alternative to excessive chemical fertilizer usage, as it enhances the quality and yield of crops and horticultural products. For instance, PGPR has been shown to improve the drought tolerance of medicinal plants (Eshaghi-Gorgi *et al.*, 2022) and restore plant growth under biotic and abiotic stress by reducing ethylene levels, increasing auxin biosynthesis, and promoting nitrogen fixation in the roots (Glick *et al.*, 2007; Zahir *et al.*, 2008). The concept of induced systemic tolerance has been proposed, referring to the physical and chemical changes induced by microorganisms in plants that result in enhanced tolerance to abiotic stress (Yang *et al.*, 2009).

Holbrook (2015) described how drought stress reduces water content in plants, causing loss of turgor and stomatal closure, resulting in decreased growth. Additionally, excessive reactive oxygen species are generated, causing lipid peroxidation of the cell membrane and damage to enzymes, proteins, and nucleic acids (Ahmadi *et al.*, 2019). Plants respond to drought stress by developing various strategies, including maintaining high tissue water potential, growing a deeper root system to optimize water absorption, increasing cell wall flexibility, and osmotic adjustment (Tardieu *et al.*, 2017; Polle *et al.*, 2019; Leuschner *et al.*, 2019). Abiotic stress has been found to increase the biosynthesis of secondary metabolites in plants, with recent studies showing an increase in the production and accumulation of phenolic compounds, anthocyanins, and flavonoids in lettuce and pea plants subjected to water stress (Kumar & Sharma, 2018; Xu *et al.*, 2019; Sharma *et al.*, 2022).

PGPR-induced tolerance to water stress has been reported in several plant species. The mechanisms of drought resistance in plants induced by rhizobacteria involve several physiological and biochemical changes, such as alterations in plant hormone levels, enhanced antioxidant defense, and metabolic adjustments involving the accumulation of organic solutes such as sugars, amino acids, polyamines, and volatile organic compounds. These mechanisms have been observed as action strategies in PGPR-mediated tolerance to salinity and drought (Kaushal & Wani, 2016).

Among the most widely used PGPR genera around the world is *Bacillus*, known for its ability to form endospores, providing resistance to adverse environmental conditions such as water stress; in addition, this genus has great potential to stimulate plant growth through the synthesis of plant hormones such as indoleacetic acid and gibberellins,

the solubilization of phosphates and the production of different metabolites with antagonistic activity, such as lipopeptides including phengyzine and surfactin, which stimulate induced systemic resistance (Xie *et al.*, 2017).

Based on the above, this study is aimed to determine the effect of PGPR on physiological variables of growth, stomatal conductance (Gs), and efficiency of the photosystem PSII in *Melissa officinalis* plants subjected to water stress, elucidating its potential use as biofertilizers.

## MATERIAL AND METHODS

### Experiment Location

The experiment was conducted in the greenhouse of the Biological Control Laboratory at the Pedagogical and Technological University of Colombia, located at 5°33'15" N and 73°21'30" W, with an average temperature of 12°C.

### Plant Material

Lemon balm seeds were disinfected in a 70% ethanol solution for one minute. Germination was carried out in peat moss, and the seedlings were irrigated with distilled water until they were transplanted into 1-liter pots 60 days after sowing. One lemon balm seedling was planted per pot and watered to field capacity for 85 days. Afterward, the treatments with plant growth-promoting rhizobacteria (PGPR), previously isolated and cultivated in a Nutrient Agar medium, were applied.

**Experimental design.** The PGPR were applied directly to the plant roots at concentrations of  $1 \times 10^6$  CFU. In a completely randomized design with four replicates per treatment and the following six treatments:

Treatment 1: *Bacillus cereus* without water stress (BCW).

Treatment 2: *Bacillus cereus* with water stress (BCD).

Treatment 3: *Bacillus amyloliquefaciens* without water stress (BAW).

Treatment 4: *Bacillus amyloliquefaciens* with water stress (BAD).

Treatment 5: Without bacterial inoculation and without water stress (absolute control) - SBW.

Treatment 6: Without bacterial inoculation and with water stress (relative control) - SBD.

### Application of Water Stress

After applying plant growth-promoting rhizobacteria (PGPR), the plants were subjected to drought stress at two levels: field capacity and 50% of field capacity, following the methodology of Eshaghi-Gorgi *et al.* (2022). The pots were weighed daily to measure water lost through leaching and evapotranspiration, and this water was replaced

accordingly. The plants were fertilized once with Nutriponic® at a dose of 5 cc L<sup>-1</sup>. The water stress treatments lasted for 90 days, after which the plants were harvested.

### Measurement of Growth and Stress Parameters

At the end of the experiment, growth variables such as plant height, root length (cm), and fresh and dry weights of shoots and roots were measured according to the methodology of Ghorbani *et al.* (2011). Physiological variables were also assessed. Chlorophyll content was measured using a SPAD-502 Plus chlorophyll meter (SPAD units), a portable, non-destructive device for determining leaf chlorophyll content. Stomatal conductance (gs) was measured using a Decagon Devices SC-1 porometer. The equipment was calibrated before use, and one leaf per plant was selected and placed in the porometer, ensuring a proper seal to avoid leaks.

### Fluorescence Measurements

Chlorophyll fluorescence is a valuable tool for assessing the physiological status of plants and determining the impact of stress on their photosynthetic apparatus (Jiménez-Suancha *et al.*, 2015). Fluorescence measurements were taken from the upper third of the leaves between 10:00 and 11:00 AM using a JUNIOR-PAM fluorometer (WALZ Photosynthesis Instruments, Germany). Maximum photochemical efficiency (Fv/Fm) was calculated as the ratio of variable fluorescence (Fv) to maximum fluorescence (Fm), following Equation 1 (Baker, 2008). Additionally, the quantum yield of photochemical energy conversion ( $Y(II) = (Fm' - Fs')/Fm'$ ), regulated quantum yield of non-photochemical energy dissipation ( $Y(NPQ) = 1 - (Y(II) + Y(NO))$ ), and non-regulated quantum yield of non-photochemical energy dissipation ( $Y(NO) = 1 / (NPQ + 1 + qL(Fm/Fo - 1))$ ) were calculated as stress indicators.

$$Fv/Fm = (Fm - F_0)/Fm \text{ Equation (1)}$$

### Statistical Analyses

The data were analyzed using R software version 4.3.0. A Shapiro-Wilk normality test and Bartlett's test for homogeneity of variances were performed. Following these tests, an Analysis of Variance (ANOVA) was conducted, and a Tukey test was applied when significance was observed ( $p < 0.05$ ).

## RESULTS AND DISCUSSION

**Plant Growth.** “The height of lemon balm plants did not differ significantly between the control treatment and the treatment that was subjected to water stress (50% field capacity). This contrasts with the findings of Eshaghi-Gorgi *et al.* (2022), who reported a 28.9% reduction in height in lemon balm plants subjected to water stress. However, the



present study found higher average numbers of leaves than those reported by Eshaghi-Gorgi *et al.* (2022), who found a 24.1% reduction in the number of leaves under water stress compared to non-stressed plants.”

“Drought stress (50% field capacity) reduced the number of leaves ( $P < 0.05$ ), fresh shoot weight, and fresh root weight in plants without inoculation of plant growth-promoting rhizobacteria (PGPR) (Table 1). In plants not subjected to water stress, the use of *Bacillus cereus* significantly increased the number of leaves, fresh root weight, and shoot weight compared to plants without PGPR inoculation and those treated with *Bacillus amyloliquefaciens* (ANOVA,  $P < 0.05$ ). Although there was no statistically significant difference in root length between the treatments (*t*-test,  $P \geq 0.05$ ), plants treated with *Bacillus cereus* showed the highest value. Similarly, plant height was not affected by any of the analyzed treatments, but its values were higher in plants with *Bacillus cereus* inoculation.

The application of PGPR restored both shoot and root fresh weight compared to non-inoculated and water-stressed plants (Table 1). In plants not subjected to water stress, the shoot fresh weight increased by 28% with the application of *B. cereus* and 34.3% with *B. amyloliquefaciens*, compared to non-inoculated plants. The number of leaves showed a significant decrease ( $P < 0.05$ ) in water-stressed plants without PGPR application, while water-stressed plants with PGPR inoculation did not show a significant difference compared to non-water-stressed plants (Table 1).

**Table 1.** Effect of Plant Growth-Promoting Rhizobacteria (PGPR) on Physiological Growth Parameters in Lemon Balm Plants Subjected to Drought Stress.

Drought stress	PGPR	Number of laves	Aerial part fresh weight (g)	Root fresh weight (g)	Plant height (cm)	Root length (cm)	Dry shoot weight	Dry roots weight
100% Field capacity	<i>Bacillus cereus</i>	158.00±23.46 <sup>a</sup>	13.62±5.41 <sup>a</sup>	10.10±3.40 <sup>a</sup>	6.40±1.32 <sup>a</sup>	67.62±35.86 <sup>a</sup>	3.21±0.75 <sup>a</sup>	2.89±0.53 <sup>a</sup>
	<i>Bacillus amyloliquefaciens</i>	118.00±41.32 <sup>ab</sup>	8.95±0.51 <sup>ab</sup>	6.12±2.84 <sup>abc</sup>	5.65±1.06 <sup>a</sup>	49.62±7.39 <sup>a</sup>	2.69±0.31 <sup>a</sup>	2.24±0.55 <sup>a</sup>
	Without inoculation	136.50±35.02 <sup>ab</sup>	9.81±0.29 <sup>ab</sup>	7.66±1.06 <sup>ab</sup>	5.87±0.37 <sup>a</sup>	45.62±13.55 <sup>a</sup>	1.83±0.32 <sup>a</sup>	1.85±0.34 <sup>a</sup>
50% Field capacity	<i>Bacillus cereus</i>	115.00±34.71 <sup>ab</sup>	8.32±4.27 <sup>ab</sup>	5.51±3.07 <sup>abc</sup>	6.55±1.56 <sup>a</sup>	47.32±15.94 <sup>a</sup>	2.52±0.19 <sup>a</sup>	2.15±0.19 <sup>a</sup>
	<i>Bacillus amyloliquefaciens</i>	114.50±13.33 <sup>ab</sup>	5.34±0.51 <sup>b</sup>	3.96±0.62 <sup>bc</sup>	5.375±0.99 <sup>a</sup>	45.62±9.96 <sup>a</sup>	2.33±0.08 <sup>a</sup>	2.35±0.11 <sup>a</sup>
	Without inoculation	94.75±14.43 <sup>b</sup>	3.16±1.34 <sup>b</sup>	2.27±1.39 <sup>c</sup>	5.40±1.63 <sup>a</sup>	41.72±14.41 <sup>a</sup>	1.69±0.04 <sup>a</sup>	1.19±0.15 <sup>a</sup>

The values represent the mean of 4 replicates ± SD. Values with different letters indicate a significant difference (Tukey,  $P < 0.05$ ).

The inoculation of *B. cereus* significantly increased root fresh weight, shoot fresh weight, and the number of leaves in plants grown at field capacity (Table 1). Plants treated with *B. cereus* also showed higher values for these variables than plants treated with *B. amyloliquefaciens* or without PGPR inoculation. In plants subjected to water stress, *B. cereus* and *B. amyloliquefaciens* both had a positive effect on root fresh weight, shoot fresh weight, and the number of leaves (Table 1). Root characteristics are important indicators of plant adaptation to environmental conditions, including water stress. However, research on root characteristics is lacking for many cultivated species (Bardgett *et al.*, 2014).

The decrease in growth observed in lemon balm under drought stress can be explained by the alteration of several physiological aspects of the plant under water stress conditions, such as reduced water potential, water uptake, and stomatal closure (Blankenship, 2015). All these changes represent the plant's attempt to cope with the unfavorable period of low water availability (Kapoor *et al.*, 2020). Medicinal plants have been reported to show growth and dry weight reductions similar to those found in this study (Saheri *et al.*, 2020; Abbaszadeh *et al.*, 2020; Eshaghi-Gorgi *et al.*, 2022).

The decrease in the number of leaves is a common physiological response of plants to water stress conditions (Bhargavi *et al.*, 2017). Leaves are the primary organs for photosynthesis, and under water scarcity, the production of various photosynthetic products, including triose phosphates, sugars, and amino acids, decreases. This reduction in photosynthetic output can ultimately lead to the shedding of leaves to conserve resources.

Additionally, reducing leaf area is a strategy to mitigate the impact of drought, as a decrease in leaf area results in less water loss through transpiration. This reduction in leaf area is due to cell expansion inhibition, which is a consequence of cellular turgor loss (Bangar *et al.*, 2019).

The reduction in the water stress effect on plants due to PGPR application can be explained by various physiological responses at the cellular level, such as increased activity of ACC (1-aminocyclopropane-1-carboxylic acid) deaminase enzymes, leading to a decrease in ethylene levels in the plant (Glick *et al.*, 2007). Specifically, the *Bacillus* genus has been used for its ability to form endospores, providing tolerance to adverse conditions such as water stress. Its application is associated with the synthesis of plant hormones and the production of metabolites such as lipopeptides, including fengycin and surfactin, which stimulate plant resistance to both biotic and abiotic stresses (Xie *et al.*, 2017).

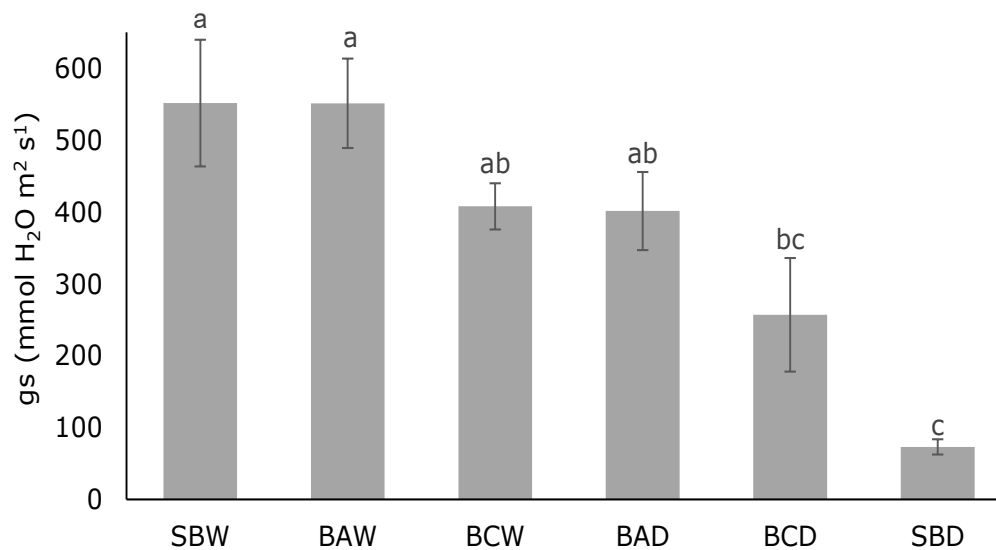
Similar results to those found in this study have been reported in other works (Ganjeali *et al.*, 2018; Mutumbam *et al.*, 2018; Zakerian *et al.*, 2020; Eshaghi-Gorgi *et al.*

al., 2022; Salazar-Garcia *et al.*, 2022). These studies have indicated that inoculation with PGPR, arbuscular mycorrhizal fungi, or a combination of these, can improve the growth and biomass of medicinal plants subjected to water stress. For example, Zhang *et al.* (2019) reported that *Glycyrrhiza uralensis* subjected to drought stress and inoculated with *Bacillus pumulis* showed enhanced drought tolerance due to improved integrity of chloroplast and mitochondrial cellular structure, leading to increased chlorophyll content, more efficient photosynthetic rate, and better relative water content.

Various mechanisms of inducing drought and salinity stress tolerance in plants regulated by PGPR action have been reported, including increased production of plant hormones such as indoleacetic acid and gibberellins, accumulation of osmolytes such as proline and glycine, and accumulation of total soluble solids in plants (Etesami *et al.*, 2018). All these physiological processes at the cellular and organ levels in plants inoculated with PGPR can explain the improvement in growth parameters of lemon balm subjected to water stress and inoculated with *B. cereus* and *B. amyloliquefaciens* in the present study.

**Stomatal Conductance.** Drought stress reduced stomatal conductance (gs) (Figure 1). The treatment that showed the greatest decrease in gs was the treatment without PGPR inoculation and with water stress (SBD), while the treatment with water stress but inoculated with *B. amyloliquefaciens* (BAD) exhibited stomatal opening behavior similar to the treatment without water stress and inoculated with *B. cereus* (BCW). The treatments with the highest average gs values were the treatments without water stress, inoculation (SBW), and without water stress but with inoculation of *B. amyloliquefaciens* (BAW). The application of *B. amyloliquefaciens* prevented stomatal closure in lemon balm plants subjected to water stress, explaining why the gs values were similar to those of plants at 100% of field capacity.





**Figure 1.** Stomatal conductance of *M. officinalis* under drought conditions and PGPR inoculation. Averages with different letters show statistically significant differences according to Tukey test ( $P \leq 0.05$ ). Vertical bars on each average indicate standard error ( $n=4$ ). Abbreviations of treatments explained in Materials and Methods.

Drought stress causes stomatal closure, reduced leaf area, and suppression of photosynthesis (Zare *et al.*, 2011; Nezhadahmadi *et al.*, 2013). Among these physiological changes in the plant, the decrease in  $\text{CO}_2$  conductance due to stomatal closure under drought conditions is a major factor contributing to reduced photosynthesis (Singh *et al.*, 2018). This reduction in photosynthesis explains the observed decrease in fresh weight, number of leaves, and  $g_s$  in non-inoculated plants subjected to water stress.

The treatment with water stress but inoculated with *B. amyloliquefaciens* showed statistically similar stomatal conductance values to the treatments without water stress (Figure 1). This indicates that bacteria of the *Bacillus* genus can help plants to activate resistance mechanisms to water stress conditions. Studies conducted on pepper plants inoculated with *Bacillus butanolivorans* and subjected to water stress have shown similar behavior to the present study (Kim *et al.*, 2022).

Stomatal closure during water stress requires signaling by the hormone ABA. It has been found that some rhizobacteria regulate the ABA status in the plant (Karadeniz *et al.*, 2006), *B. amyloliquefaciens* can alter the plant's endogenous ABA signaling, preventing a reduction in stomatal conductance ( $g_s$ ).

**Maximum quantum efficiency of photosystem II.** The maximum quantum efficiency of photosystem II ( $F_v/F_m$ ) (equation 1) did not show significant differences between treatments (Table 2). This parameter indicates the maximum potential efficiency with

which the light absorbed by the PSII antenna is converted into chemical energy in the first electron acceptor (QA) (Blankenship, 2015). When Fv/Fm decreases, the excitation rate of the PSII reaction centers is affected, which in turn affects the production of NADPH and ATP.

All evaluated treatments presented fluorescence values of chlorophyll below 0.8, which could indicate abiotic stress (Jeyakumar *et al.*, 2005) even on control plants. The fact that all treatments including the control, had a Fv/Fm values lower than 0.8 might indicate that leaves were starting the senescence process, in which case the maximum potential efficiency of photosystem II also decreases (Bresson *et al.*, 2018).

The Fv/Fm values of chlorophyll found in this study were lower than those reported by Pellegrini *et al.* (2011) in lemon balm plants subjected to ozone stress (0.806-0.817) but higher than those reported by Rodriguez *et al.* (2014) in papaya plants subjected to water stress due to flooding (0.48-0.55 for treatments with 48 hours or more of inundation). These comparisons suggest that the Fv/Fm values observed in this study are within the expected range for plants subjected to stress conditions.

**Quantum yield Y(II), Y(NPQ), and Y(NO).** The parameters for non-photochemical quenching quantum yield Y(NPQ) and Y(NO) complement the quantum yield of photochemical energy conversion Y(II), and the sum of these is equivalent to unity:  $Y(II) + Y(NPQ) + Y(NO) = 1$ . These parameters provide insight into the plant's capacity to cope with excess excitation energy (Baker, 2008). Thus, a reduction in Y(II) indicates a decrease in quantum efficiency and closure of the reaction centers, accompanied by an increase in the quantum yield of non-regulated non-photochemical energy loss Y(NO) (Mathur *et al.*, 2021).

In the present study, the parameters Y(II), Y(NPQ), and Y(NO) did not show significant differences between treatments, but rather showed a similar trend to that reported by Mathur *et al.* (2021), whereby Y(NO) increased when Y(II) decreased (Table 2).

In addition to photochemical adjustments, plants manifest other mechanisms to cope with water deficit, especially under mild water stress. For example, stomatal closing (Figure 1) and photorespiration increase are earlier responses than photochemical or enzyme changes (Flexas *et al.*, 2012). The above might mean that stressed plants irrigated until 50% field capacity might have undergone mild and not severe water deficit.

**Table 2.** Effect of Plant Growth-Promoting Rhizobacteria (PGPR) on Fluorescence Parameters in Lemon Balm Plants Subjected to Drought Stress.

Drought stress	PGPR	Fv/Fm	Y(II)	Y(NPQ)	Y(NO)
100% Field capacity	<i>Bacillus cereus</i>	0.7032 <sup>a</sup>	0.3997 <sup>a</sup>	0.1857 <sup>a</sup>	0.4145 <sup>a</sup>
	<i>Bacillus amyloliquefaciens</i>	0.7582 <sup>a</sup>	0.4537 <sup>a</sup>	0.1800 <sup>a</sup>	0.4335 <sup>a</sup>
	Without inoculation	0.7620 <sup>a</sup>	0.4317 <sup>a</sup>	0.1685 <sup>a</sup>	0.3662 <sup>a</sup>
50% Field capacity	<i>Bacillus cereus</i>	0.7865 <sup>a</sup>	0.4250 <sup>a</sup>	0.1415 <sup>a</sup>	0.4025 <sup>a</sup>
	<i>Bacillus amyloliquefaciens</i>	0.7572 <sup>a</sup>	0.4085 <sup>a</sup>	0.1892 <sup>a</sup>	0.3997 <sup>a</sup>
	Without inoculation	0.7377 <sup>a</sup>	0.4475 <sup>a</sup>	0.1712 <sup>a</sup>	0.3840 <sup>a</sup>

The values represent the mean of 4 replicates  $\pm$  SD. Values with different letters indicate a significant difference (Tukey,  $P < 0.05$ ).

## CONCLUSIONS

Growth-promoting rhizobacteria (PGPR) alleviate the effects of water stress in lemon balm plants at 50% of field capacity, improving growth and development parameters such as number of leaves, shoot fresh weight, root fresh weight, and stomatal conductance. PGPR application prevented stomatal closure in plants subjected to water stress. The maximum quantum yield of PSII (Fv/Fm) showed similar values in plants with PGPR application plus water stress and in plants without water stress, which may indicate that the rhizobacteria prevent damage to the photosystem caused by water absence in the plant. Native strains of *Bacillus cereus* and *Bacillus amyloliquefaciens* are viable alternatives to be used in areas facing water stress issues in medicinal plants such as lemon balm.

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**Conflict of interest:** The manuscript was prepared and reviewed with the participation of the authors, who declare that there exists no conflict of interest that puts at risk the validity of the presented results.

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