Rice seedlings showed a higher heat tolerance through the foliar application of biostimulants

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Abstract

The use of biostimulants is an agronomic tool to improve plant tolerance to abiotic stress in plants. This study explored the effect of foliar biostimulants sprays such as brassinosteroids (BR), amino acids (AA), nitrophenolates (NP) or a biostimulant based on botanical extracts (BE) on leaf gas exchange parameters [photosynthesis (P_N), stomatal conductance (g_s) and transpiration (E)], leaf photosynthetic pigments, lipid peroxidation of membranes and proline content of two commercial rice genotypes ['Fedearroz 67' and 'Fedearroz 60'] under heat stress conditions. The established treatments were: i) plants without heat stress and foliar applications of biostimulants (C), ii) plants under heat stress and without foliar applications of biostimulants (HT), and iii) plants with heat stress and three foliar applications with BR (1 mL·L^{-1}), AA (30 mL·L^{-1}), NP (15 mL·L^{-1}) or BE (15 mL·L^{-1}). The results showed that the application of BR, AA, NP or BE increased the values of P_N (~14.5 μmol CO$_2$·m^{-2}·s^{-1}), g_s (~0.46 mmol·m^{-2}·s^{-1}) and E (~43.9 H$_2$0 day^{-1}·plant^{-1}) compared to plants (both genotypes) not treated with biostimulants under heat stress (9.9 μmol CO$_2$·m^{-2}·s^{-1} for P_N, 0.31 mmol·m^{-2}·s^{-1} for g_s, and 27.3 H$_2$0 day^{-1}·plant^{-1} for E). Foliar biostimulant sprays also caused a lower malondialdehyde and proline production in rice genotypes under heat stress. In conclusion, the biostimulants BR, AA, NP, or BE can be considered an agronomic strategy to help mitigate the adverse effects of heat stress in rice areas where periods of high temperatures are expected during the day in Colombia.

Introduction

Rice (*Oryza sativa* L.) is one of the most important cereal crops globally, especially in developing countries (Wang *et al.*, 2015). It is considered a staple food because it is an essential source of calories that feeds almost half of the world's population (Hirabayashi *et al.*, 2014; Bahuguna *et al.*, 2017). In Colombia, rice crops occupied 561,073 ha with a production of 2,564,250 t and an average yield of 4.9 t ha^{-1} in 2019 (Fedearroz, 2020).

Global climate change has generated an increase in the earth's surface temperature in recent decades, and the global mean air temperature is forecast to increase by approximately 2 to 3 °C by 2050 (IPCC, 2014; Wang *et al.*, 2019). Additionally, heat waves or extreme temperature events are expected to be more intense, frequent, and prolonged than what has been observed in recent years (Hatfield and Prueger, 2015). High daytime temperature events would impact crop productivity (Hatfield and Prueger, 2015; Fan *et al.*, 2018). In Colombia, 65% of the rice-growing areas are expected to increase temperatures between 2 and 2.5 °C by 2050 (Ramirez-Villegas *et al.*, 2012).

High daytime temperatures (heat stress) are among the main environmental factors that cause serious adverse effects on rice production (Nigam *et al.*, 2015; Wang *et al.*, 2019). The optimal average temperature for rice growth varies from 25 to 30 °C during the day and from 20 to 22 °C for nighttime highs (Chen *et al.*, 2017a; Kilasi *et al.*, 2018). When the temperature exceeds these ranges, it can cause physiological, biochemical and molecular changes in plants such as an increase in reactive oxygen species (ROS) and lipid peroxidation of membranes (Zafar *et al.*, 2018), alteration in leaf gas exchange parameters (photosynthesis, stomatal conductance, and transpiration) and plant water relations (Sahu, 2013; Vivitha *et al.*, 2018).

Photosynthesis (P_N) is one of the most susceptible physiological processes to heat stress in rice plants, showing a decrease in its values with an increase in daytime temperatures above the optimal levels (28 and 22 °C for day and night, respectively) (Radhakrishna *et al.*, 2018). Sonjaroon *et al.* (2018) observed a reduction of 57% in P_N in rice plants subjected to a moderate period (7 days) of high temperature during the day (40 °C). Biochemical markers such as the concentration of leaf photosynthetic pigments (chlorophyll and carotenoids), lipid peroxidation of membranes (malondialdehyde (MDA) production) and accumulation of compatible solutes (proline) have been used to characterize the response of rice plants to stress conditions caused by high daytime temperatures (Sánchez-Reinoso *et al.*, 2014; Thussagunpanit *et al.*, 2015a). According to Hussain et al. (2019), an increase in MDA content and a decrease in chlorophyll concentration indicate a negative impact on the integrity of the cell membrane and the photosynthetic efficiency of plants under heat stress conditions. On the other hand, the accumulation of low-molecular-weight compatible solutes such as proline is considered a primary strategy for protecting and acclimatizing plants to heat stress conditions (Harsh *et al.*, 2016; Shin *et al.*, 2016).

The development of new management practices to induce tolerance to abiotic stress in plants, including heat stress, is acquiring great importance (Baninasab and Ghobadi, 2011; Nguyen *et al.*, 2018). In this regard, the application of biostimulants under unfavorable environmental conditions can induce tolerance to abiotic stress in different crops (Povero *et al.*, 2016; Bulgari *et al.*, 2019). According to du Jardin (2015), a plant biostimulant is "any substance or microorganism applied to plants with the aim to enhance nutrition efficiency, abiotic stress tolerance and/or crop quality traits, regardless of its nutrients content". Biostimulants can be classified as of natural origins such as free amino acids, algae, and botanical extracts, effective microorganisms, humic substances, and chitosan; or synthetic, in which plant growth regulators, phenolic compounds, inorganic salts, and essential elements can be found (Przybysz *et al.*, 2014; du Jardin, 2015). Biostimulants' positive effects on plants include growth promotion, modulation of development and quality traits, nitrate assimilation, hormonal activity, and higher tolerance to environmental stress (du Jardin, 2015; Francesca *et al.*, 2020). Some of the biostimulants studied against different types of abiotic stress have been brassinosteroids (Thussagunpanit

et al., 2015b), amino acids (Li *et al.*, 2016), nitrophenolates (Przybysz *et al.*, 2014) and botanical extracts (Farooq *et al.*, 2017).

Brassinosteroids (BR) are polyhydroxylated steroidal plant hormones; they are involved in plant growth regulation through their participation in metabolic processes such as photosynthesis, antioxidant activity, osmolyte accumulation, nitrogen metabolism and plant water relations under stress conditions (Ahanger *et al.*, 2018; Anwar *et al.*, 2018). Exogenous applications of BR have been reported to help mitigate the adverse effects of high daytime temperatures in rice (Thussagunpanit *et al.*, 2015a and b), maize (Yadava *et al.*, 2016) and tomato (Zhou *et al.*, 2014) by modulating the components of the antioxidant defense system and inducing heat shock proteins (HSP).

Nitrophenolates (NP) are phenolic compounds that participate in various plant metabolic processes. For example, they increase the concentration of endogenous auxins, promote growth and development, stimulate the absorption of nutrients and improve the photosynthetic and antioxidant activity (Djanaguiraman *et al.*, 2010; Valero *et al.*, 2014). NP can generate a faster and more effective activation of plant defense mechanisms (as they increase the antioxidant system enzymes' capacity and activity) under stress conditions (Przybysz *et al.*, 2014; Kazda *et al.*, 2015). Gulluoglu *et al.* (2006) reported that the foliar application of NP decreased the negative effects of heat stress in soybean (*Glycine max* L.) plants by favouring growth (dry mass accumulation) and yield parameters (yield, number of pods and grain weight).

Commercial products of amino acids (AA) can be constituted by active ingredients such as arginine, proline, tryptophan, glycine, or cysteine. AA can increase tolerance to different types of abiotic stress by activating physiological processes such as scavenging of ROS, osmoprotection, increased nutrient availability, and chelation of metals in the soil (Lucini *et al.*, 2015; Van Oosten *et al.*, 2017). The foliar AA sprays generated a better photosynthetic efficiency (F_v/F_m) and higher levels of chlorophyll and carotenoids in *Lolium perenne* L. plants under heat stress (Botta, 2013). Finally, botanical extracts (BE) have also been considered as a useful alternative for the mitigation of abiotic stress in recent years. They can help to improve various physiological processes that stimulate plant growth and development under abiotic stress conditions (Bulgari *et al.*, 2015; Van Oosten *et al.*, 2017). Zhang *et al.* (2010) also observed the positive effect of foliar applications of seaweed (*Ascophyllum nodosum* Jol.) BE on the chlorophyll content and activity of the enzyme nitrate reductase (NR) of *Agrostis stolonifera* L. plants under high-temperature conditions.

Increases in the intensity and frequencies of high daytime temperature events in Colombia are estimated for the next few years (Ramirez-Villegas *et al.*, 2012). Agronomic management strategies to mitigate the negative effect of heat stress conditions have focused mainly on the evaluation and development of rice genotypes under stress scenarios. These genotypes have been studied to identify physiological response mechanisms that allow strengthening plant breeding programs in Colombia (Restrepo-Díaz and Garces-Varon, 2013; Sánchez-Reinoso *et al.*, 2014). Additionally, crop sowing times have been identified to avoid unfavorable climatic conditions in susceptible phenological stages of the crop (Garcés-Varon and Restrepo-Díaz, 2015; Garces-Varón and Puente, 2019). On the other hand, it has been widely reported that foliar applications of biostimulants decrease the negative impact of different types of abiotic stress in cereals (Przybysz *et al.*, 2014; Thussagunpanit *et al.*, 2015b; Li *et al.*, 2016; Farooq *et al.*, 2017). However, scientific studies on agronomic strategies to mitigate the adverse effect of specific periods of heat stress such as foliar applications of biostimulants (BR, AA, NP, or BE) from a physiological and biochemical point of view remain scarce in the country. This study hypothesizes that foliar applications of four biostimulants can help mitigate the negative effects caused by high daytime temperatures in two rice genotypes (susceptible ('Fedearroz 60') and moderately tolerant ('Fedearroz 67')). For this reason, the objective of this research was to evaluate the effect of foliar applications of four biostimulants (brassinosteroids (BR), amino acids (AA), nitrophenolates (NP) or botanical extracts (BE)) on leaf gas exchange parameters (photosynthesis, stomatal conductance, and transpiration), photosynthetic pigment content, and biochemical responses (MDA production and proline content) of two commercial rice genotypes subjected to a prolonged period of heat stress.

Materials and Methods

General growth conditions and biological material

An experiment was carried out from December 2015 to March 2016 under glass greenhouse and laboratory conditions at the Faculty of Agricultural Sciences of the Universidad Nacional de Colombia, Bogotá campus (4°35'56" N, 74°04'51" W). Initially, seeds of cultivars 'Fedearroz 60' ('F60') (genotype susceptible to high daytime temperatures (Sánchez-Reinoso *et al.*, 2014)) and 'Fedearroz 67' ('F67') (genotype widely cultivated by farmers in Colombia for the last five years) were sown in 1 L plastic pots containing loam soil in the glass greenhouse. The environmental conditions of the greenhouse during the experiment were: 28/20 °C day/night temperature, 60-80% relative humidity and a natural photoperiod of 12 h (1500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of photosynthetically active radiation (PAR) at noon). After seed germination (7 days after sowing (DAS)), the seedlings of both cultivars were watered daily with 50 mL of a nutrient solution prepared from a complete liquid fertilizer (Nutriponic`, Walco SA, Colombia) at a dose of 2.5 mL·L^{-1} H$_2$O until the end of the experiment (80 DAS). The concentration of the nutrient solution was as follows: 2.08 mM Ca (NO$_3$)$_2$·4H$_2$O, 1.99 mM MgSO$_4$·7 H$_2$O, 2.00 mM NH$_4$H$_2$PO$_4$, 10.09 mM KNO$_3$, 46.26 nM H$_3$BO$_3$, 0.45 nM Na$_2$MoO$_4$·2H$_2$O, 0.32 nM CuSO$_4$·5H$_2$O, 9.19 nM MnCl$_2$·4H$_2$O, 0.76 nM ZnSO$_4$·7H$_2$O, and 19.75 nM FeSO$_4$·H$_2$O. In general, the plants were always kept at field capacity to avoid water deficit conditions during the experiment.

Heat stress treatments and foliar applications of biostimulants

At 57 DAS, when rice plants showed collar formation on the fifth leaf of the main stem (stage V5) (Counce and Keisling, 2000), they were transferred to growth chambers (MLR-351H, Sanyo, Bensenville, Illinois, USA) to determine the different treatments of daytime temperatures. Rice plants were initially divided into two groups to establish heat stress treatments caused by high daytime temperatures. The first group of plants was established with the initial environmental conditions (28/20 °C day/night) (control without heat stress (C)). The second group was exposed to a temperature of 40 °C between 11:00 and 16:00 for 15 days. The exposure period and the high daytime temperature were selected based on preliminary studies (Restrepo-Diaz and Garces-Varon, 2013; Sánchez-Reinoso *et al.*, 2014). Finally, a 12 h photoperiod and relative humidity of 60-70% were established in the chambers during the period of heat stress.

The group of plants subjected to conditions of heat stress (40 °C) was also subdivided into other five groups to establish foliar treatments with biostimulants, which were as follows: brassinosteroids (BR) at a dose of 1 mL·L^{-1}, amino acids (AA) at a dose of 30 mL·L^{-1} (Globafol, Valagro SpA, Atessa, Italy), nitrophenolates (NP) at a dose of 15 mL·L^{-1} (Atonik`, Arysta Lifescience SA, Bogotá, Colombia), a commercial product based on botanical extracts, gibberellins, auxins and cytokinins (BE) at a dose of 15 mL·L$^{-1}$ (Biozyme` TF, Arysta Lifescience SA, Bogotá, Colombia) and plants without application of biostimulants (HT). Rice plants of treatments C and HT were only sprayed with distilled water. At the end of the experiment (80 DAS), a total of eight treatment groups were obtained depending on the heat stress condition and foliar application of biostimulants, which are summarized as follows: i) plants without stress due to high daytime temperatures (40 °C) and without foliar applications of biostimulants (control without heat stress and foliar application (C)); ii) plants subjected to heat stress and without foliar applications of biostimulants (heat stress without foliar application (HT)); iii) plants with heat stress and with three foliar applications of BR, AA, NP or BE. The concentrations of all biostimulants (BR, AA, NP, or BE) were selected from the doses of the commercial products recommended in rice-producing areas of Colombia. Finally, Table 1 summarizes the characteristics of the biostimulants used in this study.

Biostimulants' applications were carried out at three different times during the experiment: i) foliar application 54 DAS, ii) foliar application 65 DAS, and iii) foliar application 75 DAS. In general, each biostimulant application was carried out between 07:00 and 09:00, using a Style 1.5 compression sprayer (Matabi, Spain) with an application volume of 15 mL H$_2$O per plant, wetting the upper and lower surfaces of leaves. All foliar applications carried a surfactant adjuvant (INEX-A, Cosmoagro, Colombia) at a dose of 0.1%

(v/v). The treatments were arranged in a completely randomized design with five replicates, and each replicate consisted of two plants. Finally, the experiment lasted 80 days.

Table 1. The chemical name of the active ingredient, trade name, manufacturer, and biological activity of the biostimulants applied in rice plants (*Oryza sativa* L.) of the genotypes 'Fedearroz 67' and 'Fedearroz 60' under heat stress conditions

Chemical name of the active ingredient	Trade name (Manufacturer)	Biological activity
(25 R) – 3β. 5α – dihydroxy-spirostan-6-one	Biomex DI-31 (Minerales exclusivos SA, Bogotá, Colombia)	Regulates plant development by modulating various signaling pathways
Plant-based amino acids (arginine, proline, tryptophan, glycine, cysteine), nitrogen and soluble potassium	Globafol (Valagro SpA, Atessa, Italy Italy)	Regulates the synthesis of proteins and enhances the photosynthetic activity of plants
Sodium p- nitrophenolate, Sodium o-nitrophenolate, Sodium 5 nitroguaiacolate	Atonik® (Arysta Lifescience SA, Bogotá, Colombia)	Induces the activity of naturally synthesized auxins and the enzyme nitrate reductase
Plant-based extracts, gibberellins, indoleacetic acid and zeatin	Biozyme® TF (Arysta Lifescience SA, Bogotá, Colombia)	Regulates cell division in meristems, as well as cell elongation

Leaf gas exchange and water use efficiency

One mature leaf per plant was selected to carry out gas exchange readings. The leaves were previously adapted to the surrounding environment, and the leaf surface was not touched to avoid stomatal closure before measurements. Photosynthesis (P_N), stomatal conductance (g_s), and intercellular CO_2 concentrations inside the leaf (C_i) were estimated using a portable photosynthesis meter (LI-COR 6200, Lincoln, NE, USA) between 10:00 and 15:00 h. During the readings, the conditions of the LiCor chamber were as follows: photosynthetically active radiation of 700 µmol·m^{-2}·s^{-1}, leaf temperature 27 ± 5 °C, and leaf-air vapor pressure difference 1.8 ± 0.5 kPa. With the values of P_N, g_s, C_i and ambient CO_2 concentration (C_a) we calculated, respectively, the stomatal limitation (1- C_i/C_a = L_S) (Yin *et al.*, 2006), the intrinsic water use efficiency given by the ratio (P_N/g_s=WUE$_i$) and the carboxylation efficiency (P_N/C_i) (Flexas *et al.*, 2001). Two measurements were carried out per plant. Finally, the total plant transpiration (E) was obtained using the gravimetric technique described by Sánchez-Reinoso *et al.* (2014), which consisted of covering pots with plastic to avoid water loss due to evaporation and weighing pots daily before and after irrigation between 57 and 80 DAS. The transpiration rate was expressed in mg $H_2O·$ g^{-1}FW·h^{-1}.

Leaf photosynthetic pigments

The equations described by Wellburn (1994) were used to estimate chlorophyll total (TChl) and carotenoid (Cx+c) content from 50 mg of the tissue sample from the same leaves used to measure the gas exchange variables. The tissue samples were homogenized in 3 mL of 80% acetone and then centrifuged (Model 420101, Becton Dickinson Primary Care Diagnostics, MD, USA) at 5000 rpm for 10 minutes to remove particles. The supernatant was diluted to a final volume of 6 ml by adding 80% acetone (Sims and Gamon, 2002). Chlorophyll content was determined at 663, and 646 nm and carotenoids were determined at 470 nm using a spectrophotometer (Spectronic BioMate 3 UV-vis Thermo, Madison, WI). Leaf photosynthetic pigments were determined at 80 DAS.

Proline and malondialdehyde content

The same leaves collected for the determination of leaf photosynthetic pigments were used for the proline and MDA content in all treatments. The method described by Bates *et al.* (1973) was used to estimate

the proline content in each of the evaluated treatments. Leaves were collected from the upper third of the plant; samples of 300 mg of this plant material were homogenized in liquid nitrogen and stored for further analysis. Subsequently, 10 mL of a 3% aqueous solution of sulfosalicylic acid was added to the samples and Whatman paper (No. 2) was used to filter them. Then, 2 mL of the filtrate was reacted with 2 mL of ninhydrin acid and 2 mL of glacial acetic acid. The mixture was placed in a water bath at 90 °C for 1 h and incubation on ice was used to stop the reaction. The resulting solution was dissolved in 4 mL of toluene and vortexed (V-1, BOECO, Germany) for 30 s. Finally, the absorbance readings were determined at 520 nm with the same spectrophotometer used in the quantification of leaf photosynthetic pigments (Spectronic BioMate 3 UV-Vis, Thermo, Madison, WI, USA). The proline content was calculated with the fresh weight of the sample using a standard calibration curve (equation 1).

$$\frac{\mu mol\ Proline}{fresh\ vegetal\ material} = \frac{\left[\dfrac{\left(\dfrac{\mu g\ Proline}{mL} \times mL\ Toluene\right)}{\dfrac{115.5\ \mu g}{\mu mol}}\right]}{\left[\dfrac{g\ sample}{5}\right]} \qquad (1)$$

The thiobarbituric acid (TBA) method described by Hodges *et al.* (1999) was used to estimate membrane lipid peroxidation (malondialdehyde (MDA)). Samples of 300 mg of leaves from the upper third of the plants from the different treatments were macerated and stored in liquid nitrogen. The samples were centrifuged (Model 420101, Becton Dickinson Primary Care Diagnostics, MD, USA) at 5000 rpm for 10 min, and absorbances were estimated at 440, 532 and 600 nm with a spectrophotometer. Finally, the extinction coefficient ($157\ M\ mL^{-1}$) was used to obtain the MDA concentration. Proline and MDA readings were performed at 80 DAS.

Heat tolerance coefficient (HTC)
The heat tolerance coefficient (HTC) was calculated to estimate rice plants' photosynthetic responses to the impact of high daytime temperatures and foliar applications of biostimulants. HTC was calculated by adjusting the equation described by Liu *et al.* (2010) (equation 2).

$$HTC = \frac{Net\ photosinthesis\ of\ plants\ subjected\ to\ heat\ stress}{Net\ photosinthesis\ of\ plants\ without\ heat\ stress\ (control\ plants)}$$

Statistical analysis
Data were analysed using a factorial arrangement where the first factor corresponded to the rice genotypes ('F60' and 'F67') and the second factor was the biostimulants used in foliar applications (BR, AA, NP or BE). Each treatment group consisted of 10 plants. A principal component analysis was carried out using the InfoStat 2016 program (analytical software, Universidad Nacional de Cordoba, Argentina) to select the best biostimulants used under heat stress conditions. In all cases, an analysis of variance (ANOVA) was carried out and when significant differences ($P \le 0.05$) were observed, a post hoc Tukey test was used for the comparison of means. Percentage values were transformed using the arcsine function. Data were analysed using the Statistix v 9.0 program (Analytical Software, Tallahassee, FL, USA). Figures were performed using Sigmaplot software (version 12.0; Systat Software, San Jose, CA, USA).

Results

Table 2 summarizes the analysis of variance (ANOVA) of the effect of two rice genotypes ('F60' and 'F67') subjected to heat stress conditions and treated with foliar applications of biostimulants (BR, AA, NP or BE) on the photosynthetic rate (PN), stomatal conductance (gs), transpiration (E), stomatal limitation (LS), carboxylation efficiency (PN/Ci), intrinsic water use efficiency (WUEi), leaf photosynthetic pigments (TChl and Cx+x), malondialdehyde (MDA) and proline content at 80 DAS.

Table 2. F-statistical probability (P>F) of the effects of foliar applications of four biostimulants (B) [brassinosteroids (BR), amino acids (AA), nitrophenolates (NP) or botanical extracts (BE)] on the physiological and biochemical variables of rice plants (*Oryza sativa* L.) of the genotypes (G) 'Fedearroz 67' and 'Fedearroz 60' under a prolonged period (15 days) of high daytime temperatures (40 °C) at 80 days after sowing (DAS)

| Variable	Abbr.	Variation source			CV (%) [z]
		Genotype (G)	Biostimulant (B)	G x B	
Photosynthesis	P_N	$F = 56.2$; $df = 1$; $P = 0.0003$	$F = 112.1$; $df = 5$; $P = 0.0000$	$F = 5.7$; $df = 5$; $P = 0.0000$	6.33
Stomatal conductance	g_s	$F = 8.8$; $df = 1$; $P = 0.0046$	$F = 105.1$; $df = 5$; $P = 0.0000$	$F = 10.6$; $df = 5$; $P = 0.0000$	9.23
Plant transpiration	E	$F = 1.7$; $df = 1$; $P = 0.1955$	$F = 27.3$; $df = 5$; $P = 0.0000$	$F = 2.7$; $df = 5$; $P = 0.0299$	14.3
Stomatal limitation	L_S	$F = 4.3$; $df = 1$; $P = 0.0433$	$F = 32.1$; $df = 5$; $P = 0.0000$	$F = 3.5$; $df = 5$; $P = 0.0080$	8.89
Carboxylation efficiency	P_N/C_i	$F = 14.8$; $df = 1$; $P = 0.0004$	$F = 151.0$; $df = 5$; $P = 0.0000$	$F = 8.9$; $df = 5$; $P = 0.0000$	9.23
Intrinsic water use efficiency	WUE_i	$F = 212.2$; $df = 1$; $P = 0.0000$	$F = 22.4$; $df = 5$; $P = 0.0000$	$F = 3.4$; $df = 5$; $P = 0.0105$	4.43
Total chlorophyll	TChl	$F = 221.4$; $df = 1$; $P = 0.0000$	$F = 272.4$; $df = 5$; $P = 0.0000$	$F = 0.8$; $df = 5$; $P = 0.5241$	3.11
Carotenoids	Cx+c	$F = 238.2$; $df = 1$; $P = 0.0000$	$F = 167.6$; $df = 5$; $P = 0.0000$	$F = 0.1$; $df = 5$; $P = 0.9866$	3.00
Proline		$F = 48.8$; $df = 1$; $P = 0.0000$	$F = 112.6$; $df = 5$; $P = 0.0000$	$F = 10.1$; $df = 5$; $P = 0.0000$	8.39
Malondialdehyde	MDA	$F = 305.5$; $df = 1$; $P = 0.0000$	$F = 212.1$; $df = 5$; $P = 0.0000$	$F = 3.6$; $df = 5$; $P = 0.0078$	4.91

[z]CV = Coefficient of variation

Leaf gas exchange parameters and intrinsic water use efficiency (WUEi)

Differences were observed in the interaction between the rice genotypes used ('F60' and 'F67') and the biostimulants (BR, AA, NP or BE) under a condition of high daytime temperature on P_N ($P=0.0000$), g_s ($P=0.0000$), E ($P=0.0299$), L_S ($P=0.0080$), P_N/C_i ($P=0.0000$), and WUE_i ($P=0.0105$) at 80 DAS (Table 2). In general, heat stress caused lower values of P_N, g_s, E, and P_N/C_i in rice plants of both genotypes ('F60' and 'F67') without foliar application of biostimulants (heat stress (HT)). However, an opposite trend was observed for L_S and WUE_i in these same plants (higher values) (Figures 1 and 2). A positive response could be seen when heat-stressed 'F67' and 'F60' rice plants were sprayed with biostimulants. In this regard, the foliar application of BR caused higher P_N values (17.2 µmol $CO_2 \cdot m^{-2} \cdot s^{-1}$ for 'F67' and 14.6 µmol $CO_2 \cdot m^{-2} \cdot s^{-1}$ for 'F60'). Plants with AA sprays showed intermediate readings (15.3 µmol $CO_2 \cdot m^{-2} \cdot s^{-1}$ for 'F67' and 14.1 µmol $CO_2 \cdot m^{-2} \cdot s^{-1}$ for 'F60'), whereas NP and BE exhibited mean values of 14.8 µmol $CO_2 \cdot m^{-2} \cdot s^{-1}$ for 'F67' and 12.4 µmol $CO_2 \cdot m^{-2} \cdot s^{-1}$ for 'F60'. Finally, plants only subjected to HT showed values of 11.3 µmol $CO_2 \cdot m^{-2} \cdot s^{-1}$ for 'F67' and 8.4 µmol $CO_2 \cdot m^{-2} \cdot s^{-1}$ for 'F60' (Figure 1A).

The variable g_s was favoured by foliar sprays of BR (0.54 mmol·m^{-2}·s^{-1} for 'F67' and 0.64 mmol·m^{-2}·s^{-1} for 'F60') or AA (mean values 0.45 mmol·m^{-2}·s^{-1}) in both genotypes subjected to heat stress conditions. On the other hand, foliar applications of NPs (0.43 mmol·m^{-2}·s^{-1}) only favoured g_s in plants of genotype 'F67' under conditions of high diurnal temperatures compared to HT plants of both genotypes (mean values 0.31 mmol·m^{-2}·s^{-1}) (Figure 1B). An increase in the E values of 'F60' plants subjected to heat stress was mainly observed in the treatment with BR sprays (48.6 H$_2$0 day^{-1}·plant^{-1}) compared to HT plants of the same genotype (30.1 H$_2$0 day^{-1}·plant^{-1}). The transpiration of 'F67' plants under heat stress was also stimulated with the foliar application of BE, NP, or BR showing mean values of 47.1 H$_2$0 day^{-1}·plant^{-1} compared to HT plants without biostimulant sprays (24.4 H$_2$0 day^{-1}·plant^{-1}) (Figure 1C). On the other hand, the foliar application mainly of BR (mean values 0.166) or AA (mean values 0.201) favoured L_S in plants of both rice genotypes under heat stress compared to HT plants (mean values 0.305) (Figure 1D).

Figure 2 shows that P_N/C_i and WUE$_i$ showed opposite trends between them. P_N/C_i was favoured by foliar applications of BR, AA, NP or BE in both genotypes ('F67' and 'F60'), observing that foliar treatments with these biostimulants helped improve the carboxylation of plants under heat stress (Figure 2A). However, foliar applications mainly of BR caused a decrease in WUE$_i$ (28.9 µmol·mmol^{-1} for 'F60' and 31.7 µmol·mmol^{-1} for 'F67') compared to control plants under heat stress (HT) with values of 35.1 µmol·mmol^{-1} for 'F67' and 30.1 µmol·mmol^{-1} for 'F60' (Figure 2B). Finally, the plants without heat stress and foliar application of biostimulants (C) always showed higher P_N, g_s, E and P_N/C_i, and lower L_S and WUE$_i$ in both rice genotypes ('F67' and 'F60') (Figures 1 and 2).
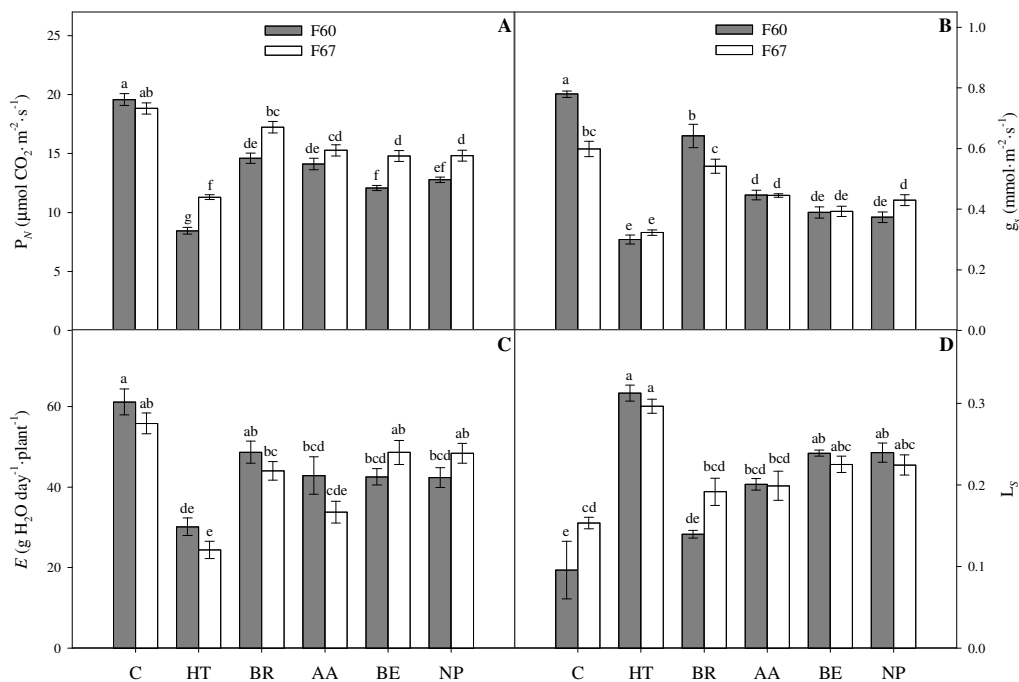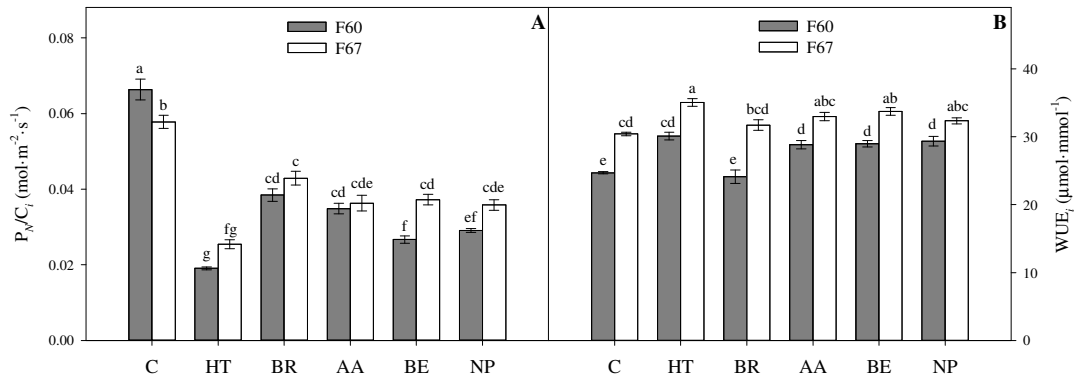

Figure 1. Leaf photosynthesis (P_N) **(A)**, stomatal conductance (g_s) **(B)**, plant transpiration (E) **(C)**, and stomatal limitation (L_S) **(D)** of rice plants (*Oryza sativa* L.) of the genotypes 'Fedearroz 67' ('F67') and 'Fedearroz 60' ('F60') under heat stress and foliar application of four different biostimulants at 80 days after sowing (DAS)

Each bar represents the mean of 10 plants ($n = 10$) ± standard error. Bars followed by different letters indicate statistically significant differences according to the Tukey test (P ≤ 0.05)

C: control plants without heat stress (28 °C); HT: plants with a prolonged period (15 days) of high daytime temperatures (40 °C); BR: heat-stressed plants sprayed with brassinosteroids; AA: heat-stressed plants sprayed with amino acids; NP: heat-stressed plants sprayed with nitrophenolates; and BE: heat-stressed plants sprayed with botanical extracts

Figure 2. Carboxylation efficiency (P_N/C_i) **(A)** and intrinsic water use efficiency (WUE_i) **(B)** of rice plants (*Oryza sativa* L.) of the genotypes 'Fedearroz 67' ('F67') and 'Fedearroz 60' ('F60') under heat stress and foliar application of four different biostimulants at 80 days after sowing (DAS)

Each bar represents the mean of 10 plants (*n* = 10) ± standard error. Bars followed by different letters indicate statistically significant differences according to the Tukey test (P ≤ 0.05)

C: control plants without heat stress (28 °C); HT: plants with a prolonged period (15 days) of high daytime temperatures (40 °C); BR: heat-stressed plants sprayed with brassinosteroids; AA: heat-stressed plants sprayed with amino acids; NP: heat-stressed plants sprayed with nitrophenolates; and BE: heat-stressed plants sprayed with botanical extracts

Leaf photosynthetic pigments

Table 2 shows that differences were only obtained for the factors genotype and biostimulant in the treatments evaluated on the content of total chlorophyll (TChl) (*P*=0.000 and 0=000, respectively) and carotenoids (Cx+c) (*P*=0.000 and 0=000, respectively) at the end of the experiment (80 DAS). The content of leaf photosynthetic pigments (TChl and Cx+c) was affected when rice plants were stressed by heat and were not treated with any biostimulant (1.07 mg·mg⁻¹·FW for TChl and 0.52 mg·mg⁻¹·FW for Cx+c) compared to plants with foliar sprays of BR, AA, BE or NP, with mean values of 1.41 mg·mg⁻¹·FW and 0.58 mg·mg⁻¹·FW for TChl and Cx+c, respectively. Plants without heat stress and biostimulant sprays (C) showed the highest values of TChl (1.78 mg·mg⁻¹·FW) and Cx+c (0.72 mg·mg⁻¹·FW) (Figures 3A and B).
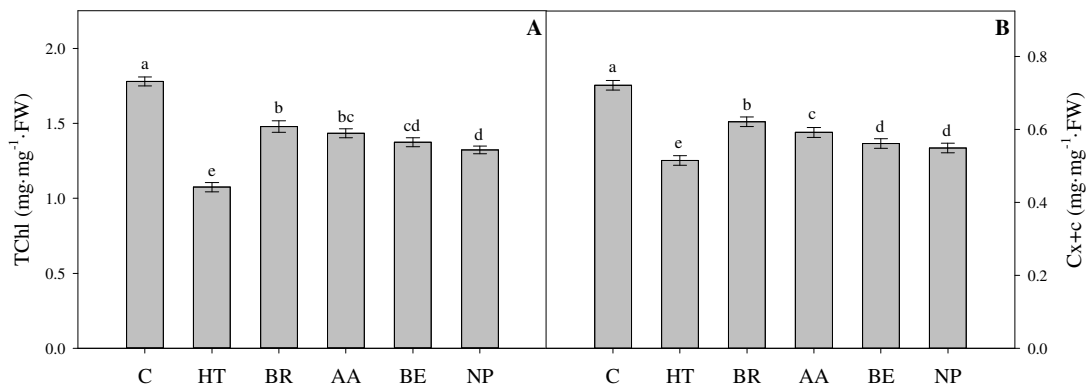
Figure 3. Total chlorophyll content (TChl) **(A)** and carotenoids (Cx+c) **(B)** of rice plants (*Oryza sativa* L.) under heat stress and the foliar application of four different biostimulants at 80 days after sowing (DAS)

Each bar represents the mean of 10 plants (*n* = 10) ± standard error. Bars followed by different letters indicate statistically significant differences according to the Tukey test (P ≤ 0.05)

C: control plants without heat stress (28 °C); HT: plants with a prolonged period (15 days) of high daytime temperatures (40 °C); BR: heat-stressed plants sprayed with brassinosteroids; AA: heat-stressed plants sprayed with amino acids; NP: heat-stressed plants sprayed with nitrophenolates; and BE: heat-stressed plants sprayed with botanical extracts

Leaf proline content and malondialdehyde (MDA) production

Figure 4 shows the proline content and malondialdehyde (MDA) production. Differences between the rice genotypes ('F67' and 'F60') and biostimulant sprays were observed in the proline content (P=0.0008) and MDA production (P=0.0078) of rice plants under heat stress conditions at 80 DAS (Table 2). The highest values of these biochemical variables were recorded in plants of both genotypes under heat stress and without foliar application of biostimulants (HT), with mean values of 9.9 μmol·g^{-1}·FW for proline and 12.4 μmol·g^{-1}·FW for MDA. In contrast, the exogenous use of biostimulants (BR, AA, BE or NP) caused a reduction in the proline content of around 22%, 34%, 33%, and 42%, respectively, in 'F67' and 'F60' plants subjected to heat stress (Figure 4A). On the other hand, a decrease in lipid peroxidation (MDA) was also observed with the foliar application of BR, AA, BE or NP, with mean values of 8.4 μmol·g^{-1}·FW and 10.5 μmol·g^{-1}·FW for 'F67' and 'F60' plants, respectively, under high daytime temperatures (Figure 4B). Finally, the accumulation of proline (mean values 4.3 μmol·g^{-1}·FW) and MDA (mean values 5.9 μmol·g^{-1}·FW) was always lower in plants without heat stress (C) in both genotypes evaluated (Figures 4A and B).
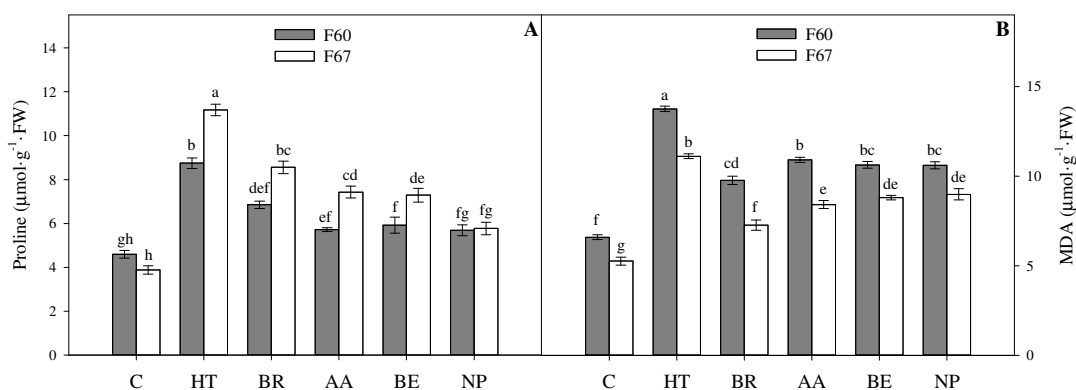
Figure 4. Proline content **(A)** and malondialdehyde (MDA) **(B)** production of rice plants (*Oryza sativa* L.) of the genotypes 'Fedearroz 67' ('F67') and 'Fedearroz 60' ('F60') under heat stress and foliar application of four different biostimulants at 80 days after sowing (DAS)

Each bar represents the mean of 10 plants (n = 10) ± standard error. Bars followed by different letters indicate statistically significant differences according to the Tukey test (P ≤ 0.05)

C: control plants without heat stress (28 °C); HT: plants with a prolonged period (15 days) of high daytime temperatures (40 °C); BR: heat-stressed plants sprayed with brassinosteroids; AA: heat-stressed plants sprayed with amino acids; NP: heat-stressed plants sprayed with nitrophenolates; and BE: heat-stressed plants sprayed with botanical extracts

Biplot analysis of physiological and biochemical responses to heat stress amelioration with biostimulant sprays

The principal component analysis (PCA) showed that the evaluated variables explained 91.3% of the physiological response of the rice genotypes ('F67' and 'F60') under conditions of heat stress and foliar treatment with four different biostimulants at 80 DAS. In Figure 5, the vectors of the physiological (P_N, g_s, E, L_s, P_N/C_i, WUE_i) and biochemical (TChl, MDA and proline) variables show angles close to the origin, which indicates that there is a high correlation between the plants' behaviour and these variables. Likewise, it was observed that plants of both genotypes under heat stress and without the application of biostimulants (HT) form a single group (I). It can be inferred that this group showed the highest effects due to the imposed heat stress condition. In contrast, the group of plants without the heat stress condition and biostimulant applications (C) (Group IV), was located in the sector opposite to group I. On the other hand, the foliar application of biostimulants showed two differential effects in the analysis: i) plants of both genotypes ('F67' and 'F60') with heat stress and foliar treatment with AA, BE or NP (Group II) displayed an intermediate behaviour, showing a slight positive effect of these biostimulants on heat stress mitigation; ii) plants of both

genotypes ('F67' and 'F60') with heat stress and foliar treatment with BR (group III) displayed a positive effect on the physiological and biochemical response of plants, showing a behaviour close to group IV (C) (Figure 5).
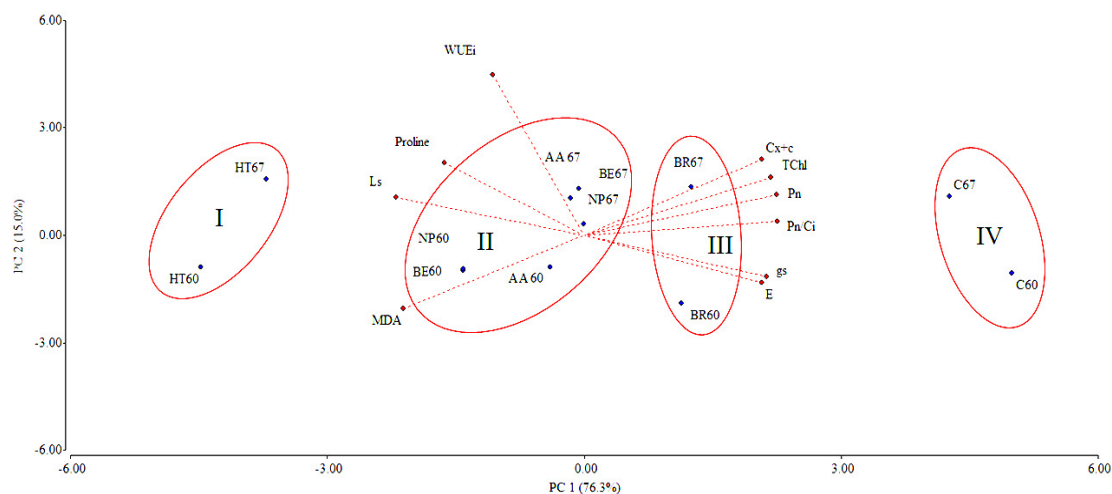
Figure 5. Principal component analysis (PCA) at 80 days after sowing (DAS) in rice plants (*Oryza sativa* L.) of the genotypes 'Fedearroz 67' ('F67') and 'Fedearroz 60' ('F60') under heat stress and foliar treatments with biostimulants

HT: plants subjected to heat stress without foliar application of biostimulants; C: control plants without heat stress conditions and foliar application of biostimulants; BR: heat-stressed plants sprayed with brassinosteroids; AA: heat-stressed plants sprayed with amino acids; NP: heat-stressed plants sprayed with nitrophenolates; BE: heat-stressed plants sprayed with botanical extracts; P_N: net photosynthetic rate; g_s: stomatal conductance; E: total transpiration; L_s: stomatal limitations; P_N/C_i: carboxylation efficiency; WUE_i: intrinsic water use efficiency; TChl: total chlorophyll; Cx+c: carotenoids; MDA: malondialdehyde; PC: principal component.

Heat tolerance coefficient (HTC)

The heat tolerance coefficient (HTC) based on P_N also corroborated the observations of the biplot analysis. Foliar applications of the biostimulants BR, AA, BE, or NP help tolerate the stress caused by high temperatures in rice genotypes 'F67' and 'F60'. The highest HTC values were observed in stressed plants of both genotypes and treated with foliar applications of BR (0.91 for 'F67' and 0.76 for 'F60'), followed by AA (0.84 for 'F67' and 0.74 for 'F60'), NP (0.80 for 'F67' and 0.67 for 'F60') and BE (0.80 for 'F67' and 0.64 for 'F60'). On the other hand, rice plants without application of biostimulants and subjected to high daytime temperatures showed the lowest values for 'F67' (0.59) and 'F60' (0.43) (Table 3).

Correlation between physiological parameters and heat tolerance coefficient (HTC)

Highly significant correlations were observed in the leaf gas exchange properties (plant transpiration (E) and stomatal conductance (g_s)) and total chlorophyll content (TChl) with the heat tolerance coefficient (HTC) in rice plants under heat stress conditions (Figure 6). A high positive correlation ($r^2 = 0.92$ and 0.96, respectively) between E and TChl and the HTC was registered in the rice genotype 'F60', with the highest values of E and TChl in relation to the HTC in the treatments with foliar applications of BR or AA (Figures 6A and C). Regarding plants of genotype 'F67', the correlations also showed that the treatments with foliar applications of BR showed the highest values of g_s ($r^2 = 0.85$) and TChl ($r^2 = 0.97$) in relation to the HTC (Figures 6B and D).
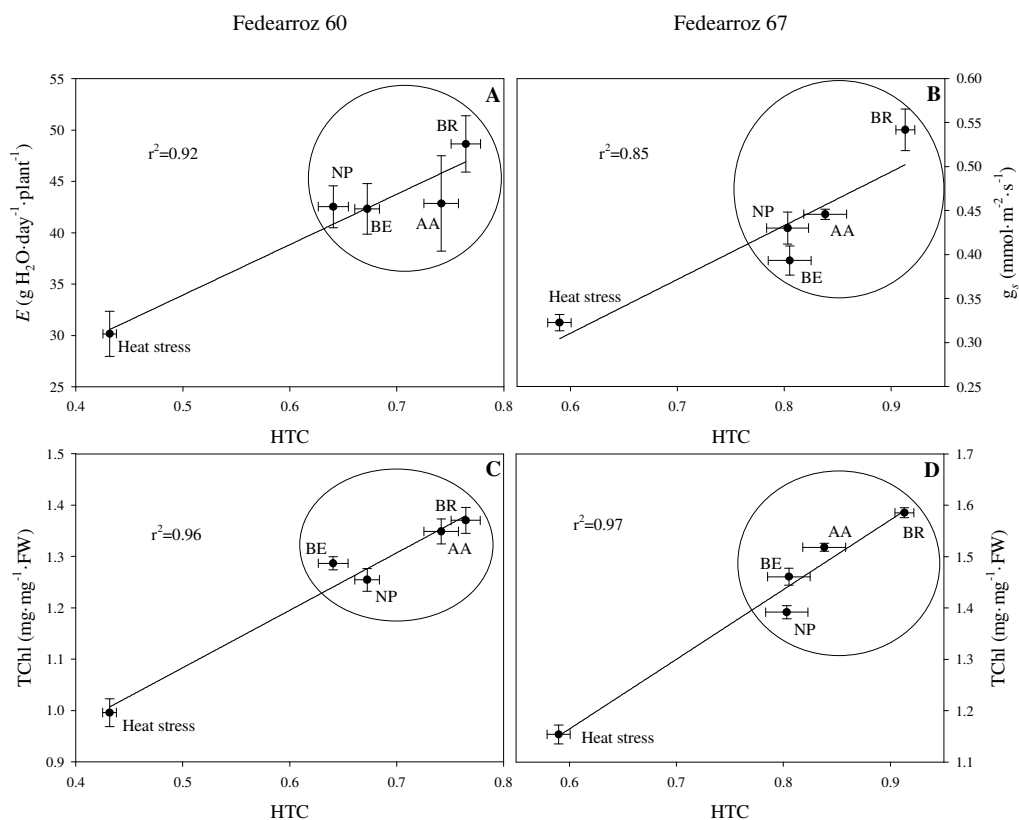
Figure 6. Correlation between plant transpiration (E) **(A)**, stomatal conductance (g_s) **(B)** or total content of chlorophyll (TChl) **(C, D)** and the heat tolerance coefficient (HTC) in rice plants (*Oryza sativa* L.) of the genotypes 'Fedearroz 60' **(A, C)** and 'Fedearroz 67' **(B, D)** under heat stress and foliar treatments with biostimulants at 80 days after sowing (DAS)

Each point represents the mean of ten plants. Vertical and horizontal bars represent ± standard error per treatment ($n = 10$). Circles represent the group of treatments with the highest tolerance to heat stress

BR: heat-stressed plants sprayed with brassinosteroids; AA: heat-stressed plants sprayed with amino acids; NP: heat-stressed plants sprayed with nitrophenolates; BE: heat-stressed plants sprayed with botanical extracts

Table 3. Effect of foliar applications of four biostimulants [brassinosteroids (BR), amino acids (AA), nitrophenolates (NP) or botanical extracts (BE)] on the heat tolerance coefficient (HTC) of rice plants (*Oryza sativa* L.) of the genotypes 'Fedearroz 67' and 'Fedearroz 60' exposed to a prolonged period (15 days) of heat stress (40 °C) at 80 days after sowing (DAS)

Genotype	Biostimulant	HTC
'Fedearroz 60'	Heat stress	0.43 g[Z]
	BR	0.76 c
	AA	0.74 cd
	BE	0.64 ef
	NP	0.67 de
'Fedearroz 67'	Heat stress	0.59 f
	BR	0.91 a
	AA	0.84 b
	BE	0.80 bc
	NPs	0.80 bc
P-value		0.000
CV (%) [Y]		4.69

[Z] Data represent the mean of ten plants per treatment ($n = 10$). Means followed by the same letter do not show significant differences at $P \leq 0.05$. [Y] CV: Coefficient of variation.

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Discussion

This study showed that plants of both rice genotypes ('F67' and 'F60') without the application of biostimulants and under conditions of heat stress (HT) showed physiological effects such as a reduction in the leaf gas exchange properties (P_N, g_s, and E), low leaf chlorophyll content, and increased MDA and proline contents (Figures 1-4). Abiotic stress due to high daytime temperatures causes adverse effects on physiological and biochemical parameters that affect the growth and development of cultivable plants (Fahad *et al.*, 2017; Wassie *et al.*, 2019). Exposure of plants to high-temperature stress decreases the exchange properties and the concentration of leaf photosynthetic pigments (total chlorophyll (TChl) and carotenoids (Cx+c)) (Chen *et al.*, 2017b; Ahammed *et al.*, 2018). Heat stress also affects the integrity of the membrane (higher lipid peroxidation (MDA)) and causes an increase in the accumulation of compatible solutes such as proline (Harsh *et al.*, 2016; Ahammed *et al.*, 2018). These physiological and biochemical variables have been used for phenotyping or evaluating management strategies to confer tolerance to rice genotypes under periods of heat stress (Restrepo-Diaz and Garces-Varon, 2013; Sánchez-Reinoso *et al.*, 2014; Sharma *et al.*, 2017).

Agronomic management practices such as the foliar application of biostimulants have been used to improve plant tolerance responses, since they can regulate and/or modify physiological and biochemical processes in plants to stimulate growth, mitigate the limitations induced by abiotic stress and increase yield (Bulgari *et al.*, 2019; Drobek *et al.*, 2019). Our results showed that foliar applications of biostimulants (BR, AA, NP, or BE) helped to mitigate the negative effects caused by heat stress conditions by favouring the physiological and biochemical responses in seedlings of both rice genotypes (Table 3; Figures 1-6). The beneficial role of foliar sprays of these compounds on the physiological and biochemical parameters of plants under periods of high diurnal temperatures has been widely documented for BR in rice (Thussagunpanit *et al.*, 2015a; Sonjaroon *et al.*, 2018), AA in maize (Matysiak *et al.*, 2020) and mung bean (Priya *et al.*, 2019), NP in soybean (Gulluoglu *et al.*, 2006), and BE in tomato (Carmody *et al.*, 2020).

In this study, it was observed that BR mitigated the negative effects of heat stress in rice genotypes 'F67' and 'F60', through an improvement in the gas exchange parameters and biochemical markers, and increased the heat tolerance coefficient (HTC). Sonjaroon *et al.* (2018) also observed that rice plants with foliar BR treatments and under heat stress showed an increase in physiological variables such as P_N, g_s, E and L_S compared to plants without BR application. It has been reported that BR can induce and improve thermotolerance in both heat-tolerant and heat-susceptible genotypes (Ahammed *et al.*, 2020). The positive response on the gas exchange parameters of plants under heat stress conditions may be associated with the fact that BR participate in the accumulation of carbon metabolites such as soluble sugars, starch or sucrose, and the induction of chlorophyll biosynthesis (Sadura and Janeczko, 2018; Tanveer, 2019). On the other hand, BR applications decreased lipid peroxidation (MDA) values and proline content, since this plant hormone regulates the activity of antioxidant enzymes (ascorbate peroxidase (APX), catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR), dehydroascorbate reductase (DHAR) and monodehydroascorbate reductase (MDHAR)) and favours the expression of stress response genes that can encode phytochelatins, osmolytes, organic acids, metallothioneins, and heat shock proteins (HSP) (Rajewska *et al.*, 2016).

Foliar applications of AA (arginine, proline, tryptophan, glycine, and cysteine) also favoured gas exchange parameters (P_N, g_s, E, L_S, P_N/C_i and WUE_i) in plants of genotypes 'F67' and 'F60' under heat stress conditions. The positive effect of foliar AA applications in creeping bentgrass (*Agrostis stolonifera*) plants has been reported, improving P_N, g_s, E, and relative water content under heat stress conditions (Li *et al.*, 2016). AA help to mitigate the effects of stress caused by high daytime temperatures because they participate in the metabolism of nitrogen and the balance of the C/N ratio (Price *et al.*, 2012). Additionally, these compounds favor the synthesis of glutamate receptors (GLRs), which help mediate processes such as accumulation of soluble sugars, stomatal movements (stomatal opening), and stimulation of photosynthesis (Teixeira *et al.*, 2018). On the other hand, the exogenous application of AA (arginine, proline, tryptophan, glycine, and cysteine) in plants under conditions of heat stress also increased the content of leaf photosynthetic pigments

(TChl and Cx+c) and decreased the concentration of MDA and proline. Vijayakumari1 and Puthur (2016) also found that the application of AA γ-aminobutyric acid caused a significant decrease in the proline content in pepper (*Piper nigrum* L.) plants under conditions of heat stress. Regarding oxidative damage, the mitigating effect of foliar applications of AA in rice plants could be because these compounds participate in the biosynthesis of chlorophyll (Ali *et al.*, 2019), regulation of the activity of antioxidant enzymes such as SOD, CAT, APX and GR and the accumulation of non-enzymatic antioxidants (ascorbic acid (AsA) and glutathione (GSH)) (Li *et al.*, 2016).

The exogenous use of NP helped the evaluated physiological and biochemical parameters of rice genotypes exposed to a high daytime temperature condition. Przybysz *et al.* (2014) observed that foliar applications of NP also caused an increase in the photosynthetic efficiency, *E* and water uptake, and a decrease in the stomatal resistance of *Arabidopsis thaliana* L. plants under drought conditions. Likewise, Djanaguiraman *et al.* (2009) reported that cotton (*Gossypium hirsutum* L.) plants treated with a commercial product based on NP (Atonik) also had lower lipid peroxidation of membranes (MDA) and a higher TChl during the leaf senescence process (oxidative stress). Additionally, Swaefy and El-Ziat (2017) concluded that foliar applications of NP decreased proline values in calendula (*Calendula officinalis*) plants under salinity conditions. It has been reported that NP can increase the photosynthetic plant capacity by reducing stomatal resistance, guaranteeing a more effortless and higher flow of CO_2 to the chloroplast, increasing the maximum photochemical efficiency of PSII, and promoting chlorophyll biosynthesis or slowing down its degradation (Przybysz *et al.*, 2014; Kazda *et al.*, 2015). These compounds are also involved in the capture of free radicals by phenolic compounds (inhibiting lipid and protein oxidation) and ROS scavenging through increased peroxidase activity (Djanaguiraman *et al.*, 2010; Szparaga *et al.*, 2018).

BE applications on plants of both rice genotypes ('F67' and 'F60') under conditions of stress caused by high daytime temperatures decreased the adverse effects on gas exchange parameters. Rashid *et al.* (2018) recorded that quinoa plants treated with *Moringa oleífera* BE increased gas exchange parameters (P_N and g_s) and water use efficiency under heat stress conditions. This positive response may be associated with the fact that BE are rich in glycine betaine, which delays the loss of photosynthetic activity by inhibiting chlorophyll degradation. Additionally, they participate in chlorophyll biosynthesis and regulate S and N metabolism (Jannin *et al.*, 2013; Yao *et al.*, 2020). BE also caused lower lipid peroxidation and proline synthesis, and a higher concentration of leaf photosynthetic pigments in heat-stressed plants. Zhang and Ervin (2008) and Zhang *et al.* (2010) observed that applications based on seaweed BE promoted higher TChl values and a lower concentration of MDA in creeping bentgrass (*Agrostis stolonifera* L.) plants subjected to moderate periods of heat stress (>14 days). BE have been reported to help plants under abiotic stress because they improve enzymatic activity (SOD, APX, and CAT) and non-enzymatic antioxidant metabolism, such as phenolic compounds. They also favor the primary metabolism of plants by increasing the levels of free amino acids, proteins, carbohydrates, and leaf photosynthetic pigments (Mansori *et al.*, 2016; Santaniello *et al.*, 2017; Yakhin *et al.*, 2017). This was observed in the present study with a higher content of TChl and Cx+c and lower lipid peroxidation of membranes (MDA).

Finally, the positive effects of the foliar application of the different biostimulants used (BR, AA, NP or BE) on rice plants ('F67' and 'F60') under conditions of high temperatures during the day are the result of the combination of physiological (P_N, g_s, *E*, L_s, P_N/C_i and WUE_i) and biochemical (TChl, Cx+c, MDA and proline) attributes which are reflected in a higher HTC (Table 3). This indicates that these biostimulants are signaling molecules that can interact with cellular transcription factors to regulate different physiological and biochemical processes involved in tolerance to abiotic stress conditions in plants (Van Oosten *et al.*, 2017; Bulgari *et al.*, 2019; Kerchev *et al.*, 2020).

Conclusions

In the current study, the results obtained in this study showed that biostimulants' foliar application (BR, AA, NP, or BE) helped to ameliorate the negative effects of heat stress in rice genotypes 'F67' and 'F60'. Foliar applications of biostimulants helped the leaf gas exchange parameters (net photosynthesis, stomatal conductance, total transpiration, carboxylation efficiency, and water use efficiency), and concentration of leaf photosynthetic pigments (chlorophylls and carotenoids). The use of these biostimulants also showed a significant reduction in the lipid peroxidation of membranes (MDA) and proline content, suggesting an antioxidant activity in rice plants subjected to heat stress. This research allows us to conclude that BR, AA, NP or BE are compounds that can generate a beneficial effect on rice plants under conditions of heat stress. Foliar applications of these biostimulants can be considered an agronomic strategy to mitigate the adverse effects caused by heat stress conditions in rice-producing areas where episodes of increases in mean daytime temperatures could be expected.

Authors' Contributions

Conceptualization: GGV and HRD; Data curation: CCCA and HRD; Formal Analysis: CCCA and HRD; Funding acquisition: GGV and HRD; Investigation: EHQC and ADSR; Methodology: EHQC, ADSR and HRD; Project administration; ADSR and HRD; Resources: GGV; Software: ADSR and HRD; Supervision: ADSR and HRD; Validation: EHQC and ADSR; Visualization: EHQC and CCCA; Writing - original draft: CCCA; Writing - review and editing: GGV and HRD. All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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