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#### **ORIGINAL ARTICLE**

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# **On-farm foliar application of a humic biostimulant increases the yield of rice**

Biostimulants play a crucial role in enhancing crop yields while promoting sustainability and environmental responsibility. Evaluating the efficacy of biostimulants on-farm requires rigorous, multiyear trials conducted across various locations and with different cultivars. This study was conducted in Uruguay from 2015 to 2023 to assess the impact of a single application of a humic biostimulant (HB) during the R3 phenological stage on irrigated rice (*Oryza sativa* L.). The study encompassed 103 farms situated in diverse cropping zones, each characterized by distinct cultivars, soil qualities, radiation, and temperature conditions across the East and North regions. Results revealed that the HB treatment elicited an average yield increase of 7.4% across all sites. Notably, 93% (97) of the trials exhibited a positive yield response, with an average increase of 8.5%, while only six trials (all in the eastern zone) showed a negative response to the HB treatment. A combined analysis of variance indicated that the biostimulant's effect did not significantly differ between production zones, years, or rice cultivars when negative responses were excluded. Furthermore, relationships with environmental variables were nonsignificant, underscoring the positive effect of the biostimulant regardless of location. These findings hold significant implications for Uruguay's rice sector, that is, integrating HBs into standard management practices could substantially boost irrigated rice yields in



rice-producing areas.

**Abstract**

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# **1 INTRODUCTION**

Rice (*Oryza sativa* L.) is the primary food source for more than one-half of the world's population and is key for global food security (Tarang et al., [2020\)](#page-12-0). Uruguay is among the top eight high-quality rice exporters in the world (Rebollo et al., [2023\)](#page-11-0). A satellite estimate revealed an increase in irrigated rice area, reaching over 158,000 ha (MGAP-DIEA,

[2023\)](#page-11-0). The main zones include the North, Central, and East of Uruguay, accounting for 70%, 20%, and 10% of the cultivated area (Rebollo et al., [2023\)](#page-11-0). The most grown cultivars in the season 2022–2023 were INIA Merín, Gurí INTA CL, INIA Olimar, INOV CL, and INIA Tacuarí with 44%, 22%, 10%, 4%, and 3.5% of the total area with an average yield of 9.6 t ha<sup>-1</sup> (Almeida & Bica, [2023\)](#page-10-0). The grain yield potential of irrigated rice-producing countries included in the Global Yield Gap Atlas [\(www.yieldgap.org\)](http://www.yieldgap.org) varies from 7 to 15 t ha<sup>−</sup>1. Rice yields in Uruguay are increasing but still have not

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**Abbreviations:** HB, humic biostimulant; PB, plant biostimulants.

yet reached 80% of the yield potential of 15.8 t ha<sup>-1</sup>, thus being a yield gap of 4.3 and 4.9 t ha<sup>-1</sup> for the North and East zones, respectively (Carracelas et al., [2019\)](#page-11-0).

The grain yield of a rice cultivar depends on interactions between the genotype, its responses to environmental conditions, and management practices (Shrestha et al., [2012\)](#page-12-0). The temperature is considered a critical factor affecting grain yield, and each growth phase has its threshold of low and high temperature. The critical temperature for tillering is 45– 48˚C (Goswami et al., [2006\)](#page-11-0), and temperatures above 35˚C negatively affect the growth of roots and lead to spikelet sterility (Hussain et al., [2019\)](#page-11-0). However, the responses of rice to high-temperature stress vary with the extent of temperature increase and duration. Temperatures below 20˚C delay germination and seedling establishment, hamper tiller formation, affect flowering, cause panicle sterility, and reduce grain yield (Hussain et al., [2019\)](#page-11-0). Incidents of high temperatures during sensitive stages of the crop affect its performance (Korres et al., [2017\)](#page-11-0).

Increasing food production for a fast-growing world population without compromising natural resources is one of the greatest challenges for agriculture (Canellas et al., [2015\)](#page-10-0). Plant biostimulants (PB) are gaining interest as innovative "green" products to increase yield in optimal and suboptimal growing conditions (Sible et al., [2021\)](#page-12-0). However, there is great variability in the effectiveness of PB in different crops and cultivars (Li et al., [2022\)](#page-11-0). Among the types of biostimulants, humic biostimulant (HB) has been applied to seeds, soil, and, to a lesser extent, leaves of crops to stimulate growth, nutrient absorption, product quality, yield, and tolerance to abiotic stress (Canellas et al., [2015\)](#page-10-0). The effects measured by bioassays, immunological tools, and genomics under controlled conditions are being explained by signaling of endogenous genes responsible for the biosynthesis of protective compounds, attenuating oxidation processes caused by water stress and high temperature (Fleming et al., [2019;](#page-11-0) Yakhin et al., [2017\)](#page-12-0). Foliar-applied HB induced higher grain yields in soybean (Izquierdo et al., [2023;](#page-11-0) Prado et al., [2016\)](#page-11-0) and winter wheat (Bezuglova et al., [2019\)](#page-10-0), while in maize production fields, the foliar application on 35 demonstration strips increased grain yields by 6.5% (Olk et al., [2022\)](#page-11-0).

Modern rice cultivars show compensation among sequentially developed yield components and this phenotypic plasticity has adaptive value to provide stability across environments because of compensatory growth of yield components such as tiller and panicle number, spikelet number per panicle, and filling percentage of spikelets (Kumar et al., [2016\)](#page-11-0). The number of panicles  $m^{-2}$  and grains panicle<sup>-1</sup> were increased by a seaweed extract applied three times on an Indian cultivar, and the yield response varied from 4.15% to 9.14% (Arun et al., [2019\)](#page-10-0). Furthermore, treatment with HB increased panicles plant<sup>-1</sup> and yield plant<sup>-1</sup> by 12.7% and 13.17%, respectively, in the treated plants compared to the control

# **RIGHTSLINK**

#### **Core Ideas**

- ∙ Humic biostimulant applied in phenology stage R3 on rice generates a yield increase of 7.4%.
- ∙ The increase in yield due to the humic biostimulant is independent of environmental factors.
- ∙ The use of humic biostimulants can be incorporated into agricultural management practices in rice-producing areas.

(Mulyatni et al., [2017\)](#page-11-0). Consecutive HB foliar sprays in rice during maximum tillering and booting stages increased the average yield per plant by 15% (Talha et al., [2020\)](#page-12-0). However, there is a paucity of results on field foliar applications of HB in multiple farm locations and different years in crops, specifically in rice.

On-farm experimentation provides the appropriate methodology to test new products and practices in farmers' fields, and the data from these tests can be used to validate simulation models and determine the economic profitability of new technology (Laurent et al., [2020\)](#page-11-0). Hence, this series of on-farm experiments evaluated the efficacy of HB under field conditions with all the inherent variation present in rice cropping systems. The use of HB in Uruguay is not yet part of the agronomic management of rice, and field foliar application of HB could be implemented as an agricultural practice only after long-term validation at the farm level. Therefore, the objective of this study was to evaluate the effect of a foliar applied HB on grain yield and its components in the main rice cultivars grown in different environmental conditions in the two main producing areas of Uruguay for about one decade.

# **2 MATERIALS AND METHODS**

#### **2.1 On-farm trials**

During the summer seasons (December–March) from 2015 to 2023, 103 on-farm trials were installed on commercial rice farms. A total of 77 and 26 trials were in the East and North zones and 17 cultivars were used during the study period (Figure [1\)](#page-2-0). The on-farm trials were airplane sprayed at the R3 stage with the HB (PromoBacter, BIOCIS). Previous research in Uruguay showed that the application of HB at a dose of 4 L  $ha^{-1}$  during the R3 phenology stage of rice, which is characterized by panicle exertion, apex on flag leaf neck, and panicle emergence (Velázquez et al., [2015\)](#page-12-0), considerably increased the root dry weight and yield  $(+761 \text{ kg ha}^{-1})$  (Izquierdo et al., [2021\)](#page-11-0). The HB treatment was applied once at a dose of 4 L ha<sup> $-1$ </sup> using a volume of water of 15 L ha<sup> $-1$ </sup>. On each farm, strip was maintained without application to have untreated plots as

<span id="page-2-0"></span>

**FIGURE 1** Main irrigated rice production zones and varieties used by farmers in 103 farm trials from 2015 to 2023. Map of harvested area adapted from Institute of Agricultural Research, INIA (National Agriculture Research Institute) database, Uruguay, 2017. The number of trials for each zone and the number of trials for each cultivar in each zone are in parentheses.

control. At crop harvest, a square iron frame with a side of 1 m was used to obtain 5–10 rice plant samples from an area of 1  $m<sup>2</sup>$  each. The samples were taken randomly from five crop rows of each trial's untreated and treated field plants. Yield components data on panicles m<sup>-2</sup>, grains panicle<sup>-1</sup>, weight (g) of 1000 grains, and yield (kg m<sup>-2</sup>) adjusted to 14% humidity were recorded. The complete dataset is shown in Table S1.

## **2.2 Source of the HB**

The HB is a commercial product, used in previous research (Izquierdo et al., [2023\)](#page-11-0), and obtained from wheat and maize crop residues mixed with horse and cow manure and vermicomposted 6 months by the earthworm *Einsenia foetida*. The extraction, pH stabilization, and dilution of the HB were carried out following agro-industrial methods under Uruguay's license and production registry. The final product, PromoBacter (BIOCIS), had the following composition: total humic extracts 5.72% w/v, humic acids 4.05% w/v, fulvic acids 1.22% w/v, density  $\sim$  0.003 g m L<sup>-1</sup>, and pH 6.8.

#### **2.3 Environmental indices**

#### **2.3.1 Soil productivity index**

Each trial site was associated with its farm productivity index based on predominant soils, slope, geological material, and

soil fertility estimated on aerial photo-cartographic maps (scale 1:20.000) by the National Commission for Agroeconomic Study (CONEAT) available at [https://dgrn.mgap.gub.](https://dgrn.mgap.gub.uy/js/visores/dgrn/) [uy/js/visores/dgrn/.](https://dgrn.mgap.gub.uy/js/visores/dgrn/) The CONEAT index shows the relationship between the agricultural productivity of a property and the units, quality, and suitability of the soil that compose it. The index was calculated through topographic photo interpretation, field verification, and the establishment of a national cartographic base. The range of values is from 0 to 300, in which an index value below 100 indicates low productivity (Lanfranco & Fraga, [2011\)](#page-11-0).

#### **2.3.2 Climatic parameters**

Daylight duration (h day<sup>-1</sup>) (Ld), radiation (cal cm<sup>-2</sup> day<sup>-1</sup>) (Rd), days with a minimum temperature equal to or below  $15^{\circ}$ C (T15), days with a maximum temperature equal to or over 34°C (T34), and thermal unit day<sup>-1</sup> (TU) of each trial site were obtained from GRAS/INIA database [\(http://www.inia.](http://www.inia.uy/gras/Clima/Banco-datos-agroclimatico) [uy/gras/Clima/Banco-datos-agroclimatico\)](http://www.inia.uy/gras/Clima/Banco-datos-agroclimatico). TU = [*t*˚ max + *t*<sup>°</sup> min)/2] − 10<sup>°</sup>C, where 10<sup>°</sup>C is the base temperature for rice. Considering that rice's critical physiological temperature period is between the phenological stages R3 and R6, the data cover the months of January, February, and March from 2015 to 2023, respectively. The means of climatic parameters and soil productivity for each year of evaluation in the different zones (North and East) are shown in Figure S1.

#### **2.4 Crop management**

The management practices were decided at each site by the field owners and managers, including cultivar, planting date, irrigation, seed and fertilizer rates, pest management, weed control, and harvesting practices following extended good agricultural practices (ACA, [2018\)](#page-10-0). The application of HB to the treated crop strip at each site is the only variation in management between the two treatments.

#### **2.5 Statistical analysis**

To assess treatment effects on grain yield and its yield components, mean effect sizes were calculated for each site. The effect size for each paired treated and untreated trial was calculated using the natural log of the response ratio (Equation 1) to normalize the data and facilitate the statistical analysis (Laurent et al., [2019\)](#page-11-0) as follows:

Effect size = 
$$
\ln\left(\frac{\text{Treatment}}{\text{Control}}\right)
$$
. (1)

Mean effect sizes and their 95% confidence intervals (CIs) from all 103 on-farm trials were estimated by bootstrapping 10,000 replicates in the "DescTools" package (Signorell et al., [2021\)](#page-12-0). The complete dataset is available for download in the Supporting Information associated with this manuscript. This approach offers an intuitive way to interpret treatment effects, since CIs provide a range of possible effect sizes in which the true treatment effect is likely to lie (Carey et al., [2022\)](#page-10-0). In some cases, to aid interpretation, we back-transformed the effect sizes and associated CIs into percentage multiplying the effect size and CI by 100, respectively. The effect of the zone, cultivars, and year on mean effect size was determined by three-way analysis of variance (ANOVA) using 88 sites, which presented at least three data response for each cultivar, using the "aov" function. The Pearson's correlation procedure was used to analyze the relationships between the different grain yield components in the "Performance Analytics" packages. To explore the relationship between yield change and the environmental variables, quadratic (only in radiation variable) and linear regression models were performed. Additionally, simple and multiple linear regression analysis was performed to find the best models to explain the yield response using different predictor variables (grain yield component). The goodness of fit of the model was evaluated using Akaike information criterion (AIC) and Bayesian information criterion (BIC). The smaller values of AIC and BIC indicate a better fit of the model (Table [4\)](#page-9-0). All statistical analyses were performed in RStudio software.

#### **3 RESULTS AND DISCUSSION**

#### **3.1 Yield response in on-farm trials**

Among the 103 on-farm trials evaluated, the yield response varied from −27.1% in the eastern zone (2023; Figure [2\)](#page-4-0) to +27.3% in the northern zone (2015; Figure [3\)](#page-5-0). The mean yield response was 6.66% (IC: 5.31–7.99) and 9.46% (IC: 7.43–11.46) in the East and North zones, respectively. About 93% (97) of the trials had a positive grain yield response with an average of 8.5%, while a total of six trials located in the eastern zone had a negative grain yield response to treatment (Figure [2\)](#page-4-0). Adverse results have been associated with an incorrect biostimulant application, timing, and concentration rate in field trials and commercial farming (de Santiago et al., [2010;](#page-11-0) Fleming et al., [2019\)](#page-11-0). On the other hand, these may be due to natural variation, data management problems, equipment problems, greater tiller induction in cultivars susceptible to *Perycularia* without appropriate fungicide control, or caused by flooding or extreme heat (Izquierdo et al., [2023\)](#page-11-0). All responses (including negative ones) were considered in the analysis because they often show an important source of yield variability (Laurent et al., [2019\)](#page-11-0). The application of biostimulants increases grain yield by an average of 13.6% with a CI of 2.2 in cereals (Li et al., [2022\)](#page-11-0). In our study, a single foliar application of a humic-type biostimulant resulted in a positive mean yield response indicating the great potential that the application of the biostimulant could have in rice-producing areas of Uruguay.

# **3.2 ANOVA between zones, years, and cultivars**

A humic acid foliar spray increased grain yield and its components as well as the macronutrient content of rice plants (El-Gohary et al., [2010\)](#page-11-0). In our study and among the 88 trials used for ANOVA, significant differences were found in grain yield effect size between production zones (Table [1\)](#page-6-0). A database across the Uruguayan main production zones revealed that the average rice yield was 8491 kg ha−<sup>1</sup> in the Eastern region and 8380 kg ha−<sup>1</sup> in the Northern region and a yield gap of 18% in both zones (Tseng et al., [2021\)](#page-12-0). Moderate but not significant effect sizes were found between zones for panicles m<sup>-2</sup>, grains panicle<sup>-1</sup>, and 1000-grain weight (Table [1\)](#page-6-0). The mean yield response in the northern zone was 10.5%, while in the eastern zone it was 6.3% (Table [2\)](#page-6-0). However, when the negative responses were eliminated from the analysis, there were no significant differences between production zones (data not shown) due to the majority of sites with negative responses falling into the eastern zone due to data management problems and equipment problems.

<span id="page-4-0"></span>

**FIGURE 2** Mean effect of the humic biostimulant on the yield response (%), and their confidence interval (black triangle), in the 77 sites located in the eastern zone of Uruguay. The mean effect for the zone is shown (black circle).

# **3.3 Yield response in the most used cultivars**

Diverse genotypic responses to the application of biostimulants have been documented in cereals (Li et al., [2022\)](#page-11-0). There were no significant differences between the mean yield

responses for the different cultivars (Table [2\)](#page-6-0). However, the highest and lowest percentage of grain yield response was found in cultivars Merin (10.4%) and XP (2.8%), respectively. In addition, the year did not have a significant effect, where the highest and lowest mean grain yield responses were found in 2015 (12.6%) and 2023 (5.6%) due to heavy rains

<span id="page-5-0"></span>

**FIGURE 3** Mean effect of the humic biostimulant on the yield response (%), and their confidence interval (black triangle), in the 26 sites located in the northern zone of Uruguay. The mean effect for the zone is shown (black circle).

during grain filling. Overall, the mean yield response was 7.4% across all sites with a CI of 5.7 (Table [2\)](#page-6-0). Since the CI does not include the zero value, the foliar application of the HB has a significant effect on grain yield as compared to the untreated (Laurent et al., [2019\)](#page-11-0). The CIs for the six cultivars most used by farmers indicated that Merin, Gurí, and Tacuarí presented a significant and positive mean yield response to the treatment compared to the control across the trials. While for the cultivars Olimar, El Paso 144, and INOV, there is a probability that the treatment will produce a null or negative effect in new trials (Figure [4\)](#page-7-0).

# **3.4 Environmental factors and trade-off between yield components**

Rice tillering is a key component of grain yield and is conditioned by soil, plant density, climate, and genotype (Adriani et al., [2016;](#page-10-0) Ao et al., [2010\)](#page-10-0). In our study, grain yield was positively and significantly correlated with the number of panicles  $m^{-2}$  and grains panicle<sup>-1</sup>, and these correlations were slightly increased by treatment (Table [3\)](#page-7-0). The correlation coefficient for grain yield on 1000-grain weight was negative and very low. The results consistently show that the field-foliar

**Trait Source of variation** *df* **Mean square**  $F$  **value**  $Pr(>F)$ Yield 20029\* 2012 2020 1 0.030527 4.97 0.029\* Year 8 0.004192 0.682 0.705 Cultivar 7 0.003255 0.53 0.809 Residuals 71 0.006143 Panicles m<sup>-2</sup> Zone 201 2008 2009 1 0.007539 0.883 0.351 Year 8 0.004212 0.493 0.857 Cultivar 7 0.006772 0.793 0.596 Residuals 71 0.008538 Grains panicle<sup>-1</sup> Zone 1 0.005259 0.38 0.539 Year 8 0.006389 0.462 0.879 Cultivar 7 0.012543 0.907 0.506 Residuals 71 0.013832 1000-Grain weight Zone 1 0.0001842 0.208 0.6499 Year 8 0.0008809 0.994 0.4482 Cultivar 7 0.0016402 1.851 0.0908 Residuals 71 0.0008861

<span id="page-6-0"></span>**TABLE 1** Analysis of variance of the yield and yield components response size to a humic biostimulant (HB) treatment for two rice production zones, 9 years, and the most used cultivars (eight) in Uruguay.

Abbreviation: *df*, degrees of freedom.

\*Significant at 0.05.

**TABLE 2** Means of yield response and their confidence intervals for each zone, cultivar, and year.

			Yield response $(\%)$	CI(95%)		
		$\boldsymbol{N}$	Mean	L.L	U.L	<i>p</i> -value
Zone	East	64	6.3	0.22684673	12.3731533	0.041
	North	24	10.5	5.84533931	15.1546607	$1.234E^{-05}$
Cultivar	<b>EEA 404</b>	$\overline{7}$	9.1	3.38570408	14.7142959	0.001
	Guri	10	5.1	$-5.04766147$	15.2876615	0.330
	<b>INOV</b>	11	6.5	1.58107161	11.3589284	0.009
	Merin	11	10.4	6.14200813	14.6579919	$2.338E^{-06}$
	Olimar	27	8.3	2.28052981	14.3794702	0.007
	Paso 144	11	6.8	3.78543324	9.75456676	$1.015E^{-05}$
	Tacuari	8	5.8	3.39585869	8.16414131	$2.556E^{-06}$
	<b>XP</b>	3	2.8	$-12.8407132$	18.4807132	0.739
Year	2015	5	12.6	8.01707778	17.1829222	$1.190E^{-07}$
	2016	6	8.7	3.14866663	14.2913334	0.002
	2017	7	6.9	$-0.07132953$	13.9313295	0.053
	2018	13	6.0	0.41893638	11.6210636	0.035
	2019	10	9.1	5.14740596	13.052594	$8.242E^{-06}$
	2020	9	5.8	$-0.15541418$	11.8354142	0.057
	2021	15	6.7	1.43899716	12.0010028	0.012
	2022	12	8.9	3.2455124	14.5544876	0.002
	2023	11	5.6	$-4.21705245$	15.4170525	0.266
Overall		88	7.4	1.69882515	13.1811748	0.011

*Note*: *N* denotes number of trials.

Abbreviations: CI, confidence interval; L.L, lower limit; U.L, upper limit.

<span id="page-7-0"></span>

**FIGURE 4** Yield response of six rice cultivars to the foliar application of the humic biostimulant in on-farm tests conducted in the two main rice production areas of Uruguay (2015–2023). Black square dots are the average of the response at each test site. Round black spots are the average response of the cultivar.

**TABLE 3** Pearson's correlation coefficients between grain yield and its components for humic biostimulant (HB) non-treated (above the diagonal) and treated (below the diagonal) plants over years, production zones, and genotypes during the study period (2015–2023).

	Panicles $m^{-2}$	Grains panicle $^{-1}$	1000-Grain weight $(g)$	Yield $(g m^{-2})$
Panicles $m^{-2}$	-	$-0.319*$	$-0.404*$	$0.401*$
Grains panicle <sup><math>-1</math></sup>	$-0.381*$	$\qquad \qquad \blacksquare$	$-0.212*$	$0.521*$
1000-Grain weight $(g)$	$-0.315*$	$-0.259*$	$\overline{\phantom{0}}$	$-0.046$
Yield $(g m^{-2})$	$0.418*$	$0.522*$	$-0.066$	$\qquad \qquad -$

\*Significant at 0.05.

application of the HB caused an increase in yield over years and cultivars, which agrees with Mulyatni et al. [\(2017\)](#page-11-0), Talha et al. [\(2020\)](#page-12-0), and Izquierdo et al. [\(2021\)](#page-11-0).

Variation in yield was expected among the on-farm trial locations as rice responds differently to environmental conditions. Although rice production is irrigated in Uruguay,

rainfall has been a major problem when El Niño/Southern Oscillation events of high rainfall and cloudiness occur (Magrin et al., [2014\)](#page-11-0). Cloudiness affects production negatively in high-yield years conditions (Ferrero et al., [2017\)](#page-11-0). Temperatures above or below the optimal for growth reduce growth and grain yield (Dubey et al., [2018;](#page-11-0) Hussain et al., [2019;](#page-11-0) Macedo, [2014\)](#page-11-0).



**FIGURE 5** Linear regressions of the yield effect size of eight rice cultivars to the soil productivity index CONEAT (National Commission for Agroeconomic Study), heliophany, radiation, number of days with temperature below 15˚C, thermal units (*e*), and number of days with temperature over 34˚C from 2015 to 2023 over two main production zones in Uruguay. T15: sum days with a minimum temperature equal to or below 15˚C; TU: sum thermal units  $[(t^* \max + t^* \min/2) - 10^{\circ} \text{C}]$ ; and T34: sum days with a maximum temperature equal to or over 34°C.

In this study, the effect size response for grain yield by site was poorly related to the CONEAT soil capacity index, which indicates a relatively constant and positive effect of the biostimulant independent of the location (Figure 5). In addition, grain yield response was not affected by climatic conditions as evidenced by the very low and nonsignificant regression coefficients with the climatic parameters obtained over years and cultivar for heliophany, radiation, number of days with temperature below 15˚C, thermal units, and number of days with temperature over  $34^{\circ}$ C (Figure 5).

It is known that cereal crops compensate between grain yield components to achieve its total grain yield and the response to HB was no exception. A total of 25 out of 103 tests showed a negative response in panicles  $m^{-2}$  (Figure [6a\)](#page-9-0), which was compensated by the increase in the number of grains per panicle (Figure [6b,f\)](#page-9-0). On the other hand, the HB does not have a significant effect on the grain's weight  $(R^2 = 0.0002, p-value = 0.02097, Figure 6c)$  $(R^2 = 0.0002, p-value = 0.02097, Figure 6c)$ . Although some responses to HB are absorbed by the compensatory plasticity of the yield components, especially by grains panicle<sup>-1</sup>, the general yield response to treatment was positive and the grain's size (weight) response was poorly associated with the yield response (Figure [6c\)](#page-9-0). A multiple regression model with a high prediction value fits well for grain yield response to treatment (Table [4\)](#page-9-0).

The specific modes of action of biostimulants are currently being investigated. The characterization of the prime state in maize seedlings subjected to humic acid showed that regulatory stress-responsive genes were positively modulated (Canellas et al., [2020\)](#page-10-0), whereas the gene humic-induced expression in wheat depended on genotype (Arslan et al., [2021\)](#page-10-0). In addition, the application of vermicompost extracts resulted in the activation of the antioxidant enzymatic function and the increase of reactive oxygen species (ROS) scavenging enzymes to block toxic oxygen radicals produced in plants under stress (Calderín García et al., [2012\)](#page-10-0), and increased the dry weights of roots of different plant species by 22% in response to exogenous application of HS (Rose et al., [2014\)](#page-11-0).

Numerous published data, mainly on vegetables, demonstrate the positive response to biostimulants. However, scanty research has been published using an HB with foliar spraying in rice fields (Izquierdo et al., [2021\)](#page-11-0). Foliar applications are favored because they can be merged with conventional

<span id="page-9-0"></span>

**FIGURE 6** Simple linear regression models among the response (%) to humic biostimulant (HB) treatment of grain yield on panicles m<sup>−</sup><sup>2</sup> (a), grains panicle<sup>-1</sup> (b), and 1000-grain weight (TGW) (c); panicles m<sup>-2</sup> on TGW (d) and grains panicle<sup>-1</sup> (f); and grains panicle<sup>-1</sup> on TGW (e) across years, locations, and cultivars.

**TABLE 4** Simple and multiple linear regressions to find the best regression models to explain grain yield response using the yield component as predictor variables.

<b>Model</b>	<b>Equation</b>	$R^2$	<i>p</i> -value	<b>AIC</b>	<b>BIC</b>
$y = \beta_0 + \beta_1 x_1 + \varepsilon$	$y = 6.580 + 0.197x_1$	0.042	0.020	709.8	717.7
$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \varepsilon$	$y = 0.906 + 0.932x_1 + 0.826x_2$	0.797	$2.2e-16$	550.8	561.4
	$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \varepsilon$ $y = 0.302 + 0.960x_1 + 0.894x_2 + 0.623x_3$	0.848	$2.2e-16$	521.8	535.0

*Note*:  $x_1$ : panicles m<sup>-2</sup>;  $x_2$ : grains panicle<sup>-1</sup>;  $x_3$ : 1000-grain weight.

Abbreviations: AIC, Akaike information criterion;BIC, Bayesian information criterion; *df*, degrees of freedom.

spraying practices. A review shown that new stress-tolerant cultivars, water and pest management, mechanization, and seed systems had the most effect on grain yield (Mishra et al., [2022\)](#page-11-0). These technologies could be suited or are already used for large farms in the United States, Japan, southern Brazil, and Uruguay. However, the review does not mention biostimulation as part of the integrated management of rice

cultivation. The Uruguayan rice sector has incorporated innovations, greater input requirements, and capital goods but not the use of biostimulants. In the case of biostimulants, the physiological, chemical, or molecular variables of interest, such as canopy structure and growth, photosynthesis, water relations, and leaf biochemistry, can be monitored with current field phenotyping systems (Rouphael et al., [2018\)](#page-12-0). However,

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<span id="page-10-0"></span>field, long-term, and on-farm research as in our experiments is necessary to validate the use of HBs for managing rice production sustainably.

# **4 CONCLUSION**

The utilization of HBs sourced from vermicompost contributes to the local circular economy. This technology presents a straightforward and sustainable alternative, seamlessly integrating into pest and disease management plans to enhance rice grain yields. Research indicates a favorable grain yield response to this treatment, particularly pronounced in Uruguay's Northern region compared to the Eastern zone. The yield increase attributed to the biostimulant may be linked to a higher panicle density per square meter, with negligible influence from environmental variables. Consistent results across multiple years, locations, and rice genotypes in Uruguay's primary production areas endorse the broader adoption of HBs, thereby fostering both technical and sustainable rice agriculture. This accessible technology holds promise for bridging yield gaps domestically and internationally. Further agricultural investigations targeting physiological mechanisms and biostimulant gene signaling, along with field assessments of parameters like leaf chlorophyll evolution and nitrogen dynamics during reproductive phases, promise deeper insights into the underlying mechanisms driving the response to HBs in rice cultivation.

#### **AUTHOR CONTRIBUTIONS**

**Juan Izquierdo**: Conceptualization; formal analysis; methodology; software; validation; writing—original draft. **Osvin Arriagada**: Data curation; formal analysis; methodology; software; validation; visualization; writing—review and editing. **Gustavo García-Pintos**: Conceptualization; data curation; funding acquisition; investigation; project administration; resources; supervision. **Rodomiro Ortiz**: Writing—review and editing. **Martín García-Pintos**: Investigation. **Marcelo García-Pintos**: Investigation.

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### **DATA AVAILABILITY STATEMENT**

The complete dataset underlying this article is available for download in the supplementary materials associated with this manuscript.

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