

# A bioproduct based on *Solieria chordalis* enhances heat tolerance, photosynthetic pigments and yield of maize plants

*Um bioproduto à base de Solieria chordalis aumenta a tolerância ao calor, os pigmentos fotossintéticos e a produtividade do milho*

Vinicius Salvati Campagnaro<sup>1</sup>(ORCID 0009-0002-5900-4762), Kamille Daleck Spera<sup>2</sup>(ORCID 0000-0001-7688-1228), Pedro Henrique Gorni<sup>3</sup>(ORCID 0000-0002-3866-9215), Rafael Dal Bosco Ducatti<sup>4</sup>(ORCID 0000-0001-8916-6557), André Rodrigues dos Reis<sup>5</sup>(ORCID 0000-0002-6527-2520)

<sup>1</sup>Campagnaro Planejamentos e Assessoria Agropecuária LTDA, Sidrolândia, MS, Brazil.

<sup>2</sup>São Paulo State University, Assis, SP, Brazil.

<sup>3</sup>Escola Superior de Agronomia de Paraguaçu Paulista, Paraguaçu Paulista, SP, Brazil.

<sup>4</sup>Evolution Agrobusiness LTDA – Dugan Agribusiness LTDA, Luís Eduardo Magalhães, BA, Brazil. Author for correspondence: rducatti@alunos.utfpr.edu.br; r.ducatti@dugan.com

<sup>5</sup>São Paulo State University, Tupã, SP, Brazil.

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## ABSTRACT

The use of bioproducts derived from seaweed has been gaining prominence in agricultural production systems due to their bioactive properties and effects. These products exhibit phytostimulating characteristics that enhance plant growth and improve yield parameters in several important crops. Maize, one of the world's widely cultivated crops, benefits significantly from the application of seaweed-derived bioproducts due to its susceptibility to various environmental stresses. The aim of this study was to evaluate the foliar application of a bioproduct based on *Solieria chordalis* (Rhodophyta) on the biosynthesis of photosynthetic pigments and yield of 27 different maize hybrids grown in a Brazilian ecoregion denominated "Cerrado". This region experiences various types of abiotic stresses such as dry spells and high temperatures. The experiment was conducted at a research station located in Sidrolândia, Mato Grosso do Sul, Brazil, during the second maize harvest season of 2023. The results showed that a singular foliar application of a red seaweed-based product (1.0 L ha<sup>-1</sup>) at the V<sub>6</sub> phenological stage enhanced photosynthetic pigments and yield of most maize hybrids. Foliar application of the red seaweed-based product increased the primary metabolism, thereby boosting the yield of maize plants under field conditions.

**KEYWORDS:** Chlorophyll *a*. Chlorophyll *b*. Carotenoids. Primary Metabolism. Red Seaweed Extract. Rhodophyta.

## RESUMO

A utilização de bioprodutos derivados de algas marinhas vem ganhando destaque nos sistemas de produção agrícola devido às suas propriedades e efeitos bioativos. Esses produtos apresentam características fitoestimulantes que melhoram o crescimento das plantas e melhoram os parâmetros de rendimento em diversas culturas de interesse agrônomo. O milho, uma das culturas mais cultivadas no mundo, é significativamente beneficiado pela aplicação de bioprodutos derivados de algas marinhas devido à sua susceptibilidade a vários estresses ambientais. O objetivo deste estudo foi avaliar a aplicação foliar de um bioproduto produzido a partir da macroalga vermelha *Solieria cordalis* (Rhodophyta) na biossíntese de pigmentos fotossintéticos e na produtividade de 27 híbridos de milho na região do Cerrado brasileiro, a qual sofre diversos tipos de estresses abióticos, como verânicos e altas temperaturas. O experimento foi conduzido em uma estação experimental localizada na cidade de Sidrolândia, Mato Grosso do Sul, Brasil, durante a segunda safra de milho de 2023. Os resultados mostraram que uma única aplicação foliar de um produto à base de algas vermelhas (1,0 L ha<sup>-1</sup>) no estágio fenológico V<sub>6</sub> aumentou os pigmentos fotossintéticos e o rendimento da maioria dos híbridos de milho. A aplicação foliar do produto à base de algas vermelhas elevou o metabolismo primário das plantas culminando em um maior rendimento em condições de campo.



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**PALAVRAS-CHAVE:** Clorofila *a*. Clorofila *b*. Carotenóides. Extrato de Algas Marinhas Vermelha. Metabolismo Primário. Rhodophyta.

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## INTRODUCTION

According to the Food and Agriculture Organization of the United Nations (FAO), maize (*Zea mays*) was the second most cultivated crop in 2022, covering approximately 203 million hectares worldwide. Brazil accounted for 10.3% of this area, ranking third behind China and the United States of America, which together represented 37.0% of the total cultivated area (FAO 2024). In the 2022/2023 harvest season, global maize production reached 1.15 billion metric tons and Brazil also ranked third, with a production of 137 million metric tons, equivalent to 11.9% of the total global production (FAS:USDA 2024).

These statistics underscore Brazil's significant contribution to the world's maize supply. Maize serves as a staple crop extensively utilized in food/feed, fuel, cosmetics, pharmaceuticals, and chemical/industrial products (MAINTRA & SINGH 2021). However, abiotic factors profoundly influence maize development and yield (HOU et al. 2021). Despite maize being classified as a C<sub>4</sub> crop with excellent photosynthetic efficiency and low susceptibility to saturation under high solar radiation (ERENSTEIN et al. 2022), conditions such as drought, heat stress, salinity, and soil fertility can substantially reduce crop productivity (COSTA et al. 2021a, ADEM et al. 2023, KIM & LEE 2023).

The use of products to stimulate crop development and induce the production of greater amounts of compounds for better protection against biotic and abiotic stressors has increased since the discovery of metabolic pathways involved in the specialized metabolism of plants (DUCATTI et al. 2024). Among these products, commonly referred to as biostimulants or elicitors, are macro seaweeds. Biostimulants play an essential role in antioxidant activity, activating enzymatic and non-enzymatic mechanisms to maintain metabolic homeostasis under stressful conditions (LANZA & REIS 2021). These products are beneficial for plants, increasing crop productivity by reducing reactive oxygen species (ROS) and enhancing the synthesis and accumulation of proteins, sugars, amino acids, flavonoids, and photosynthesis, among other functions (GOLUBKINA et al. 2018, DUCATTI et al. 2021a).

The aim of this study was to evaluate the foliar application of a biostimulant based on *Solieria chordalis* on the biosynthesis of photosynthetic pigments and yield of 27 maize hybrids grown in a Brazilian ecoregion denominated "Cerrado".

## MATERIALS AND METHODS

### Experimental area, design, and hybrids

The experiment was conducted at a research station situated in Sidrolândia, Mato Grosso do Sul, Brazil, during the second maize harvest season of 2023. It was developed as a completely randomized experimental design consisting of a factorial scheme of 27 maize hybrids (Table 1) and the application or not of a red seaweed-based biostimulant. Each treatment consisted of four replications (totaling 216 plots).

The red seaweed-based biostimulant (1.0 L ha<sup>-1</sup> – EvoRed/VitaPlex®) was foliar applied at the V<sub>6</sub> phenological stage (NLEYA et al. 2016) along with a nonionic

adjuvant ( $0.05 \text{ L ha}^{-1}$ ) using a self-propelled sprayer calibrated to deliver  $100.0 \text{ L ha}^{-1}$  of solution. At sowing (March 2<sup>nd</sup> 2023), the area received  $250.0 \text{ kg ha}^{-1}$  of fertilizer applied in-furrow, consisting of 16.0% N, 16.0%  $\text{K}_2\text{O}$ , and 16.0%  $\text{P}_2\text{O}_5$ .

**Table 1.** List of hybrids used for the development of the trials.

Genotype	Hybrid name	Hybrid type	Growth cycle
G1	AS1868 PRO4	Single-cross	Early
G2	DKB260 PRO4	Single-cross	Super Early
G3	AG8701 PRO4	Single-cross	Early
G4	S9504 VIP3	Single-cross	Super Early
G5	B2702 VYHR	Single-cross	Super Early
G6	AS1820 PRO4	Single-cross	Early
G7	AG9035 PRO4	Single-cross	Super Early
G8	SHS2050 PRO4	No info	Semi Early
G9	AS1800 PRO3	Single-cross	Super Early
G10	K7373 VIP3	Single-cross	Super Early
G11	K7300 VIP3	Single-cross	Super Early
G12	GNZ7788 VIP3	Single-cross	Early
G13	SHS8010 VIP3	No info	Early
G14	MG540 PWU	Single-cross	Early
G15	K7510 VIP3	Single-cross	Early
G16	2M91 PRO4	Single-cross	Early
G17	GNZ7710 VIP3	Single-cross	Super Early
G18	2M76 PRO3	Single-cross	Early
G19	B2782 PWU	Triple-cross	Early
G20	FS575 PWU	Single-cross	Early
G21	DKB360 PRO	No info	Early
G22	S9801 VIP3	No info	Early
G23	FS403 PWU	Single-cross	Super Early
G24	FS505 PWU	Single-cross	Early
G25	COD052 PWU	No info	No info
G26	MG635 PWU	Triple-cross	Early
G27	BX35R726 PWU	No info	No info

Thirty-five days after crop emergence, at the  $V_4$  phenological stage, an additional application of  $150.0 \text{ kg ha}^{-1}$  of fertilizer was broadcasted using a formulation containing 33.0% N and 12.0% S.

Each plot consisted of 13 rows, each measuring 12 meters in length (equivalent to  $93.6 \text{ m}^2$  per plot). Sowing lines were spaced 0.6 meters apart and plant density was kept the same across all hybrids (60 thousand plants per hectare). The meteorological conditions encountered during the development of the experiment are outlined in Table 2.

**Table 2.** Meteorological conditions during the development of the trials. Sidrolândia – Mato Grosso do Sul – Brazil. 2023.

Month	Total precipitation (mm)	Mean lowest temperature (°C)	Mean highest temperature (°C)	Mean relative humidity (%)	Radiation (Kj.m <sup>-2</sup> )
February	216.6	23.50	24.67	78.79	1332.59
March	126.2	24.59	25.94	74.20	1490.24
April	267.2	22.70	24.01	74.61	1400.45
May	107.0	21.08	22.44	68.62	1291.90
June	86.8	18.29	19.79	67.29	1319.40
July	6.0	20.78	22.30	59.54	1260.89

Data from a meteorological station.

### Photosynthetic pigments

At the VT phenological stage, leaves from each plot were harvested, carefully folded in aluminum foil, immediately placed in liquid nitrogen (on-site), and then stored in an ultra-freezer for subsequent quantification of Chlorophyll *a* (*Chl a*), Chlorophyll *b* (*Chl b*), and carotenoids (*Car*). Leaf discs (0.2 g) were collected in the middle third of the leaf blade of the last fully developed leaf and added in 80% acetone. Readings were performed on a spectrophotometer at 470 nm, 647 nm, and 663 nm according to the methodology proposed by LICHTENTHALER (1987). Contents were determined based on fresh weight.

### Yield

Parcels were harvested once the hybrids reached a humidity level below 18%. Each hybrid was harvested at different times, aligning with their respective developmental cycles. During harvesting, only the two central rows of each parcel were utilized, minimizing the potential for interference between adjacent parcels as per DUCATTI (2023). The grains were weighed, and their final weight was adjusted to a standardized humidity level of 15%. Subsequently, these weights were extrapolated to represent yield per hectare.

### Statistical analysis

All assumptions of ANOVA were thoroughly verified for data collected across all assessments. Parametric methods employing ANOVA were utilized to ascertain the presence of significant differences among the means of each treatment/variable. When significant, means were further scrutinized using Tukey's Honestly Significant Difference (HSD) test at a significance level of 5%. Correlations between yield, *Chl a*, *Chl b*, and *Car* were evaluated via Pearson's correlation coefficient. All statistical analyses were conducted using the AgroEstat software program (BARBOSA & MALDONADO Jr. 2015).

## RESULTS

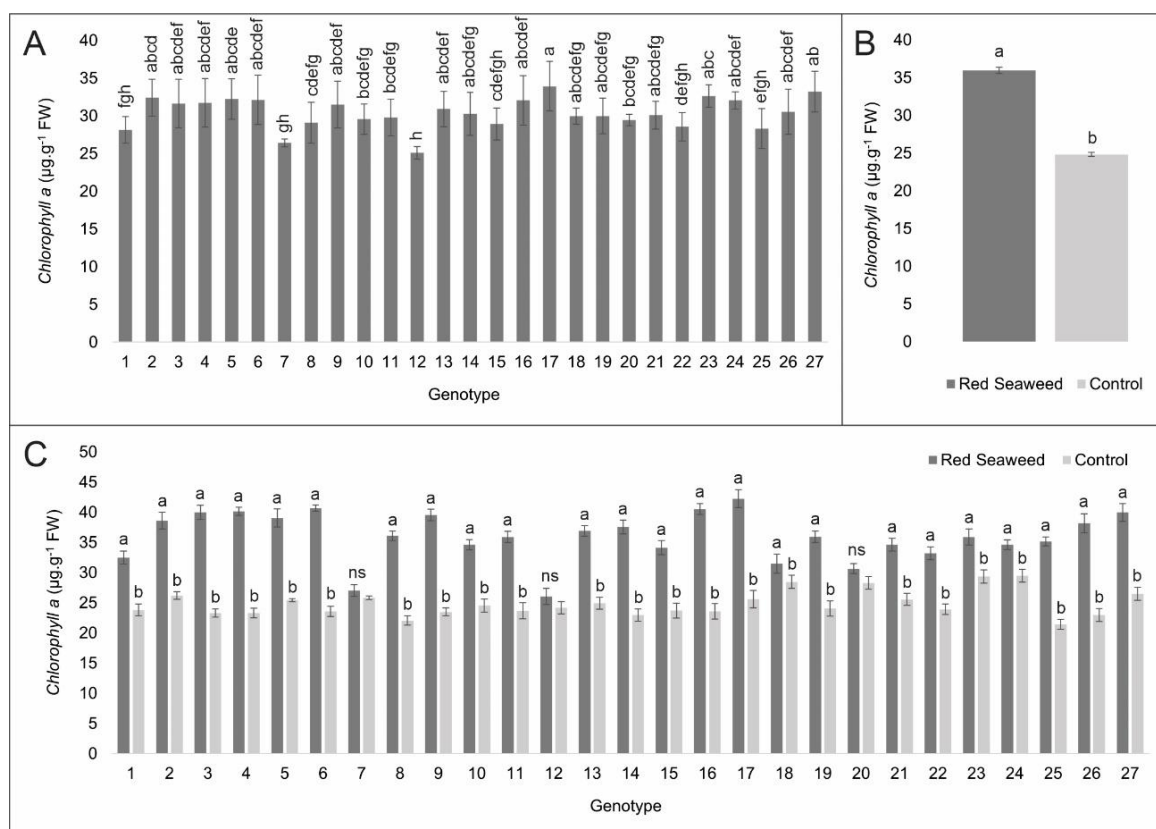
### Chlorophylls and Carotenoids

The foliar application of the red seaweed-based biostimulant exhibited an increase on the total amounts of *Chl a*, *Chl b*, and *Car* for both factors, the hybrids and the treatment receiving the biostimulant (Table 3).

**Table 3.** ANOVA for the quantification of Chlorophyll a (*Chl a*), Chlorophyll b (*Chl b*), and Carotenoids (*Car*) in maize plants treated with a sole application of a biostimulant based on *Soleria chordalis*.

Variable analyzed	Source of variation	Degrees of freedom	Sum of squares	Mean squares	F value	p value
<i>Chl a</i>	Hybrid (H)	26	875.11	33.65	7.56	<0.0001
	Biostimulant (B)	1	6728.02	6728.02	1511.01	<0.0001
	Interaction H x B	26	1292.97	49.72	11.17	<0.0001
<i>Chl b</i>	Hybrid (H)	26	320.81	12.33	3.98	<0.0001
	Biostimulant (B)	1	1309.65	1309.65	422.11	<0.0001
	Interaction H x B	26	126.39	4.86	1.57	0.0494
<i>Car</i>	Hybrid (H)	26	3879.81	149.22	23.87	<0.0001
	Biostimulant (B)	1	10553.78	10553.78	1688.01	<0.0001
	Interaction H x B	26	2316.88	89.11	14.25	<0.0001

As indicated in Table 3, all hybrids were significantly influenced by the treatments. To provide a deeper understanding of these interactions, Tukey's HSD test was employed to compare the mean amounts of *Chl a* and *b*, and *Car* among treatments and hybrids. These analyses are presented in Figures 1, 2, and 3.

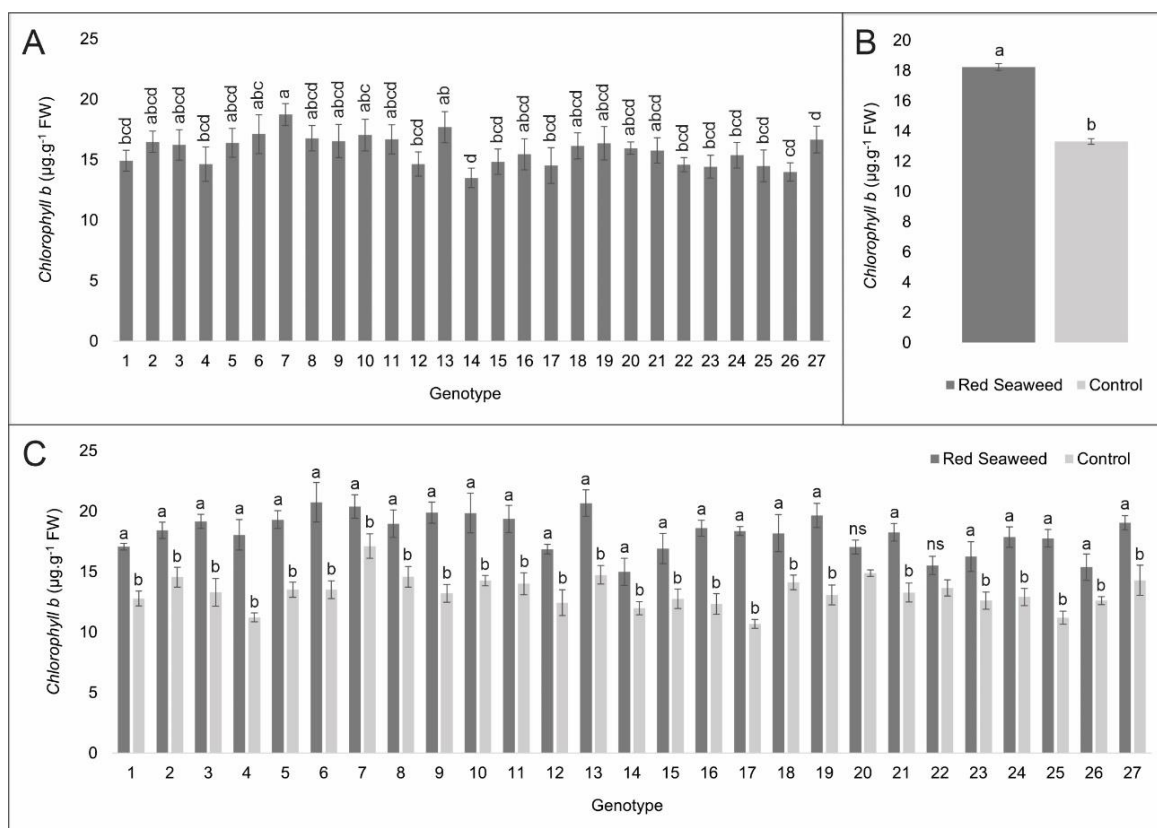


**Figure 1.** Mean *Chlorophyll a* (µg.g<sup>-1</sup> of fresh weight) according to the hybrids (A), the use of the red seaweed biostimulant (B), and the interaction between the hybrid and the use of the red seaweed biostimulant (C). Different letters indicate statistical difference according to Tukey's HSD at 5%. Bars represent the standard error. \*ns: not significant.

*Chl a* content varied from 19.4 to 46.6 µg g<sup>-1</sup> of fresh weight. Notably, hybrid 12 was the hybrid with the lowest contents of *Chl a*, even though this hybrid was statistically similar to hybrids 01, 07, 15, 22, and 25 (Figure 1A). Hybrids 07, 12, and 20 were the only ones not significantly affected by the biostimulant application (Figure

1C). However, on average, the utilization of the biostimulant led to an increase of over 45% in *Chl a* content (Figura 1B).

*Chl b* pigment ranged from 8.8 to 24.5  $\mu\text{g g}^{-1}$  of fresh weight across treatments. Nevertheless, few hybrids could be outlined as for their mean values for this pigment (Figure 2A). In regard to the application of the red seaweed bioproduct, only hybrid numbers 20 and 22 did not exhibit a significant response to it (Figure 2C). On average, the utilization of the biostimulant resulted in an increase of approximately 37% in this pigment (Figure 2B).



**Figure 2.** Mean *Chlorophyll b* ( $\mu\text{g.g}^{-1}$  of fresh weight) according to the hybrids (A), the use of the red seaweed biostimulant (B), and the interaction between the hybrid and the use of the red seaweed biostimulant (C). Different letters indicate statistical difference according to Tukey's HSD at 5%. Bars represent the standard error. \*ns: not significant.

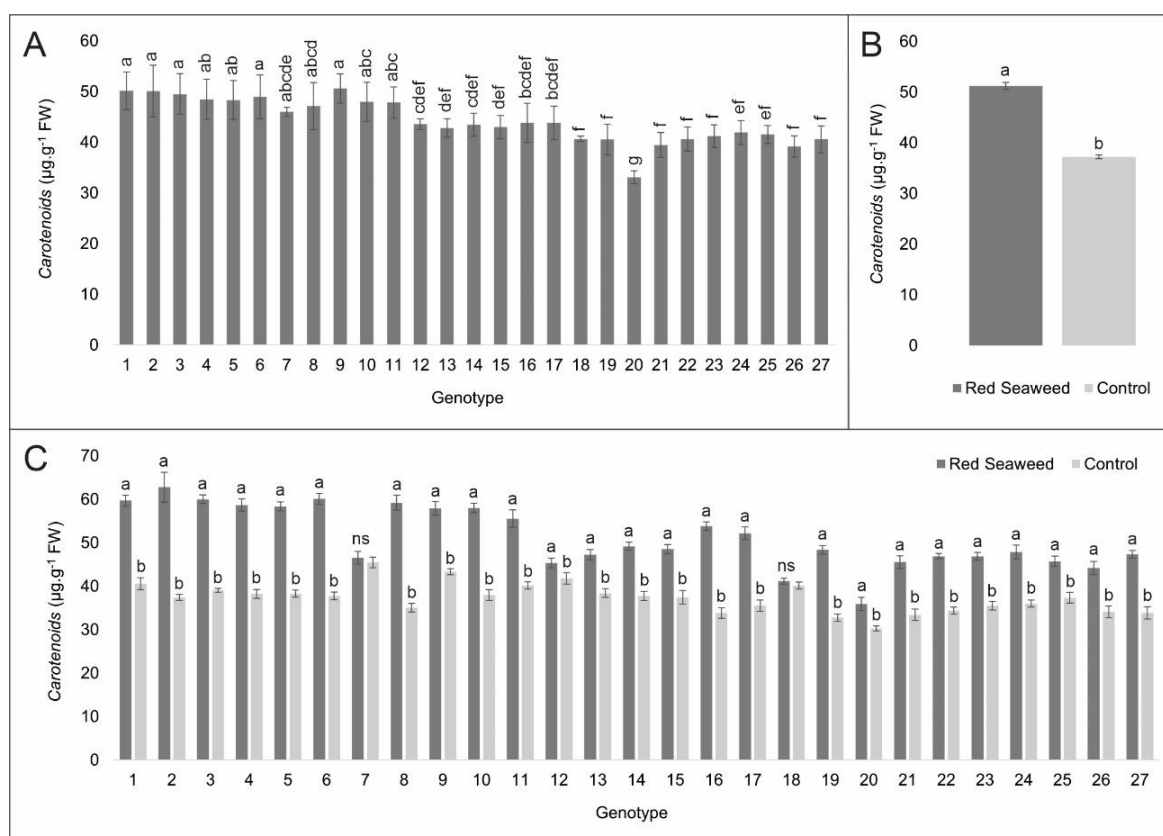
The *Car* content showed an average increase of 37.5% when the biostimulant was sprayed on plants (Figure 3B). However, hybrids 07 and 18 did not exhibit a significant response to the application of the product (Figure 3C). The hybrid that exhibited a lowest content of *Car* in comparison with the others was hybrid 20 (Figure 3A), yet, it was responsive to the application of the red seaweed bioproduct (Figure 3C).

### Yield

Following a pattern similar to that observed for the photosynthetic pigments, yield was significantly influenced by both factors, the hybrid type and the application of the biostimulant, as illustrated in Table 4.

To elucidate the distinctions among the means of each treatment, Tukey's HSD test was employed, and the analysis is presented in Figure 4. The hybrids 03, 07, 16,

and 23 were the only genotypes that did not exhibit a response to the application of the red seaweed biostimulant (Figure 4C). However, hybrids 03, 16, and 23 did respond positively to the application of the biostimulant regarding the analyzed photosynthetic pigments (Figures 1, 2 and 3). On average, an increase of approximately 25% in yield was observed (Figure 4B), with yield values ranging from 4.47 to 11.0 t ha<sup>-1</sup>.



**Figure 3.** Mean Carotenoids (µg.g<sup>-1</sup> of fresh weight) according to the hybrids (A), the use of the red seaweed biostimulant (B), and the interaction between the hybrid and the use of the red seaweed biostimulant (C). Different letters indicate statistical difference according to Tukey's HSD at 5%. Bars represent the standard error. \*ns: not significant.

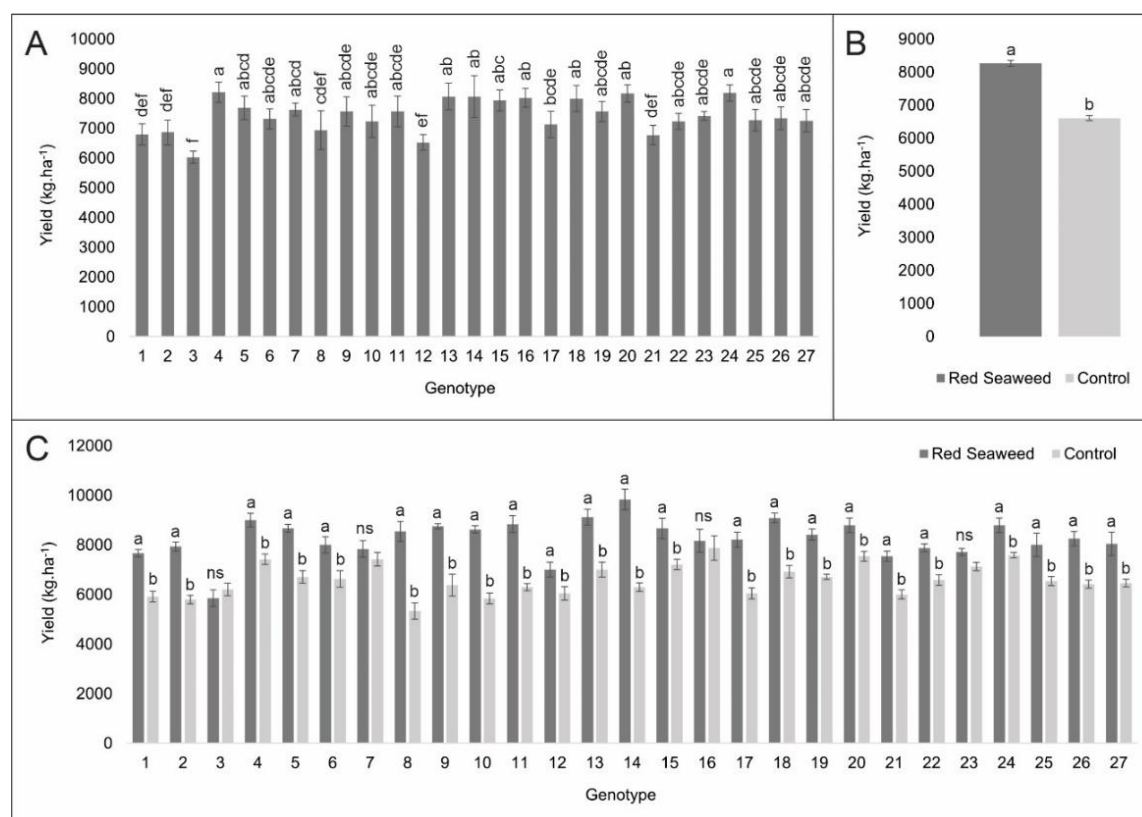
**Table 4.** ANOVA for the quantification of yield in maize plants treated with a sole application of a biostimulant based on *Soleria chordalis*.

Variable analyzed	Source of variation	Degrees of freedom	Sum of squares	Mean squares	F value	p value
Yield	Hybrid (H)	26	17938.40	689.93	8.05	<0.0001
	Biostimulant (B)	1	41588.64	41588.64	485.35	<0.0001
	Interaction H x B	26	10780.25	414.62	4.84	<0.0001

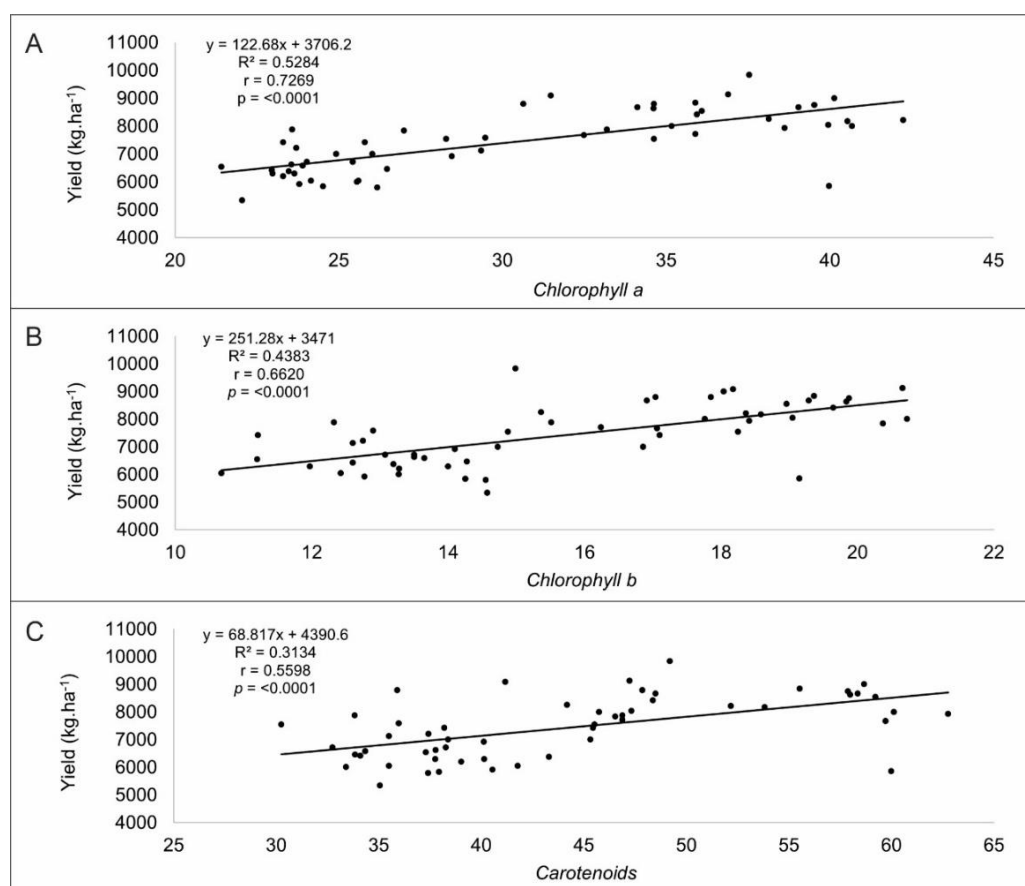
## Correlations

To assess the influence of photosynthetic pigments on yield, Pearson's correlations were conducted for the parameters analyzed. These correlations are depicted in Figures 5ABC. All photosynthetic pigments exhibited significant correlations with yield. *Chl a* showed the highest correlation at 72.6%, followed by *Chl b* at 66.2%, and *Car* at 55.9%.





**Figure 4.** Mean yield (kg.ha<sup>-1</sup>) according to the hybrids (A), the use of the red seaweed biostimulant (B), and the interaction between the hybrid and the use of the red seaweed biostimulant (C). Different letters indicate statistical difference according to Tukey's HSD at 5%. Bars represent the standard error. \*ns: not significant.



**Figure 5.** Correlations between "Yield x Chlorophyll a" (A), "Yield x Chlorophyll b" (B), "Yield x Carotenoids" (C).  $R^2$  = Coefficient of determination,  $r$  = Pearson's correlation,  $p$  = correlation significance.



## DISCUSSION

With the ongoing climatic variations induced by global warming, crops are encountering increasingly significant challenges (stresses) throughout their growth cycles. These stresses pose a threat to the sustainability of crop production, as the utilization of agricultural inputs rises while crops may experience substantial yield reductions ranging from 7% to 29% in attempts to adapt to these challenges and ensure survival (AHMAD et al. 2022, REZAEI et al. 2023).

Maize has been a focal point of intensive research endeavors aimed at identifying strategies to maintain or even enhance productivity under projected climate changes (SADDIQUE et al. 2020). AHMAD et al. (2020) demonstrated that climate adaptations could potentially elevate maize productivity by 12% to 17%, while SADDIQUE et al. (2020) reported increases of up to 31%, depending on the specific strategy employed.

Temperature greatly impacts the development of maize plants. SUNOJ et al. (2016) showed that a lower diurnal temperature amplitude increased leaf night respiration and considerably reduced carbohydrate and biomass accumulation. As demonstrated by these authors, a thermal amplitude lower than 2 °C and higher than 18 °C negatively impacts the vegetative growth of maize and might cause large yield reductions. As demonstrated in Table 2, the thermal amplitude found during the experiment may have greatly affected yields of untreated plants compared to the treated ones.

The use of biostimulants represents a strategy that farmers can employ to mitigate crop damage, alleviate stresses, and potentially enhance yield. As elucidated by DUCATTI et al. (2024), biostimulants encompass any substances or stimuli, including physical factors such as touch and sound, capable of directly or indirectly stimulating the primary and specialized metabolism of plants, thereby influencing their development and outcomes. Seaweed extracts are among the substances exhibiting biostimulating properties.

As evidenced by SHUKLA et al. (2021), seaweed-based products significantly impact the defense and growth mechanisms of various commercially cultivated crops. These mechanisms confer enhanced resilience against drought and elevated temperatures (SHUKLA et al. 2018, JACOMASSI et al. 2022, REPKE et al. 2022).

Indeed, seaweed extracts possess the capability to directly influence the synthesis of defense compounds such as chaperones, heat-shock proteins, antioxidant enzymes, non-enzymatic metabolites, and proteins involved in enhancing resistance to biotic and abiotic stresses (ALI et al. 2021). However, it has also been demonstrated that these extracts can augment the activity of Photosystem II, the Krebs cycle, and the Calvin cycle by enhancing the activity of the Ribulose-1,5-bisphosphate carboxylase/oxygenase enzyme (RuBisCO). Additionally, seaweed extracts promote the synthesis of polyphenolic and terpenoid compounds, which play pivotal roles in plant development and primary metabolism (DUCATTI et al. 2021a, MELO et al. 2023).

Chlorophylls, carotenoids, and other compounds involved in photosynthesis represent examples of terpenoids produced by plants (PICHERSKY & RAGUSO 2016). The significance of these compounds for plant growth and development is closely tied to their roles in absorbing photons and transferring electrons to the

production of ATP and NADPH required for the light-independent stage of photosynthesis (MARTINS et al. 2023).

*Chlorophyll a* primarily absorbs light in the red-blue spectrum (430 – 662 nm) and stands as the most prevalent and abundant form of chlorophyll found in plants (LI et al. 2018). Conversely, *Chlorophyll b* absorbs light in the blue-green spectrum (453 – 642 nm) and serves primarily to shield *Chlorophyll a* from excessive light (MARTINS et al. 2023).

Similarly, carotenoids absorb photons in the blue-green spectrum (400 – 550 nm) to augment the antenna system of plants and enhance photosynthetic efficiency. Moreover, under conditions of excessive light, these molecules are crucial for shielding chlorophylls from photodegradation by absorbing electrons from highly excited triplet-chlorophylls, thus preventing the generation of ROS and mitigating chlorophyll degradation (SWAPNIL et al. 2021). Additionally, carotenoids function as thermal energy dissipation molecules, aiding in the regulation of plant temperature (HEBER et al. 2006). Seaweed extracts contribute to augmenting the total content of chlorophyll and carotenoids in plants, as evidenced by the findings of the present study. This enhancement enables plants to better withstand elevated temperatures and excessive light while improving their efficiency in converting CO<sub>2</sub> and water into ATP and NADPH (MARTINS et al. 2023).

JANNIN et al. (2013) had previously demonstrated the potential of seaweed extracts from *Ascophyllum nodosum* in increasing the total chlorophyll content in *Brassica napus*. Furthermore, other studies have also showcased the effects of various seaweed species in elevating the total chlorophyll content in diverse commercially cultivated crops such as wheat (*Triticum aestivum*), beans (*Phaseolus vulgaris*), and tomato (*Solanum lycopersicum*) (LATIQUE et al. 2013, CARRASCO-GIL et al. 2021, DUCATTI et al. 2023a, DUCATTI et al. 2024).

The positive effects observed across most hybrids regarding their yield can be attributed to the beneficial impact of the application of the red seaweed-based bioproduct (*Solieria chordalis*) in increasing the total content of chlorophylls and carotenoids. This elevation in photosynthetic pigments facilitated a heightened uptake and conversion of CO<sub>2</sub> and water into ATP and NADPH, which are essential for sugar synthesis in the Calvin cycle. Furthermore, the increased carotenoid content facilitated greater heat dissipation and protection of chlorophyll against photodegradation. These factors collectively contributed to the enhanced productivity of maize, as depicted in Figure 5ABC.

As can be observed in Table 2, the second harvest season of 2023 (February to July) had an accumulated rainfall of approximately 800 mm, most of it accumulated in the vegetative and initial reproductive phase of the hybrids tested. According to EMBRAPA (2006) the water needs for maize production falls between 400 to 700 mm during their whole cycle. On the other hand, COSTA et al. (2021b) states that the water footprint for maize production in the Brazilian “Cerrado” is of, on average, 512 mm for a production of 10 t.ha<sup>-1</sup>. Therefore, water was not a big factor negatively influencing yields for the 2023 maize second harvest season.

## CONCLUSION

The use of the red seaweed bioproduct in a single application was effective in helping plants to increase their photosynthetic pigments, hence, protecting them against high temperatures. It is believed that the water-use-efficiency increased due to higher amounts of photosynthetic pigments, which would make plants less sensitive to dry spells. In turn, a yield increase of 25% was observed.

## AUTHOR CONTRIBUTIONS

Conceptualization, methodology, and formal analysis, **VSC, KDS, PHG and RDBD**; software, validation, and investigation, **VSC, KDS and PHG**; resources and data curation, **ARR and VSC**; writing-original draft preparation, **KDS and PHG**; writing-review and editing, **RDBD**; visualization, **RDBD**; supervision and project administration, **ARR**. All authors have read and agreed to the published version of the manuscript.

## INSTITUTIONAL REVIEW BOARD STATEMENT

Not applicable for studies not involving humans or animals.

## INFORMED CONSENT STATEMENT

Not applicable because this study did not involve humans.

## DATA AVAILABILITY STATEMENT

The data can be made available under request.

## CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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