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RESEARCH

Ascophyllum nodosum-derived biostimulant promotes physiological conditioning to increase soybean yield in a semiarid climate

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Abstract

The use of algae-based biostimulants as biopromoters for the growth, flowering, and fruiting phases has been intensified in recent years, despite a lack of information about the ideal quantity for maximum efficiency by the plant. This study aimed to select the concentration and application timing of *Ascophyllum nodosum*-derived biostimulant capable of modulating the physiology and increasing the yield of soybean plants from a semiarid region. The trials were carried out in a greenhouse by applying leaf spraying of biostimulant at 0, 0.5, 1.0 and 1.5 L ha^{-1}, as a single or double application, in different developmental stages (V4, V4 + R1, R1, R1 + R4) of Extrema soybean cultivar. Growth, water content, pollen viability, gas exchange and grain yield were investigated. The biostimulant promoted a better physiological conditioning and productive responses, depending on the dose and application timing. *Ascophyllum nodosum*-based biostimulant at 1.5 L ha^{-1}, applied 0.75 L ha^{-1} at V4 + 0.75 L ha^{-1} at R1 (T12), was the most significant treatment, improving the net photosynthesis, water status and plant growth, which resulted in a 12% increase in grain yield. In conclusion, the dosage and timing of application play pivotal roles in eliciting physiological and productive responses through biostimulants. Seaweed-based biostimulants emerge as essential components to optimizing the cultivation and yield of soybean plants, serving as efficient and sustainable biological regulators.

Keywords Biostimulant · *Glycine max* · Growth regulators · Plant performance · Semiarid region · Seaweed extract

Introduction

Soybean is an important leguminous crop widely cultivated in countries like Brazil, United States, Argentina and China, corresponding to the largest producers responsible for over 86% of global production in 2022/23 season (AGROSTAT 2022; USDA 2023). Brazil is the world's largest soybean producer, with a planted area exceeding 43 million ha (CONAB 2023; USDA 2023). The crop is commonly cultivated in semiarid regions and is relatively susceptible to environmental stress.

In last decades, climate change has led to significant alterations in environmental conditions worldwide, such as temperature and precipitation patterns (Malhi, et al. 2021). Environmental stresses such as drought and high temperatures have affected arable areas and decreased the yield of numerous plant species (Leite-Filho et al. 2021; Shahzad et al. 2021). The situation is particularly challenging for the soybean crop due to severe damage to morphological, physiological, and biochemical pathways, impacting all stages of plant development and yield, resulting in economic losses (Chaudhry and Sidhu 2021; Silva et al. 2021; CONAB 2023; USDA 2023).

The increasing demand for techniques to mitigate the damage caused by climate variations has forced producers to select cultivars adapted to adverse environments, even if they present lower productivity capacity (Hassan et al. 2021). Biostimulants can constitute an alternative strategy to overcome environmental limitations, given their composition of active principles capable of activating plant metabolic processes (Hasanuzzaman et al. 2018; Hidangmayum et al. 2019; Khan et al. 2021; Raza et al. 2021; Tiwari et al. 2021). They consist of a mixture of plant growth regulators containing numerous chemical compounds, such as

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amino acids, nutrients, polyamines, secondary compounds and several other bioactive compounds (Gandhi et al. 2024; Vaghela et al. 2023). The biostimulants not only contribute to crop yield but can also alleviate the deleterious effects of specific abiotic stresses (Shahrajabian et al. 2021; Cocetta et al. 2022; Gupta et al. 2022; Gandhi et al. 2024).

Numerous studies have evidenced the contributions of biostimulants based on the seaweed *Ascophyllum nodosum* (ANE) to the performance of species under stressful conditions (Shukla and Prithiviraj 2021; Shahzad et al. 2023). In tomato, the biostimulant protected against membrane damage and reactive oxygen species (ROS), resulting in reduced oxidative stress and improved photochemical performance under drought and salt stress (Hernández-Herrera et al. 2022; Villa et al. 2023). In stressed-watermelon plants, the biostimulants supported the root system, leaf biomass, and increased leaf number (Bantis and Koukounaras 2022). In soybean, ANE-biostimulants were able to improve photosynthetic efficiency by dissipating energy excess and increasing the enzymatic antioxidant system under drought (Rosa et al. 2021). ANE-treated stressed plants displayed faster rehydration associated with elevated water content and stomatal conductance, and enhanced ROS scavenging (Shukla et al. 2018).

There are no data on ANE-biostimulants improving performance of soybean cultivars from semiarid regions, particularly regarding the timing of application and sampling procedures, which are crucial factors in eliciting positive responses in plants (Andreotti et al. 2022; Ali et al. 2023). These biostimulants aspects become critical in production of crops from semiarid regions that utilize cultivars with diverse levels of resilience and productive potential.

Our working hypothesis was that the *Ascophyllum nodosum*-based biostimulant at specific dosage level and application time improves the physiological and productive performance of soybean plants. This hypothesis was tested by cultivating a semiarid-adapted soybean cultivar (Extrema) using three ANE-biostimulant dosage levels applied at three distinct development stages. The photosynthetic performance, water status, growth and grain yield trials were assessed in different stages of plant development.

Material and methods

Study site, experimental conditions and treatments

The experiment was carried out in a greenhouse at the Federal University of Piauí (UFPI), Campus Professora Cinobelina Elvas, Bom Jesus, Piauí, Brazil (located at 9°05′02.6"S, 44°19′32.8"W, and 277 m above sea level), between June and September 2022. During the trials, temperature and relative humidity inside the greenhouse were monitored using a digital thermo-hygrometer. Figure 1 shows the

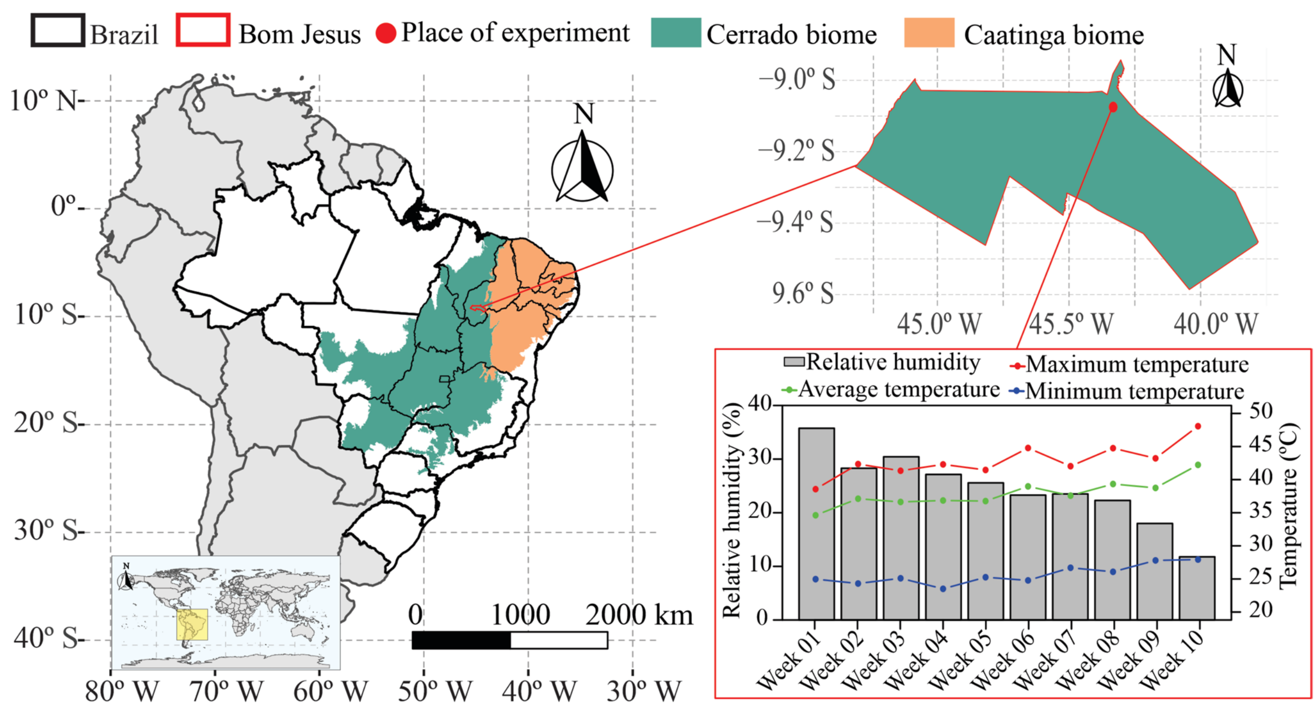

Fig. 1 Coordinates of greenhouse location and dynamics of mean, maximum and minimum temperature, and air relative humidity during the trials with soybean plants (Extrema cultivar) from June to September 2022

environmental data during the study, encompassing the highest temperature peaks and low air humidity, characteristic of semiarid regions.

During the trials, soil samples of typical dystrophic Yellow Latosol from the UFPI experimental area were collected from the 0–0.20 m layer and analysed (Online Resource 1). The soil was corrected according to the recommendations for the cerrado soil fertilization (Sousa and Lobato 2004), and thereafter used to fill plastic pots of 11 dm^3. Sowing was done adding five seeds from Extrema cultivar per pot. Thinning procedures were carried out at 7 and 14 days after sowing to maintain only one plant per pot. Irrigation management was conducted daily using the pot weighing method (Veihmeyer and Hendrickson 1931), with water replacement performed according to the amount required to reach 60% of field capacity (FC) during the vegetative stage and 80% FC during the reproductive stage (Miranda et al. 2023).

ANE biostimulant containing the PSI-494 biomolecule complex was provided by Brandon Bioscience (Tralee, Ireland). The ANE was manufactured using a proprietary process under high temperatures and alkaline conditions, and the composition was the same cited in Carmody et al. (2020): ash (35.81 ± 0.87% w/w); total carbohydrates (63.52 ± 0.55% w/w); polyphenols (0.55 ± 0.06% w/w); other organic compounds (0.12 ± 0.03% w/w); and low macronutrient content with N (0.3–0.4% w/w), P (0.1–0.2% w/w) and K (2–3% w/w).

For all treatments, 2.0 mL diluted solution containing the biostimulant was sprayed on the leaves of each plant, taking into account the crop stage (Vegetative 4 – V4, Reproductive 1 – R1, Reproductive 4 – R4), application timing and dose considering a population of 220,000 plants ha^{-1} and a spray volume of 120 L ha^{-1} in the field (Fig. 2). The application volume of 2.0 mL per plant was determined based on preliminary tests.

The design was completely randomized, with sixteen treatments and four replications. The treatments consisted of a combination of the biostimulant dose (0.5, 1.0 and 1.5 L ha^{-1}) and application timings, which occurred in a single application or double applications depending on the crop development

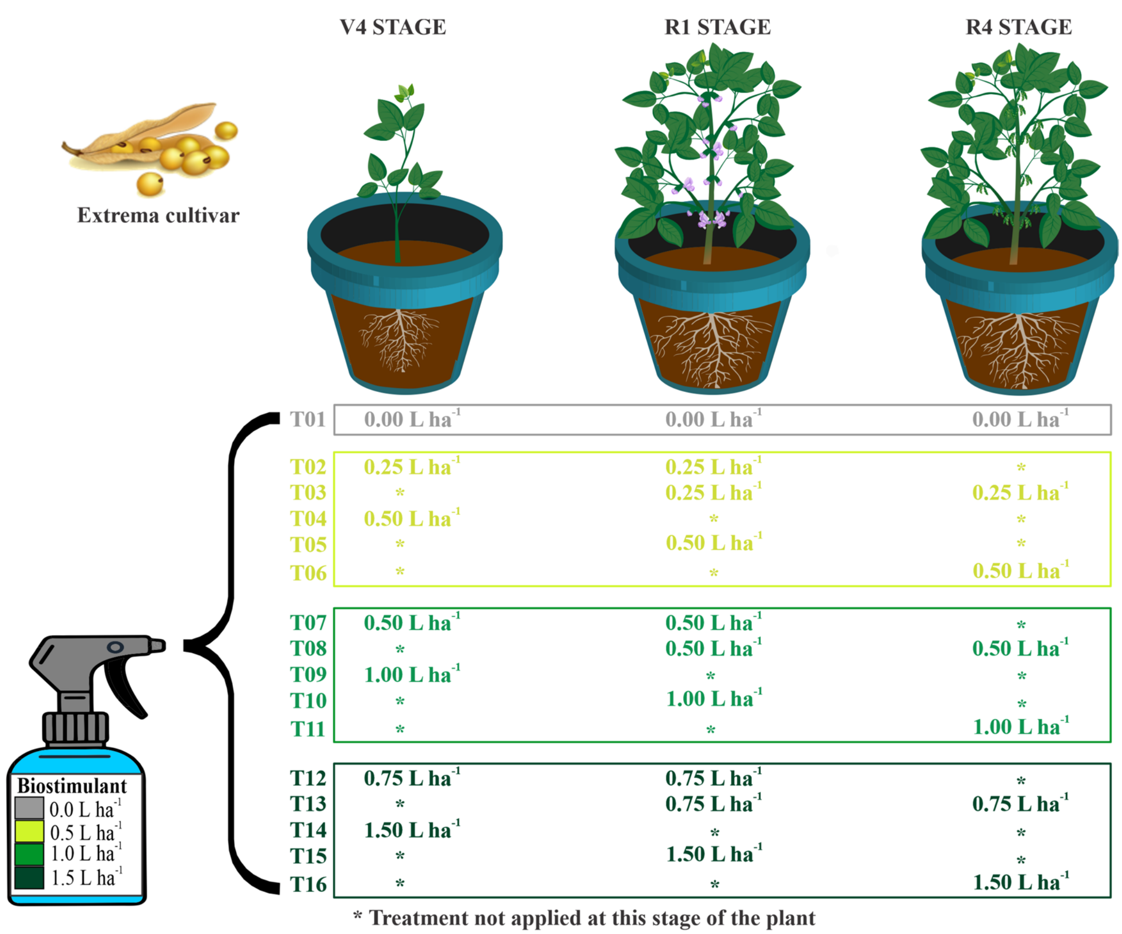

Fig. 2 Summary of *Ascophyllum nodosum*-based biostimulant treatments on different stages of soybean crop (Extrema cultivar) under greenhouse conditions. The plants from T01 were sprayed with distilled water, constituting the negative control. The biostimulant was applied at doses 0, 0.5, 1.0 and 1.5 L ha^{-1} when the plants achieve the stages V4, R1 and R4

stage (V4, V4-R1, R1, R1-R4, and R4) (Fig. 2). The applications were made at 30 (V4), 40 (R1) and 60 days (R4) after sowing (DAS). A group of plants was sprayed with distilled water (2.0 mL per plant), constituting the control treatment (T1). Registers of entire experiment are documented by photos (Online Resource 2).

The assays were performed in specific time-points when the plants reached the V4, R2, R4, R6 and/or R8 stages, as described in Table 1. The time-point were defined corresponding to seven days after biostimulant applications at V4, R1, and R4 (as detailed in Fig. 2), or during later reproductive stage and yield trials.

Photosynthetic parameters

Gas exchange measurements were conducted on the first fully expanded leaf using an infrared gas analyzer (IRGA, Model GFS3000; Walz) between 8:30 and 10:30 a.m. on completely sunny days. The assays of net photosynthesis (*A*), stomatal conductance (gs), and transpiration (*E*) were performed with a photosynthetic photon flux density (PPFD) of 1,000 μmol photons m^{-2} s^{-1} and an internal CO_2 concentration of 400 ppm.

Water status

The relative water content (RWC) was measured at V4, R4 and R6 stages. A total of 10 discs with a diameter of 1.0 cm^2 were extracted from fully expanded leaves of each treatment. Initially, the fresh weight (FW) of the discs was measured and then the samples were immersed in distilled water for 24 h to determine the turgid weight (TW). The material was then placed in an oven at 60 °C for 72 h to obtain the dry weight (DW), and the RWC was calculated following Čatský (1960), using the following formula:

$$RWC(\%) = \frac{(FW - DW)}{(TW - DW)} \times 100$$

Biometric measurements and yield

The plant height was measured in the R2 reproductive stage, post-application of the biostimulant at R1 stage, from the first visible internode above the ground to the last node of the apex, using a measuring tape. The number of flowers and pods were counted manually.

Soybean yield was estimated at the end of the experiment (R8), 94 days after planting, in kilogram per hectare (kg ha^{-1}), according to the formula:

$$\text{Yield (kg ha}^{-1}) = \frac{220{,}000 \times NPP \times NGP \times WTG}{1000}$$

where *NPP* is number of pods per plant, *NGP* is number of grains per plant, and *WTG* is weight of one thousand grains.

Pollen viability

To verify the influence of the biostimulant on plant fertility, four floral buds at R2 stage were collected during the pre-anthesis stage, between 9:00 and 10:00 a.m., in each replication. The material was used to prepare slides using the anther crushing technique (Guerra and Souza 2002). The identification of viable pollen was performed using the Alexander reactive stain (Alexander 1980). In each slide, 200 pollen grains were counted using an optical microscope with a 40 × objective. Pollen grains with purple coloring were classified as viable, while those without internal coloring were considered non-viable. The percentage of viable pollen (VP) was determined by the equation:

$$VP(\%) = \frac{N^{\underline{o}} \text{ of viable pollen}}{200} \times 100$$

Statistical analyses

Attending the normality and homogeneity, the data were subjected to analysis of variance, followed by mean comparison using the Scott-Knott test ($p \leq 0.05$) (Borges and Ferreira 2003). To provide a comprehensive overview, the data were initially standardized (Cao et al. 1999) and then a principal component analysis (PCA) was performed to characterize the variables that most discriminated the structural characteristics in each treatment (Hair Junior et al. 2009). Parametric tests, figures and PCA were performed using the statistical software R (R Core Team 2023).

Table 1 Time-point of physiological and yield assays in greenhouse experiments with soybean plants treated with extract of *Ascophyllum nodosum* (ANE-biostimulants) in different vegetative and reproductive stages as detailed in Fig. 2. DAS – days after sowing

Biostimulant application		Analysis time-point		
DAS	Stage	DAS	Stage	Assay
30	V4	37	V4	Gas exchange and RWC
40	R1	47	R2	Plant height, Number of flowers and Pollen viability
60	R4	67	R4	Gas exchange, RWC and Number of pods
-	-	74	R6	Gas exchange, RWC and Number of pods
-	Harvest	94	R8	Yield trials

Results

Physiological responses to biostimulant treatments during V4 stage

The net photosynthesis of soybean plants remained unaltered by biostimulant treatment in a double application manner during stages V4-R1 (T02) and single application at 1.0 (T09) and 1.5 L ha^{-1} (T14) at V4; however, it was reduced in treatments with a single dose (T04) or double application (T07 and T12) (Fig. 3a). At the evaluation time (stage V4), only 50% of the dose was applied for treatments T07 and T12 (equivalent to 0.5, and 0.75 L ha^{-1}, respectively), while a complete dose (0.5 L ha^{-1}) was applied for treatment T04 (Fig. 2).

The biostimulant at rates of 1.0 and 1.5 L ha^{-1} (T09 and T14, respectively) significantly increased the transpiration rate of plants compared to the other treatments (Fig. 3b). In contrast, the application of 0.5 to 0.75 L ha^{-1} at V4 [T04, T07 (first application rate at V4 time-point) and T12 (first rate at V4)] significantly reduced the stomatal conductance (gs) of soybean plants compared to the control, whereas the application of 1.5 L ha^{-1} (T14) resulted in an increased gs at the V4 stage (Fig. 3c).

Biostimulant at 0.5 (T2 and T4) and 1.0 L ha^{-1} (T7 and T9) reduced the relative water content (RWC) compared to control plants, while the dose of 1.5 L ha^{-1} (T12 and T14) did not significantly alter the RWC of plants at vegetative stage 4 (Fig. 3d). The intrinsic water use efficiency (*A/gs*) was higher in T04 and T12, surpassing all other treatments and the control; whereas the instantaneous water use efficiency (*A/E*) did not exhibit statistical differences (Fig. 3e and f).

Reproductive and biometric responses of biostimulant application at R2 stage

At R2 stage, the biostimulant significantly decreased the number of flowers, with the more prominent effects in a single application at 0.5 (T4) and 1.5 L ha^{-1} (T15) treatments, except for T13 plants as compared to the control (Fig. 4a).

Although the biostimulant application promoted negative effects in the number of flowers (T04 and T15), in most cases it promoted a higher pollen viability to the remaining flowers compared to the control, reaching values equal to or higher than 99% (T04, T07, T08, T14, and T15). Thus, except for T2, biostimulant application significantly increased pollen viability in soybean plants, regardless of the dose and application timing, as compared to the control (Fig. 4b). In general, soybean plant height at the R2 stage was slightly stimulated by treatments T05, T10, T13, and T15, while the remaining treatments did not result in any significant alteration compared to the control (Fig. 4c).

Physiological performance and number of pods at R4 and R6 stage

At R4 stage, net photosynthesis was not changed by biostimulant application but there was a reduction in plants from treatments T05, T06, T07, T08, T09, T10, and T16 compared to the control T01 (Fig. 5a). In contrast, transpiration rate was increased in plants treated with biostimulant at T03, T04, T07, T08, T09, T10, and T15 compared to the control (Fig. 5b), but no significant changes were observed in stomatal conductance (Fig. 5c).

Soybean plants at the R4 stage exhibited a higher relative water content under biostimulant treatment at 1.0 (T11) and 1.5 L ha^{-1} (T12 and T15) in the R4 and R1 stages, respectively, as compared to the control (Fig. 5d). Conversely, soybean plants had a higher number of pods when the biostimulant was applied at 1.5 L ha^{-1} in double application at V4-R1 (T12) or R1-R4 (T13), not differing from T02 and T05 but surpassing the control and other treatments (Fig. 5e).

The intrinsic and instantaneous water use efficiencies of biostimulant-treated plants were similar or little reduced as compared to the control treatments. The decreases were statistically significant in plants from 1.0 L ha^{-1} dose, except for T11 (Fig. 5g and f).

At the R6 stage, soybean plants showed elevated rates of net photosynthesis when the biostimulant was applied as T04, T09, T10, and T14, as compared to control and other treatments (Fig. 6a). In contrast, transpiration and stomatal conductance rates were stimulated in plants from treatments T04, T05, T06, T07, T09, T10, T14, and T16 compared to the control, except for stomatal conductance in T06 (Fig. 6b and c).

In general, except for 1.5 L ha^{-1} dose, the biostimulant significantly increased the relative water content of soybean plants compared to the control (T01), with particular emphasis on treatment T07 (Fig. 6d). In contrast, the number of pods in soybean plants either remained the same or was reduced compared to the control treatment when the biostimulant was applied at 1.0 (V4-R1) or 1.5 L ha^{-1} (R1-R4 or R1) (Fig. 6e). Intrinsic and instantaneous water use efficiency did not exhibit significant changes at this stage (Fig. 6f and g).

Soybean yield

The biostimulant application at 0.5 (T02, T03, T04), 1.0 (T07, T08, T11), and 1.5 L ha^{-1} (T12, T13, T14, T15) promoted significant increase in the number of grains per pod,

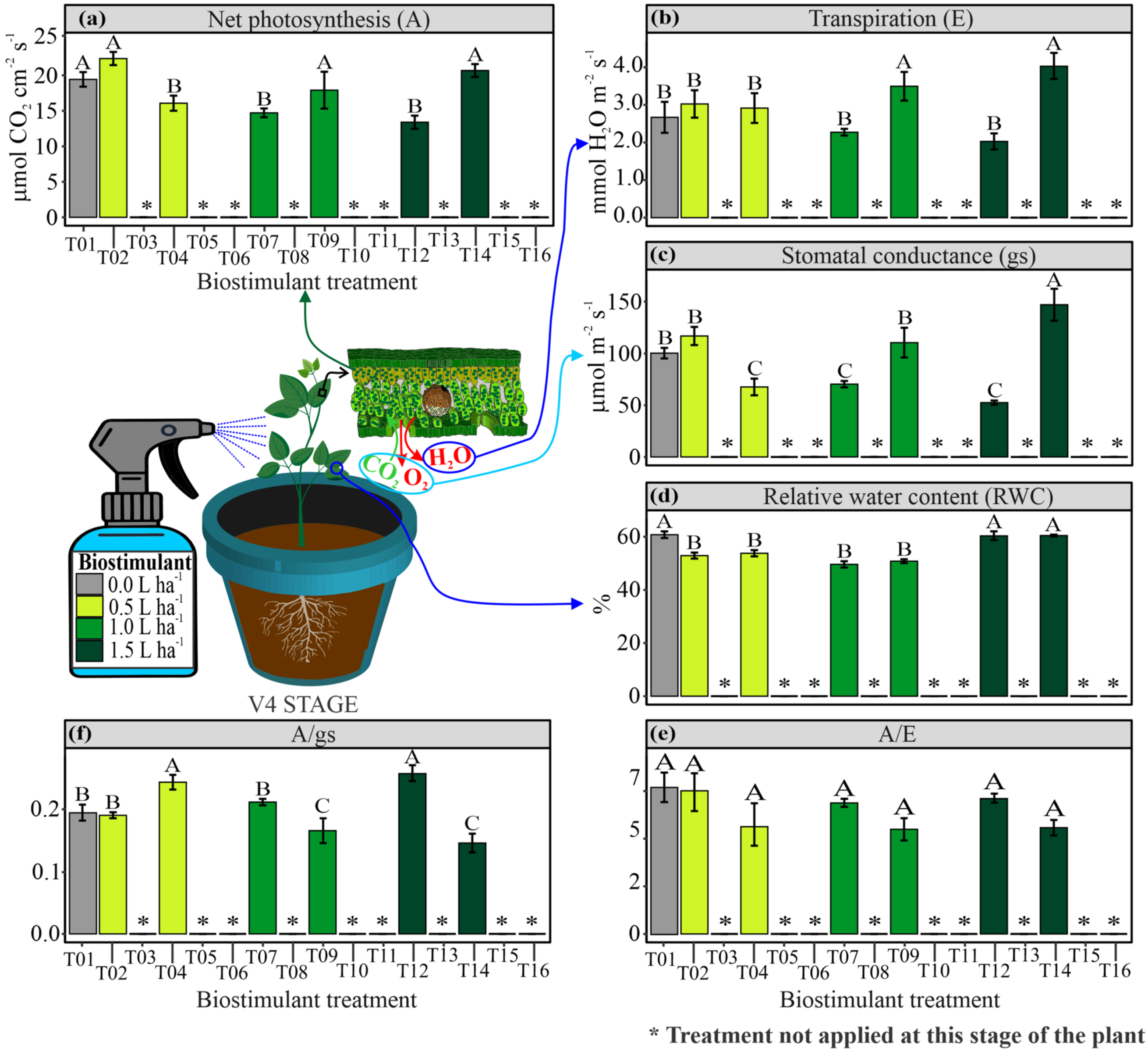

Fig. 3 Physiological parameters of soybean plants at V4 stage in response to different dosage of biostimulant. **a** Net photosynthesis-*A*. **b** Transpiration-*E*. **c** Stomatal conductance –g.s. **d** Relative water content-RWC. **e** Instantaneous water use efficiency -*A/E*. **f** Intrinsic water use efficiency -A/gs rate of soybean plants at V4 stage (Extrema cultivar) after application of biostimulant at 0.5, 1.0 and 1.5 L ha^{-1}, as detailed in Fig. 2. The vertical lines represent mean ± error, $n = 4$ (number of independent repetitions). Different uppercase letters represent significant differences due to biostimulant treatments according to the Scott−Knott test ($p < 0.05$). * Treatment was not initiated in the V4-stage time-point according to Fig. 2

while T06 resulted in a significant reduction (Fig. 7a). A similar pattern was observed for grain weight, with plants treated with the biostimulant at 0.5 (T02, T03, T06), 1.0 (T07, T09), and 1.5 L ha^{-1} (T12, T13, T14) showing a significant gain compared to the control (Fig. 7b).

The soybean yield gain as affected by biostimulant treatment was dependent on the dose and application stage (Fig. 7c). The highest yield data were observed only when the biostimulant was applied at 1.5 L ha^{-1} as a double application between the V4-R1 stages (T12), resulting in a value 12% higher than the control.

Principal component analysis (PCA)

Principal Component Analysis (PCA) accounted for 72.38% of the total variation, with 45.01% attributed to component 1 and 27.3% to component 2 (Fig. 8). The results indicated distinct clustering patterns among

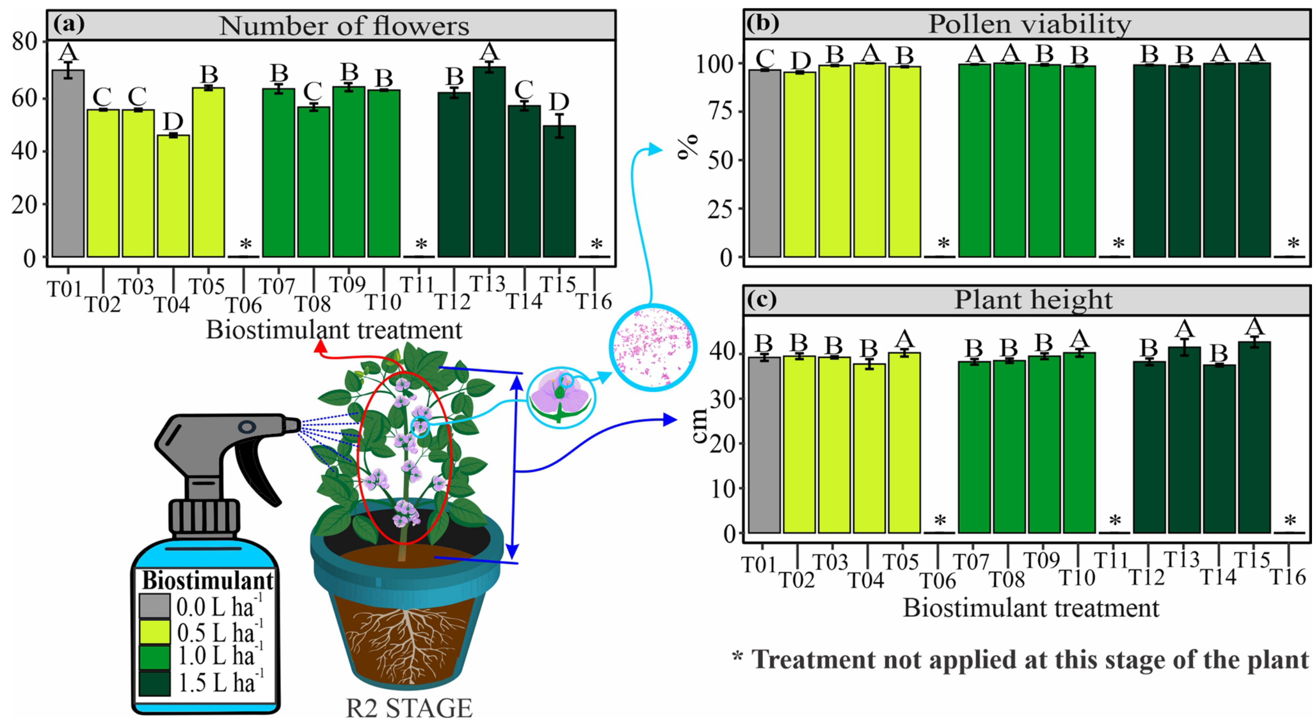

Fig. 4 Morphometrical and pollen viability parameters of soybean plants at R2 stage in response to different dosage of biostimulant. **a** Number of flowers. **b** Plant height. **c** Pollen viability of soybean plants at R2 stage (Extrema genotype) after application of biostimulant at 0.5, 1.0 and 1.5 L ha^{-1}, as detailed in Fig. 2. The vertical lines represent mean ± error, $n = 4$ (number of independent repetitions). Different uppercase letters represent significant differences due to biostimulant treatments according to Scott−Knott test ($p < 0.05$). * Treatment was not initiated in the R2-stage time-point

soybean plant stages, with overlaps observed between application timing and biostimulant dosage.

Regarding PC2, an overlap was noted between group 4 and group 1, where the application of 1.5 L ha^{-1} at V4-R1 or R1-R4, and 0.5 L ha^{-1} (at V4-R1), exhibited stronger correlations with productivity factors and instantaneous water use efficiency across all assessed stages. A higher correlation was observed between these variables and their respective treatments. However, in terms of PC1, these two groups were distinctly separated, displaying variations in the grouped variables.

In group 2, treatments applied solely during the reproductive period (either in a single or double application) clustered together and showed stronger associations with stomatal conductance. Conversely, in group 3, applying all three doses at the V4 stage, along with a 1.0 L ha^{-1} dose at V4-R1, exhibited weaker correlations with productivity, photosynthetic factors, and variables related to flowering. Nonetheless, photosynthetic parameters evaluated at R6 (A, E, RWC, and gs) showed significant influence following application at the V4 stage.

Discussion

Ascophyllum nodosum-based biostimulants are widely marketed globally and their effectiveness may vary depending on the application method, dosage and adopted crop (Online Resource 3) (Deolu-Ajayi et al. 2022). The potential of ANE-based biostimulants in the performance of semiarid-typical soybean cultivars remains to be explored, particularly concerning the optimum dosage and the most effective application stage. The optimal performance of the 95R95IPRO soybean cultivar was observed when the *A. nodosum*-based biostimulant was applied at 1.0 L ha^{-1}, a response associated with higher rates of net photosynthesis and stomatal conductance, even in plants under stress (Repke et al. 2022). Here, soybean plants from semiarid-adapted Extrema cultivar displayed improved physiological conditioning when treated with *A. nodosum*-based biostimulant at 1.0 and 1.5 L ha^{-1}, but the productive performance was improved only by 1.5 L ha^{-1} treatment (Figs. 3, 5, 6, 7 and 8).

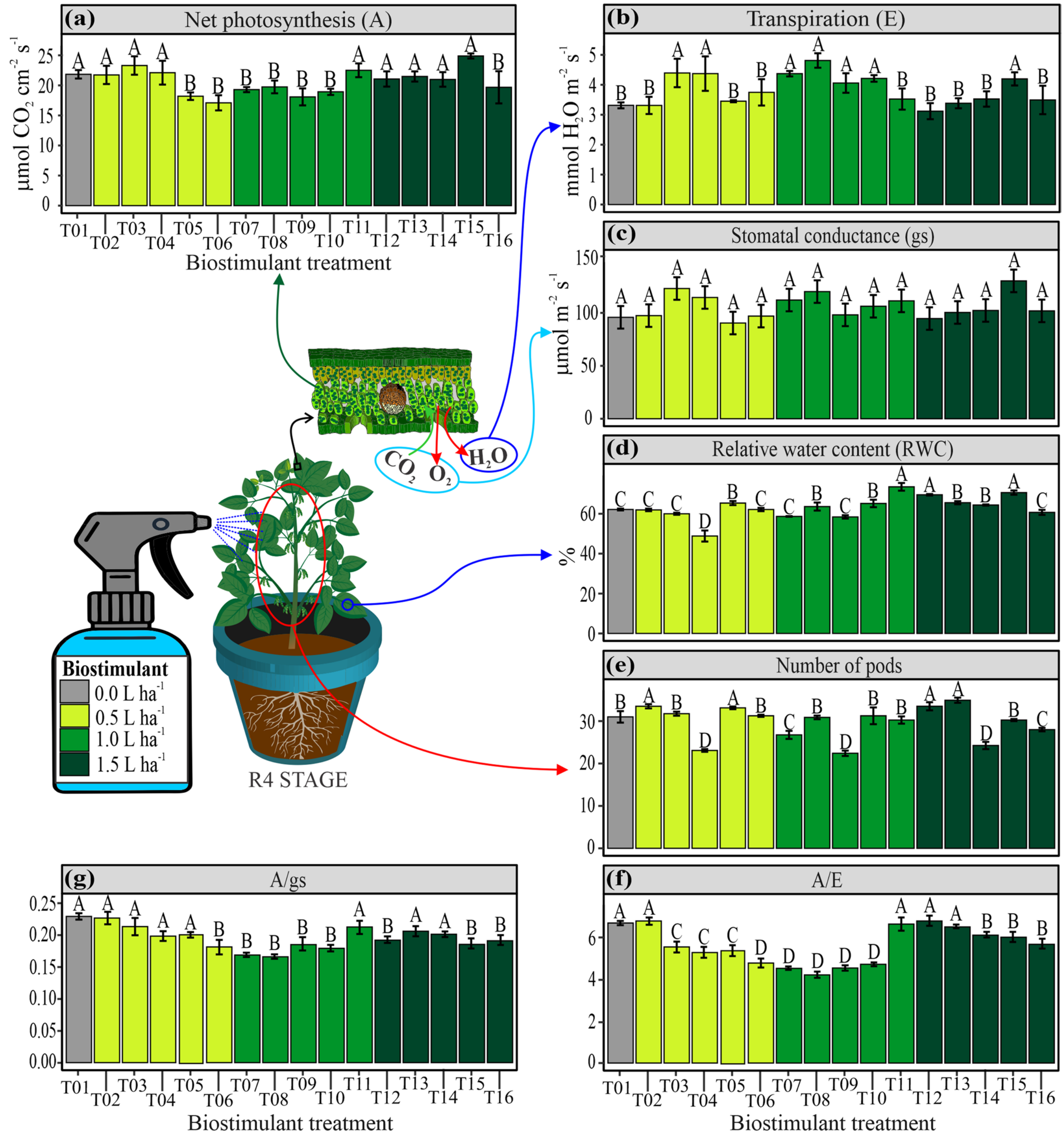

Fig. 5 Physiological and early productive parameters of soybean plants at R4 stage in response to different dosage of biostimulant. **a** Net photosynthesis -*A*. **b** Transpiration -*E*. **c** Stomatal conductance -*g*. **d** Relative water content -RWC. **e** Number of pods. **f** Instantaneous water use efficiency -*A/E*. **g** Intrinsic water use efficiency -A/gs of soybean plants (Extrema cultivar) at R4 stage, after treatments with biostimulant as detailed in Fig. 2. The vertical lines represent mean ± error, $n = 4$ (number of independent repetitions). Different uppercase letters represent significant differences due to biostimulant treatments according to the Scott−Knott test ($p < 0.05$)

At the vegetative stage V4, T14-treated plants exhibited stomatal conductance higher than those from control treatment, which was associated with elevated transpiration rate (Fig. 3). Although a higher instantaneous water use efficiency was not observed, treatments T12 and T14 showed unchanged relative water content, suggesting a restrict control of thermal balance, as transpiration process is determinant for heating dissipation and cooling the leaf

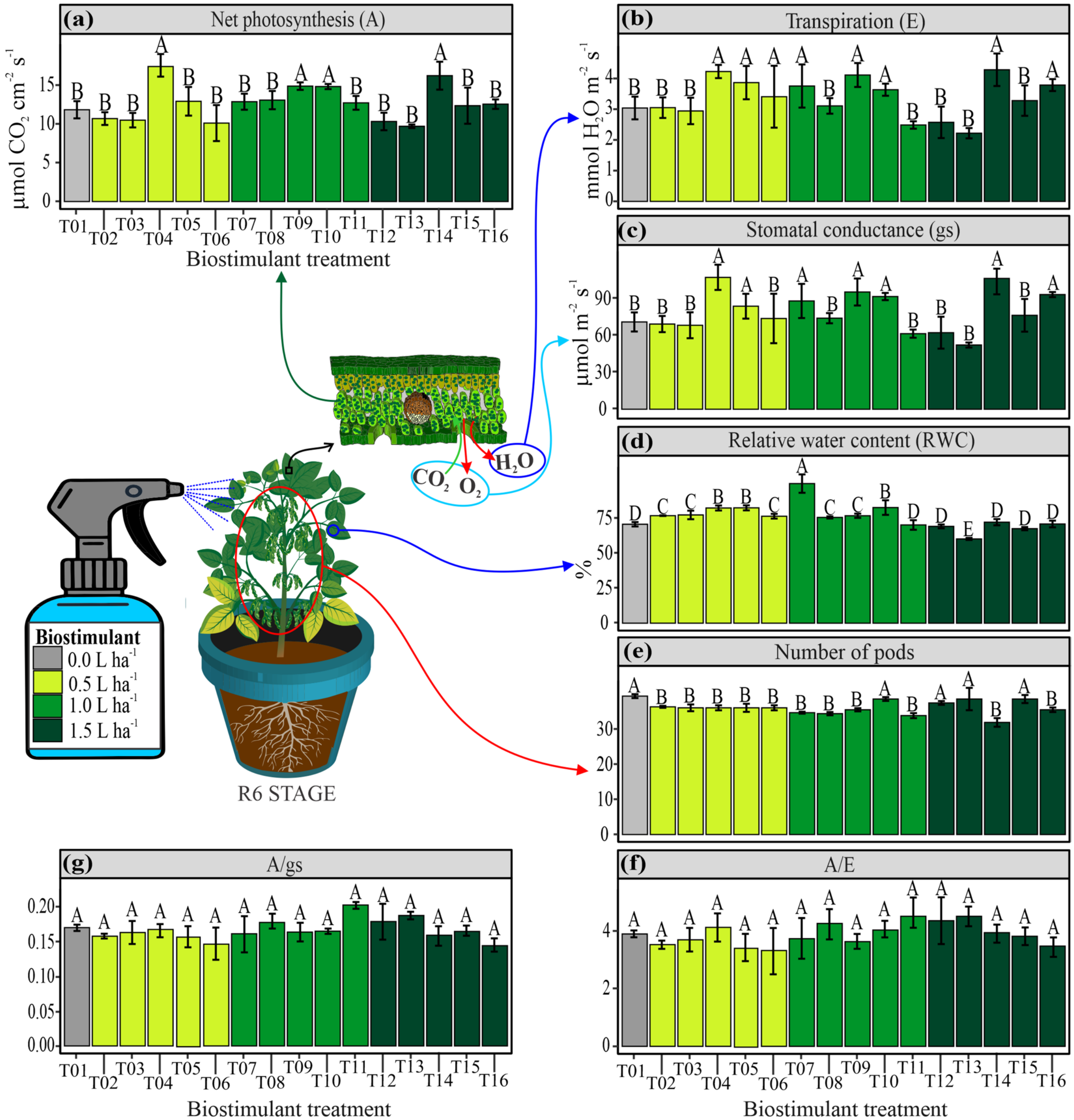

Fig. 6 Physiological and productive parameters of soybean plants at R6 stage in response to different dosage of biostimulant. **a** Net photosynthesis -*A*. **b** Transpiration -*E*. **c** Stomatal conductance -gs. **d** Relative water content -RWC. **e** Number of pods. **f** Instantaneous water use efficiency -A/E. **g** Intrinsic water use efficiency -A/gs of soybean plants, Extrema cultivar, at R6 stage, after treatments with biostimulant as detailed in Fig. 2. The vertical lines represent mean ± error, $n=4$ (number of independent repetitions). Different uppercase letters represent significant differences due to biostimulant treatments according to the Scott−Knott test ($p<0.05$)

(Ranawana et al. 2023). The biostimulant at 1.5 L ha^{-1} also elicited significant responses in the physiological conditioning of soybean plants, as it significantly increased the relative water content (RWC) at the V4 (T12 and T14) and R4 (T11, T12, T15) stages (Figs. 3d and 5d). The results indicated a better balance of water potential, involving tight control of stomatal conductance, which directly influenced the water consumption, transpiration and leaf temperature regulation (Hassan et al. 2020; Liao et al. 2022; Rahimi et al. 2022; Petrík et al. 2023).

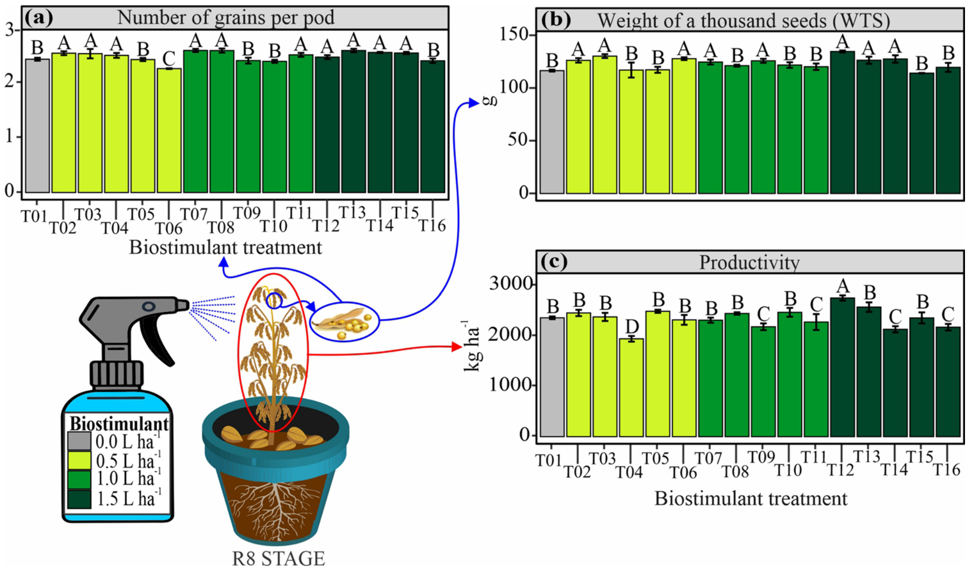

Fig. 7 Productive parameters of soybean plants at R8 stage in response to different dosage of biostimulant. **a** Number of grains per pod. **b** Weight of a thousand seeds. **c** Yield of soybean plants (Extrema genotype) after treatment with biostimulant at 0.5, 1.0 and 1.5 L ha^{-1} in the V4, R1 and R4 stages, as detailed in Fig. 2. The vertical lines represent mean ± error, $n = 4$ (number of independent repetitions). Different uppercase letters represent significant differences due to biostimulant treatments according to the Scott−Knott test ($p < 0.05$)

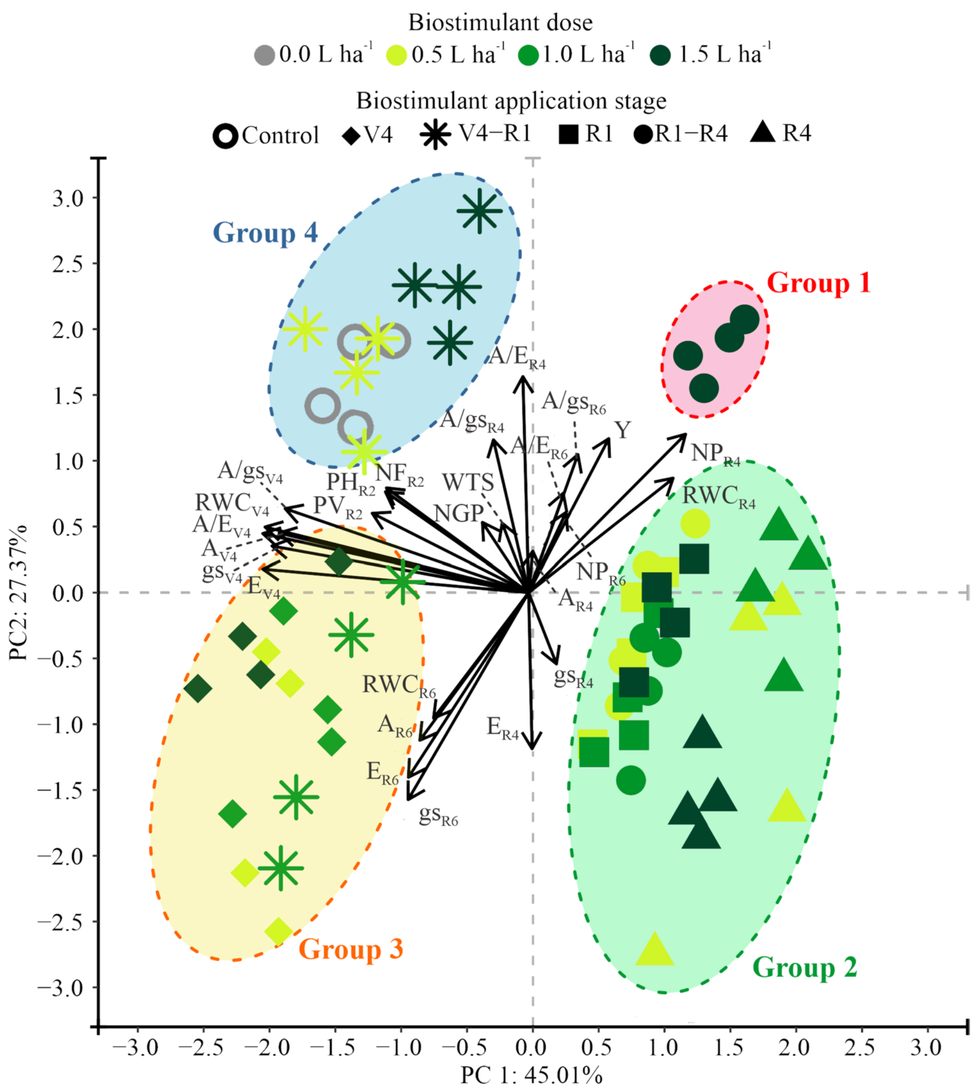

Fig. 8 Principal component analysis (PCA) of the mean parameters of soybean plants (Extrema cultivar) evaluated at different stages (V4, R2, R4, R6 and R8) after application of the biostimulant, as detailed in Fig. 2 and Table 1. PCA was composed by data of net photosynthesis (*A*), transpiration (*E*), stomatal conductance (gs), relative water content (RWC), instantaneous water use efficiency (*A/E*) and intrinsic water use efficiency (A/gs), plant height (H), number of flowers (NF), pollen viability (VP), number of pod (NP), number of grains per pod (NGP), weight of a thousand seeds (*WTS*) and yield (Y). Group 1 consisted of plants treated with 1.5 L ha^{-1} at the R1 to R4 stages. Group 2 comprised plants treated with doses of 0.5, 1.0, and 1.5 L ha^{-1} either as a single dose or applied at the R1 and R4 stages. Group 3 included plants treated at the V4 stage with all doses, with treatment split at the V4 to R1 stage at 1.0 L ha^{-1}. Group 4 was comprised of the negative control (T01) and doses of 0.5 and 1.5 L ha^{-1} rated applied at the V4 and R1 stages

Growth morphology directly impacts flowering and grain yield in soybean cultivation, particularly due to increased pod formation in internodes of plants (Xue et al. 2022). Herein, biostimulant application modulated the relationship between vegetative and reproductive stages. Soybean plants exhibited increased height when treated with the biostimulant at 0.5 (T05), 1.0 (T10), and 1.5 (T13 and T15) L ha^{-1} (Fig. 4c). The height increase was associated with a higher number of flowers in T13 at the R2 stage (Fig. 4a) and a higher number of pods in T13 at R4 (Figs. 5e and 6e). Treatment T12 also stimulated the number of pods at the R4 and R6 stages (Figs. 5e and 6e), but there was no direct relationship with the height of the plants. This phenomenon likely resulted from the timing of the biostimulant application, which was split in T12 (0.75 + 0.75 L ha^{-1}) at the V4 and R1 stages, compared to the double application at the R1 and R4 stages in T13. In several plant species, this response was attributed to fluctuations in gibberellin levels (Mosa et al. 2022; Zhao et al. 2022).

The results also suggest that the induction of the photosynthetic machinery at the R6 stage by the *A. nodosum*-based biostimulant was crucial for plant production. It supplied seeds with assimilates and increased fertilization rates, resulting in a higher number of grains per pod (Figs. 4, 5, 6, 7 and 8). This metabolic adjustment was essential for reserve accumulation in the seeds, leading to an increase in thousand-seed weight (Fig. 7b) and higher plant productivity (Fig. 7c). These findings align with previous studies that emphasized the biostimulant's role in maintaining photosynthetic metabolism at the R6 stage, thereby enhancing plant performance, as observed by Repke et al (2022).

Numerous studies have evidenced that biostimulants play a role in the hydrolysis and biosynthesis of molecules, as well as in the accumulation of reserves during seed formation (Sunmonu et al. 2016), contributing to pod production and plant yield. In this study, soybean plants exhibited a higher number of pods when exposed to 0.5 (T02 and T05) and 1.5 (T12 and T13) L ha^{-1} at the R4 stage (Fig. 5e). The increased pod number was not directly associated with number of flowers, which was even lower than the control (Fig. 4a). These findings suggest that, in these application setups, the biostimulant increased fertilization rates likely due to the rise in viable pollen (Fig. 4b), resulting in a higher number of pods (Fig. 5e), as previously demonstrated by Sun et al. (2022).

The biostimulant applied in a single dose at 0.5 L ha^{-1} in V4 (T04) and at 1.0 L ha^{-1} or 1.5 L ha^{-1} in V4 (T9 and T14) or R4 (T11 and T16) promoted significant decrease in soybean yield (Fig. 7). The low productivity likely occurred due to negative feedback, where the single-dose application during the V4 and R4 stages was insufficient to elicit productive responses in the plants (Shukla et al. 2018; Shahrajabian et al. 2021; Andreotti et al. 2022). In the V4 stage, the activation of physiological responses occurred but did not sustain the production; while the application in R4 stage may have been too late to promote plant response (Andreotti et al. 2022; Mosa et al. 2022). Another explanation was the specific application stage, highlighting that soybean plants seem to exhibit greater sensitivity to biostimulant when spraying during R1 stage. Accordingly, to increase plant height with the biostimulant was under foliar spray at the R1 stage (T05, T10, T13 and T15) (Fig. 7c).

The data reinforce recent findings that the algae-based biostimulants modulate soybean yield depending on dosage and application timing (Repke et al. 2022). Here, a typical semiarid cultivar displayed the highest yield treated in a double application at different stages, at a rate of 1.5 L ha^{-1} (Fig. 7c), specifically in the form of T12 (0.75 L ha^{-1} at V4 + 0.75 L ha^{-1} at R1), with productivity values 12% higher than the control (Fig. 7c).

Conclusion

The algae-based biostimulant through foliar spray promotes better physiological performance in soybean plants under greenhouse conditions in a timing and dose-dependent manner. The superior performance of biostimulant-treated plants was achieved in a double application at 1.5 L ha^{-1}, divided into 0.75 L ha^{-1} at V4 + 0.75 L ha^{-1} at R1 (T12), which reflected in improved number of pods, number of grains per pod, weight of a thousand seeds and grain yield. Further studies are necessary to investigate the role of biostimulant at molecular and biochemical levels in cultivated crops, employing as a strategy to activate plant responses against environmental stresses. The findings providing crucial information to support soybean cultivation in semiarid regions as an eco-friendly and sustainable alternative.

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Data availability The datasets generated and analyses during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests The authors declare no competing interests.

References

AGROSTAT - Estatística de Comércio Exterior do Agronegócio Brasileiro (2022) Indicadores da agricultura. https://indicadores.agricultura.gov.br/agrostat/index.htm. Accessed on: November 28, 2023

Alexander MP (1980) A versatile stain for pollen fungi, yeast and bacteria. Stain Technol 55:13–18

Ali A, Hussain T, Tantashutikun N, Hussain N, Cocetta G (2023) Application of smart techniques, internet of things and data mining for resource use efficient and sustainable crop production. Agriculture 13:397

Andreotti C, Rouphael Y, Colla G, Basile B (2022) Rate and timing of application of biostimulant substances to enhance fruit tree tolerance toward environmental stresses and fruit quality. Agronomy 12:603

Bantis F, Koukounaras A (2022) *Ascophyllum nodosum* and silicon-based biostimulants differentially affect the physiology and growth of watermelon transplants under abiotic stress factors: the case of drought. Horticulturae 12:1177

Borges LC, Ferreira DF (2003) Power and type I error rates of Scott-Knott, Tukey and Student-Newman-Keuls's tests under residual normal and non-normal distributions. Rev Mat Stat (São Paulo) 67–83

Cao Y, Williams DD, Williams NE (1999) Data transformation and standardization in the multivariate analysis of river water quality. Ecol Appl 9:669–677

Carmody N, Goñi O, Łangowski Ł, O'Connell S (2020) *Ascophyllum nodosum* extract biostimulant processing and its impact on enhancing heat stress tolerance during tomato fruit set. Front Plant Sci 11:807

Čatský J (1960) Determination of water deficit in disks cut out from leaf blades. Biol Plantarum 2:76–78

Chaudhry S, Sidhu GPS (2021) Climate change regulated abiotic stress mechanisms in plants: a comprehensive review. Plant Cell Rep 41:1–31

Cocetta G, Landoni M, Pilu R et al (2022) Priming treatments with biostimulants to cope the short-term heat stress response: a transcriptomic profile evaluation. Plants 11:1130

CONAB - Companhia Nacional de Abastecimento (2023) Acompanhamento da safra Brasileira, safra 2022/23, 4º levantamento.https://www.conab.gov.br/info-agro/safras/graos. Accessed on: November 09, 2023.

Deolu-Ajayi AO, Meer IMD, Werf AVD, Karlova R (2022) The power of seaweeds as plant biostimulants to boost crop production under abiotic stress. Plant Cell Environ 45:2537–2553

Gandhi G, Biswas K, Vaghela P, Nayak J, Nair A, Moradiya K, Gopalakrishnan VAK, Veeragurunathan V, Ghosh A (2024) In-depth metabolite characterization of seaweed-based plant biostimulants: insights into bioactive components. Algal Res 81:103574

Guerra M, Souza MJ (2002) Como observar cromossomos: um guia de técnicas em citogenética vegetal, animal e humana. FUNPEC, São Paulo. 131 p

Gupta S, Doležal K, Kulkarni MG, Balázs E, Van Staden J (2022) Role of non-microbial biostimulants in regulation of seed germination and seedling establishment. Plant Growth Regul 97:271–313

Hair Junior JF, Black WC, Babin BJ, Anderson RE, Tatham RL (2009) Análise multivariada de dados. Bookman, Porto Alegre. 688 p

Hasanuzzaman M, Nahar K, Anee TI, Khan MI, Fujita M (2018) Silicon-mediated regulation of antioxidant defense and glyoxalase systems confers drought stress tolerance in *Brassica napus* L. S Afr J Bot 115:50–57

Hassan MU, Rasool T, Iqbal C, Arshad A, Abrar M, Abrar MM, Habib-ur-Rahman M, Noor MA, Sher A, Fahas S (2021) Linking plants functioning to adaptive responses under heat stress conditions: a mechanistic review. J Plant Growth Regul 41:2596-2613

Hassan MU, Aamer M, Chattha MU, Hassan MU, Rasool T, Iqbal C, Arshad A, Abrar M, Abrar MM, Habib-ur-Rahman M, Noor MA, Sher A, Fahad S (2020) The critical role of zinc in plants facing the drought stress. Agriculture 10:396

Hernández-Herrera RM, Sánchez-Hernández CV, Palmeros-Suárez PA, Ocampo-Alvarez H, Santacruz-Ruvalcaba F, Meza-Canales ID, Becerril-Espinosa A (2022) Seaweed extract improves growth and productivity of tomato plants under salinity stress. Agronomy 12:2495

Hidangmayum A, Dwivedi P, Katiyar D, Hemantaranjan A (2019) Application of chitosan on plant responses with special reference to abiotic stress. Physiol Mol Biol Plant 25:313–326

Khan M, Ashfaque F, Chhillar H, Irfan M, Khan NA (2021) The intricacy of silicon, plant growth regulators and other signaling molecules for abiotic stress tolerance: an entrancing crosstalk between stress alleviators. Plant Physiol Biochem 162:36–47

Leite-Filho AT, Soares-Filho BS, Davis JL, Abrahão GM, Börner J (2021) Deforestation reduces rainfall and agricultural revenues in the Brazilian Amazon. Nat Commun 12:2591

Liao Z, Zeng H, Fan J, Lai Z, Zhang C, Zhang F, Wang H, Cheng M, Guo J, Li Z, Peng Wu (2022) Effects of plant density, nitrogen rate and supplemental irrigation on photosynthesis, root growth, seed yield and water-nitrogen use efficiency of soybean under ridge-furrow plastic mulching. Agric Water Manag 268:107688

Malhi GS, Kaur M, Kaushik P (2021) Impact of climate change on agriculture and its mitigation strategies: a review. Sustainability 13:1318

Miranda RS, Fonseca BSF, Pinho DS, Batista JYN, Brito RR, Silva EM, Ferreira WS, Costa JH, Lopes MS, Sousa RHB, Neves LF, Penha JAF, Santos AS, Lima JJP, Paula-Marinho SO, Alcântara Neto F, Aguiar ES, Santos CP, Gomes-Filho E (2023) Selection of soybean and cowpea cultivars with superior performance under drought using growth and biochemical aspects. Plants 12:3134

Mosa WFA, Sas-Paszt L, Głuszek S, Górnik K, Anjum MA, Saleh AA, Abada HS, Awad RM (2022) Effect of some biostimulants on the vegetative growth, yield, fruit quality attributes and nutritional status of apple. Horticulturae 9:32

Petrík P, Petek-Petrik A, Mukarram M, Schuldt B, Lamarque LJ (2023) Leaf physiological and morphological constraints of water-use efficiency in C3 plants. AoB Plants 15:plad047

R Core Team (2023) A language and environment for statistical computing. R foundation for statistical computing, Vienna, Austria. http://www.R-project.org/

Rahimi A, Mohammadi MM, Moghaddam SS, Heydarzadeh S, Gitari H (2022) Effects of stress modifier biostimulants on vegetative growth, nutrients, and antioxidants contents of garden thyme (*Thymus vulgaris* L.) under water deficit conditions. J Plant Growth Regul 41:2059–2072

Ranawana SRWMCJK, Bramley H, Palta JA, Siddique KHM (2023) Role of transpiration in regulating leaf temperature and its application in physiological breeding. In: Masthigowda MH, Gopalareddy K, Khobra R, Singh G, Singh GP (eds) Translating physiological tools to augment crop breeding. Springer, Singapore, pp 91–119

Raza A, Tabassum J, Mubarik MS, Anwar S, Zahra N, Sharif Y, Hafeez MB, Zhang C, Corpas FJ, Chen H (2021) Hydrogen sulfide: an emerging component against abiotic stress in plants. Plant Biol 24:540–558

Repke RA, Silva DMR, Santos JCC, Silva MA (2022) Increased soybean tolerance to high-temperature through biostimulant based on *Ascophyllum nodosum* (L.) seaweed extract. J Appl Phycol 34:3205–3218

Rosa VR, Santos ALF, Silva AA, SAB MPV, Germino GH, Cardoso FB, Silva MA, (2021) Increased soybean tolerance to water deficiency through biostimulant based on fulvic acids and *Ascophyllum nodosum* (L.) seaweed extract. Plant Physiol Biochem 158:228–243

Shahrajabian MH, Chaski C, Polyzos N, Petropoulos SA (2021) Biostimulants application: a low input cropping management tool for sustainable farming of vegetables. Biomolecules 11:698

Shahzad A, Ullah S, Dar AA, Sardar MF, Mehmood T, Tufail MA, Shakoor A, Haris M (2021) Nexus on climate change: agriculture and possible solution to cope future climate change stresses. Environ Sci Pollut Res 28:14211–14232

Shahzad R, Harlina PW, Gallego PP, Flexas J, Ewas M, Leiwen X, Karuniawan A (2023) The seaweed *Ascophyllum nodosum*-based biostimulant enhances salt stress tolerance in rice (*Oryza sativa* L.) by remodeling physiological, biochemical, and metabolic responses. J Plant Interact 18: 2266514

Shukla PS, Prithiviraj B (2021) *Ascophyllum nodosum* biostimulant improves the growth of *Zea mays* grown under phosphorus impoverished conditions. Front Plant Sci 11:601843

Shukla PS, Shotton K, Norman E, Neily W, Critchley AT, Prithiviraj B (2018) Seaweed extract improve drought tolerance of soybean by regulating stress-response genes. AoB Plants 10:51

Silva EHF, Antolin LAS, Zanon AL, Andrade-Junior AS, Souza HA, Carvalho KS, Vieira-Junior NA, Marin FR (2021) Impact assessment of soybean yield and water productivity in Brazil due to climate change. Eur J Agron 129:126329

Souza DMG, Lobato E (2004) Cerrado: correção e adubação. Embrapa Informação Tecnológica, Brasília. 416 p

Sun X, Sun X, Pan X, Zhang H, Wang Y, Ren H, Wang F (2022) Identification and fine mapping of a quantitative trait locus controlling the total flower and pod numbers in soybean. Agronomy 12:790

Sunmonu TO, Kulkarni MG, Van Staden J (2016) Smoke-water, karrikinolide and gibberellic acid stimulate growth in bean and maize seedlings by efficient starch mobilization and suppression of oxidative stress. S Afr J Bot 102:4–11

Tiwari RK, Lal MK, Kumar R, Chourasia KN, Naga KC, Kumar D, Das SK, Zinta G (2021) Mechanistic insights on melatonin-mediated drought stress mitigation in plants. Physiol Plantarum 172:1212–1226

USDA - United States Department of Agriculture (2023) Foreign Agricultural Service. Table 11 Soybean Area, Yield, and Production. https://apps.fas.usda.gov/psdonline/reporthandler.ashx?reportId=906&templateId=1&format=html&fileName=Table%2011%20Soybean%20Area,%20Yield,%20and%20Production. Accessed on: November 28, 2023

Vaghela P, Gandhi G, Trivedi K, Anand KGV, Chavda D, Manna M, Seth T, Seth A, Shanmugam M, Ghosh A (2023) Underpinning beneficial maize response to application of minimally processed homogenates of red and brown seaweeds. Front Plant Sci 14:1273355

Veihmeyer FJ, Hendrickson AH (1931) The moisture equivalent as a measure of the field capacity of soils. Soil Sci 32:181–194

Villa VV, Marques PAA, Rezende R, Wenneck GS, Terassi DdS, Andrean AFBA, Nocchi RCdF, Matumoto-Pintro PT (2023) Deficit irrigation with *Ascophyllum nodosum* extract application as a strategy to increase tomato yield and quality. Agronomy 13:1853

Xue Y, Zhang Y, Shan J, Ji Y, Zhang X, Li W, Li D, Zhao L (2022) Growth repressor *GmRAV* binds to the *GmGA3ox* promoter to negatively regulate plant height development in soybean. Int J Mol Sci 23:1721

Zhao C, Ma J, Zhang Y, Yang S, Feng X, Jun Y (2022) The miR166 mediated regulatory module controls plant height by regulating gibberellic acid biosynthesis and catabolism in soybean. J Integr Plant Biol 64:995–1006

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