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Application of Plant Growth Regulators Mitigates Water Stress in Basil

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Abstract: Abiotic stresses, such as water limitation, are significant limiting factors in basil production. One alternative to mitigate the harmful effects of this stress on plants is using plant growth regulators. This study's objective is to evaluate different doses of plant regulators in basil under water deficiency conditions. A randomized block experimental design in a factorial scheme with two factors was used: the first factor referred to the water regimes of 50% and 100% stomatal conductance, the second to different doses of the plant regulator mixture: 0 mL L^{-1} (control), 3 mL L^{-1}, 6 mL L^{-1}, 9 mL L^{-1}, and 12 mL L^{-1}. Each treatment consisted of 12 pots per repetition. Biometric parameters, chlorophyll *a* fluorescence, and gas exchange were analyzed. The plant regulator positively influenced basil plants under water deficiency, with the most pronounced effects observed at the 12 mL L^{-1} dose: a 17% increase in the number of leaves, a fourfold increase in CO_2 assimilation and carboxylation efficiency, and a sevenfold increase in water use efficiency. Therefore, the application of plant regulators on basil is recommended to mitigate the negative effects of water stress, with the most significant results observed at a dose of 12 mL L^{-1}.

Keywords: bioregulator; chlorophyll *a* fluorescence; gas exchange; *Ocimum basilicum* L.

1. Introduction

Basil (*Ocimum basilicum* L.) is an annual, erect, branched, herbaceous, aromatic, and medicinal plant, standing 30 to 50 cm tall, belonging to the Lamiaceae family [1]. Its significance stems from its essential oil components, including methyl chavicol, methyl cinnamate, linalool, and eugenol [2,3]. Widely utilized in the food, therapeutic, cosmetic, and agricultural sectors, basil is a versatile ingredient [4].

With over 150 species worldwide, basil exhibits significant morphological and chemical diversity [5]. Consequently, genotype selection and abiotic factors, such as water

availability, are crucial for optimizing biomass and chemical compound production [6,7]. Water deficiency, a primary limitation of basil, adversely affects physiological processes, growth, and biomass production in aromatic and medicinal plants [8,9]. Plants regulate CO_2 absorption by adjusting stomatal opening, which involves short-term water loss [10]. Water scarcity reduces leaf water potential and stomatal conductance, obstructing CO_2 flow and diminishing photoassimilate accumulation, ultimately limiting productivity [11,12].

Water availability is vital for plant growth and product success, influencing physiological and biochemical processes such as increased abscisic acid synthesis, alterations in cell wall content and elasticity, reduced leaf emission, and decreased leaf area [13,14]. It also increases water movement resistance in vascular tissues and roots [10].

To mitigate the problems caused by water deficit in basil cultivation, plant regulators offer a potential solution. Bioregulators can enhance leaf area expansion, root system development, and antioxidant defenses [9]. Research on major crops like soybeans and sugarcane has shown promising results with Stimulate® (Campinas, Brazil), a bioregulator containing auxin, gibberellin, and cytokinin, but studies on its application in medicinal and aromatic plants are limited [9,15].

Bioregulators have shown protective effects under water stress in soybean cultivation [16]. For instance, positive effects on plant height and root growth were observed in young eucalyptus plants treated with different doses of Stimulate® under water stress [17]. Conversely, Bulegon et al. [18] reported no significant results for photosynthetic parameters in soybeans under water stress treated with Stimulate®. However, important findings were noted regarding antioxidant enzyme activity in basil plants treated with varying concentrations of Stimulate®, which enhanced antioxidant capacity under water deficiency [9]. Given the relatively novel nature of plant regulator use in agriculture, further research is essential to better understand and optimize this technology.

This research hypothesizes that the appropriate dose of bioregulator can improve photosynthetic efficiency and increase basil tolerance to water deficiency. To test this hypothesis, biometric parameters, chlorophyll *a* fluorescence, and gas exchange were measured. This research was conducted with the objective of evaluating the effect of different doses of plant regulator on basil under water deficiency conditions.

2. Materials and Methods

2.1. Installation and Experimental Conditions

The experiment was conducted in a greenhouse at the Department of Biostatistics, Plant Biology, Parasitology, and Zoology of Biosciences Institute (BBVPZ), São Paulo State University "Júlio de Mesquita Filho"–UNESP, Botucatu, SP, Brazil (22°53′13″ S and 48°29′50″ W), between August and September 2019. The region's climate is classified as mesothermal Cwa, humid subtropical, with rainy summers and dry winters [19].

The basil cultivar used in this study was "Alfava Basilicão". We chose to analyze only this variety to control variables and focus specifically on the response of this cultivar to the plant growth regulator and water deficiency conditions. Additionally, "Alfava Basilicão" is widely cultivated in Brazil and exhibits representative species characteristics, making the results obtained relevant and applicable to common agricultural practices in the country.

Sowing took place in trays, and seedlings were kept in a controlled greenhouse environment for 30 days.

Sprinklers performed irrigation with a 3 m^3 h^{-1} flow rate. The soil used for transplanting was collected from the Lageado Farm at the School of Agricultural Sciences in Botucatu and was subjected to chemical analysis for subsequent correction, fertilization, and liming.

Before setting up the experiment, soil samples were collected for chemical analysis for fertility purposes, and fertilization and liming were carried out following recommendations (Table 1). As there are no specific recommendations for basil cultivation, fertilization was adapted based on the recommendations of [20] for mint cultivation.

Table 1. Chemical analysis of the soil from the experimental area.

pH	OM *	P	S	Al^{3+}	H^+Al^{3+}	K	Ca	Mg	SB *	CEC *	V% *
CaC_2	g dm^{-3}	mg dm^{-3}					$mmol_c$ dm^{-3}				
4.0	11	1	18	10	63	1.25	5	1	7	70	10

* OM = Organic matter; SB = Sum of bases; CEC = Cation exchange capacity; V% = Base Saturation.

The experiment was conducted in pots and, following the chemical analysis of the soil, 210 g of limestone, 0.2 g of nitrogen (N), 2.7 g of phosphorus (P), and 0.07 g of potassium (K) were applied. The sources of fertilizers used were urea (45% N), single superphosphate (18% P), and potassium chloride (60% K).

At 30 days after sowing, seedlings were transplanted into 8.5 L capacity pots in a greenhouse. The experiment followed a randomized complete block design in a factorial scheme (2 × 5). The first factor consisted of two water regimes, maintaining 50% and 100% of stomatal conductance, and the second factor involved five doses of a plant growth regulator mixture: 0 (control), 3, 6, 9, and 12 mL L^{-1}. Each treatment was replicated with 12 pots, each containing 2 plants. The evaluations were focused on four central pots per treatment, with the remaining pots considered as border plants, totaling 120 pots for the entire experiment.

The mixture of plant growth regulators used the commercial product Stimulate®, manufactured by Stoller do Brasil Ltda. (Campinas, Brazil), consisting of 90 mg L^{-1} kinetin (Kt, cytokinin), 50 mg L^{-1} indole-3-butyric acid (IBA, auxin), and 50 mg L^{-1} gibberellic acid (GA3, gibberellin). Stimulate® was applied via foliar spray 20 days after transplanting (DAT) the seedlings, (at the moment when water deficiency was induced), using a manual CO_2 pressurized sprayer with a pressure of 4 kgf cm^{-2}, a flow rate of 0.2 L min^{-1}, and an open cone nozzle. Subsequent applications were made every 15 days until reaching 60 DAT.

Water stress was defined by measuring the rate of stomatal conductance in fully expanded leaves using an infrared gas analyzer (IRGA), model 6400 XT, from LICOR (Lincoln, NE, USA). Under water stress conditions, stomatal conductance is influenced by water potential, solar radiation, temperature, and transpiration. The rate of stomatal conductance was measured in irrigated plants, which showed values of 250 to 300 mmol m^{-2} s^{-1}. After that, irrigation was suspended, and daily evaluations of stomatal conductance were performed. When stomatal conductance values reached 50% of the initial measurement, the plants were considered under water stress and were maintained this way for seven days, being rehydrated on the eighth day. Seven cycles of water stress were performed over a period of 60 days.

After exposure to water deficiency, the plant regulator was applied every 15 days. Morphophysiological evaluations were conducted 60 days after treatment.

2.2. Biometric Measurements

Functional growth analysis was used to determine leaf area and total shoot dry mass. Collections were made at the end of 60 days, with the separation and weighing of leaves and stems, which were then placed in a forced air circulation oven at 40 °C until reaching a constant mass to obtain the dry mass. Leaf area was measured using a bench leaf area meter, model LICOR 3100. The number of leaves, plant height, and stem diameter were also recorded using a graduated ruler for height (cm) and a digital caliper for diameter (mm).

2.3. Physiological Measurements

Chlorophyll a Fluorescence

Chlorophyll *a* fluorescence measurements were performed using a fluorometer attached to a photosynthesis analyzer (IRGA), model LI-6400, from LICOR, by the saturated pulse method [21], using the nomenclature recommended in [22]. Analyses were carried out in the morning, between 9:00 a.m. and 11:00 a.m. under artificial light. The maxi-

mum photochemical yield of photosystem II (PSII), initial fluorescence (F_o), maximum fluorescence (F_m), and variable fluorescence (F_v) were measured, allowing the calculation of the following parameters: potential quantum efficiency of PSII (F_v/F_m), representing the quantum yield of the photochemical phase of photosynthesis; quantum efficiency of the antennae (F_v'/F_m'), representing the efficiency of electron excitation capture by the open reaction centers of PSII; photochemical quenching coefficient (qP), reflecting the carbon photosynthetic metabolism; non-photochemical quenching coefficient (NPQ), representing all other forms of energy dissipation, mainly as heat; and apparent electron transport rate (ETR). For ETR calculation, the fraction of excitation energy distributed to PSII was considered 0.5, and the fraction of absorbed photosynthetically active photon flux density (PPFD) by the leaf was 0.84 [23]. The leaves were wrapped in aluminum foil for 15 min for these analyses to adapt to darkness.

2.4. Gas Exchange

Gas exchange measurements were conducted using an infrared gas analyzer (IRGA), model 6400, manufactured by LICOR (Lincoln, NE, USA), for CO_2 and water vapor. The photosynthetically active radiation (PAR) used for basil cultivation was set at 800 µmol m^{-2} s^{-1}, determined via a curve generated with IRGA before the analyses. The ambient CO_2 concentration during assessments ranged from 350 to 400 µmol mol^{-1} air. Assessments were carried out between 9 a.m. and 11 a.m., with PAR standardized on fully expanded leaves from two plants per plot. The measured parameters included CO_2 assimilation rate (A), transpiration rate (E), stomatal conductance (g_s), and internal CO_2 concentration (C_i). Additionally, water use efficiency (WUE) was determined by the A/E ratio, while the carboxylation efficiency of the enzyme ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) (CE) was determined by the A/C_i ratio [24].

2.5. Statistical Analysis

The data were subjected to the Shapiro–Wilk normality test ($p \geq 0.05$) and heterogeneity using Minitab statistical software, version 20.1. After verifying normality and homogeneity, means were subjected to analysis of variance (F-test) and regression analyses using the Sisvar® statistical program, version 5.8. Build 92 [25] and SigmaPlot® software, version 14.5, were used for graph construction.

3. Results

3.1. Biometric Analysis

At 60 days after transplanting (DAT), plant height and dry mass were not influenced by the significant interaction of the studied factors, nor by the isolated factors. The number of leaves, leaf area, and stem diameter were influenced by the significant interaction of the studied factors (Table 2).

Table 2. Summary of the analysis of variance of biometric parameters: plant height (PH—cm), number of leaves (NL), leaf area (LA—cm^2), and stem diameter (SD—mm) of basil cv. Basilicão subjected to water deficiency and different doses of the plant regulator mixture (0, 3, 6, 9, and 12 mL L^{-1}).

Source of Variation	PH	NL	LA	SD
Water Deficiency (WD)	80.0 ^{ns}	88.18 **	21.52 **	7.23 *
Plant Regulator (PR)	34.5 ^{ns}	22.71 **	6.86 **	6.35 **
WD × PR	44.1 ^{ns}	34.77 **	10.87 **	8.27 **
CV (%)	10.5	7.50	9.37	7.87

* Significance at 1% probability by F-test; ** significance at 5% probability by F-test; ^{ns} = not significant at the 5% probability level. CV = coefficient of variation.

A greater number of leaves was observed in plants under water deficiency with the application of 12 mL L^{-1} of plant regulators, showing an increase of approximately 17% compared to those grown under normal irrigation conditions (Figure 1A). There was a

reduction in leaf area in basil plants under adequate water availability and the application of bioregulators, which intensified as the product dose increased (Figure 1B). The stem diameter increased proportionally with the increase in dose of the applied plant growth regulators (Figure 1C).

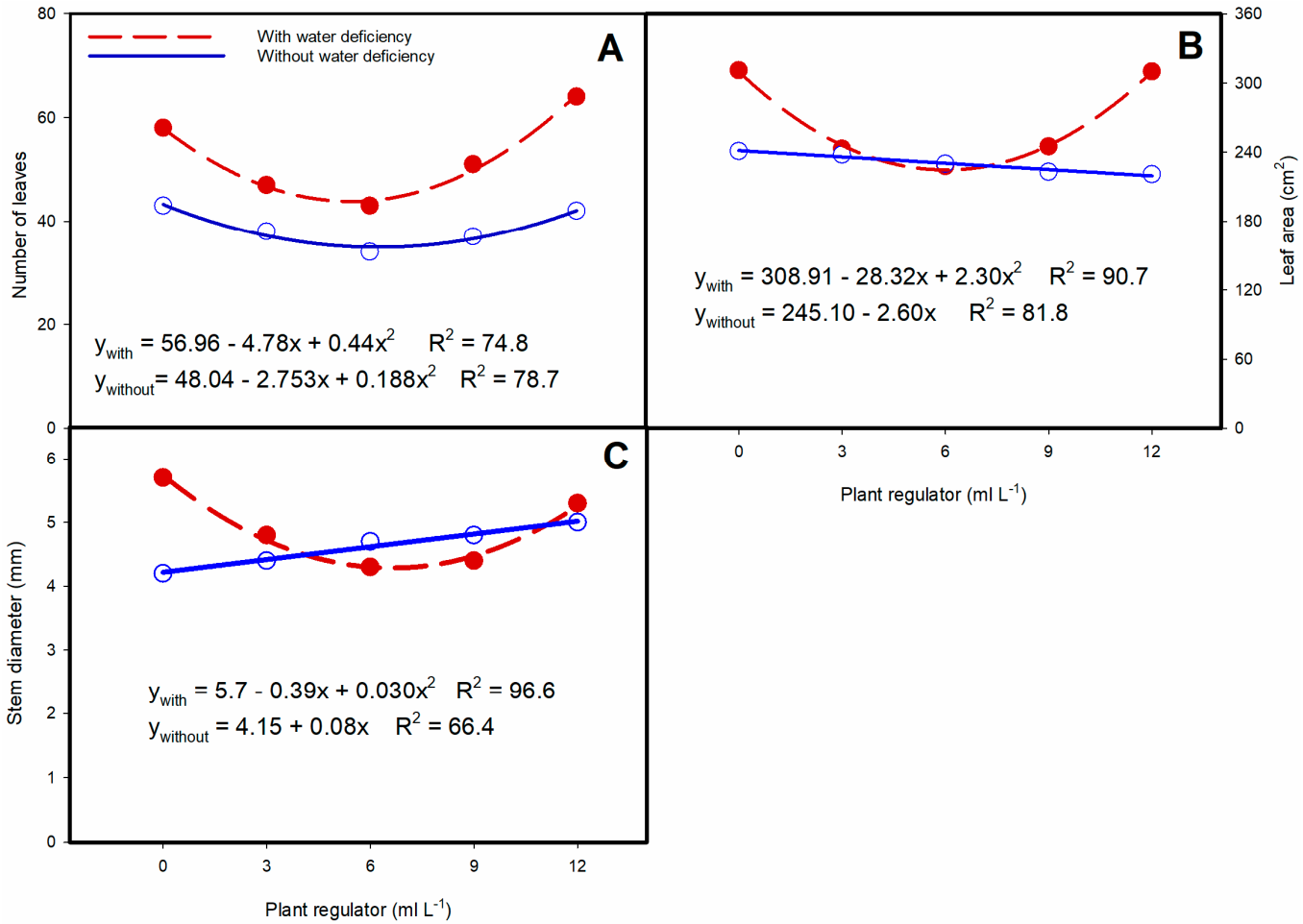


Figure 1. Biometric parameters of basil: plant height (**A**), leaf area (**B**) and stem diameter (**C**) under water deficiency and application of different doses of bioregulators at 60 days after transplanting.

3.2. Chlorophyll a Fluorescence

At 60 DAT, the quantum efficiency of PSII (F_v/F_m) and the photochemical quenching coefficient (qP) did not differ significantly among the treatments analyzed. A significant interaction was observed between the analyzed factors for antenna quantum efficiency (F_v'/F_m'), non-photochemical quenching coefficient (NPQ), and electron transport rate (ETR) (Table 3).

Table 3. Summary of variance analysis of chlorophyll *a* fluorescence parameters: quantum efficiency of PSII (F_v/F_m), photochemical quenching coefficient (qP), antenna quantum efficiency (F_v'/F_m'), non-photochemical quenching coefficient (NPQ), and electron transport rate (ETR) of basil cv. Basilicão subjected to water deficiency and different doses of a mixture of plant growth regulators (0, 3, 6, 9, and 12 mL L^{-1}).

Source of Variation	F_v/F_m	F_v'/F_m'	qP	NPQ	ETR
Water Deficiency (WD)	0.69 ^{ns}	0.53 *	1.53 ^{ns}	115.16 **	5.41 *
Plant Regulator (PR)	0.44 ^{ns}	0.30 *	0.80 ^{ns}	187.25 **	5.37 *
WD × PR	0.73 ^{ns}	0.76 **	1.69 ^{ns}	201.89 **	108.15 **
CV (%)	2.82	10.33	9.52	7.54	5.59

* Significance at 1% probability by F-test; ** significance at 5% probability by F-test; ^{ns} = not significant at the 5% probability level. CV = Coefficient of variation.

In plants with adequate water availability, an increase in F_v'/F_m' was observed, with a clear decrease under the application of 12 mL L^{-1}. On the other hand, in plants subjected to water deficiency, the application of plant regulators had a negative effect, inducing lower F_v'/F_m' in plants under the application of 12 mL L^{-1} (Figure 2A).

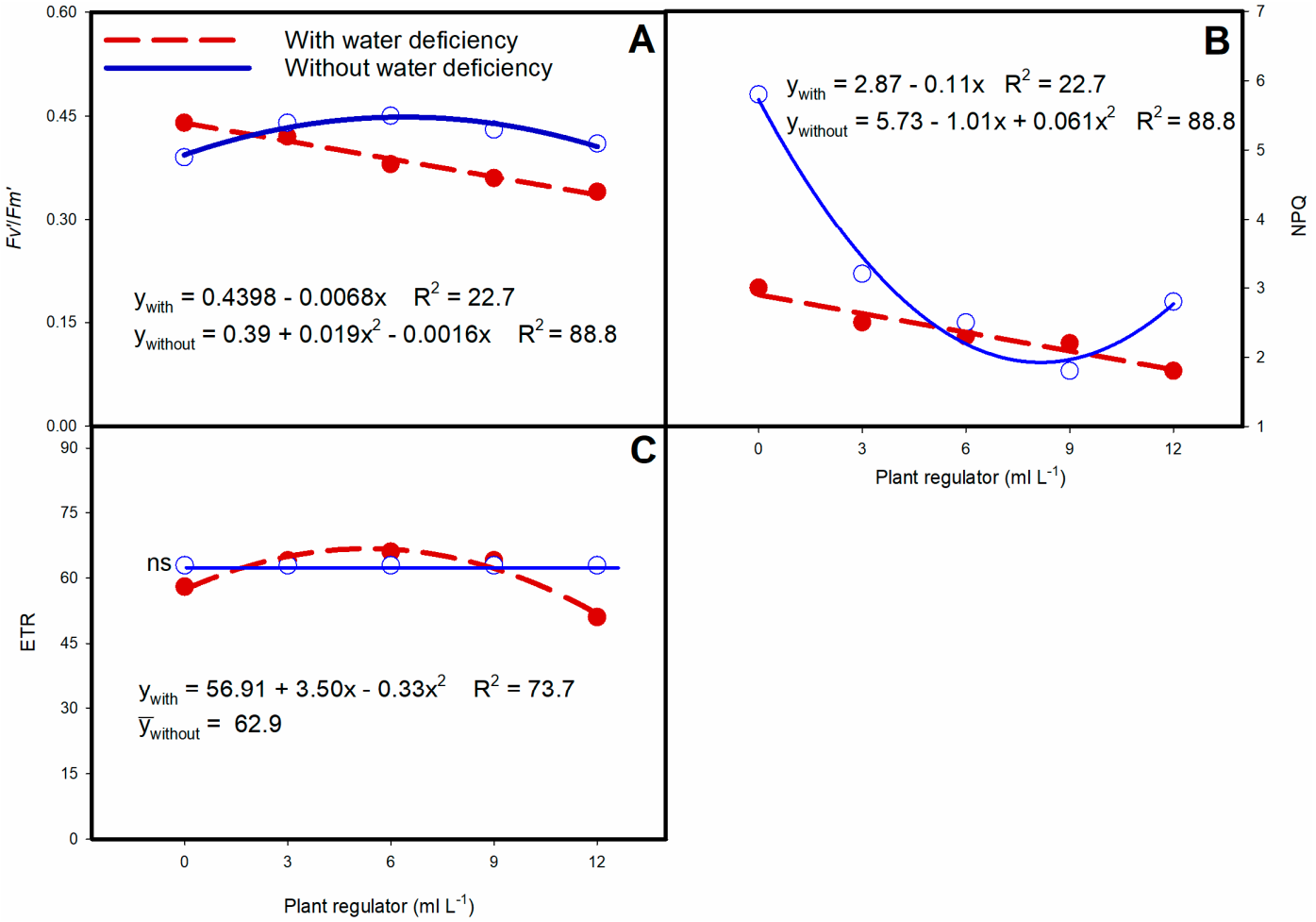


Figure 2. Chlorophyll *a* fluorescence parameters of basil: antenna quantum efficiency (F_v'/F_m') (**A**), non-photochemical quenching coefficient (NPQ) (**B**), and electron transport rate (ETR) (**C**) under water deficiency and application of different doses of bioregulators at 60 days after transplanting. ns = not significant.

Plants showed a decrease in NPQ with the reduction in bioregulator doses compared to the control, which exhibited a higher NPQ. In water-deficient plants, the effect was also decreasing, where NPQ decreased with the increase in tested doses (Figure 2B).

Plants without water deficiency showed no significance in the regression analysis, whereas water-deficient plants were influenced by the plant regulator, with lower doses of the product favoring an increase in ETR in basil plants (Figure 2C).

Gas exchange, the assimilation of CO_2 (A), stomatal conductance (g_s), internal carbon concentration (C_i), water use efficiency (WUE), and carboxylation efficiency (CE) were significantly influenced by the interaction of water deficit and plant regulators at 60 DAT (days after treatment), whereas transpiration (E) was influenced only by the application of plant regulators (Table 4).

Table 4. Summary of the analysis of variance of gas exchange parameters: assimilation of CO_2 (A—µmol CO_2 m^{-2} s^{-1}), stomatal conductance (g_s—mol m^{-2} s^{-1}), internal carbon concentration (C_i—µmol mol^{-1}), transpiration (E—mmol m^{-2} s^{-1}), water use efficiency (WUE—µmol CO_2 m^{-2} s^{-1}/ mmol H_2O m^{-2} s^{-1}), and carboxylation efficiency (CE—µmol CO_2 m^{-2} s^{-1}/µmol m^{-2} s^{-1}) of basil cv. Basilicão subjected to water deficit and different doses of plant regulator mixture (0, 3, 6, 9, and 12 mL L^{-1}).

Source of Variation	*A*	g_s	C_i	*E*	WUE	CE
Water Deficiency (WD)	258.20 **	7.52 *	0.01 ^{ns}	4.14 ^{ns}	25.82 **	154.95 **
Plant Regulator (PR)	184.90 **	3.56 *	6.37 **	4.09 *	46.06 **	143.79 **
WD × PR	153.64 **	8.18 **	3.71 *	1.68 ^{ns}	25.60 **	117.44 **
CV (%)	8.01	14.78	4.11	11.19	17.87	10.01

* Significance at 1% probability by F-test; ** significance at 5% probability by F-test; ^{ns} = not significant at the 5% probability level. CV = Coefficient of variation.

The A in plants with and without water deficiency was influenced by the application of plant regulators. Plants under water deficiency with application of 12 mL L^{-1} of the bioregulator assimilated four times more CO_2 compared to control plants (Figure 3A).

A higher g_s was observed in plants without water deficiency and application of 12 mL L^{-1} of the bioregulator (Figure 3B). C_i decreased in plants under water deficiency, compared to ideal conditions, as the dose of the applied bioregulator increased (Figure 3C). A higher E was observed in plants treated with the highest doses of the tested plant regulator (Figure 3D). WUE increased approximately seven times in plants under water deficiency with the application of 12 mL L^{-1}, compared to the control (Figure 3E).

EC demonstrated a similar effect to that observed in WUE, with a positive influence from the application of plant regulators. A fourfold increase in EC was observed in plants under water deficiency with 12 mL L^{-1} of plant regulators, compared to the control (Figure 3F).

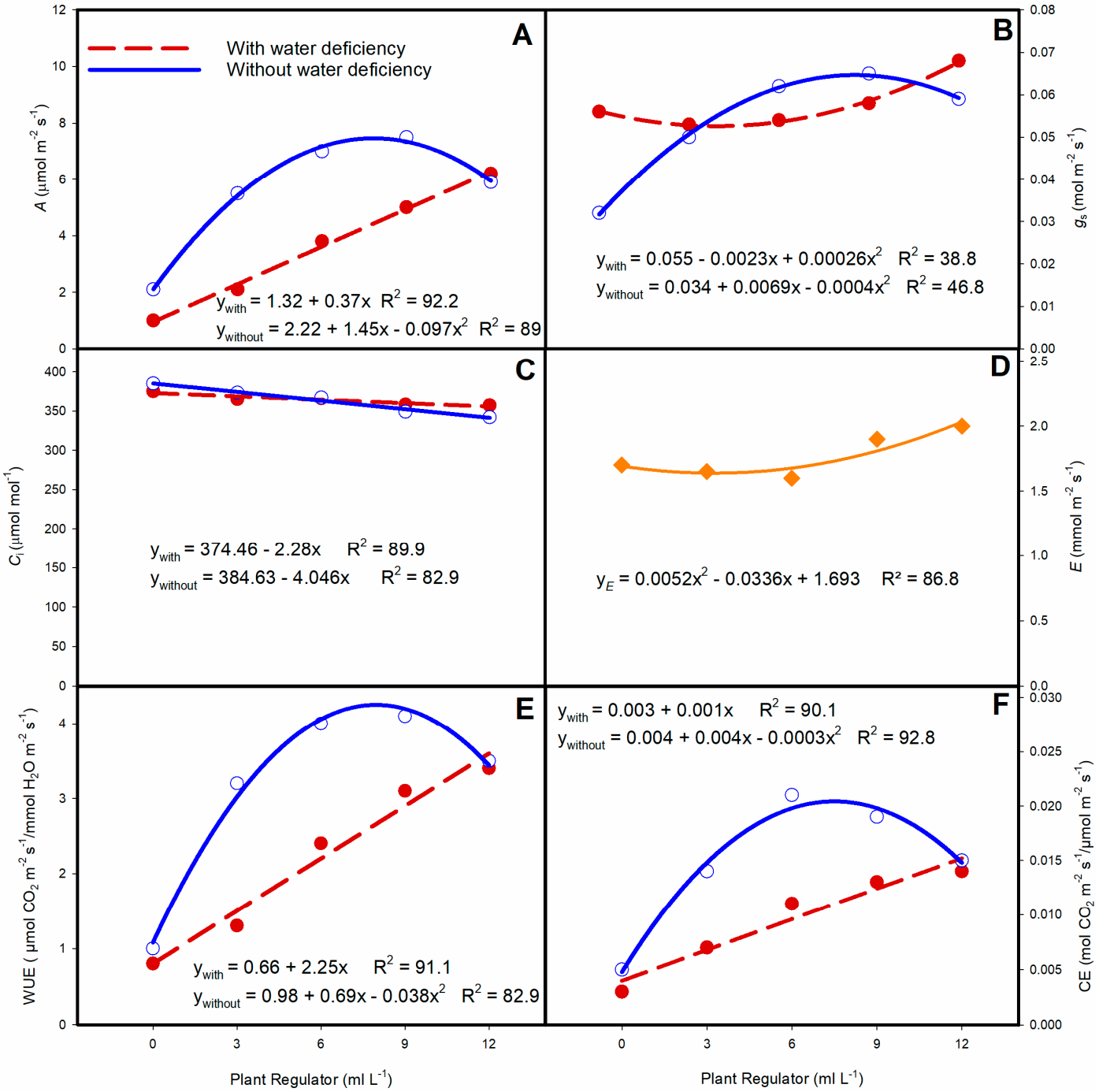


Figure 3. Gas exchange parameters of basil: assimilation of CO_2 (A—µmol CO_2 m^{-2} s^{-1}) (**A**), stomatal conductance (g_s—mol m^{-2} s^{-1}) (**B**), internal carbon concentration (C_i—µmol mol^{-1}) (**C**), transpiration (E—mmol m^{-2} s^{-1}) (**D**), water use efficiency (WUE—µmol CO_2 m^{-2} s^{-1}/mmol H_2O m^{-2} s^{-1}) (**E**), and carboxylation efficiency (CE—µmol CO_2 m^{-2} s^{-1}/µmol m^{-2} s^{-1}) (**F**) under water deficiency with application of different doses of bioregulators. Except for parameter (**D**), where a significant effect was only observed for the doses of plant growth regulator, at 60 days after transplanting.

4. Discussion

Plant growth regulators play a significant role in mitigating water deficiency in plants [26]. These natural or synthetic compounds influence various physiological and biochemical processes, allowing plants to better adapt to water stress conditions [27]. In this study, a negative effect of water deficiency was observed on most of the variables analyzed, but these deleterious effects were mitigated by the application of Stimulate®.

Despite the potential adverse effects of water deficiency on basil cultivation, as reported in this research [8–12], the non-significant effect of water deficiency and the treatments analyzed on plant height and dry mass suggest the tested basil cultivar's tolerance to stress. This also indicates that the tested plant regulator did not have a noticeable impact on stem growth. Similarly, Santos et al. [28] did not observe low sensitivity of basil to these parameters. On the other hand, the physiological effects were more pronounced, indicating that the regulation of physiological metabolism promoted by Stimulate® was crucial in ensuring that the effects on height and dry mass growth were not significantly pronounced.

In contrast, a positive effect on the increase in the number of basil leaves was observed, possibly due to the induction of this response by the phytohormones present in the plant regulators. This result is in line with the findings of [29], where a similar increase in the number of leaves in passion fruit seedlings was observed. In [30], analysis of the leaf area in popcorn maize was carried out and an increase in the number of leaves accompanied by a reduction in leaf area was observed. Conversely, [31] reported an increase in leaf area in yellow passion fruit seedlings with the application of plant regulators.

In this study, water deficiency did not have a direct impact on the leaf area of basil plants, contrasting with studies [30] on salt stress in popcorn maize, where there was a reduction in leaf area. On the other hand, a larger stem diameter was observed in plants under water deficiency with the application of plant regulators. Possibly, the composition of the regulators used, which contain auxin, gibberellin, and cytokinin, favored the increase in this variable [32].

Research developed in [33] showed that the application of plant regulators to soybean seeds resulted in an increase in stem diameter compared to the use of a nutrient complex. This result can be explained by the greater absorption and utilization of nutrients by the roots promoted by plant regulators, reflected in the increased stem thickness and aiding in lodging resistance [34].

Despite the increase in stem diameter with the application of the highest dose of the tested bioregulator (12 mL L^{-1}), doses above this threshold may cause a reduction in this variable, as evidenced in [29] where inhibition of stem diameter growth in passion fruit with doses between 30 and 150 mL L^{-1} was observed. In this study, plants subjected to water deficiency showed greater stem diameter growth under the application of lower doses of plant regulators, corroborating the results that high doses can negatively interfere with plant diameter.

Chlorophyll *a* fluorescence was analyzed to identify the possible effects of plant regulators on electron transport in the chloroplast. The F_v/F_m ratio indicates the maximum quantum efficiency of PSII, with values between 0.75 and 0.85 indicating the integrity of the photosynthetic apparatus in plants. Plants with F_v/F_m values below 0.75 indicate stress and reduced photosynthetic potential [35,36]. In the present study, all F_v/F_m values obtained were within this range.

The absence of a significant difference in F_v/F_m in this study indicates that the combination of plant regulators did not provide adequate protection to the plants against water deficit, especially with chlorophyll *a* fluorescence and the quantum efficiency of PSII, as evidenced by the decreasing values observed in plants under water stress. Similar results were reported in [37] in sunflowers subjected to water deficiency, suggesting a reduction in PSII photochemical efficiency due to stress, resulting in damage to the photosynthetic apparatus.

The NPQ revealed a significant reduction in both water-deficient and non-water-deficient plants, as did the ETR. This phenomenon can be attributed to the reduced water availability, hindering electron transport in the thylakoids of the chloroplasts, potentially inactivating this pathway [38]. However, the use of plant regulators provided higher CO_2 assimilation and stomatal conductance, indicating their potential to mitigate the negative effects caused by water deficiency.

The findings of this investigation suggest a significant improvement in the photosynthetic metabolism efficiency of basil plants under water stress when treated with plant

regulators. This phenomenon points to a beneficial effect of these compounds, which may include the ability of plant regulators to modulate photosynthetic metabolism, promote the synthesis of antioxidant molecules [9], and optimize the use of available water resources by the plant [27]. However, additional studies are necessary to fully elucidate the underlying mechanisms of this observation and its relevance to agriculture and food security.

5. Conclusions

(1) Stimulate® plant regulator effectively mitigated the effects of water deficiency in basil by significantly improving biometric parameters, chlorophyll *a* fluorescence, and gas exchange. These enhancements led to increased photosynthetic efficiency and better physiological responses to water stress, especially at doses of 9 and 12 mL L^{-1};

(2) The application of the plant growth regulator mitigated the adverse effects of water deficiency and increased the tolerance of basil plants. This study not only highlights the efficacy of Stimulate® in improving resilience to water stress but also paves the way for future investigations under various environmental and genotypic conditions. Furthermore, it encourages a detailed exploration of the underlying mechanisms of bioregulator action, contributing to the optimization of agricultural practices on a global scale.

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