

RESEARCH ARTICLE

# Biostimulants isolated or integrated with paclobutrazol as tomato development triggering factors

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## ABSTRACT

Plant biostimulants are substances with different chemistry or biological composition whose, when applied to plants, can favour their development mainly by enhance nutrient uptake or nutrition efficiency and also can alter plant hormonal balance. Paclobutrazol (PBZ) is a retardant plant growth regulator which promotes reduction on stem internodes culminating in shorter plants. The integration of both, biostimulants and PBZ, can lead better development of plants by at same time favour the nutrition performance of shorter plants. Although some farmers already have used these substances in crop field, alone or in combination, there is a great lack of scientific studies to verify the real efficiency of the biostimulants available in market. The aim of this study was to study the effect of different biostimulants on the morphometrical and physiological aspects of tomato when applied in combination or not with paclobutrazol in *Solanum lycopersicum* L., hybrid H9553. The biostimulants used were Stimulate<sup>®</sup>, Serenade<sup>®</sup>, EnerVig<sup>®</sup>, Px-Fertil<sup>®</sup>, Vorax<sup>®</sup>, Liqui-plex<sup>®</sup> and DuoOrgano +<sup>®</sup>. PBZ reduced plant height and, unlike expected, no one of the biostimulants favoured volume increase or dry mass of roots. However, all biostimulants favoured the accumulation of leaf dry mass, with no increase in the number of leaves or net photosynthetic rate per specific leaf area. When isolated, or in interaction with biostimulants, the PBZ generated stimulatory or inhibitory effect on expression of the parameters evaluated, depending on the organ and its age, and acted in synergism or antagonism with the biostimulants, depending on the substance in question.

**Keywords:** Dry mass; Photosynthesis; Plant growth regulator; *Solanum lycopersicum*

## INTRODUCTION

Plant biostimulants are substances or micro-organisms whose, when applied to plants, stimulate natural processes to enhance or benefit nutrient uptake, nutrient efficiency, tolerance to abiotic stress, and crop quality (Jardin, 2015). The interest in plant biostimulants has increased due to the growing interest of scientists, extension specialists, the private sector and producers with the intention of improving crop yields (Rouphael and Colla, 2018). The growth rate of biostimulants market is estimated at over 10% each year, with an estimated projection of US \$ 4.9 billion by 2025 (Langowski et al., 2019).

Biostimulants also have been defined as one or more than one plant growth regulator with some minerals whose, when applied in right concentrations and proportions, can favour plant development, mainly by increasing the root system, and consequently nutrient uptake, specially in soil of low fertility and water availability (Ferrini and Nicese,

2002). Some biostimulants are considered effective as earlier flowering promoter and also as promoter of increasing plant stress tolerance (Yugi and Mattson, 2015; Goni et al., 2018).

A great range of biostimulants are available in market, varying in their composition. Diverse set of biological and inorganic materials including microbial fermentations of animal or plant feedstock, living microbial cultures, macro, and micro-alga, protein hydrolysate, humic and fulvic substances, composts, manures, food, and industrial wastes prepared using widely divergent industrial manufacturing processes (Brown and Saa, 2015). Naturally, the effect of these substances on plant development depends on their composition, especially when they have plant growth regulators or substances whose triggers alteration on plant hormonal balance (Santos et al., 2014).

Although the use of biostimulants has been growing in agricultural field there is a great lack of scientific

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Received: 01 February 2020; Accepted: 28 March 2020

information about their effectiveness in promote benefits in most agricultural crops and its different cultivation condition as well as combined with plant growth regulators (Ricci et al., 2019).

For tomato, *Solanum lycopersicum* L., the use of some biostimulants promotes an increase in some aspects related to production, such as the number of racemes and fruits per plant depending on the composition and concentration of the substances (Tanaka et al., 2003), on the external quality and mass of the fruits (Reynaldo et al., 2012). However, a range of others biostimulants and condition of cultivation has not been studied, although is known that they have been used by some farmers.

Beside the biostimulants, plant growth regulators are another group of substances applied in plants which can modify not only the growth itself but also the yield capacity. Among many plant growth regulators, paclobutrazol (PBZ) is the one extensively used which act in inhibition of gibberellin synthesis, causing alterations in plant hormonal balance and promoting prominent reduction of stem elongation (Rademarcher, 2000; Lima et al., 2013; Almeida et al., 2016). The alteration in plant top architecture, when associated with their root system nutrient uptake capacity, can culminate in alteration on plant yield (Wang et al., 2019). According to Seleguini et al. (2013), the application of paclobutrazol proved to be sufficient to obtain more compact seedlings, without damaging the leaf area, in addition to allowing an increase of about 37% in the dry matter of roots.

Some studies have investigated the effect on plant development or production when it is used substances that induce reduction of plant size, such as PBZ, combined with nitrogen fertilizers (Nouriyani et al., 2012; Almeida et al., 2016). Although many biostimulants contain nitrogen and other nutrients, no studies have carried out to verify the effect of the combination of PBZ with them.

The aim of this study was to investigate the effect of different biostimulants on gas changes and growth of aerial part and root system of tomato when applied in combination or not with paclobutrazol.

## MATERIAL AND METHODS

The experiment was carried out in the experimental area of the Horticulture Sector of the Goias Federal University, located at 16° 35'S of latitude and 49° 21'W of longitude at 727m of altitude. The production of the seedlings was carried out at a commercial nursery VIVATI (Viveiro Vale do Tietê) in Abadia de Goiás city, Goiás state. The tomato hybrid (*Solanum lycopersicum* L.) used was H9553 (Heinz

Seeds Company), which has a determined growth. Four 450-cell trays were hand sowed using Golden Mix<sup>®</sup>, coconut fiber type 11 and covered with vermiculite. According to the recommendations of Lima et al. (2012), on the ninth day after sowing, a solution of paclobutrazol (PBZ), in a concentration of 0.2 ml L<sup>-1</sup>, was sprayed on the seedlings, arranged in trays, with the use of a costal sprayer, till the point of runoff.

On the day of the transplant, at 34 days after sowing (DAS), twelve seedlings were selected by treatment (with and without PBZ) (Fig 1A) and evaluated the stem base diameter (mm), number of leaves (un), height (cm), root length (cm), fresh (g) and dry (g) mass of shoot and root. The data were submitted to analysis of variance by the 5% F test probability. When a significant difference was found, the mean test (Tukey at 5% of probability) was used.

Before the transplanting, according to the soil analysis (Table 1), the substrate was on saturation base of 70% (Silva et al., 2012). For planting fertilization N-P-K 4-30-16, 1,500 kg ha<sup>-1</sup> was used followed the technical recommendations for the crop, as a result of the soil analysis (Comissão de Fertilidade de Solos de Goiás, 1988). The subsequent fertilization occurred seven days after transplanting (DAT) with application of 5 kg ha<sup>-1</sup> of MAP (10% N and 46% P<sub>2</sub>O<sub>5</sub>) and at 14 DAT with application of 5 kg ha<sup>-1</sup> of MAP and 66.6 kg ha<sup>-1</sup> of Ammonium Nitrate, both were diluted in water and distributed in all pots.

Minutes before transplanting the seedlings were immersed until the stem base in solution with the biostimulants for 10 seconds. The treatments of biostimulants were:

1. Stimulate<sup>®</sup> by Stoller Interprises Inc. used at 1.5 mL L<sup>-1</sup>, consisting of cytokinin (90 mg L<sup>-1</sup>), gibberellin (50 mg L<sup>-1</sup>) and auxin (50 mg L<sup>-1</sup>).
2. Serenade<sup>®</sup> by Bayer S.A used at 2 mL L<sup>-1</sup>, containing microorganisms, and recommended as soil conditioner and rooting agent.
3. EnerVig<sup>®</sup> by Oxiquímica Agrociência Ltda. used 2 mL L<sup>-1</sup>, containing cuprum (17.02 g L<sup>-1</sup>), manganese (17.02 g L<sup>-1</sup>), iron (22.02 g L<sup>-1</sup>) and zinc (33.92 g L<sup>-1</sup>).
4. Px-Fertil<sup>®</sup> by Fertilizantes Tanaka Indústria e Comercio Ltda. used at 2 mL L<sup>-1</sup>, based on fish extracts.
5. Vorax<sup>®</sup> by Microquímica Indústria Química Ltda. used at 2 mL L<sup>-1</sup>, containing L-glutamic acid, algae extract, glycine, betaine and nitrogen.
6. Liqui-plex<sup>®</sup> by Alltech Crop Science used at 2 mL L<sup>-1</sup>, based on nutrients and amino acids.
7. DuoOrgano+<sup>®</sup> by Casa Bugre Indústria e Comércio de Fertilizantes Ltda. used at 2 mL L<sup>-1</sup>, containing microorganisms, nitrogen (10,90 g L<sup>-1</sup>), phosphorus (16,35 g L<sup>-1</sup>), potassium (10,90 g L<sup>-1</sup>), cobalt (0,10 g L<sup>-1</sup>) and molybdenium (0,10 g L<sup>-1</sup>).

A randomized block design with three replicates, in a 2 x 8 factorial scheme was applied. The treatments consisted of two types of seedlings treated with and without plant growth regulator (PBZ) and seven biostimulants treatments, in addition to the control treated with water only. For each treatment, performed in triplicate, there were 25 plants (1 per pot).

After selection and visual standardization seedlings about 20 cm high, treated with and without PBZ, respectively were transplanted. They were visually evaluated, as recommended by Lima et al. (2012).

The plants were cultivated in 8 L polyethylene pots, having as substrate a homogeneous mixture of soil (Dystrophic Red Latosol) and sand, in a ratio of 2:1, respectively. All material was pre-sterilized in an autoclave at 121°C, under pressure 1.1 Kgf cm<sup>-2</sup> for 20 minutes.

The plants were cultivated in a greenhouse with a drip irrigation system with two emitters per pot, keeping the substrate always moist. The cultivation environment had nebulization and evaporative adiabatic cooling system and was covered with thermo-reflective mesh. With the aid of a LICOR quantummeter-photometer-radiometer model LI-185, Nebraska, USA, the PAR radiation (photosynthetically active radiation) was quantified at peak radiation time, 12 p.m., inside the greenhouse, observing the value of 1,062 μmol m<sup>-2</sup>s<sup>-1</sup>. During the experiment the humidity varied from 33% to 74%, evaluated at 12 p.m. and 7 a.m., respectively, using a digital thermohygrometer. In 24h temperature varied from 31.2°C to 23.8°C.

During the cultivation of the plants, the insecticide Neonicotinoid Imidacloprido® was used, as recommended by the manufacturer, to control the fly (*Bemisia tabaci*). The first application was made nine DAT and the subsequently three days of each other, totalling four applications. At seven DAT, the first evaluation was performed, quantifying only number of leaves (un) and height (cm). At 14 DAT, the second evaluation was carried out, counting the number of lateral branches (un), height (cm) and number of leaves (un).

The final evaluation was performed at the end of the vegetative stage, when more than 80% of the plants had open flowers at 21 DAT. The variables evaluated in all

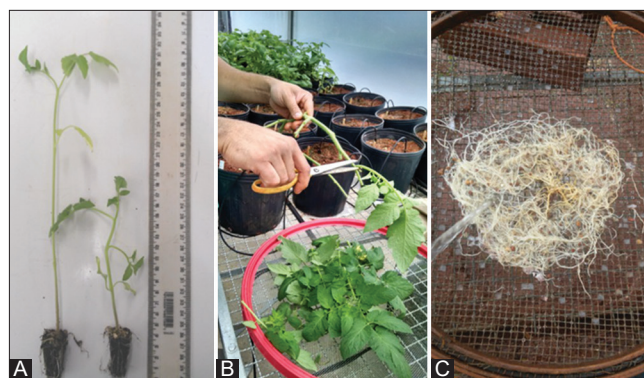
plants were height (cm), stem base diameter (mm), number of leaves (un), number of lateral branches (un), dry mass of leaves, stem and root (g) (Fig 1B and 1C) and root volume (cm<sup>3</sup>). Chlorophyll fluorescence analysis and gas exchange (net photosynthesis, stomatal conductance and transpiration) were performed between 9 am and 12 pm on leaves of the third node from the base to the apex of the plant, in five plants per treatment and block.

Hansatech PEA MK2 Fluorometer, Kings Lynn, England, was used to analyze the potential photochemical efficiency of photosystem II (*Fv/Fm*). For this evaluation leaves were acclimated to the dark for 20 min and exposed for 5 s of saturating actinic light (2500 μmol m<sup>-2</sup> s<sup>-1</sup>). For photosynthetic rate (*A*, μmol m<sup>-2</sup>s<sup>-1</sup>), stomatal conductance (*g<sub>s</sub>*, mol H<sub>2</sub>O m<sup>-2</sup>s<sup>-1</sup>), transpiration rate (*g<sub>s</sub>*, mmol H<sub>2</sub>O m<sup>-2</sup>s<sup>-1</sup>) an infrared gas analyser LI-6400XTR, Biosciences, Nebraska, USA, was used adjusted to a constant chamber temperature of 24°C and an attached LED light source to provide 1000 μmol photons m<sup>-2</sup>s<sup>-1</sup>. The leaves were placed in the 6 cm<sup>2</sup> chamber and were recorded after the stabilization of the data.

The data were submitted to analysis of variance by the 5% F test probability. When the significant difference was found, the mean test (Tukey at 5% probability) was used.

## RESULTS

At 34 DAS, a significant effect of PBZ was observed, which promoted a reduction of plant height and root



**Fig 1.** Seedlings without and with the application of PBZ on the day of transplanting (A); Evaluation of the aerial part (B) and the root system (C) of tomato plants at 21 DAT.

**Table 1: Analysis of the substrate used in the experiment**

Clay	Silt	Sand	MO	pH	P(Mehl)	K	Ca	Mg	H+Al
----- % -----				(CaCl <sub>2</sub> )	---- mg/dm <sup>3</sup> ----		-----cmolc/dm <sup>3</sup> -----		
32	15	53	0.3	6	2.1	150	1.3	0.55	1.7
Al	CTC	M	V	Ca/Mg	Mg/K	Ca/K	Ca/CTC	Mg/CTC	K/CTC
-- cmolc/dm <sup>3</sup> --		----- % -----		-	-	-	----- % -----		
0	3.9	0	56.8	2.4	1.4	3.4	33	14	9.8

MO: organic matter; M: Saturation by aluminium; V: Base saturation

length by approximately 50% and 18%, respectively. PBZ also promoted a reduction of 31.5% of the fresh mass and 50% of the aerial part dry mass and 40% of the dry mass of roots (Table 2).

At seven DAT, there were differences for the height and leaf number (Table 3) regarding the application of PBZ. Plants treated with PBZ had a reduction of 5% in their height and of 47.4% in the number of leaves (Table 4). For these parameters there was no effect of biostimulants, as well as their interaction with PBZ and biostimulants (Table 3). At 14 DAT, there was an effect on the height parameter with PBZ, as there was for the interaction PBZ x Biostimulants, but no difference for leaf number and number of lateral branches in relation of PBZ, biostimulants or interaction (Table 3).

At 14 DAT, regardless of the biostimulant, all the plants had height reduction with PBZ, however, among the

**Table 2 : Means for height (H), root length (RL), leaf number (LN), stem diameter (SD), fresh shoot mass (FSM), fresh root mass (FRM), dry shoot mass (DSM) and dry root mass (DRM), at 34 DAS for industrial tomato seedlings H9553 treated with different biostimulants**

Parameters	No PBZ	With PBZ	F	VC (%)
H	22.62 <sup>a1</sup>	11.12 <sup>b</sup>	187.27 <sup>**</sup>	7.05
RL	17.40 <sup>a</sup>	13.80 <sup>b</sup>	11.57 <sup>*</sup>	9.60
LN	2.83 <sup>a</sup>	2.60 <sup>a</sup>	1.44 <sup>ns</sup>	9.91
SD	2.25 <sup>a</sup>	2.43 <sup>a</sup>	3.97 <sup>ns</sup>	5.32
FSM	1.65 <sup>a</sup>	1.13 <sup>b</sup>	37.04 <sup>**</sup>	8.60
FRM	0.59 <sup>a</sup>	0.50 <sup>a</sup>	1.42 <sup>ns</sup>	20.73
DSM	0.18 <sup>a</sup>	0.09 <sup>b</sup>	77.44 <sup>**</sup>	10.80
DRM	0.05 <sup>a</sup>	0.03 <sup>b</sup>	11.76 <sup>*</sup>	19.91

<sup>1</sup>Means followed by the same letter in the column do not differ statistically from each other, by the Tukey test at 5% of error probability. \*\* Significant by 1% F test; \* Significant by 5% F test; ns: not significant by the 1% and 5% F tests. PBZ: paclobutrazol; VC: variance coefficient

**Table 3 : Summary of variance analysis (F test) for height (H) and number of leaves (LN), at 7 and 14 days after transplanting (DAT), and number of lateral branches (NLB) at 14 DAT, for industrial tomato H9553**

Parameters.	DAT	PBZ (P1)	Biostimulants (P2)	P1XP2	CV%
H	7	7.45 <sup>*</sup>	1.29 <sup>ns</sup>	0.19 <sup>ns</sup>	6.47
LN		884.70 <sup>**</sup>	1.58 <sup>ns</sup>	0.62 <sup>ns</sup>	6.62
H	14	315.44 <sup>**</sup>	1.51 <sup>ns</sup>	3.26 <sup>*</sup>	6.73
LN		2.28 <sup>ns</sup>	0.64 <sup>ns</sup>	1.54 <sup>ns</sup>	13.03
NLB		0.91 <sup>ns</sup>	0.49 <sup>ns</sup>	1.37 <sup>ns</sup>	19.40

\*\* Significant by 1% F test; \* Significant by 5% F test; ns: Not significant by F test at 1% and 5%. PBZ: paclobutrazol

**Table 4: Means of the Height and number of leaves at seven DAT for industrial tomato H9553**

PBZ	Height (cm)	Number of leaves (un.)
without PBZ	4.39 <sup>a</sup>	22.73 <sup>a</sup>
With PBZ	4.17 <sup>b</sup>	12.67 <sup>b</sup>

Means followed by the same letter in the column do not differ statistically from each other, by the Tukey test at 5% error probability

plants treated with PBZ, those submitted to treatment with DuoOrgano<sup>®</sup> had a lower height than the others, as well as those from treatment with Vorax<sup>®</sup> which had a higher height than the others biostimulants (Table 5).

At 21 DAT, we observed an isolated effect of the PBZ for the height, stem diameter, root volume, net photosynthesis, stomatal conductance and transpiration. For biostimulants, isolated effect was observed only for the dry leaf mass parameter. Significance for PBZ x biostimulants was observed only for leaf dry matter, dry stem mass and transpiration. There was no variation for other parameters (Table 6).

At 21 DAT there was a reduction of 21.8% in the height of the plants treated with PBZ, however there was an increase of 15% and 3.4% for root diameter and root volume, respectively. The use of PBZ also induced an increase in the rates of net photosynthesis and stomatal conductance (Table 7).

**Table 5: Height at 14 DAT for industrial tomato H9553.**

Biostimulants	Height (cm)	
	Without PBZ	With PBZ
Stimulate <sup>®</sup>	32.95 <sup>aA1</sup>	23.28 <sup>abB</sup>
Serenade <sup>®</sup>	34.36 <sup>aA</sup>	22.86 <sup>abB</sup>
Liqui-plex <sup>®</sup>	32.11 <sup>aA</sup>	22.91 <sup>abB</sup>
Duo-organo+ <sup>®</sup>	32.35 <sup>aA</sup>	20.74 <sup>bB</sup>
Px-fertil <sup>®</sup>	35.93 <sup>aA</sup>	23.32 <sup>abB</sup>
Vorax <sup>®</sup>	31.15 <sup>aA</sup>	26.71 <sup>aB</sup>
Enervig <sup>®</sup>	34.61 <sup>aA</sup>	22.50 <sup>abB</sup>
Water	32.55 <sup>aA</sup>	25.38 <sup>abB</sup>

<sup>1</sup>Means followed by the same lowercase letter in the column and capital letter in the line do not differ statistically from each other by the Tukey test at 5% error probability. PBZ: paclobutrazol

**Table 6: Summary of the variance analysis with the values of F for height (H), number of leaves (LN), number of lateral branches (LBN) stem diameter (SD), root volume (RV), leaves dry mass (LDM), steam dry mass (SDM), root dry mass (RDM), potential photochemical efficiency of photosystem II (Fv/Fm), net photosynthesis (A), stomatal conductance (gs) and transpiration (E) at 21 DAT for industrial tomato H9553**

Parameters	PBZ (P1)	Biostimulants (P2)	P1 X P2	VC%
H	85.15 <sup>**</sup>	0.83 <sup>ns</sup>	1.83 <sup>ns</sup>	9.20
LN	0.47 <sup>ns</sup>	1.06 <sup>ns</sup>	0.96 <sup>ns</sup>	9.36
LBN	0.69 <sup>ns</sup>	0.47 <sup>ns</sup>	1.01 <sup>ns</sup>	10.48
SD	25.23 <sup>**</sup>	1.69 <sup>ns</sup>	1.36 <sup>ns</sup>	9.60
RV	11.54 <sup>**</sup>	1.41 <sup>ns</sup>	2.11 <sup>ns</sup>	3.41
LDM	4.04 <sup>ns</sup>	3.27 <sup>*</sup>	8.10 <sup>**</sup>	16.73
SDM	1.07 <sup>ns</sup>	1.08 <sup>ns</sup>	2.99 <sup>*</sup>	19.3
RDM	1.60 <sup>ns</sup>	1.62 <sup>ns</sup>	1.18 <sup>ns</sup>	16.76
Fv/Fm	0.12 <sup>ns</sup>	0.51 <sup>ns</sup>	0.57 <sup>ns</sup>	0.37
A	4.61 <sup>*</sup>	0.31 <sup>ns</sup>	2.17 <sup>ns</sup>	12.70
g	6.63 <sup>*</sup>	1.64 <sup>ns</sup>	0.95 <sup>ns</sup>	31.35
E	17.48 <sup>**</sup>	1.68 <sup>ns</sup>	2.72 <sup>*</sup>	8.33

\*\*Significant by 1% F test; \*Significant by 5% F test; ns: not significant by the 1% and 5% F tests. PBZ: paclobutrazol; VC: variance coefficient

At 21 DAT, without PBZ, all biostimulants promoted an increase in dry leaf mass (LDM) compared to control, and among biostimulants, treatment with Vorax® promoted the smallest increase. When using PBZ, LDM and stem dry mass (SDM) increased in the control treatment, and when comparing the effect of treatments with Stimulate®, Liqui-plex® and EnerVig® reduced the LDM. Treatment with Liqui-plex® also reduced the SDM when associated with the use of PBZ, this effect also occurs when compared to treatment without PBZ. When PBZ was used, the transpiration rates of plants from treatments with Serenade®, EnerVig® and Liqui-plex® increased in relation to treatments without PBZ (Table 8).

## DISCUSSION

In all evaluations, and regardless of the use of biostimulants, PBZ reduced plant height. This is a well-known effect of the PBZ on tomato and other species (Nascimento et al., 2003; Almeida et al. 2016). The regulatory action of PBZ on the growth of plants and organs occur, because it is a substance that inhibits the synthesis of gibberellin by blocking the activity of cytochrome P-450 monooxygenases and, consequently, inhibiting the ent-caurene oxidation to ent-caurenoic acid in its biosynthetic route (Hedden and Graebe, 1985; March et al., 2013; Carvalho et al., 2019). Gibberellin is the preponderant hormone to induce cell elongation in the stems, so the growth of these organs is impaired. The enzymes affected by PBZ during gibberellin biosynthesis are also important in the formation of abscisic acid, ethylene and brassinosteroids (Norman et al., 1986; Chen et al., 2019), as well as stimulates cytokinin synthesis (Upreti et al., 2013). Facing of all these interferences it is

assumed that the PBZ not only changes the physiological effects dependent on gibberellin, but also interferes in the hormonal balance of the plants more broadly.

The isolated reduction in the number of leaves promoted by the PBZ only at seven DAT can be due to the inhibition of cell division in the meristems or difficulty of cellular differentiation because the alteration on the plant hormonal balance, as discussed for potatoes (Mustafa and Ennylisan, 2014). However, the effect of PBZ depends on the age of the plant, because the reduction caused by PBZ in the aerial and root dry mass at seven DAS as well as the reduction in the number of leaves at seven DAT was not maintained in the other subsequent evaluations. In contrast to the 21 DAT, PBZ besides not interfering in the number of leaves, in relation to the control, it still induced an increase in the dry mass of leaf and stem, and also induced a stem larger diameter and root volume, denoting that there was good functionality of meristems and that the lowest height of the plant was only due to the reduction in the length of the internodes.

Although it was not the aim of this work, some crops have a productive gain due to the use of PBZ (Hua et al., 2014; Almeida et al., 2016) and the physiological basis for this gain may be associated with factors observed here at 21 DAT when interacting PBZ and biostimulants. It was observed that while there was an increase in the leaf and stem dry mass using PBZ, compared to the treatment just with water, there was also no change in the number of leaves and lateral branches, whereas there was an increase in the net photosynthesis per leaf area. This means that there is more biomass allocated in photosynthesizing organ when using PBZ, favouring higher rates of net photosynthesis. Also

**Table 7: Height (H), stem diameter (SD) and root volume (RV), net photosynthesis (A) and stomatal conductance (g) as a function of the application of paclobutrazol (PBZ), at 21 days after transplanting (DAT) for industrial tomato H9553**

PBZ	H	SD	RV	A	g
	cm	mm	cm <sup>3</sup>	µmol CO <sub>2</sub> m <sup>-2</sup> s <sup>-1</sup>	mmol H <sub>2</sub> O m <sup>-2</sup> s <sup>-1</sup>
Without PBZ	41,18 <sup>a</sup>	6,75 <sup>b</sup>	168,26 <sup>b</sup>	14,00 <sup>b</sup>	1,371 <sup>b</sup>
With PBZ	32,19 <sup>b</sup>	7,76 <sup>a</sup>	173,98 <sup>a</sup>	15,15 <sup>a</sup>	1,733 <sup>a</sup>

<sup>1</sup>Means followed by the same letter in the column do not differ statistically from each other, by the Tukey test at 5% of error probability

**Table 8: Dry mass of leaves (LDM), dry matter of stem (SDM) and transpiration rate at 21 DAT for industrial tomato H9553**

Biostimulants	LDM (g)		SDM (g)		Transpiration (mol H <sub>2</sub> O m <sup>-2</sup> s <sup>-1</sup> )	
	Without PBZ	With PBZ	Without PBZ	With PBZ	Without PBZ	With PBZ
Stimulate®	4.52 <sup>abcA1</sup>	3.51 <sup>bA</sup>	2.22 <sup>aA</sup>	1.75 <sup>abA</sup>	10.01 <sup>aA</sup>	9.55 <sup>aA</sup>
Serenade®	5.79 <sup>aA</sup>	4.98 <sup>abA</sup>	2.58 <sup>aA</sup>	2.20 <sup>abA</sup>	9.93 <sup>aB</sup>	11.60 <sup>aA</sup>
Liqui-plex®	4.45 <sup>abcA</sup>	3.44 <sup>bA</sup>	2.26 <sup>aA</sup>	1.51 <sup>bB</sup>	8.77 <sup>aB</sup>	11.32 <sup>aA</sup>
Duo-organo+®	4.18 <sup>abcA</sup>	4.33 <sup>abA</sup>	1.87 <sup>aA</sup>	1.98 <sup>abA</sup>	9.07 <sup>aA</sup>	10.18 <sup>aA</sup>
Px-fertil®	4.92 <sup>abA</sup>	5.01 <sup>abA</sup>	2.34 <sup>aA</sup>	2.11 <sup>abA</sup>	9.75 <sup>aA</sup>	10.94 <sup>aA</sup>
Vorax®	3.52 <sup>bcB</sup>	6.19 <sup>aA</sup>	1.89 <sup>aA</sup>	2.46 <sup>abA</sup>	10.89 <sup>aA</sup>	10.24 <sup>aA</sup>
Enervig®	3.96 <sup>abcA</sup>	3.78 <sup>ba</sup>	2.36 <sup>aA</sup>	1.71 <sup>abA</sup>	8.79 <sup>aB</sup>	10.83 <sup>aA</sup>
Água	2.59 <sup>cB</sup>	6.16 <sup>aA</sup>	1.79 <sup>aB</sup>	2.62 <sup>aA</sup>	9.27 <sup>aA</sup>	9.93 <sup>aA</sup>

<sup>1</sup>Means followed by the same lowercase letter in the column and capital letter in the line do not differ statistically from each other, by the Tukey test at 5% error probability. PBZ: paclobutrazol

associated with this information, it is noticed that there was no change in the dry mass of roots, thus the same biomass is allocated in an organ of absorption of water and nutrients to supply plants that have lower height. It is also known that PBZ triggers mechanisms that lead to increased nitrogen uptake and transport in plants (Nouriyani et al., 2012). In this sense, it is presumed that there is a greater availability of water and nutrients from the roots to the aerial part, which reverts in greater stem biomass, evidenced in the increase of its diameter, and increase of photosynthetic foliar mass. In addition to the photoassimilates from this higher photosynthetic rate, they can be allocated to other organs of productive interest, as well as the possibility of reallocation of photoassimilates because PBZ interferes in source-sink relationship (Ribeiro et al., 2007). It was also observed that the highest transpiration rate occurred in plants treated with PBZ, exactly the treatment in which the highest volume of roots occurred, which shows that the greater the absorptive area, the greater the loss of transpired water, leaving in the aerial part of the plant the nutrients carried by this larger volume of water moved.

Since the  $F_v/F_m$  ratio was not altered by the use of PBZ, but there was an increase in the net photosynthetic rate associated to the increase in the stomatal conductance rate, it is possible to infer that the  $CO_2$  influx may be the limiting factor to the lower photosynthetic rate of plants not treated with PBZ, and that the use of this product does not interfere in the photochemical efficiency of photosystem II. Although PBZ can increase the efficiency of nitrogen uptake and transport by the plant (Nouriyani et al., 2012), it is possible to associate it with the best photosynthetic performance (Bolton and Brown, 1980; Flores et al., 2018; Xia et al., 2018) due to the possibility of increasing the chlorophyll levels and key proteins in the photosynthetic process, such as rubisco (ribulose 1,5-bisphosphate carboxylase/oxygenase), which concentrates an average proportion of 20% of total leaf nitrogen in C3 plants, being in good nutritional conditions (Evans and Seeman, 1984; Long et al., 2016).

Due the complex composition of the biostimulants, it is not possible to indicate the mechanisms of action by which they trigger their physiological effects, which may be as diverse as their responses, which influences since seed germination till tolerance to pathogens, but it is possible to verify their isolated effect or when in interaction with PBZ on plant development.

The effects of biostimulants on plants are different, and their effectiveness is expected to depend on the genotype and organ in question, as well as on cultivation conditions. The association of biostimulants with plant growth regulators further increases the complexity of

plant responses, and their interaction study is important even though some biostimulants already contain growth regulating substances or potential to induce changes in plant hormonal balance (Yakhin et al., 2017).

The main mechanism attributed to biostimulants favouring plant development is the proportional increase of the root system in relation to the aerial part, culminating in greater absorption of nutrients (Jardin, 2015). However, in this work no effect of biostimulants on root volume or dry mass of roots was observed. On the other hand, without the use of PBZ, all biostimulants promoted an increase in the dry mass of leaves in relation to the control, without, however, affecting the number of leaves and the photosynthetic rates.

When comparing the effect of biostimulants between plants treated and not treated with PBZ, the treatment with Vorax® was the only one that promoted an increase in the dry mass of leaves in PBZ treated plants in relation to the plants not treated with PBZ. Although it was the least biostimulant effective in increasing dry mass of leaves in plants not treated with PBZ. This suggests that there is a synergistic effect of this biostimulant and PBZ in triggering increase of leaf dry mass, and that although it contains nitrogen it is not possible to infer that it would be a direct effect of this nutrient because other biostimulants also have nitrogen but did not promote the same effect. Since this effect was not observed for dry stem mass it is also possible to infer that the action of the biostimulant depends on the organ in question. In other species, regardless of whether it is associated with PBZ, treatment with Vorax® has promoted desirable effects, such as seedling development (Röder et al., 2018).

When PBZ was used, plants from treatments with Stimulate®, EnerVig® and Liqui-plex® had a decrease in leaf dry mass in relation to the control treatment, which also occurred when comparing the plants not treated with PBZ. This response evidences an antagonistic interaction between PBZ and these biostimulants, since when only the biostimulants were used alone, there was only an increase in the dry mass of the leaves in relation to the control treatment plants.

As occurred in leaves, there was an antagonistic interaction between PBZ and Liqui-Plex® when the stem dry mass was analysed, being the only biostimulant that induced decrease of this parameter in plants treated with PBZ in relation to plants not treated with PBZ, as compared to plants treated only with PBZ and water. Unlike in leaves, treatments with Stimulate® and EnerVig® did not interfere on stem dry mass in interaction with PBZ, indicating once again that the effect depends on the organ in question. The isolated effect of PBZ inducing increase in plant mass is

already known for other species (Setia et al., 1995) as also Stimulate® promoting decrease or increase in biomass depending the species and the organ (Soares et al., 2017). The Liqui-Plex®, isolated, have been studied focused on yield resulting an increase of pepper (Antonious et al., 2017) and onion (Santos et al., 2018). As well we did not see effect of EnerVig® on tomato stem biomass, Cavalcanti Filho et al. (2018) studying this biostimulant, combined with others, also did not see its effect on coffee stem seedlings development.

There was also interaction between PBZ and the treatments with EnerVig® and Liqui-plex®, whose presence of PBZ caused an increase in transpiration rates, a factor not limiting to the development of the plants under the conditions of the experiment in question, but to be considered in other specific situations of soil water availability. It is known that PBZ promotes increase of gas changes in leaves, including transpiration (Berova and Zlatev, 2000). When compared treatments with and without PBZ, its use overcome the lower transpiration rates caused by these two biostimulants.

## CONCLUSIONS

The use of PBZ reduces plant height and, unlike expected, no one of the biostimulants favours volume increase or dry mass of roots. However, all biostimulants favour the accumulation of leaf dry mass, with no increase in the number of leaves or net photosynthetic rate per specific leaf area. When isolated, or in interaction with biostimulants, the PBZ generates stimulatory or inhibitory effect on expression of the parameters evaluated, depending on the organ and its age, and acts in synergism or antagonism with the biostimulants, depending on the active substance in question.

### Authors' contributions

Talles Silva, carried out the experiment and scientific writing; Hyrandir Melo, Monita Tarazi, Luis Cunha Junior and Luiz Campos, contributed with experiment evaluation, scientific writing and with the orthographic and grammatical revision of the work; Abadia Nascimento, contributed to scientific writing and guided the conduct of the work.

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