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Morphophysiology and quality of yellow passion fruit seedlings submitted to inhibition of gibberellin biosynthesis

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ABSTRACT. The aim of this study was to verify if a growth reduction of yellow passion fruit seedlings' growth morphophysiology and quality could be changed by paclobutrazol applied through seedling immersion. The experiment was conducted in a greenhouse, with seedlings grown in polyethylene tubes (290 cm³), with substrate. At 40 days after sowing, the seedlings were immersed in an aqueous solution of paclobutrazol at concentrations of 0, 50, 100, 150, and 200 mg L⁻¹. The experiment was conducted in a randomized block design, with five treatments (paclobutrazol concentrations) and four replicates. At 15 and 30 days after treatment, growth characteristics were evaluated. At the end of the assay, destructive evaluations related to mass determination, total leaf area, and seedling quality index were performed. Paclobutrazol treatment induced restrictions in seedling growth, except for fresh and dry mass of root and total fresh mass. Based on these characteristics, the increase in values induced by paclobutrazol was verified. The seedling quality, defined by the major value of the Dickson quality index and a smaller robustness index, was higher when submitted to paclobutrazol treatment.

Keywords: *Passiflora edulis*; plant growth regulator; triazole; Dickson quality index.

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Introduction

Brazil produces the largest volume of yellow passion fruit fruits (*Passiflora edulis* Sims), covering a cultivated area of 41,800 ha, with production of 593,429 t of fruit in 2019; however, productivity remains at a low level (14.8 t ha⁻¹) (Sidra, 2020). Currently, the advances in this productive chain are limited by aspects related to crop productivity and sustainability, which are highly associated with water stress and susceptibility to pathologies. The importance of the introduction of new genotypes of passifloraceas is indisputable, and although Brazil is the main center of diversity of the genus *Passiflora*, the identification and insertion of sources of resistance in breeding programs are complex actions, restricting the availability of new materials to farmers (Gonçalves et al., 2018). In this way, alternatives that relate to drought tolerance and lower susceptibility to viruses, such as hardening of fruits, are important for the evolution of this productive chain.

Despite the high water capacity of roots in the deeper layers of the soil profile, vigorous growth of *Passiflora edulis* Sims is a factor that determines high sensitivity to drought (Souza et al., 2018). Therefore, planting of *Passiflora edulis* Sims seedlings in field conditions is determined by water use efficiency related to water absorption capacity by the roots and restriction of leaf transpiration through the lower growth of the shoot.

One of the practices adopted to attenuate the symptoms of the virus that causes hardening of fruits is the use of late seedlings (Gonçalves et al., 2018). Plants with higher numbers of healthy adult leaves, in full metabolic activity and with adequate mechanical resistance to different weather conditions, represent greater support to adaptability to field conditions after transplanting. In spite of the effectiveness verified in the field, late seedlings are conducted in plastic bags, with higher volume of substrate and time of permanence in the nursery, raising the cost of production and making transport logistics difficult in comparison to seedlings produced in tubes. The greater period of conduction of seedlings in the nursery causes stretching of shoots due to light restriction, resulting in instability and susceptibility of seedlings to mechanical and physiological stress during transplanting practices.

An immediate strategy that allows modulation of environmental stresses is the management of vegetative propagation through seedlings with growth regulator. Based on the knowledge of natural defense mechanisms of plants, protocols for the use of plant growth regulators are being developed, resulting in biochemical and structural strategies aiming at increasing the vigor of seedlings. Such changes in the nursery phase generate greater vigor in the development after transplanting.

The biosynthesis of phenolic compounds and flavonoids is involved in the biochemical mechanisms of plant defense. The exogenous application of phenolic compounds such as salicylic acid has been effective in the attenuation of symptoms, and it is recommended as management for this virus (Silva et al., 2016). The action of jasmonates, induced by phytophagous insects, has also been linked to biochemical defense mechanisms (biosynthesis of secondary compounds such as nicotine, phenolic compounds, and flavonoids). However, the restriction of vegetative vigor induced by negative interaction between jasmonates and gibberellins has been postulated as a structural defense mechanism (Guo, Major, & Howe, 2018). Triazoles such as paclobutrazol (PBZ) act as stress protectants by maintaining the relative water content, membrane stability index, photosynthetic activity, and photosynthetic pigments and protect the photosynthetic machinery by enhancing the levels of osmolytes, antioxidant activities and endogenous hormones, thereby enhancing the yield (Soumya, Kumar, & Pal, 2017).

These studies reaffirm the hypothesis that restriction of vegetative growth of *Passiflora edulis* Sims induced by inhibitors of gibberellin biosynthesis may intervene as a defense mechanism of the plants, resulting in seedlings with greater capacity for field installation. Given the above, the objective was to verify if the morphophysiology is affected by paclobutrazol, to characterize this effect, and to analyze if the application of this regulator increases the quality of yellow passion fruit seedlings grown in tubes.

Material and methods

The experiment was performed from April to August of 2019. After extraction and drying under shade in environment conditions, the seeds of yellow passion fruit (*Passiflora edulis* Sims) were sowed in polyethylene tubes 290 cm³ in size and containing substrate composed of pine bark, vermiculite, charcoal powder, water, and phenolic foam, and were maintained in a greenhouse. Fifteen days later, emergence thinning performed, keeping the most vigorous plants. The experimental design was defined as a randomized blocks, with each tray having 54 seedlings per plot. Eight seedlings in the central position were submitted to non-destructive evaluations; four plants were collected for evaluations of fresh and dry weight.

At 40 days after emergence, the trays were immersed up to substrate levels over one and a half minute until saturation in paclobutrazol solution with the following concentrations: 0, 50, 100, 150 and 200 mg L⁻¹. At 15 days after immersion in PBZ, the following seedling characteristics were evaluated: height (H), basal (BSD) and median diameter (MSD) (measured by digital pachymeter), number of leaves (NL), and SPAD index, through a portable chlorophyll meter (SPAD 502, Minolta, Japan). SPAD index was determined from the average value of three readings, made in the second mature leaf.

At the end of the experiment, at 30 days after PBZ treatment, all characteristics previously described were evaluated, along with total leaf area (TLA), specific leaf area (SLA), individual leaf area (ILA), shoot (SFW), root (RFW), and total fresh weight (TFW), shoot (SDW), root (RDW), and total dry weight (TDW), shoot-root ratio (SRR), shoot (SDWP), root (RDWP), and total dry weight percentage (TDWP), Dickson quality index (DQI), and robustness (RI).

Dry mass was obtained after weighing the fresh mass and drying the plants in a stove with air forced circulation at 65°C for 48 hours. After this process, samples were weighed with a precision balance to determine the dry masses of passion fruit seedlings, percentages of dry matter were calculated, and through the relationship between the masses of shoot and root, the shoot:root ratio was determined. The total leaf area was defined through the use of a leaf area integrator, LI-3100, LI-COR, USA. Then, the relationship was calculated between the total leaf area and total leaf dry mass, defining the specific leaf area. Individual leaf area was calculated by the use of the relationship between total leaf area foliar and number of leaves. From the obtained data, the robustness index and Dickson quality index were estimated. RI was obtained by the ratio between the height and basal stem diameter of the seedling. The DQI was calculated by the use of the following equation:

$$DQI = [TDW / (H/BSD + SDW/RDW)]$$

Leaf gas exchange determination was performed, and the following characteristics were registered: potential net photosynthesis (A) ($\mu\text{mol m}^{-2} \text{s}^{-1}$), transpiration (E) ($\text{mmol of H}_2\text{O m}^{-2} \text{s}^{-1}$), stomatal conductance (g_s) ($\text{mol m}^{-2} \text{s}^{-1}$) and internal concentration of CO_2 (C_i) ($\mu\text{mol mol}^{-1}$). These evaluations were taken on the first true leaf, identified from the apex of the main shoot, between 8 to 11:00 AM, with the open system of a portable infra-red gas analyzer (LCPro, ADC Bio Scientific Limited, Hoddesdon), with an air flow of 300 mL min^{-1} and were accomplished using an actinic light source adjusted at $1200 \mu\text{mol of photons m}^{-2} \text{s}^{-1}$. Leaf water potential (Ψ_w) and relative water content (RWC) were obtained from the same leaf from which gas exchanges were measured. Leaf water potential (MPa) was determined through the use of a pressure chamber (PMS1000, PMS Instrument Company, Albany) from 4:00 to 6:00 AM.

Data were tested for homogeneity of variance (Cochran) and normality (Lilliefors) and then analyzed by a one-way analysis of variance (ANOVA). We then conducted a one-way ANOVA of regressions relating the measured traits to PBZ concentrations. Best-fit models were chosen based on the significance level ($p \leq 0.05$ or $p \leq 0.1$) and the highest coefficient of determination ($R^2 \geq 70$) associated with the biological value. All statistical analyses were conducted using SAEG, version 9.1.

Results and discussion

The great effect of paclobutrazol (PBZ) was verified regarding seedling morphology and quality for all characteristics evaluated at 15 and 30 days after growth regulator application (Table 1). However, water status and leaf gas exchange were not affected by growth restriction (Table 2).

Table 1. Summary of the variance analysis and coefficient of variation (CV) of the height of the seedling (H); basal stem diameter (BSD); median stem diameter (MSD); number of leaves (NL) and leaf greening (SPAD); total (TLA), individual (ILA), and specific leaf area (SLA); shoot (SFW), root (RFW), and total fresh weight (TFW); shoot:root ratio (SRR); shoot (SDW), root (RDW), and total (TDW) dry weight; shoot (SDWP), root (RDWP), and total dry weight percentage (TDWP); robustness index (RI); Dickson quality index (DQI); leaf water potential (Ψ_w); relative content of water (RWC); CO_2 concentration in the substomatic cavity (C_i); stomatal conductance (G_s); transpiration (E) and potential net photosynthesis (A) of *Passiflora edulis* Sims submitted to different paclobutrazol (PBZ) concentrations, evaluated at 15 and/or 30 days after application of growth regulator.

Mean squares											
15 DAA						30 DAA					
SV	DF	H	BSD	MSD	NL	SPAD	H	BSD	MSD	NL	SPAD
PBZ	4	**	**	*	*	**	**	**	*	**	**
Bl	3	ns	ns	*	ns	ns	ns	ns	ns	ns	ns
Res	12	0.1	0.6	0.5	0.08	12.8	0.5	2.0	0.9	0.1	7.5
CV (%)		7.2	2.7	2.1	4.8	7.1	11.9	4.6	2.7	4.7	5.3
30 DAA											
SV	DF	TLA	ILA	SLA	SFW	RFW	TFW	SRR	SDW	RDW	TDW
PBZ	4	*	*	*	**	**	**	**	**	**	**
Bl	3	ns	ns	*	ns	**	ns	ns	ns	ns	ns
Res	12	1396.6	22.9	132.6	1.5	0.7	1.9	0.08	0.1	0.02	0.2
CV (%)		15.5	14.1	7.4	14.8	7.2	6.9	16.1	15.6	9.3	11.0
SV	DF	SDWP	RDWP	TDWP	RI	DQI	Ψ_w	RWC	C_i	G_s	E
PBZ	4	*	**	**	**	*	ns	ns	ns	ns	ns
Bl	3	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Res	12	3.2	2.2	2.5	0.09	0.03	0.7	3.2	66.3	0.05	0.14
CV (%)		6.2	11.6	8.2	12.9	13.5	22.9	1.9	2.7	38.7	19.2

*Significant at 5% probability and **Significant at 1% probability by F test. S.V.: Source of variation; DF: degrees of freedom; Bl: block; Res: residue; C.V.: coefficient of variation in percentage.

Table 2. Pearson's correlation coefficient properties (r) between leaf gas exchange characteristics: stomatal conductance (g_s), transpiration (E), and CO_2 concentration in the substomatic cavity (C_i), water status: leaf water potential (Ψ_w), relative content of water (RWC), and specific leaf area (SLA) of *Passiflora edulis* Sims submitted to different paclobutrazol (PBZ) concentrations.

	SLA	Ψ_w	RWC	C_i	E	g_s
Ψ_w	0.1841	-	-	-	-	-
RWC	-0.4660*	-0.1649	-	-	-	-
C_i	-0.0888	-0.1250	0.1573	-	-	-
E	-0.1076	0.1085	0.2885	-0.0823	-	-
g_s	-0.0885	0.0587	0.3725	0.2491	0.7757**	-
A	-0.1022	0.1798	0.2089	-0.5710**	0.7621**	0.6076**

*Significant at 5% probability and **Significant at 1% probability by F test.

Leaf gas exchange of passion fruit plants is susceptible to many factors, such as salinity, drought, moistness, substrate and fertilization, and microorganism interaction, among others. For the present study, besides the occurrence of growth restriction gas exchange of yellow passion fruit, the leaves remained unchanged.

It must be considered that passion fruit plants were maintained in a greenhouse, in optimized environmental conditions; thus, no differences could be expressed for paclobutrazol treatment when physiological characteristics related to water status or foliar gas exchanges were evaluated. Another important fact to be reported is the restriction of root volume, depending on the container in which the plants were grown. The tube reduces the expression of one of the main mechanisms of tolerance to water restriction of yellow passion fruit and also of the application of paclobutrazol, related to the elevation of the capacity for water absorption based on the vigor of root growth.

For the present study, in spite of the lack of association between growth restriction and physiological alterations, the interrelation observed for gas exchanges measures was validated by means of positive (between stomatal conductance and potential net photosynthesis, between stomatal conductance and transpiration, and between potential net photosynthesis and transpiration) and negative correlations (between potential net photosynthesis and CO_2 concentration in the substomatal cavity, (C_i) (Table 2). In this study, the high stomatal density of yellow passion fruit, previously characterized by Sánchez, Fischer, and Corredor (2013), was related to the close correlation between stomatal conductance, potential net photosynthesis, and transpiration in plants submitted to the application of paclobutrazol in the present study.

Although there was no quantitative effect, there was a tendency for an increase in the relationship between photosynthesis and paclobutrazol concentrations through a linear model (Figure 1A).

Changes in leaf morphology, such as an increase in leaf blade thickness, were related to the elevation of the photosynthetic capacity of the plants due to the optimization of stomata closing mechanisms under water restriction conditions (Souza et al., 2018). For the present study, although the seedlings were not exposed to any type of stress, the positive relationship between paclobutrazol concentrations and potential net photosynthesis is an important indication of the benefits of triazole for passion fruit plants.

For the present study, it was verified that even though the yellow passion fruit plants under the effect of paclobutrazol did not express a correlation between specific leaf area and the gas exchange parameters evaluated, a negative correlation occurred between specific leaf area and relative water content. Thus, the tendency for higher water status resulting from treatment with paclobutrazol resulted in availability of structural water to foment greater leaf thickness (Figure 1B).

The leaves of yellow passion fruit are characterized by high stomatal density (106 stomates per mm^2) and low stomatal control ability (Sánchez et al., 2013). Factors that increase stomatal density induce the optimization of stomatal mechanisms, increasing stomatal conductance, transpiration, and water economy. Souza et al. (2018) and Sánchez et al. (2013) verified the positive correlation between stomatal density and stomatal closure on yellow passion fruit leaves.

The effect of paclobutrazol on seedling morphology was characterized by decreases in almost all characteristics in comparison to the control treatment, except for SPAD index and total and root fresh weight (Figures 1F and 2D). The most expressive effect occurred for height evaluated at 30 DAA (decrease of 55% in relation to the control) (Figure 2A). Among the alterations caused by PBZ in different crop species described in the literature, height reduction is the main effect (Berberich, Snyder, Geneve, & Williams, 2006; Pricinotto & Zucareli, 2014).

The lower height of *P. edulis* seedlings verified in this study was associated with a reduction in the internode length. Berberich et al. (2006) verified in the Blue Bouquet variety of yellow passion fruit that the average length of branches was reduced by PBZ application (50 to 100 ppm) due to an internode shrinkage, and any alteration in number was observed.

Gibberellin biosynthesis inhibition caused by PBZ and, eventually, stimulation of other plant hormones results in physical and metabolic alterations at the cellular level (Rademacher, 2015). PBZ treatment can result in a reduction in the cell elasticity coefficient, restricting cell elongation (Saxena, Singh, & Rajan, 2017), mainly due to lignin accumulation (Kamran et al., 2018). Together, these responses can reflect an internode growth restriction. As a rule, stem reduction in plants submitted to PBZ results in an increased

diameter (Kamran et al., 2018); however, in the present case, the basal diameter of the stem, evaluated at 15 and 30 DAA (Figure 2B), and the medium diameter of the stem, evaluated at 30 DAA (Figure 2C), were reduced in seedlings submitted to PBZ.

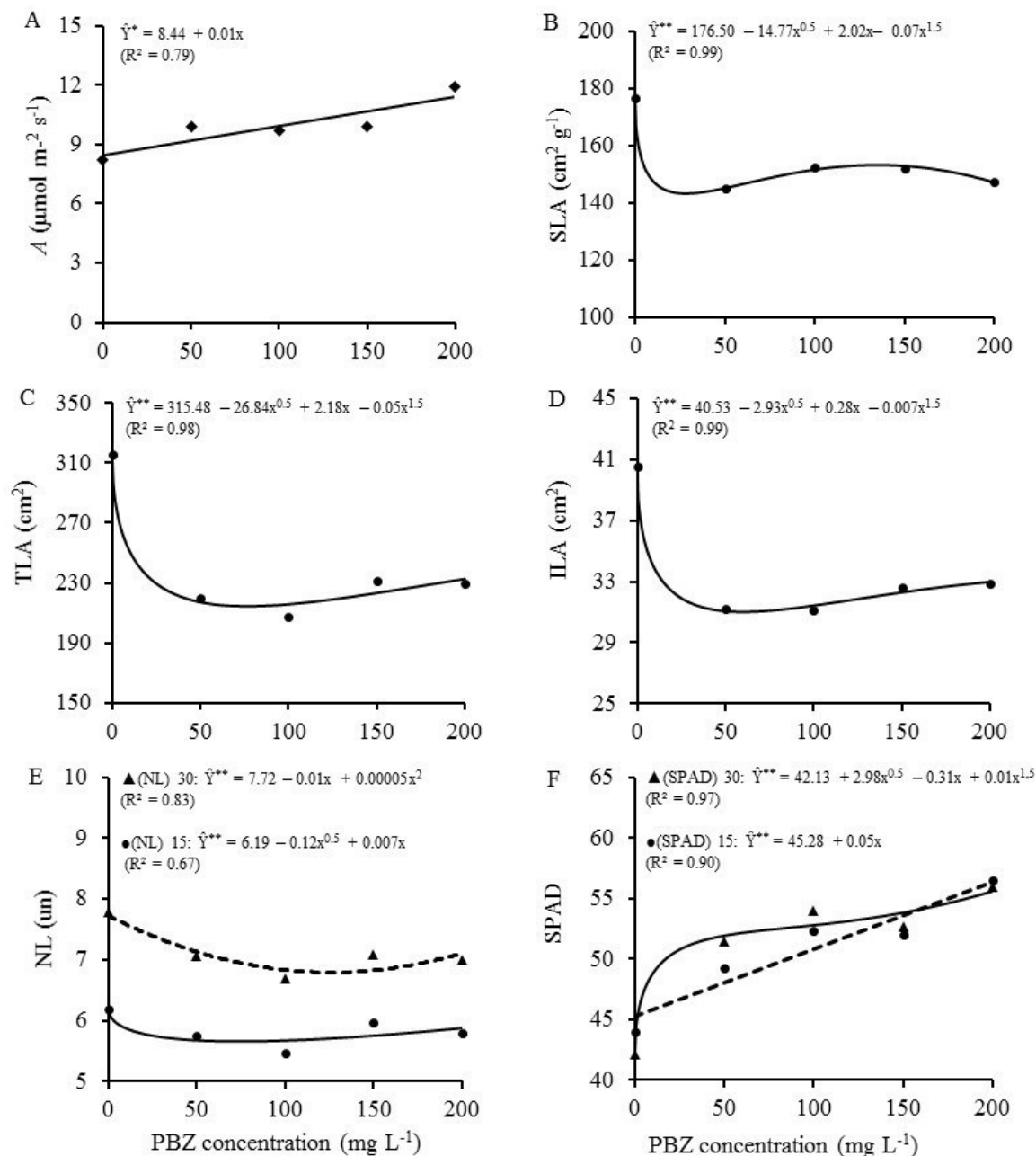


Figure 1. (A) Potential net photosynthesis (A), (B) specific leaf area (SLA), (C) total leaf area (TLA), and (D) individual leaf area (ILA) at 30 days after application (DAA) of growth regulator, (E) number of leaves (NL), and (F) leaf greening (SPAD) at 15 (●) and 30 (▲) DAA of growth regulator in *Passiflora edulis* Sims seedlings.

For total leaf area, individual leaf area, and specific leaf area, decreases in seedlings treated with PBZ were verified in comparison to control plants (0 mg L⁻¹) (Figure 1C, D, and B).

The smallest individual leaf area and total leaf area observed in seedlings treated with PBZ were related to reductions in the cellular elongation capacity (Figure 1E). In studies on the application of paclobutrazol in cucurbitaceae seedlings, Flores et al. (2018) verified the reduction in leaf area. Individual leaf area reduction was a main factor related to the specific leaf area decrease (Figure 1B). Specific leaf area expresses a relationship between total leaf area and total leaf dry biomass, which indicates leaf thickness. The highest leaf thickness was observed in potato leaves submitted to PBZ, which was related to the larger epidermis cells and more elongated palisade cells (Araújo et al., 2019).

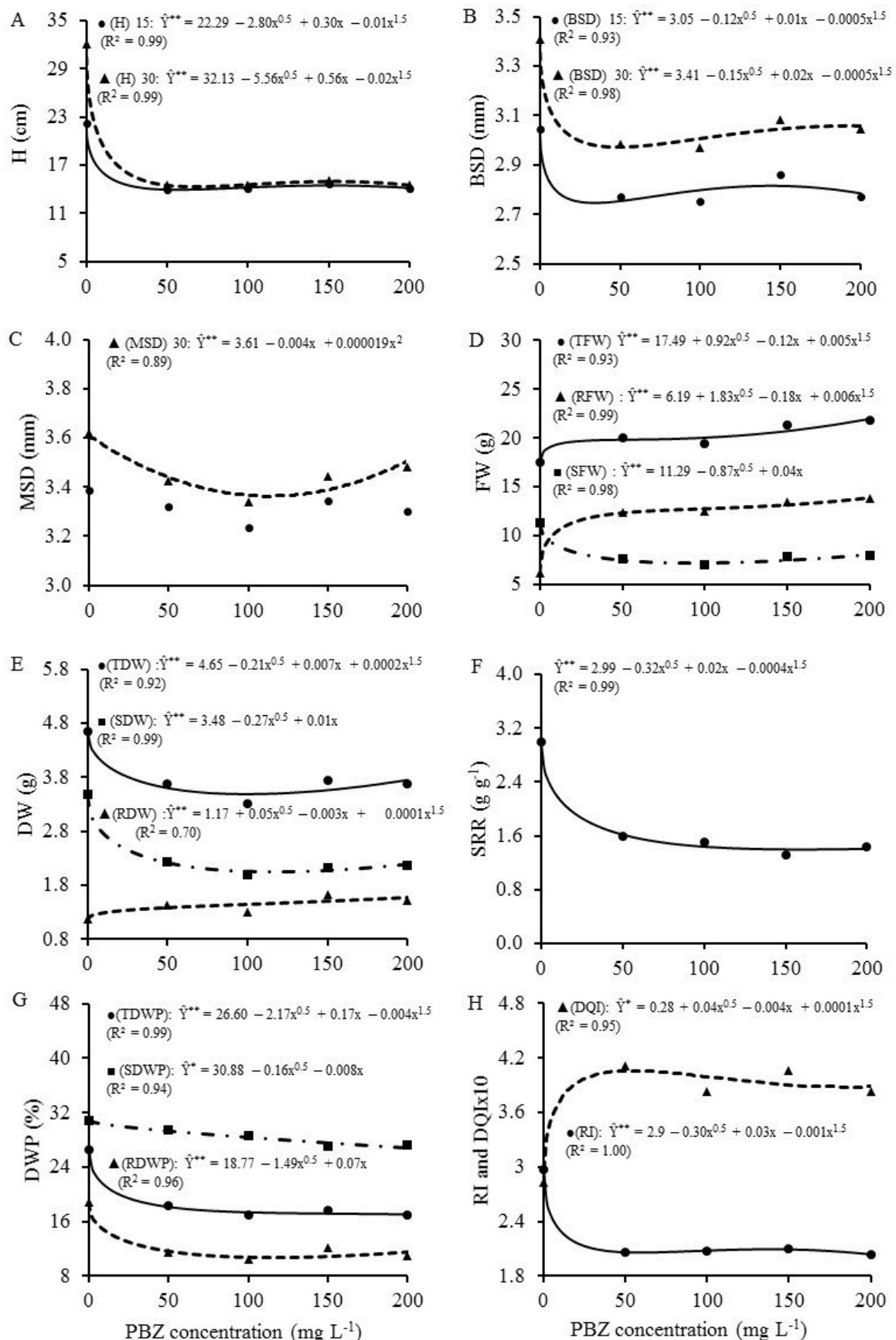


Figure 2. (A) Height of seedlings (H) at 15 (●) and 30 (▲) days after application of growth regulator (DAA), (B) basal stem diameter (BSD) at 15 (●) and 30 (▲) DAA, (C) median stem diameter (MSD) at 15 (●) and 30 (▲) DAA, (D) fresh weight (FW) shoot (■), root (▲), and total (●), (E) dry weight (DW) shoot (■), root (▲), and total (●), (F) shoot-root ratio (SRR), (G) dry weight percentage (DWP) shoot (■), root (▲), and total (●), (H) (RI and DQI x10) Dickson quality index (▲), and robustness index (●) of *Passiflora edulis* Sims seedlings submitted to different concentrations of paclobutrazol.

Leaf limbus of passion fruit species with palisade parenchyma and thicker epidermis is related to the greater speed of the stomatal closure mechanism in a water restriction condition (Gonçalves et al., 2018). The anatomical characteristics of the leaf blade structure are closely related to the ability to resist CABMV virus. Thicker epidermis and more compact palisade parenchyma or abundance of sclerenchyma tissues in the leaf limbus confer mechanical resistance to virus penetration and inoculation with aphids (Gonçalves et al., 2018). The application of paclobutrazol in red cedar seedlings resulted in greater development of sclerenchyma tissues in the leaves, making them thicker and resistant to mechanical damage (Rodrigues et al., 2016). In this way, the reduction in specific leaf area (increase in leaf blade thickness) by the application of paclobutrazol could contribute to elevated seedling vigor.

To determine the association of the SPAD index with PBZ concentration, evaluated at 15 and 30 DAA, cubic root and linear models were established (Figure 1F). The values of seedlings submitted to PBZ treatment were higher in comparison to control plants. The higher SPAD index caused by PBZ treatment is related to two processes: hormonal changes and cellular elongation restriction. Due to an increase in the endogenous cytokinin level (biosynthesis elevation and oxidation reduction) caused by PBZ, the activity levels of antioxidant enzymes can be elevated, delaying senescence and restricting protein degradation, thus promoting chlorophyll biosynthesis. The cell elongation restriction effect induced by PBZ results in increases in chlorophyll due to the smaller leaf area, which results in greater greening of leaves (Brito, Matsumoto, Santos, Gonçalves, & Ribeiro, 2016).

The increases in fresh mass of root and total fresh mass and the reduction fresh mass of shoot were verified in plants submitted to growth restriction. The increase in fresh mass of root strongly influenced the increase in the total fresh mass. Decreases in total and shoot dry mass occurred simultaneously with the increase in root dry mass in plants treated with PBZ (Figure 2E). According to Rademacher (2015), these changes are related to the effect of growth restriction in changing the sink-source process, resulting in root growth stimulation rather than shoot growth. Martins et al. (2019) proposed that modulation of gibberellin metabolism provides an important signaling mechanism that mitigates plant stress via coordinated suppression of stem growth and stimulation of lateral roots.

Although it was not quantified, through visual observations, root tissue thickening in plants treated with PBZ was observed (Figure 3). Morphological and anatomical evaluations of *Raphanus sativus* root submitted to paclobutrazol showed that the root diameter became larger after treatment, which was consistent with the increase in xylem development (Jabir et al., 2017). In consequence, elongation and radial cell division stimulation were observed with an increase in the diameter. The radial growth of roots was associated with a greater flux and accumulation of water and nutrients.

Regarding biomass partition, the shoot:root ratio was reduced for plants submitted to growth restriction (Figure 2F). According to Posse et al. (2018), values between 2.2 and 2.4 for this ratio indicate seedlings of good quality for passion fruit plantlets. Considering that the higher quality of seedling obtained by greater recipients as postulated by the 'mudão' technique is highly associated with better conditions of root growth, the decrease in the shoot:root ratio in conditions with abundant shoot growth can be considered a good quality indicator (Faleiro et al., 2019). Morgado, Bruckner, Rosado and Santos (2017) verified passion fruit seedlings with values of up to 1.0, indicating good plantlet vigor. Therefore, in the present work, the balanced mass distribution between stem and root induced by PBZ can result in greater survival of yellow passion fruit seedlings in field conditions. Stem, root, and total dry mass percentages were reduced by growth restriction induced by PBZ (Figure 2G).

The maintenance of water content of leaf tissue represents a potential reservoir, which is extremely important in stress conditions. Jungklang, Saengnil, and Uthaibutra (2017) verified that PBZ treatment in *Curcuma alismatifolia* induced tolerance of water stress in plants, but this was related to maintenance of cell turgidity, fresh weight, and relative water content of leaves, but not for roots.

Seedling quality can be evaluated through the use of the robustness index (RI) and the Dickson quality index (DQI). The RI is defined by the ratio of seedling height to diameter and indicates a balance of plant growth. The lower the value of this index, the higher will be the surviving index after transplanting (Melo, Abreu, Santos, Oliveira, & Silva, 2018). According to Posse et al. (2018), RI is an important factor for determining seedling quality, mainly in regions characterized by water availability restriction. For this study, growth restriction resulted in lower RI values for all evaluated concentrations. In contrast to RI, the highest Dickson quality index (DQI) of seedlings treated with PBZ is associated with a better quality standard. Thus, however mass accumulation and morphological characteristics were predominantly reduced with PBZ application, the seedling quality was increased (Figure 2H).



Figure 3. Visual aspects of control (A), 50 mg L⁻¹ (B), 100 mg L⁻¹ (C), 150 mg L⁻¹ (D), and 200 mg L⁻¹ (E) of PBZ of *Passiflora edulis* Sims seedling roots developed in polyethylene tubes with 290 cm³, containing humidified Vivatto® substrate and maintained in a greenhouse.

Conclusion

The morphology of yellow passion fruit seedlings is changed upon application of a gibberellin inhibitor, with slight effects observed in leaf gas exchange and water status.

The restriction of gibberellin biosynthesis results in reductions in almost all morphological characteristics.

Available water is driven for enlargement of leaf and root thickness. The increases in root fresh mass and specific leaf area induced by paclobutrazol are related to its capacity of turgidity maintenance in root and leaf cells.

The decrease in shoot and increase in root growth promoted by the gibberellin inhibitor determine a more adequate morphology for the seedling quality index.

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