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Paclobutrazol as a strategy to induce reproductive precocity in sour passion fruit

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Abstract – The adoption of growth regulators can be an alternative method to modulate the quality of the propagation material and obtain reproductive precocity. The objective was to evaluate if a gibberellin biosynthesis inhibitor affects the morphophysiological, biochemical and anatomical parameters of sour passion fruit seedlings and if such alterations affect the reproductive period. Two trials were conducted. In trial I, 40 days after emergence, the seedlings were immersed up to the substrate level in paclobutrazol (PBZ) solutions at concentrations of: 0, 40, 80, 120 and 160 mg L^{-1}. At 45 days after the application of PBZ - DAA, the morphophysiology, biochemistry and anatomy were evaluated. Trail II was conducted in pots, using seedlings from trail I with 45 DAA of PBZ. At 200 days after transplanting, accumulation of shoots and roots fresh and dry mass (g), flowering speed index, total number of reproductive organs and SPAD index were evaluated. In seedlings, restriction of shoot growth induced by PBZ was associated with root increment, seedling quality, photosynthetic pigments and optimization of gas exchange. A shorter period of transition from the juvenile – vegetative phase to reproductive is observed when the seedlings were submitted to PBZ concentrations.

Index terms: *Passiflora edulis* Sims, passion fruit, gibberellin biosynthesis inhibitor, growth regulator, seedling quality, juvenility.

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Paclobutrazol como estratégia para a indução da precocidade reprodutiva de maracujazeiro azedo

Resumo – A adoção de reguladores de crescimento pode ser um método alternativo para modular a qualidade do material de propagação e obter precocidade reprodutiva. Objetivou-se avaliar se os parâmetros morfofisiológicos, bioquímicos e anatômicos de mudas de maracujazeiro azedo são afetados por um inibidor da biossíntese de giberelina e, se tais alterações afetam o período reprodutivo. Foram conduzidos dois ensaios. No ensaio I, aos 40 dias após a emergência, as mudas foram imersas até o nível do substrato em soluções de paclobutrazol (PBZ) nas concentrações de: 0, 40, 80, 120 e 160 mg L^{-1}. Aos 45 dias após a aplicação de PBZ – DAA, avaliou-se a morfofisiologia, bioquímica e anatomia. O ensaio II foi conduzido em vasos, utilizando mudas do ensaio I com 45 DAA de PBZ. Aos 200 dias após o transplantio, foram avaliados acúmulo de massas fresca e seca da parte aérea e raiz (g), índice de velocidade de florescimento, número total de órgãos reprodutivos e índice SPAD. Em mudas, a restrição do crescimento da parte aérea induzida pelo PBZ foi associada à incremento radicular, qualidade de mudas, pigmentos fotossintéticos e otimização das trocas gasosas. Menor período de transição da fase juvenil – vegetativa para reprodutiva é observado quando as mudas foram submetidas a concentrações a PBZ.

Termo para indexação: *Passiflora edulis* Sims, maracujazeiro, inibidor da biossíntese de giberelina, regulador de crescimento, qualidade de mudas, juvenibilidade.

Introduction

The sour passion fruit (*Passiflora edulis* Sims) is considered the most important species within genus *Passiflora* because it has greater economic expression (ANDERSON et al., 2022) due to the fruits organoleptic quality, juice yield and vigor, and it is widely cultivated in Brazil (PREISIGKE et al., 2020).

The incidence of diseases is a limitation of the sour passion fruit production chain, mainly viruses (CERQUEIRA et al., 2014; NASCIMENTO et al., 2006) and fungal soil diseases (FISCHER; REZENDE, 2008). Genetic improvement programs also have effective and constant participation in the search for solutions to solve this problem. The selection of resistant genotypes combined with productive potential through the study of the presence of proteins involved in the plant defense mechanism (RIBEIRO et al., 2019) and precocity in production, has been the programs focus.

The juvenile phase of passion fruit plants is considered the period of greatest vulnerability to phytopathogenic organisms, which can lead to high mortality rates and make cultivation unfeasible (FALEIRO et al., 2019). Some strategies, such as the production of tall seedlings, have been developed and used in certain regions of the country, aiming mainly at delaying the exposure of plants to the virus in the field in their most vulnerable phase (CAVICHIOLI et al., 2014), as well as precocity in production (PETRY et al., 2019). Reducing the period in the field of the juvenile phase allows exploring the maximum productivity in the first harvests, before the diseases presence (SALAZAR et al., 2016).

Although such practices have a high technological level, the protocols related to the adoption of these production models are quite specific, a fact that limits the accession to universal management in the country for *Passiflora* culture passion frut. Therefore, the innovation in broader technologies is necessary to meet the diversity of production systems.

The use of growth regulators, especially inhibitors of gibberellin biosynthesis, is a consolidated technology in many agricultural crops to promote the balance between vegetative/reproductive development (AJMI et al., 2020; AZARCON et al., 2022; AZARCON et al., 2022; HELMEY et al. al., 2021). The management of sour passion fruit seedlings with paclobutrazol results in increased vigor, with restriction of excessive vegetative growth, intensification of root growth and promotion of physiological and anatomical changes, such as increased biosynthesis of reserve carbohydrates and secondary compounds (TEIXEIRA, et al., 2019; TEIXEIRA, et al., 2021).

At each mango production cycle, PBZ is integrated into the floral induction management, promoting the accumulation of unexpanded cells and the increase of phenolic compounds in the apex of juvenile branches, resulting in the transition to the adult vegetative phase (OLIVEIRA, et al., 2020). Differently from tree species, for herbaceous plants such as sour passion fruit, the juvenile phase predominantly occurs at the beginning of the plant's development, with no rejuvenity manifested in the later stages. The intervention through plant growth regulators in the seedling phase is a way to reduce the period of youthfulness, allowing the anticipation of the crop reproductive cycle. The relationship between cytokinins (CUTRI et al., 2013) and gibberellins in the youthfulness of sour passion fruit trees has been addressed, mainly in the expression of genes such as FT/FD: FLOWERING LOCUS T and the FLOWERING LOCUS D (BARBOSA; DORNELAS, 2021).

The restriction of gibberellin biosynthesis in seedlings results in structural alterations that favor the vigor of seedlings, however, relationships with the establishment and development of this propagation material in the subsequent stages of cultivation have not yet been elucidated. This study aimed to analyze whether the morphophysiological, biochemical and anatomical parameters of sour passion fruit seedlings are affected by paclobutrazol (PBZ), and whether such changes affect the transition period from the juvenile-vegetative phase to reproductive.

Material and Methods

The experiments were conducted at the State University of Bahia Southwest, *Campus* Vitória da Conquista-BA, located at 845 meters of altitude, 14º 53' South latitude and 40º 48' West longitude, in the period between February and December 2018. The climate of the municipality, according to the Köppen-Geiger climate classification, is Cwa (tropical altitude). Essay facilities were in a greenhouse, covered by a 150 micron film and 90% transparency, blocking ultraviolet rays and the average temperature inside the environment was monitored by a datalogger (Figure 1).

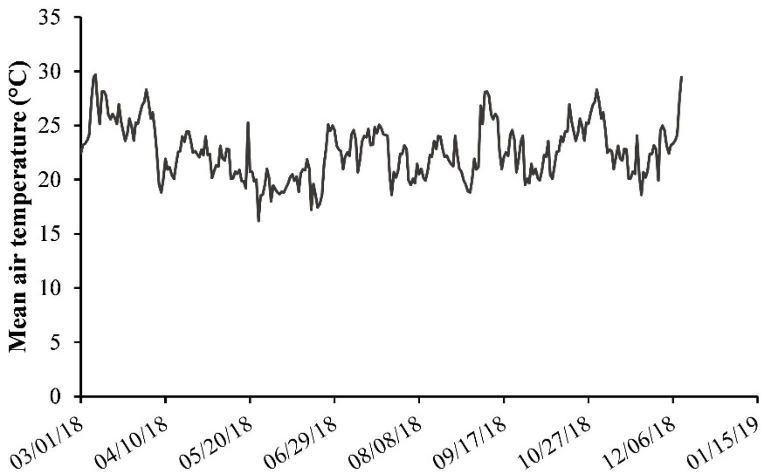

Figure 1 - Mean air temperature (°C) in the environment where the experiments were conducted, Vitória da Conquista-BA.

Essay I. Paclobutrazol via substrate in sour passion fruit seedlings

Three passion fruit seeds (*Passiflora edulis* Sims) were sown in each polyethylene tube, with a capacity of 290 cm^3 (16 cm long and 6.5 cm in diameter), containing substrate composed of biostabilized pine bark, vermiculite, charcoal, water and phenolic foam. Fifteen days after emergence, thinning was performed, and the most vigorous seedling was maintained. Irrigation was carried out daily with the aid of a watering can, keeping the substrate always close to field capacity.

The treatments consisted of four concentrations of paclobutrazol - PBZ (40, 80, 120 and 160 mg L^{-1} of active ingredient of the commercial product Cultar 250 SC® (250 g a.i. L^{-1} of PBZ), and without PBZ. At 40 days after emergence, the trays with the respective seedlings were immersed up to the substrate level, for 1 minute, until saturation in the PBZ solution was reached. (TEIXEIRA, et al., 2019).

The experimental design was in randomized blocks, with four replications, totaling 20 plots. Each experimental unit consisted of a tray containing 54 seedlings (TEIXEIRA et al., 2019), and the evaluations were carried out on the five central useful seedlings, using three seedlings for morphological evaluations and two for physiological, biochemical and anatomical evaluations and, two seedlings were selected for the implementation of essay II.

At 45 days after the PBZ application (DAA) morphological, physiological, biochemical and anatomical evaluations were performed. To evaluate the accumulation of biomass, the following parameters were measured: shoots, roots and total fresh and dry mass, with values described in grams. The shoot/root ratio, Dickson quality index and robustness index were analyzed to verify the growth balance in different tissues and seedlings quality. The SPAD index, photosynthetic pigments, gas exchange and the histochemical test for reserve polysaccharide (starch) were analyzed in order to verify the presence of carbohydrate.

The shoot and root dry mass (SDM, RDM) was obtained after weighing the shoot and root fresh mass (SFM, RFM) and drying the tissues in an oven with forced air circulation (SL 102/480, Solab, Brazil) at 65ºC until reaching constant weight. After this process, they were weighed on an analytical scale (Mark 1300, Analyzer, Brazil) with a precision of 0.01 grams. The values obtained were summed to determine the total fresh mass (TFM) and total dry mass (TDM). To evaluate the mass balance in the different plant strata, the shoot/root ratio was calculated by means of the ratio among the dry mass of each of these parts.

The robustness index (RI) was obtained through the ratio between the seedling stem height (cm) and the stem basal diameter (mm), and the Dickson quality index (DQI), calculated according to the methodology of Dickson et al. (1960), exposed in equation 1:

$$\mathrm{DQI} = \frac{\mathrm{TDM}}{\left(\frac{\mathrm{SH}}{SBD}\right) + \left(\frac{\mathrm{SDM}}{RDM}\right)} \quad (1)$$

Where: TDM= total dry mass; SH= stem height; SBD = stem basal diameter; SDM = shoot dry mass; RDM= root dry mass.

The SPAD index (Soil Plant Analysis Development) was evaluated with the aid of a portable chlorophyll meter model SPAD 502, MINOLTA®, Japan. Measurements were taken at three points on the second fully expanded leaf (D'ARÊDE et al., 2017) from the plant apex, avoiding readings in the area of the largest leaf veins (ROUPHAEL et al., 2017).

Leaf gas exchanges were evaluated in the morning between eight and eleven o'clock. The evaluations were determined using an infrared gas analyzer, LCPro, ADC, UK, coupled to an actinic light source, with 1000 µmol photons m^{-2} s^{-1} of photosynthetically active radiation (SHIMADA et al., 2017; LIMA et al., 2020), on the second fully expanded leaf. The CO_2 assimilation rate (*W* µmol $CO_2 m^{-2}s^{-1}$), stomatal conductance (g_s mol $m^{-2}s^{-1}$), transpiration rate (*E* mmol water vapor m^{-2} s^{-1}) and internal concentration of CO_2 in the leave (*Ci* μmolCO_2 $_{mol}^{-1}$ air) were determined. The intrinsic water use efficiency (*W/gs*), water use efficiency (*W/*E) and carboxylation efficiency (*W/Ci*) were determined from the ratio between the CO_2 assimilation rate and the transpiration rate, leaf internal CO_2 concentration and stomatal conductance, respectively.

The photosynthetic pigments extraction was determined based on Arnon modified methodology (1949), by eliminating the steps of maceration and centrifugation of the disks, described by Barbieri Júnior et al. (2010). The samples absorbance were measured using a spectrophotometer (700 Plus, Femto, Brazil), at wavelengths of 663 nm, 646 nm and 470 nm, to determine the values of a chlorophyll, b chlorophyll and carotenoids, respectively. The pigments concentrations (μgmL^{-1} of the extract) were calculated using specific equations (WELLBURN, 1994). Values were converted and expressed in mg g^{-1} of fresh leaf matter.

The histochemical test was performed on fragments of stem tissue (3rd internode from the apex) fixed in FAA_{50} (formaldehyde, acetic acid and 70% ethanol; 1:18 v/v). After fixing and embedding the samples in methacrylate resin (Historesin® Leica, Leica Microsystems Nussloch GmbH, Heidelberg, Germany), cross sections were cut with glass razors, in a thickness of 5 μm using a rotary microtome (RM 22355, Leica, Deerfield, Illinois, USA) and stained with Lugol's solution to stain the reserve polysaccharide (starch) (JOHANSEN, 1940). Images were obtained under a light microscope (Leica DM 2500) coupled to a digital camera (DM 2500, Leica, Germany).

Essay II. Vegetative and reproductive development of sour passion fruit plants from seedlings treated with paclobutrazol

After the completion of essay I (45 DAA of PBZ), 8 seedlings of each treatment (0, 40, 80, 120 and 160 mg L^{-1} of active ingredient) were transplanted into pots with a capacity of 20 dm^3 containing as soil substrate clayey-sandy loam texture, classified as Dystrophic YELLOW LATOSOL. The soil, with a clayey-sandy loam texture, presented the following chemical characteristics: pH (H_2O) = 5.3; P = 3.0 mg/dm^3; K+ = 0.26 cmolc/dm^3; Ca^{2+} = 1.6 cmolc/dm^3; Mg^{2+} = 1.0 cmolc/dm^3; Al^{3+} = 0.2 cmolc/dm^3; H^+ = 2.0 cmolc/dm^3; sum of bases (S.B.) = 2.86 cmolc/dm^3; Effective CEC (t) = 3.06 cmolc/dm^3; CEC at pH 7.0 (T) = 5.06 cmolc/dm^3.

Soil fertilization was carried out in a throwing solid form following the technical recommendations for the crop according to the guidelines of Embrapa Cassava and Fruticulture (BORGES and SOUZA, 2010) based on the chemical analysis of the soil. Irrigation was carried out according to the determination of the pot capacity, as described by Casaroli and Lier (2008), remaining within a range of 80% of water content.

The experimental design used was in randomized blocks, with four replications, totaling 20 plots. Two plants, each one grown in a pot (DINIZ et al., 2021; SILVA et. al., 2021), represented each experimental unit.

The system for conducting the plants was in a vertical espalier, using number 12 flat galvanized wire in each cultivation row positioned at a height of 1.8 m. Formation pruning was carried out, with the elimination of all lateral shoots, leaving only the main shoot, which was led by a string to the wire. When the main shoot exceeded 10 cm from the wire, it was pruned. After the flowers emergence and opening, artificial pollination of the flowers was performed (RAMOS et al., 2021).

At 200 days after transplanting the seedlings, the following characteristics were evaluated: shoots, roots and total fresh and dry mass, number of flower buds, flowering speed index, fruits number and SPAD index. Analyzes regarding plant growth and SPAD index were similar to those described for the seedling phase (Essay I).

The flower buds number and the fruits number were determined at the end of the essay, by counting the units per plant, and constituted the total number of reproductive organs. Pinto et al. (2005) and Jesus et al. (2016), proposed as a modification of

Maguire's equation (1962) for the speed index (NRPR). The flowering speed index (FSI) was obtained from daily counts of the first open flower emergence in each treatment according to germination methodology in *Passiflora*, as stated by equation 2 described below:

$$\mathrm{FSI} = \frac{E_1}{N_1} + \frac{E_2}{N_2} + \cdots + \frac{E_n}{N_n} \qquad (2)$$

Where: E1, E2 and En = number of flowers in full anthesis at the first, second and last count, respectively; N1, N2 and Nn = number of days after transplanting until the first, second and last count, respectively.

The number of days from emergence until the occurrence of the first flower anthesis was also determined as a way of evaluating flowering precocity.

The percentage of water content in the roots (PWCR) was calculated by the ratio between the difference between fresh mass and dry mass by the dry mass, multiplied by 100, with the percentage unit being discriminated (TEIXEIRA et al., 2021).

Data from essays I and II were subjected to tests such as homogeneity of variance (Cochran), normality (Lilliefors) and analysis of variance. Subsequently, analysis of variance of polynomial and matrix regression was performed for the relationship between characteristics of interest as function of PBZ concentrations, considering to determine the model the degree of significance ($p<0.05$) and the highest value of the coefficient of determination ($R^2 \geq 60$) associated with significant biological value. The described calculation procedures were performed using the Statistical and Genetic Analysis System program, SAEG, version 9.1 (RIBEIRO JÚNIOR, 2007).

Results

Essay I. Paclobutrazol via substrate in sour passion fruit seedlings

The growth regulator PBZ affected almost totality of the morphophysiological parameters analyzed in the sour passion fruit seedlings phase, except, the transpiration rate (*E*), water use efficiency, (*W/E*) and carboxylation efficiency (*W/Ci*). The responses to the plant regulator were not very effective when the biochemical parameters were evaluated, with an effect being verified only for b chlorophyll content (b Cl) (Table 1).

Table 1 – Abstract of variance analysis of shoot fresh and dry mass (SFM, RDM), root fresh and dry mass (RFM, RDM), total fresh and dry mass (TFM, TDM), shoot/root ratio (SRR), robustness index (RI), Dickson quality index (DQI), leaf green color intensity (SPAD), a and b chlorophyll content (a Cl; b Cl), carotenoids content (Car), CO_2 liquid assimilation rate (*W*), stomatic conductance (g_s), transpiration rate (*E*), internal CO_2 concentration (*Ci*), water use efficiency (*W/E*), carboxylation efficiency (*W/Ci*) and intrinsic water use efficiency (W/g_s) of sour passion fruit seedlings (*Passiflora edulis* Sims) at 45 days after application via cultivation substrate of paclobutrazol concentrations (PBZ).

MEDIAN SQUARES											
SV	FD	SFM	RFM	TFM	SDM x 10^{-2}	RDM x 10^{-2}	TDM x 10^{-2}	SRR x 10^{-2}	RL x 10^{-2}	DI x 10^{-3}	SPAD
BL	3	1.1^{ns}	11.2**	17.3**	2.0^{ns}	2.0^{ns}	8.0^{ns}	2.0^{ns}	7.0^{ns}	3.0^{ns}	3.8^{ns}
PBZ	4	12.7**	59.0**	22.6**	149.0**	16.0**	67.0**	314.0**	1186.0**	30.0 **	75.3**
RES	12	1.1	1.6	3.1	6.0	1.0	8.0	6.0	45.0	3.0	5.0
CV (%)		10.9	9.1	7.5	10.2	7.8	7.4	12.6	11.7	11.8	5.1

SV	FD	a Cl x 10^{-2}	b Cl x 10^{-2}	Car x 10^{-2}	*W*	g_s x 10^{-2}	*E*	*Ci* x 10^{2}	*W/E*	*W/Ci* x 10^{-5}	W/g_s
BL	3	8.0^{ns}	9.0^{ns}	3.0^{ns}	1.4^{ns}	2.0^{ns}	0.2^{ns}	0.5^{ns}	0.1^{ns}	3.3^{ns}	54.5^{ns}
PBZ	4	9.0^{ns}	56.0 **	13.0^{ns}	11.6 *	5.0 *	0.3^{ns}	4.4 *	0.6^{ns}	11.0^{ns}	184.0 *
RES	12	8.0	12.0	7.0	3.2	1.0	0.3	1.4	0.2	6.2	47.9
CV (%)		26.9	69.6	70.0	26.5	58.5	23.8	4.3	16.4	31.6	15.0

ns, * and **: not significant, significant by the "F" test at 5% and 1% of probability, respectively. SV: source of variation; FD: freedom degrees; BL: block; RES: residue; CV: coefficient of variation.

For the morphological parameters consistent with the shoot and root fresh and dry mass, total dry mass, shoot/root ratio and Dickson quality index were adjusted square root models, for total fresh mass linear model, total dry mass quadratic model and cubic root robustness index, when related to the concentrations of gibberellin biosynthesis inhibitor (Figure 2 A, B, C, D, E).

Control treatment reductions were found (0 mg L^{-1}) up to the concentrations between 107 and 120 mg L^{-1} for shoot fresh and dry mass and total dry mass, equivalent to 36.95%, 40.94% and 22.65%, respectively (Figure 2 A, B). There was stimulus for the roots fresh and dry mass due to the increase of the growth regulator concentrations, it was verified increase up to the concentration of 156.12 mg L^{-1} (17.89 g) and 116·6 mg L^{-1} (1.42 g), respectively. Such values, when compared to the observed for the control treatment were higher in 119% for the root fresh mass and 40% for the root dry mass (Figure 2 A and B). For the total fresh mass,

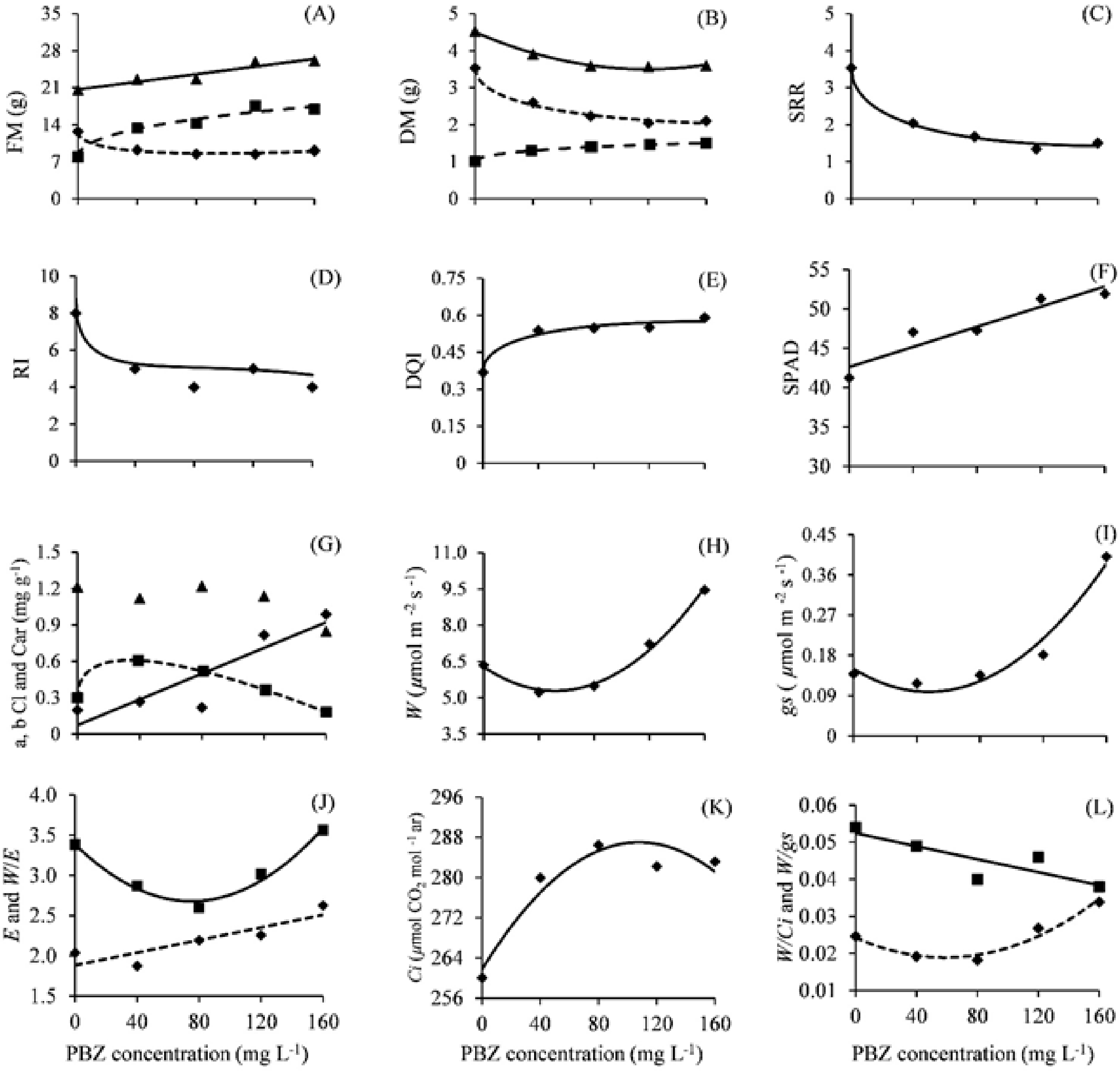

Figure 2 - Morphophysiological and biochemical characteristics of sour passion fruit seedlings (*Passiflora edulis* Sims) 45 days after application via PBZ concentrations substrate.

(A) shoot, root and total fresh mass (FM): total: ▲, shoot: ♦ and root: ■; (B) , shoot, root and total dry mass (DM): total, shoot: ♦ and root: ■; (C) shoot/root ratio (SRR); (D) robustness index (RI); (E) Dickson quality index (DQI); (F) leaf green color intensity (SPAD); (G) b chlorophyll (b Cl): ♦, a chlorophyll (a Cl ♦, carotenoid (Car):■; (H) liquid assimilation rate of CO_2 (*W*); (I) stomatic conductance (*gs*); (J) transpiration rate (*E*) and water use efficiency (*W/E*): (*E*): ♦; (*W/E*): ■; (K) CO_2 internal concentration (*Ci*); (L) carboxylation efficiency (*W/Ci*) and intrinsic efficiency of water use (*W/gs*). The regression models and determination coefficient (R2/r2) are described in Table 3.

it was observed increase due to the increase of PBZ concentrations. This result was evidenced due to the contribution of the roots fresh mass (Figure 2 A).

Regarding the biomass partition, there was decrease for the shoot/root ratio due to the increase of PBZ concentrations (Figure 2 C), due to the effect of the reduction of the shoot dry mass and increase of the root dry mass.

For the robustness index (RI) and Dickson quality index (DQI), it was observed decrease and increase, respectively, according to the increase of PBZ concentrations (Figure 2 D and E). For the relationship between RI and PBZ concentrations, minimum point occurred in 113.5 mg L^{-1} followed by a slightly accentuated curve (Figure 2 D). When the DQI was analyzed according to the regulator concentrations, the highest value point occurred for the concentration of 150 mg L^{-1}, whose increase in higher concentrations became negligible (Figure 2 E).

Due to the increase of PBZ concentrations was delineated growing linear model for the SPAD index and b chlorophyll content (Figure 2 F, G). The PBZ 160 mg L^{-1} effect on the seedlings provided 27.73% more the green color intensity compared to the control, as well as 111% more of b chlorophyll. There was a tendency to increase the carotenoid content for plants submitted up to 40 mg L^{-1} of PBZ.

Although for sour passion fruit seedlings did not occur quantitative effect for the parameters *E*, *W/E* and *W/Ci* there was an increase trend for the relationship between these characteristics and higher PBZ concentrations (Table 1 and Figure 2 J, L).

For the foliar gas exchanges, quadratic models were verified to expose the effect of PBZ concentrations, on the *W*, *gs*, *W/E*, *Ci* and *W/Ci* (Figure 2 H, I, J, K, L). For *Ci* it was observed increase of the values up to 117.25 mg L^{-1} of PBZ followed by decline in the values (Figure 2 K). On the other hand, there was slight decrease for *W, gs and W/Ci*, reaching minimal values in the concentrations of 47.5; 50.0 and 50.0 mg L^{-1}, respectively, followed by pronounced increase. The values obtained in the maximum concentration of gibberellin biosynthesis inhibitor (160 mg L^{-1}) exceed the ones found for the control treatment in 66.24%, 128% and 73.84% for *W, gs and W/Ci*, respectively (Figure 2 H, I, L).

The quantitative differential of increase trends promoted by the elevation of PBZ concentrations, between *E* and g_s, resulted in impacts on the relationship between real water use efficiency (*W/E)* and potential *(W/*g_s*)* with the PBZ concentrations (Figure 2 J, L). Decreases were observed for the ratio between *W/E* and W/g_s up to the concentration of 90 mg L^{-1} of PBZ. For higher concentrations, this decrease was maintained for *W/gs*, however, for *W/E*, the increase in PBZ concentrations resulted in value elevation, being equated in the highest concentration to the control (Figure 2 J, L).

Essay II. Vegetative and reproductive development of sour passion fruit plants coming from seedlings treated with paclobutrazol

Changes of the morphophysiological parameters of sour passion fruit seedlings induced by the PBZ application were little expressive after the transplant. Effects were observed only for shoot fresh and dry matter, total dry matter, total number of reproductive organs and water content percentage in the roots (Table 2).

For the ratio between the shoot fresh and dry mass, total dry mass of passion fruit plants and growth regulator concentrations were observed linear decreases, reaching restrictions of 22.1%, 26.9% and 26.6%, respectively, regarding to the control (Figure 3 A, B). For the roots, it was verified increase trend for fresh mass (62.3%) and for the water content percentage in the roots (80.6 %), occurring decreases for dry mass (22.2%) with the increase of PBZ concentrations (Figure 3 C, G).

In the present study, the relationship between the parameters related to the reproductive phase (number of floral buttons and fruit number (NRPR), flowering speed index (FSI) and the PBZ concentrations were described by matrix square root models (Figure 3 E, F).

Table 2 - Abstract of the variance analysis of shoot fresh and dry mass (SFM, SDM), root fresh and dry mass (RFM, RDM), total fresh and dry mass (TFM, TDM), total number of reproductive organ (NRPR), flowering speed index (FSI), percentage of water content in the roots (PWCR) and green color intensity in the leaf (SPAD) of sour passion fruit plants (*Passiflora edulis* Sims) coming from the application via PBZ concentrations substrate in the seedling phase.

		MEDIAN SQUARES									
SV	FD	SFM $x\ 10^3$	SDM $x\ 10^3$	RFM $x\ 10^4$	RDM x 10	TFM $x\ 10^4$	TDM $x\ 10^3$	NRPR	FSI	PWCR $x\ 10^4$	SPAD x 10
BL	3	4.5^{ns}	0.3^{ns}	0.5^{ns}	9.7^{ns}	1.1^{ns}	0.1^{ns}	8.3^{ns}	0.7^{ns}	2.1^{ns}	2.0^{ns}
PBZ	4	39.8^{**}	3.8^{*}	1.1^{ns}	5.0^{ns}	2.7^{ns}	5.0^{*}	177.7^{*}	1.2^{ns}	5.4^{**}	0.4^{ns}
RES	12	6.3	1.0	0.5	3.2	3.0	1.5	85.5	0.7	0.9	3.8
CV (%)		**8.7**	**14.1**	**31.9**	**17.4**	**14.6**	**14.7**	**21.9**	**105.1**	**16.7**	**9.1**

ns, * and **: not significant, significant by the "F" test at 5% and 1% of probability, respectively. SV: source of variation; FD: freedom degrees; BL: block; RES: residue; CV: coefficient of variation.

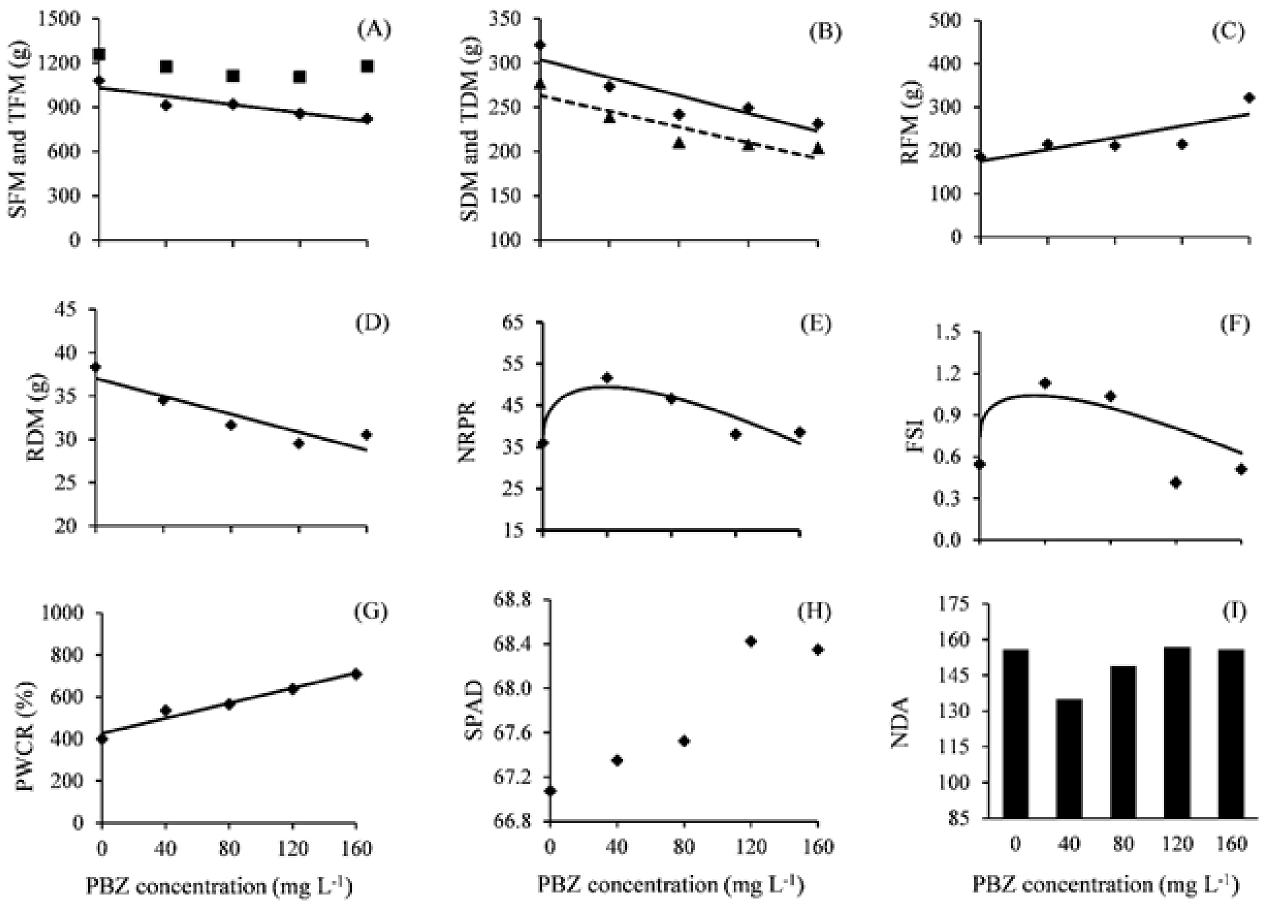

Figure 3 - Sour passion fruit plant morphology (*Passiflora edulis* Sims) coming from seedlings submitted to application via PBZ concentrations substrate.

(A) shoot fresh mass (SFM): ♦ and total fresh mass (TFM): ■; (B) shoot dry mass and total dry mass (SDM and TDM): (SDM): (TDM): ♦; (C) root fresh mass (RFM); (D) root dry mass (RDM); (E) total number of reproductive organs (NRPR); (F) flowering speed index (FSI); (G) percentage of water content in the roots (PWCR), (H) green color intensity in the leaf (SPAD) and (I) days up to the first flower antesis (NDA). * and **: significant by the 5% regression analysis and 1% of probability, respectively. The regression models and determination coefficient (R2/r2) are described in Table 3.

For the evaluation related to the FSI, it was observed an increase trend up to the concentration of 43 mg L^{-1}, however, in conditions higher than this, it has a decline of approximately 22.67% up to 139 mg L^{-1} regarding to the control plants (Figure 3 F). The number of days up to the occurrence of the first flower was also reduced for the seed-

Table 3. Regression and determination coefficient of the variables presented in Figures 1 and 2.

	Parameter	Equation	R^2/r^2
Essay I	TFM	$\hat{Y}^{**} = 20.69 + 0.036x$	$r^2 = 0.91$
	SFM	$\hat{Y}^{**} = 12.7316 - 0.877427x^{0.5} + 0.0458556x$	$R^2 = 0.98$
	RFM	$\hat{Y}^{**} = 7.87823 + 0.923367x^{0.5} - 0.0133178x$	$R^2 = 0.96$
	TDM	$\hat{Y}^{**} = 4.5016 - 0.0169x + 7 \times 10^{-5}x^2$	$R^2 = 0.99$
	SDM	$\hat{Y}^{**} = 3.53723 - 0.200258x^{0.5} + 0.00649588x$	$R^2 = 0.98$
	RDM	$\hat{Y}^{**} = 1.00171 + 0.0543889x^{0.5} - 0.00113190x$	$R^2 = 0.96$
	SRR	$\hat{Y}^{*} = 3.54763 - 0.320947x^{0.5} + 0.0121071x$	$R^2 = 0.98$
	RI	$\hat{Y}^{**} = 8.78219 - 1.15833x^{0.5} + 0.124595x - 0.00464815x^{1.5}$	$R^2 = 0.99$
	DQI	$\hat{Y}^{**} = 0.370446 + 0.0316145x^{0.5} - 0.00122165x$	$R^2 = 0.96$
	SPAD	$\hat{Y}^{**} = 38.508 + 0.0668x$	$r^2 = 0.95$
	b Cl	$\hat{Y}^{**} = 0.0729 + 0.0053x$	$R^2 = 0.80$
	Car	$\hat{Y}^{\circ} = 0.299369 + 0.1066177x^{0.5} - 0.00916494x$	$R^2 = 0.84$
	W	$\hat{Y}^{*} = 6.274 - 0.038x + 0.0004x^2$	$R^2 = 0.99$
	gs	$\hat{Y}^{*} = 0.149 - 0.002x + 2 \times 10^{-5}x^2$	$R^2 = 0.96$
	E	$\hat{Y}^{*} = 0.003x + 1.884$	$r^2 = 0.77$
	W/E	$\hat{Y}^{**} = 3.385 - 0.018x + 0.0001x^2$	$R^2 = 0.97$
	Ci	$\hat{Y}^{*} = 261.7 + 0.469x - 0.002x^2$	$R^2 = 0.97$
	W/Ci	$\hat{Y}^{*} = 0.026 - 0.0002x + 2 \times 10^{6}x^2$	$R^2 = 0.95$
	W/gs	$\hat{Y}^{**} = 0.0524 - 0.00009x$	$r^2 = 0.71$
Essay II	SFM	$\hat{Y}^{**} = 1025 - 1.416x$	$r^2 = 0.80$
	SDM	$\hat{Y}^{**} = 263.2 - 0.444x$	$r^2 = 0.82$
	RFM	$\hat{Y}^{**} = 17.58 + 0.6803x$	$r^2 = 0.66$
	RDM	$\hat{Y}^{**} = 37 - 0.0515x$	$r^2 = 0.83$
	TDM	$\hat{Y}^{*} = 303.7 - 0.505x$	$r^2 = 0.81$
	NRPR	$\hat{Y}^{**} = 36.4495 + 4.14761x^{0.5} - 0.331388x$	$R^2 = 0.83$
	FSI	$Y^{*} = 0.7475 + 0.1018x^{0.5} - 0.0088x$	$R^2 = 0.93$
	WCR	$\hat{Y}^{*} = 427.04 + 1.7937x$	$R^2 = 0.95$

ns, * and **: not significant, significant by the "F" test at 5% and 1% of probability, respectively.

lings treated with 40 and 80 mg L^{-1} of PBZ compared to the other treatments (Figure 3 I). The regression models and determination coefficient R^2/r^2 are described in Table 3.

In this study, for plants treated with PBZ, was observed the change of the leaf morphology since seedlings phase, with higher occurrence of trilobed leaves compared to the control, evidencing the regulator effect in anticipating the adult phase (Figure 4).

When analyzed the histochemical anatomical cuts, it was observed higher cellular density of starch grains in the stem tissue transversal cuts when treated with PBZ compared to the control treatment, represented for treatment 40 mg L^{-1} (Figure 5).

Discussion

Changes of the morphophysiological and biochemical parameters with the use of gibberellins biosynthesis inhibitors is consistent being observed in several agricultural cultures. One of the main purposes is to regulate the excessive growth, increase productivity (TETO et al., 2016) and modulate the plant responses against biotic and abiotic stresses (FAN et al., 2020; MOHAMMADI et al., 2017; SHALABY et al., 2022). Additionally, it has been widely employed in the management of the floral induction in fruit plants (AJMI et al., 2020). For Mango culture the PBZ is a regulator widely used in the flowering management (CAVALCANTE et al., 2020; SILVA et al., 2022).

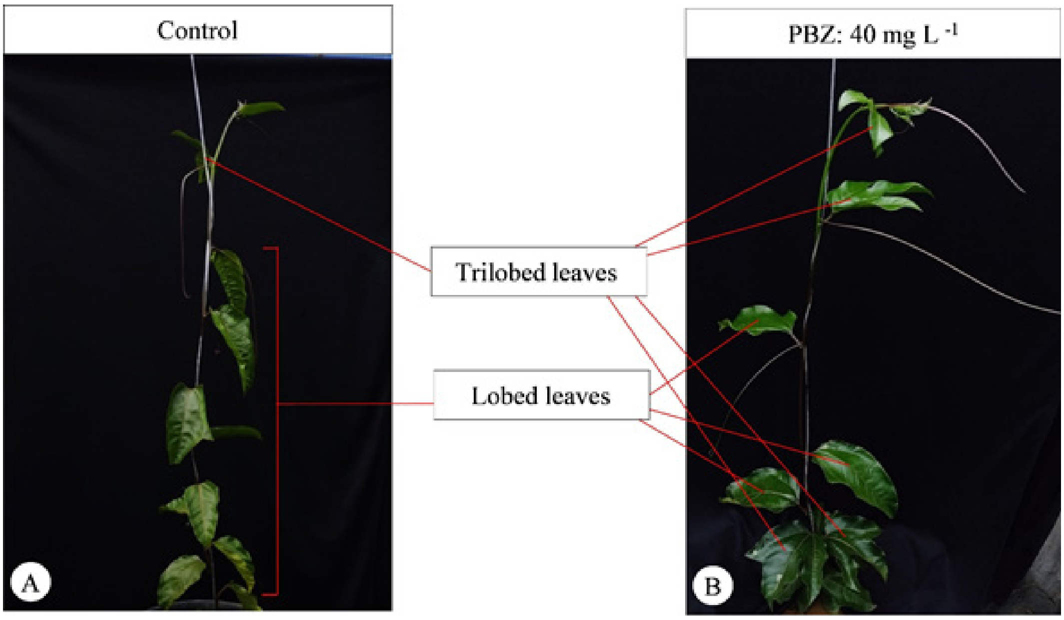

Figure 4 - Leaf morphology of sour passion fruit plants (*Passiflora edulis* Sims) coming from the application via PBZ concentrations substrate in the seedling phase.

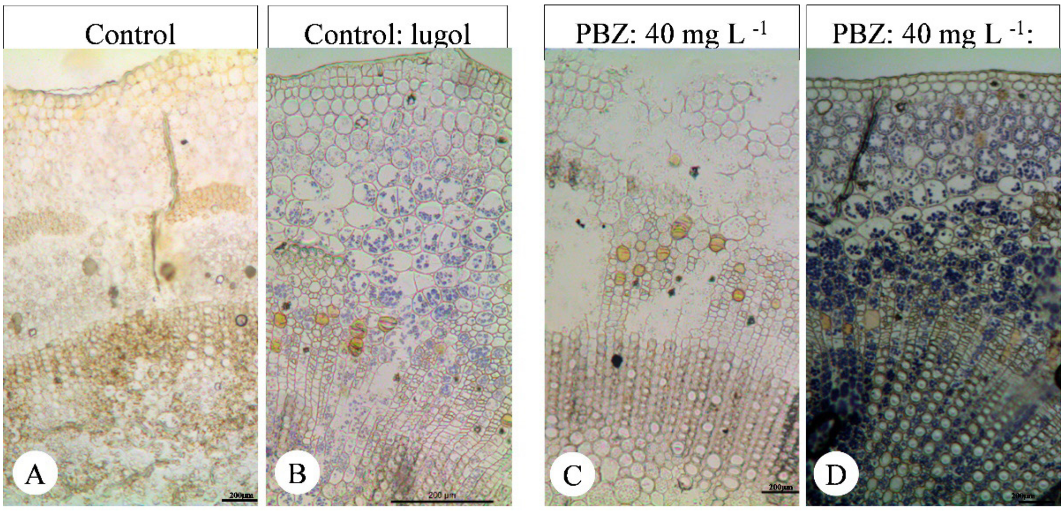

Figure 5 - Photomicrographs of stem tissue transversal cuts of sour passion fruit seedlings (*Passiflora edulis* Sims) stained with lugol solution. Purple coloration indicates starch grains.

(A) control treatment not stained; (B) control treatment stained; (C) 40 mg L^{-1} of PBZ not stained; (D) 40 mg L^{-1} of PBZ stained. Scale bar: 200 µm.

Currently, one of our research lines is to study the growth regulators in the seedlings species spread of the genus *Passiflora* in order to improve the quality and to induce resistance mechanisms to the main biotic and abiotic stresses (TEIXEIRA et al., 2019; TEIXEIRA et al., 2021; TEIXEIRA et al., 2023).

When we use growth regulators, especially triazole compounds in propagation materials, some protocols should be followed related mainly to the concentrations. The growth restriction, caused by the inhibition of endogenous gibberellin synthesis can interfere in the seedlings quality and affect the subsequent development stages. In this study, we verified that for the seedlings submitted to growth regulator intermediate concentrations the quality was high, occurring optimization of the morphophysiological and biochemical aspects, resulting in the precocity of the plant reproductive development after the transplant.

The growth regulator restricted the accumulation of fresh and dry biomass of the seedlings shoot, effect observed in other fruit plants such as apple, cherry trees, peaches and mangos (GOLLAGI et al., 2019).

The growth restriction of the shoot is the result of a set of phenological changes such as the reduction of the rod length, number of leaves, total leaf area, among others, already discussed previously for *Passiflora edulis* (TEIXEIRA et al., 2021). The lower gibberellins synthesis by PBZ and, eventually, the stimulation of other hormones in the plant result in physical and metabolic changes at cellular level (RADEMACHER, 2016), it can reduce the cells elasticity coefficient, limiting cell elongation (MARSHALL and DUMBROFF, 1999). Additionally, the PBZ promotes the accumulation of DELLA proteins, responsible for inhibiting gibberellins action (ZHENG et al., 2016). In association with these responses, it can cause growth restriction.

When the plants are submitted to PBZ, to the change of the source-drain relationship, occurring the stimulus to root growth at the expense of the smallest shoot vegetative vigor, fact observed for the present study and reported in other fruit plants (KISHORE et al., 2022). Teixeira et al. (2019) observed the largest number of cell layers and root cortex thickness and, consequently, the increase in diameter and volume in *P.edulis* seedlings using similar concentrations. Greater root development is an important factor as it is associated with greater ability to set and establish seedlings in the field, especially in conditions of water stress. **(**BINOTTI et al., 2019).

Changes in the tissues shoot/root leaves enable a redistribution of biomass promoting a balance in growth related to some parameters, such as stem height and seedlings stem diameter. For biomass partition, there was reduction for the shoot/root ratio due to the increase of PBZ concentrations, effect associated with the decrease of the shoot dry mass and increase of the root dry mass. Values lower than 2.0 for this characteristic, as verified in the present study, in concentrations above 47 mg L^{-1}, indicate better quality index (GOMES and PAIVA, 2012). The increase of the seedlings shoot dry mass and lower roots dry mass can cause a low initial stand after transplantation, due to the low instability in water supply and absorption by the root system (CALDEIRA et al., 2013).

In the roots, the increase of dry and fresh mass accumulation could be related to water availability in these organs. Morphological and anatomical changes resulting from paclobutrazol management related to the increase of water storage capacity and xylem hydraulic conductivity were considered as factors that determined the greatest roots vigor (TEIXEIRA et al., 2019). In the seedlings post transplantation phase, although there was decrease of the roots dry mass for plants originating from seedlings treated with PBZ, the fresh mass remained high. The change of root morphology verified in the seedlings was fixed in the post-transplantation stage, making the roots a water reserve organ for the sour passion fruit plants, with high percentage of water content, when compared to the control (Figure 3 G).

Growth regulators are associated with important physiological and biochemical effects in plants. Triazole compounds are related to the increase of enzymes, antioxidant molecules and optimization of foliar gas exchanges (IQBAL et al., 2020; SANTOS FILHO et al., 2022). In this study, the highest chlorophyll levels and green color intensity in the leaves (SPAD index) were observed for the seedlings treated with PBZ. Several factors are involved in the greater synthesis of chlorophyll associated with the regulator. The increase of cytokine synthesis as the effect of gibberellin biosynthesis inhibitor, results in increase of chlorophyll synthesis, maintenance of chloroplasts stability (NIVEDITHADEVI et al., 2012; NOURIYANI et al., 2012), increase of the number of chloroplasts per cells (XIA et al., 2018) and in most cases associated the pigment concentration effect, caused by the restriction of cellular elongation (BEROVA and ZLATKO, 2000). In *Betula alnoides* Buch (WANG et al., 2019) and banana tree (CHANG et al., 2019), the smaller the leaf area in plants treated with PBZ, the higher were the chlorophyll levels.

For the gas exchanges of sour passion fruit seedlings, it was verified in the present study that, the characteristics more responsive to PBZ were *W* and g_s, a similar fact to that reported by Navarro et al. (2007) and Waqas et al. (2019).

From the greater CO_2 flow, through the stomata, occurs the increase of assimilation capacity in passion fruit leaves, which results in growth increase. For the present study, this relationship is observed only for the accumulation of total fresh mass and roots fresh and dry mass due to PBZ concentrations (Figure 2 A, B). When analyzing the accumulation of total dry mass and shoot fresh and dry mass, it was verified decrease according to PBZ, while, for *W and* g_s, values higher than the control are observed.

The direction of the carbon metabolism route for defense mechanisms (metabolites synthesis with enzymatic activity – superoxide dismutase, catalase, polyphenoloxidase, synthesis of non-enzymatic metabolites such as proline and compounds derived from the phenyl alanine route, as phenolic compounds and lignin) at the expense of structural function (cellulose synthesis and hemicellulose) has been related to the effects of triazols (KAMRAN et al., 2018; LALIHYA et., 2017; REZAYIAN et al., 2018; WAQAS et al., 2019). The increase of carotenoid pigment levels verified for the present study for seedlings submitted to PBZ is an indicator of the expression of this defense mechanism related to oxygen reactive species.

In previous studies, for sour passion fruit submitted to similar concentrations, were verified increase in the phenolic compounds synthesis, lignin and calcium oxalate crystals in the stem and root (TEIXEIRA et al., 2019). Thus, the increase of g_s and *W* values in seedlings submitted to PBZ would have been directed to secondary metabolism routes, resulting in reduction of dry mass accumulation especially in the shoot.

Increased transpiration rates as a function of increased PBZ concentrations were associated with stomatal conductance increase resulting from expression of regulator-induced osmotic adjustment mechanism. The application of PBZ is associated with osmolytes elevation, especially of proline (REZAYIAN et al., 2018).

Growth regulator induced modifications in passion fruit seedlings morphophysiology, but, after transplantation, the restriction of shoot growth prevailed. However, the morphophysiological and biochemical changes in the seedlings phase with PBZ were determinants for the plant reproductive precocity, resulting in increased flowering speed index (FSI) and in the number of reproductive organs, when the seedlings were submitted to PBZ 40 mg L^{-1} concentration (Figure 3 E, F).

Cytokines have been associated with induction of transition precocity from vegetative phase to sour passion fruit reproductive stadium. Due to the interrelationship between cytokines and gibberellins in the juvenility process (BARBOSA and DORNELAS, 2021), PBZ management is an important strategy explored for many fruits. For mangoes trees treated with PBZ, an increase in the levels of abscisic acid and cytokinin and a reduction in the levels of gibberellin in the buds were observed, which provided the initiation of flowering (UPRETI et al., 2013). In fruit trees planting of *Durio zibethinus* management with PBZ in juvenile branches enabled the occurrence of flowering and fruit production in intercropped season (WIANGSAMUT and WIANGASAMUT, 2022). In pear orchards Rocha cv. cultivated in the South of Brazil, PBZ management resulted in flowering increase (CARRA et al., 2022).

For the genus *Passiflora* it was verified that the transition to the adult phase is associated with the heteroblastic process, carbohydrate elevation and increase of auxin and zeatin biosynthesis (SILVA et al., 2019). For the present study, although the intervention with growth regulator was performed in the seedlings phase, the increase of starch beads density in stem tissue as well as the precocity of the emergence of trilobed leaves in the seedlings treated with PBZ. Signs of transition anticipation from the juvenile to adult vegetative phase were considered. (SMEEKENS et al., 2010; YANG et al., 2013).

Conclusions

The balance in growth between shoots and roots by application of paclobutrazol via substrate increases the quality of sour passion fruit seedlings.

Leaf gas exchange in sour passion fruit seedlings is favored by the increase in PBZ concentration.

The greatest root growth vigor and increase in carotenes content in sour passion fruit seedlings leaves is verified for concentrations higher than 40 mg L^{-1} of PBZ.

The lowest accumulation of shoot biomass and the functionality of the roots as water reserve organs of sour passion fruit seedlings treated with paclobutrazol persists after transplanting into pots.

The restriction of gibberellin biosynthesis by means of PBZ in the seedling stage of sour passion fruit induces an accumulation of starch in the stem tissue, heteroblastic precocity and anticipation of the transition to the adult stage.

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