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Morphology of yellow passion fruit seedlings submitted to triazole induced growth inhibition

Morfologia de mudas de maracujazeiro amarelo submetidas a restrição de crescimento por triazol

Ednilson Carvalho Teixeira[1], **Sylvana Naomi Matsumoto**[2*], **Delmira da Costa Silva**[3], **Luanna Fernandes Pereira**[1], **Anselmo Eloy Silveira Viana**[2], **Alessandro de Magalhães Arantes**[4]

[1]Universidade Estadual do Sudoeste da Bahia/UESB, Vitória da Conquista, Bahia, Brasil
[2]Universidade Estadual do Sudoeste da Bahia/UESB, Departamento de Fitotecnia e Zootecnia, Vitória da Conquista, Bahia, Brasil
[3]Universidade Estadual de Santa Cruz/UESC, Departamento de Ciências Biológicas, Ilhéus, Bahia, Brasil
[4]Instituto Federal de Educação, Ciência e Tecnologia Baiano, Guanambi, Bahia, Brasil
*Corresponding author: snaomi@uesb.edu.br
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ABSTRACT

Inhibiting seedling vegetative growth may be a strategy for improving modulation against biotic and abiotic stresses after transplanting. The objective of this study was to evaluate morphological responses of yellow passion fruit (*Passiflora edulis*) seedlings to growth inhibition resulted from the application of an inhibitor of gibberellin biosynthesis. The experiment was carried out in a greenhouse using seedlings grown in 290 cm^3 polyethylene tubes. At 40 days after emergence, trays containing the seedlings were immersed in solutions at concentrations of 0, 40, 80, 120 and 160 mg (Paclobutrazol) PBZ L^{-1} up to substrate saturation. These five treatments were laid out in a randomized block design with four replicates. At 15, 30 and 45 days after PBZ application (DAA), basal stem diameter was measured; at 45 DAA, stem diameter, individual leaf area, specific leaf area, root diameter and root volume were measured, lignin content and the anatomy of leaves, stems, and roots were analyzed. Triazole-induced anatomical alterations in stems, leaves and roots cause morphological traits and intensify the specific reagent staining of phenolic compounds and lignin in yellow passion fruit (*Passiflora edulis*) seedlings.

Index terms: *Passiflora edulis*; inhibitor of gibberellin biosynthesis; propagation.

RESUMO

A restrição do vigor vegetativo de mudas pode ser uma estratégia para otimização da modulação frente à estresses bióticos e abióticos após o transplantio. O objetivo desse estudo foi verificar se os parâmetros morfológicos de mudas de maracujazeiro amarelo (*Passiflora edulis*) são afetados pela restrição do crescimento com aplicação de inibidor da biossíntese de giberelina. O ensaio foi realizado em casa de vegetação a partir de mudas cultivadas em tubetes de polietileno com capacidade de 290 cm^3. Aos 40 dias após a emergência, as bandejas com as respectivas mudas foram imersas em soluções de paclobutrazol (PBZ) com concentrações de 0, 40, 80, 120 e 160 mg L^{-1} de ingrediente ativo, até a saturação do substrato. Foi utilizado o delineamento experimental em blocos casualizados, com cinco tratamentos (concentrações de PBZ) e quatro repetições. Aos 15, 30 e 45 dias após a aplicação do PBZ (DAA), foi mensurado o diâmetro basal; aos 45 dias foram avaliados o diâmetro do caule, área foliar individual, área foliar específica, diâmetro e volume das raízes, conteúdo de lignina parte aérea e a anatomia da folha, caule e raiz. As alterações anatômicas induzidas pela restrição de crescimento por triazol condicionaram modificações na estrutura morfológica e estimulou a biossíntese de compostos secundários em mudas de maracujazeiro amarelo.

Termos para indexação: *Passiflora edulis*; inibidor da biossíntese de giberelina; propagação.

INTRODUCTION

The genus *Passiflora* is estimated to contain over 400 species of which approximately 146 are native to Brazil (Oliveira et al., 2019; Koch et al., 2019). Yellow passion fruit (*Passiflora edulis*) is the main cultivated species due to its high fruit yield, juice content, plant vigor and market acceptance (Ferreira et al., 2016).

Brazil is the world's top producer and consumer of yellow passion fruit (Faleiro; Junqueira, 2016). In 2017, 554,598 t of fruits were produced on 41,090 ha, and the Northeast region stands out with 60.92% of the country's production (Sidra, 2019). Yellow passion fruit yield ranges from 12 to 15 t ha^{-1}; however, it has a yield potential varying between 30 and 35 t ha^{-1} (Nogueira Filho et al., 2011).

Despite its economic importance, longevity and productivity of yellow passion fruit orchards are limited by poor soils, climate adversities, and, especially, diseases such as fusarium wilt (*Fusarium oxysporum f.* sp. *Passiflorae*), collar rot (*Fusarium solani*) (Fischer; Rezende, 2008), and fruit hardening (mainly cowpea aphid-borne mosaic virus) (Cerqueira-Silva et al., 2014).

Pathogen control techniques for yellow passion fruit are mostly preventive, costly, and little efficient in suppressing these diseases; therefore, it is necessary to identify techniques that minimize crop damage by inducing tolerance and improving existing management techniques, especially with regard to seedling propagation.

Seedlings are commonly treated with chemicals to have their growth and quality restricted (Benett et al., 2014). Growth regulators are applied to seedlings (leaf or substrate treatment), thereby restricting overgrowth (Seleguini et al., 2013), strengthening the sink for photosynthates in root tissues, and increasing resistance to biotic and abiotic stresses (Mohammad et al., 2017). Moreover, some inhibitors of gibberellin hormone synthesis may induce activation and regulation of processes involved in the biosynthesis of secondary metabolites for plant defense; thus, these inhibitors have a high tolerance-inducing potential.

O Paclobutrazol (PBZ) the chemical name [(2RS, 3RS) - 1 - (4- clorofenil) - 4, 4-dimetil-2- (1, 2, 4- triazol-1-il) - pentano-3-ol] is one of the most effective triazole that inhibits gibberellin biosynthesis (Baninasab; Ghobadi, 2011). It is used to enhance seedling quality of tomato (Seleguini et al., 2013) and cucurbits (Flores et al., 2018).

Morphological and anatomical responses to inhibitors of gibberellin biosynthesis have been reported (Kofidis; Giannakoula; Ilias, 2008; Rodrigues et al., 2016; Siqueira; Cecon; Salomão, 2008; Carvalho-Zanão et al., 2017). In *Passiflora* species, the use of triazoles has been linked to (Carvalho-Zanão et al., 2017), flowering (Nave et al., 2010), and crop yield (Berberich et al., 2006; Ataíde et al., 2006); however, no anatomical or morphological changes at seedling stage have been reported.

A better understanding of possible alterations in anatomical and morphological traits in *Passiflora edulis* seedlings treated with an inhibitor of gibberellin biosynthesis may provide information for the development of management strategies for seedling propagation under limiting conditions. The objective of this study was to verify if PBZ could conditioning morphological characteristics alterations and biosynthesis of secondary compounds related to defense mechanism in yellow passion fruit seedlings.

MATERIAL AND METHODS

The trial was carried out between March and June 2018 in a greenhouse at the State University of Southeastern Bahia, campus Vitória da Conquista, Bahia state, Brazil. The climate is tropical of altitude (Cwa in the Köppen-Geiger classification). Mean annual rainfall and mean annual temperature are 733.9 mm and 20.2 °C, respectively, with rainfall mostly occurring between November and January (Sei, 2013).

Three seeds of yellow passion fruit (*Passiflora edulis*) were sown in 290 cm^3 polyethylene tubes (16 cm in length and 6.5 cm in diameter) filled with substrate (Vivatto®) composed of pine bark, vermiculite, charcoal powder, water, and phenolic foam. 15 days after emergence, seedlings were thinned out so that the most vigorous one remained.

The treatments consisted of four concentrations of paclobutrazol (PBZ) (CULTAR 250 SC® ,active ingredient PBZ 250 g L^{-1}) namely 40, 80, 120 and 160 mg L^{-1} of active ingredient and a control grown without PBZ. At 40 days after seedling emergence, the trays were immersed into PBZ solutions, according to the treatment, up to substrate level for one minute so that the substrate was saturated with the PBZ solution.

A randomized block design was used with four replicates, totaling 20 experimental units. Each unit was composed of a tray containing 54 seedlings of which the eight central ones were used for evaluations: six for assessing morphological traits and two for anatomical analyses.

At 15, 30 and 45 days after PBZ application (DAA), basal stem diameter was measured; at 45 DAA, stem diameter, individual leaf area, specific leaf area, root diameter and root volume were measured, and the anatomy of leaves, stems, and roots were analyzed.

Basal stem diameter was measured at substrate level; stem diameter, at the fourth internode counting from the base of the stem; and root diameter, at two cm from the root cap. These measurements were taken using a digital caliper (DS-60, Western, China) with 0.01 mm resolution; values were expressed in mm.

Individual leaf area was calculated by the ratio of total leaf area to the number of leaves. Total leaf area was measured in all seedling leaves, in cm^2, by means an area integrator (LI 3100, LI-COR, USA).

Specific leaf area was determined by the ratio of total leaf area to leafs dry matter, maintaining 65 °C in a stove with forced air circulation. Root volume was determined using the water displacement method (Costa et al., 2018).

For anatomical analyses, we collected from one plant of each treatment replicated three times (medium third part of leaf blade between the margin and midrib), stem (third internode counting from the apex), and root (close to the base of the stem) samples. After rising the samples in running water, they were fixed in FAA_{50} [formaldehyde, acetic acid and 70% ethanol; 1:1:18 (v/v)] (Johansen, 1940) for 48 h and stored in 70% ethanol (Berlyn; Miksche, 1976). The fixed samples were embedded in methacrylate resin (Historesin® Leica, Leica Microsystems Nussloch GmbH, Heidelberg, Germany). Cross and longitudinal sections were cut at a thickness of 5 µm using a rotary microtome (RM 22355, Leica, Deerfield, Illinois, USA) with glass blades. Sections were stained with toluidine blue at pH 4.5 (O'Brien; McCully, 1981).

Structural analyses and images were made on a light microscope (Leica DM 2500) coupled with a digital camera (DM 2500, Leica, Germany).

We analyzed digital images using the software Leica Application Suite V3 to determine the thicknesses of the abaxial and adaxial epidermal tissues, palisade parenchyma, spongy parenchyma, mesophyll, and leaf thickness (mesophyll + epidermis), total cell density, palisade cell density, lacunous cell density, total cell density for leaves; xylem vessel diameter, xylem vessel density, number of collenchyma layers, and vascular cambium thickness for stems; cortex thickness and number cortex layers for roots. Measurements were made at 10 distinct points on each sample. Calcium oxalate crystals were identified using a polarizing light microscope.

Histochemical analyses were performed using cross sections collected from root and stem samples fixed in FAA_{50}. The cross sections were subjected to ferric chloride and phloroglucinol for staining phenolic compounds and lignin, respectively (Johansen, 1940).

At medium third part of leaf blade between the margin and midrib, stomatal density, stomatal index and number of epidermal cells per mm^2 were determined on epidermal peels manually excised with a steal blade on the abaxial side of leaves inserted at the third node counting from the apex. Leaf segments were placed on a glass slide containing water and glycerin and photomicrographed with a binocular light microscope (NO. 790081, Coleman, Santo André, Brazil, at magnification of 4x) coupled with a digital image capture system (LG color camera CCD digital). Epidermal cells and stomata were counted using the software ANATI QUANTI (Aguiar et. al., 2007).

For each treatment, four samples of seedlings leaves, previously submitted to stove with forced air circulation drying until constant weight, were ground and lignin content was quantified by acid hydrolysis (INCTY-CA F-005/1 Method) (Detmann et al., 2012).

Data were tested for homogeneity of variance (Cochran), 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 trait to PBZ concentrations. Best-fit models were chosen based on the significance level ($p<0.05$) and the highest coefficient of determination ($R^2 \geq 60$) associated with the biological value. For anatomical traits, only a one-way ANOVA of the regression was performed using the treatment means. All statistical analyses were conducted using SAEG, version 9.1.

RESULTS AND DISCUSSION

The application of PBZ at different concentrations affected most morphological traits evaluated in leaves, stems and roots. There was no treatment effect on specific leaf area (SLA) and basal stem diameter (BSD) 45 days after PBZ application (DAA) (Table 1).

Table 1: Summary of analysis of variance and coefficients of variation of individual leaf area (ILA), specific leaf area (SLA) and basal stem diameter (BSD) measured at 15, 30, 45 DAA; stem diameter (SDM) at 45 DAA, root diameter (RDM) and root volume (RVL) of yellow passion fruit seedlings (*Passiflora edulis*) after PBZ application at different concentrations.

SV	DF	MEAN SQUARES							
		ILA	SLA x 10^2	BSD (15d) x 10^{-2}	BSD (30d) x 10^{-3}	BSD (45d) x 10^{-2}	SDM (45d) x 10^{-1}	RDM x 10^{-2}	RVL
Block	3	15.9	1.5	2.0	8.0	7.0	2.3*	3.0	20.0*
PBZ	4	85.8**	5.2	8.0**	62.0**	19.0	3.8**	329.0**	76.0**
Residue	12	12.9	3.7	1.0	17.0	8.0	0.6	6.0	5.2
CV (%)		10.1	12.7	3.7	3.9	8.1	7.1	12.6	13.3

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

Responses of individual leaf area (ILA), number of epidermal cells (NEC), leaf thickness (LT), stomatal index (SMI), total cell density (TCD), palisade cell density (PCD), lacunous cell density (LCD) and lignin content (LC) in relation to PBZ concentrations were fitted to quadratic models (Figure 1A, 1B, 1D, 1F, 1H and 1I, respectively). Stomatal density (SMD), mesophyll thickness (MPT), palisade parenchyma thickness (PPT), and spongy parenchyma thickness (SPT) were fitted to linear models (Figure 1F, 1D, 1E). Abaxial epidermis thickness (ABT) were fitted to cubic model (Figure 1C) and total cell density model square root.

Individual leaf area (ILA) decreased with increasing PBZ concentration up to 115 mg L^{-1} (31.75 cm^2), remaining below the control (Figure 1A). The regulation of hormone-induced leaf expansion initially takes place in the epidermis (Becraft, 1999). Gibberellins modulate cell elongation by activating expansins (Xu et al., 2017) and xyloglucan endotransglucosylase (XET) (Jan et al., 2004). When the biosynthesis of gibberellins in epidermal cells is inhibited, leaf expansion is restricted (Li et al., 2011). This response was observed in PBZ-treated enrique's crowns (*Agapanthus praecox* ssp.) in which GA_4 and XET decreased more than 90% in epidermal cells (Yue et al., 2016). In a present study the restriction of cell elongation as a result of gibberellin synthesis inhibition can be observed by means of rising of epidermal cell density (greater number of cell in base of area) (Figure 1B).

The elongation of epidermal cells was inhibited on the abaxial leaf side in PBZ-treated seedlings, as shown by the increase in number of epidermal cells (NEC) (Figure 1B) and the reduction in abaxial epidermis thickness (ABT); (Figure 1C). Conversely, the reduction in leaf length and area in Japanese Lily (*Lilium longiflorium*) treated with PBZ was associated with decreased cell dimensions on the adaxial epidermis (Zhu et al., 2016).

The effect of PBZ on abaxial and adaxial epidermal cells is quite controversial, with increasing (Tsegaw; Hammes; Robbertse, 2005) and no pronounced effects (Rodrigues et al., 2016; Carvalho-Zanão et al., 2017) being reported. These variations depend on the species, concentrations and environment (Tinoco et al., 2011).

The decrease in abaxial epidermis thickness (ABT) in leaves of wheat seedlings treated with triadimefon (triazole) was related to a decrease in cell width (Gao; Hofstra; Fletcher, 1988). Abaxial epidermis thickness (ABT) in leaves of pot-grown jasmine coffee (*Tabernaemontana coronaria*) reduced at PBZ concentration of 50 mg L^{-1}; concentrations higher than 150 mg L^{-1} led to increased epidermis thickness (Youssef; El-Aal, 2013).

Despite decreasing abaxial epidermis thickness (ABT), it was observed an increase in leaf thickness (LT) with increasing PBZ concentration (Figure 1D). Since PBZ has been shown to inhibit the catabolism of cytokinins (Upreti et al., 2013), high concentrations of this growth regulator increases cell division, which in turn increases the number of cells composing the palisade and spongy parenchymas.

Increases in palisade parenchyma thickness (PPT) and spongy parenchyma thickness (SPT) of 28.55% and 56.0% compared to the control, respectively (Figure 1E), were related to the increase in mesophyll thickness (MPT) (Figure 1D), which is consistent with Carvalho-Zanão et al. (2018). Leaves of plants treated with inhibitors of gibberellin tend to be smaller; however, there is no alteration in cell composition, thus increasing the thickness of the leaf tissue (Souza et al., 2007).

Rodrigues et al. (2016) assessing the effect of PBZ on leaf anatomy of australian cedar (*Toona ciliata*) clones reported an increase in palisade parenchyma thickness, spongy parenchyma thickness and mesophyll thickness as well as higher leaf thickness when treated at higher PBZ concentrations. Carvalho-Zanão et al. (2017) studying leaf anatomy of Yellow Terrazza® and Red White Terrazza® rose cultivars treated with PBZ at concentrations of up to 80 mg L^{-1} reported increases in palisade parenchyma thickness, spongy parenchyma thickness and leaf thickness.

As PBZ increases leaf thickness (LT), specific leaf area (SLA) was expected to decrease, as observed in coffee shrubs (D'Arede et al., 2017); nonetheless, this response was not observed in our study. To SLA, it was considered the total leaf area in relation to weight of total leaves of shoot; by this way, it resulted in "dilution", due to a short time period of PBZ effect to induce leaf thickness.

Stomatal index (SMI) and stomata density (SMD) were higher in PBZ-treated seedlings (Figure 1F). Stomata density (SMD) ranged from 30 to 60 stomata mm^{-2}, lower than that reported by Sánchez, Fischer and Sanjuanelo (2013) (106.53 stomata mm^{-2}) for the same species. Higher stomatal index (SMI) and stomata density (SMD) were observed on the abaxial leaf surface of common bean seedling treated with 8.5 to 27 μM PBZ (Tari, 2003). In inhibiting the biosynthesis of brassinosteroids, PBZ stimulates the differentiation and development of stomata in leaves (Siddiqui; Hayat; Bajguz, 2018).

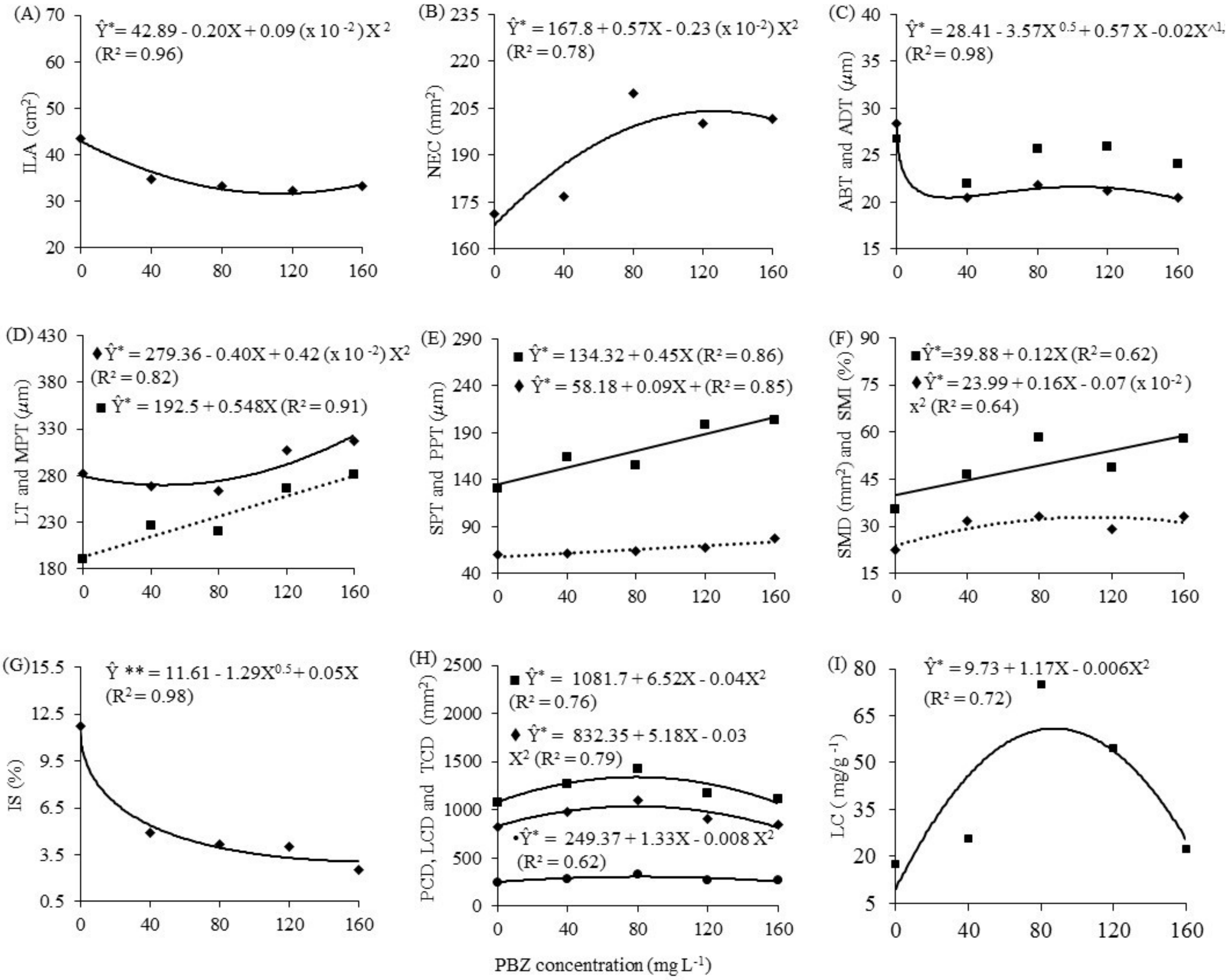

Figure 1: Leaf morphological and anatomical traits of yellow passion fruit (*Passiflora edulis*) seedlings in relation to PBZ application to substrate; (A) individual leaf area (ILA); (B) number of epidermal cells mm^{-2} (NEC); (C) ♦ abaxial epidermis thickness (ABT) and ■ adaxial thickness (ADT); (D) ♦ leaf thickness (LT) and ■ mesophyll thickness (MPT); (E) ■ spongy parenchyma thickness (SPT) and ♦ palisade parenchyma thickness (PPT); (F) ■ stomata density (SMD) and ♦ stomatal index (SMI); (G) total cell density; (H) ■ total cell density (TCD) • palisade cell density (PCD) and ♦ lacunous cell density (LCD); (I) lignin content (LC) in relation. * Significant at 5% probability and **significant at 1% probability, by F test.

It was found that the increase in leaf thickness (LT) occurred simultaneously with the decreasing of intercellular spaces and elevation of density of palisade parenchyma cells and spongy parenchyma in leaves of seedlings submitted to PBZ (Figure 1G and 1H; Figure 2). Similar result was reported for wheat grown on soil treated with PBZ at concentration of 100 mg L^{-1} (Mohamed; Allah; Rrazik, 2013).

The time of mesophyll cells elongation phase ends earlier in comparison to epidermal cells. After the beginning of spongy parenchyma cell wall dissolution, the greater the mismatch between epidermal cell elongation in relation to mesophyll cells, the major will be the intercellular spaces (Dale, 1988). Due to PBZ anticipate the end time of epidermal cellular elongation phase, the potential of mesophyll intercellular spaces formation could be reduced (Shevchuk et al., 2019).

It was observed that PBZ effect in restrict the cell elongation was differentiated to epidermal and mesophyll cells (Figure 1B and 1H). By this way, under elevated PBZ concentration the elongation restriction of epidermal cell associated to recuperation of mesophyll cells resulted in reduction of intercellular spaces (Figure 1G).

The lignin content in leaves reach a maximum value (61.1 mg g^{-1}) at 87.6 mg L^{-1} PBZ concentration (Figure 1I). PBZ modulates only the activation of enzymes related to lignin biosynthesis (Giraldo et al., 2011). Accordingly, other factors affecting substrate availability may regulate lignin biosynthesis capacity regardless of PBZ inhibition of gibberellin synthesis.

The relation between lignin content and PBZ concentration is controversial; in wheat plant submitted to PBZ it was verified a rising of lignin content (Peng et al., 2014) but Falcioni et al. (2017) observed in tobacco (*Nicotiana tabacum*) plants reduction. This fact is related to lignin biosynthesis be determined to both the precursor availability and the enzymatic activity (Falcioni et al., 2017).

Correlations between lignin accumulation and basal stem stiffness have been reported in wheat plants (Zheng et al., 2017) and rice (Weng et al., 2017; Wu et al., 2017). Lignin promotes the transport of minerals through the vascular bundles and alleviates environmental stresses such as water stress, heat stress, salinity, heavy metal toxicity, and resistance to pests and diseases (Liu; Luo; Zheng, 2018). As for pests, the increase in lignin biosynthesis is related to the population reduction of sap-sucking insects such as whiteflies and aphids, which deposit pathogens directly

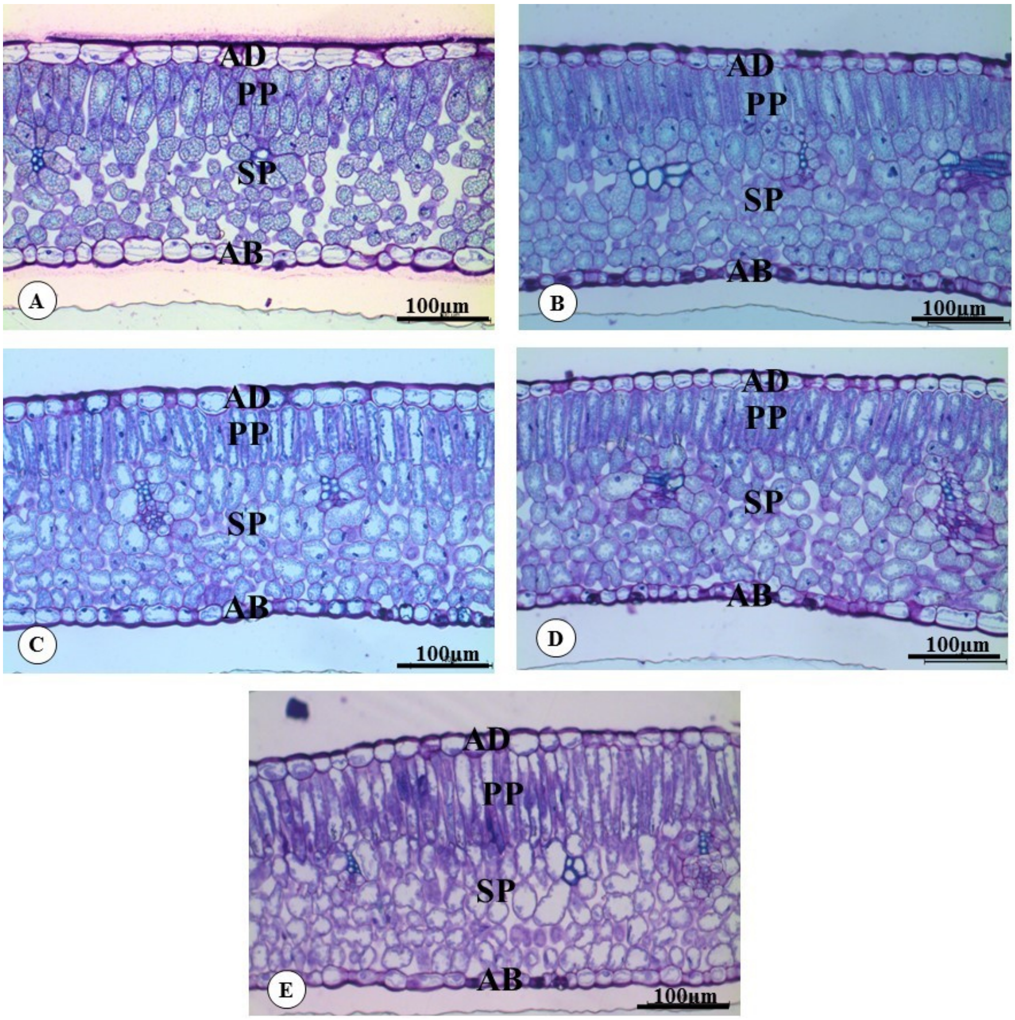

Figure 2: Photomicrographs of leaf cross sections of yellow passion fruit (*Passiflora edulis*) seedlings after application of PBZ to substrate at concentration of 0 mg L^{-1} (control) (A); 40 mg L^{-1} (B); 80 mg L^{-1} (C); 120 mg L^{-1} (D); and 160 mg L^{-1} (E). Abaxial epidermis (AB), adaxial epidermis (AD), palisade parenchyma (PP) and spongy parenchyma (SP). Bar = 100 µm.

in the vascular system from which they quickly spread throughout the plant (Taiz et al. al., 2017).

Wang et al. (2018) found that factors that increase lignin biosynthesis limit the invasion of aphid vectors and increase plant tolerance to these insects. Mandal et al. (2013), evaluating tomato varieties resistant and susceptible to pathogenic bacteria, found that resistant varieties had higher lignin content than susceptible ones.

Basal stem diameter (BSD) decreased linearly and stem diameter (SDM) increased linearly (Figure 3A, 3B) with increasing PBZ concentration. Regarding stem anatomical traits, vascular cambium thickness (VCT) and xylem vessel diameter (XDM) decreased linearly with increasing PBZ (Figure 3D, 3F); responses of the number of collenchyma layers (NCL) to PBZ concentration were fitted to a quadratic model (Figure 3C).

The PBZ effect to stem diameter was differentiated when was considered the tissue age in analysis. According to Mabvongwe et al. (2016), the PBZ effect to stem is dependent to regulator concentration and tissue age. The diameter of stem basal tissue was shorter in function to increasing, while in distal tissues (fourth internode) it was observed a rising of values (Figure 3B). Gibberellin content is a determinant factor to radial expansion of stem; in young tissues submitted to PBZ, gibberellin content is reduced, internode elongation is restricted, resulting in increasing of stem thickness (Wenzel; Willanson; Wasteneys, 2000). Paclobutrazol treatment increases the thickness of cortex, vascular bundles, and pith diameter resulting in thicker stems (Tsegaw; Hammes; Robbertse, 2005). Similarly, Rani, Misra and Singh (2018) reported an increase in stem diameter in PBZ-treated lemon trees.

High PBZ concentrations may inhibit the increase in basal stem diameter (Kamran et al., 2018); notwithstanding, PBZ treatment induced an increase stem diameter (SDM) compared to the control (Figure 3 B).

To basal diameter, due to structural consolidation of mature tissues, PBZ effect was driven to reserve availability to sink organs. Carvalho-Zanão et. al (2017) evaluating two rose cultivars treated with PBZ also reported a linear decrease in stem diameter with increasing PBZ dose. To soybeans plants submitted to triazole (Uniconazole), it was verified an intensification of starch conversion to sucrose in source organs and directing it to sink organs (Liu; Luo; Zheng, 2018). By this way, in present study, the basal diameter reduction of yellow passion fruit seedling was associated to starch decreasing.

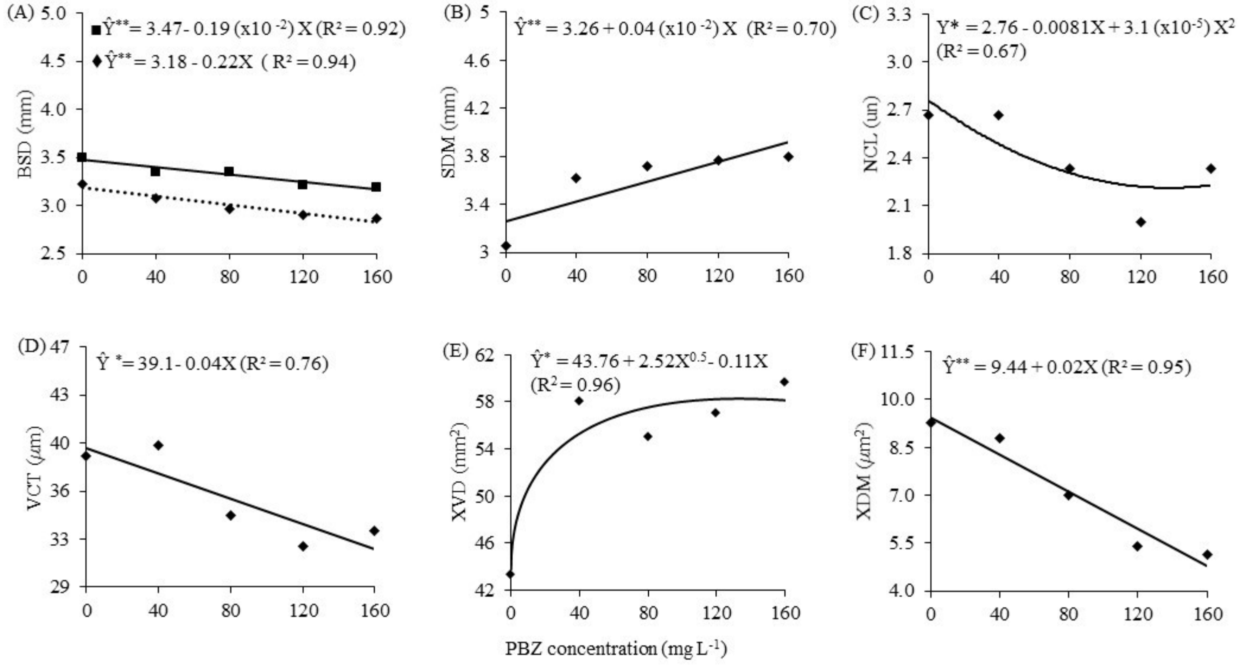

Figure 3: Morphological and anatomical responses of yellow passion fruit (*Passiflora edulis*) seedlings to substrate PBZ treatment. (A) Basal stem diameter at ♦15 and ■30 DAA; (B) stem diameter (SDM), (C) number of collenchyma layers (NCL); (D) vascular cambium thickness (VCT); (E) xylem vessel density (XVD); (F) xylem diameter (XDM) at 45 DAA. * Significant at 5% probability and **significant at 1% probability by F test.

Although the application of PBZ resulted in increased stem diameter (SDM), an opposite trend was observed for number of collenchyma layers (NCL) and vascular cambium thickness (VCT) (Figure 3C, 3D, respectively).

In contrast to our findings, inhibitors of gibberellin biosynthesis generally increases number of collenchyma layers as observed in triazole-treated coriander (prohexadione calcium and daminozide) (Kofidi; Giannakoula; Ilias, 2008) and in pot-grown Jasmine Coffee (*Tabernaemontana coronaria*) Stand plants (Youssef; El-Aal, 2013).

Although the reduction in number of collenchyma layers (NCL) was verified in this study, we verified a higher thickness of cortex (data not shown) that might have been a response to PBZ treatment, which contributed to higher stem diameter (SDM). A similar trend was also reported for soybean plants treated with 10 mg L^{-1} PBZ (El-Aal; Eid, 2017).

Vascular cambium thickness (VCT) in stems decreased linearly as a function of increasing PBZ concentration (Figure 3D). This trend was associated with increased xylem vessel density (XVD) (Figure 3E). The decrease in vascular cambium thickness (VCT) indicates increased cell division activity in collenchyma tissues, thereby increasing xylem cell differentiation due to PBZ treatment, leading to an increase in xylem vessel density.

There was a 38% increase in xylem vessel density (XVD in PBZ-treated seedlings compared to the control. In stimulating the biosynthesis of auxins and, especially, cytokinins, PBZ induces vascular cambium cells to differentiate into xylem vessels, thus increasing xylem vessel density with increasing PBZ concentration (Tsegaw, 2003).

Despite the increase in xylem vessel density (XVD), xylem vessel diameter (XDM) reduced by 44.38% in stems of PBZ-treated seedlings (Figure 3D). This reduction was attributed to PBZ-induced inhibition of cell elongation (Nazarudin; Fauzi; Tsan, 2007).

The effect of PBZ concentrations on root anatomical traits was fitted to quadratic models for root volume (RVL) and number of root cortex layers (NRCL), and to cubic models for root diameter (RDM) and root cortex thickness (RCT). For these traits, values were higher in PBZ-treated seedlings than in controls (Figure 4).

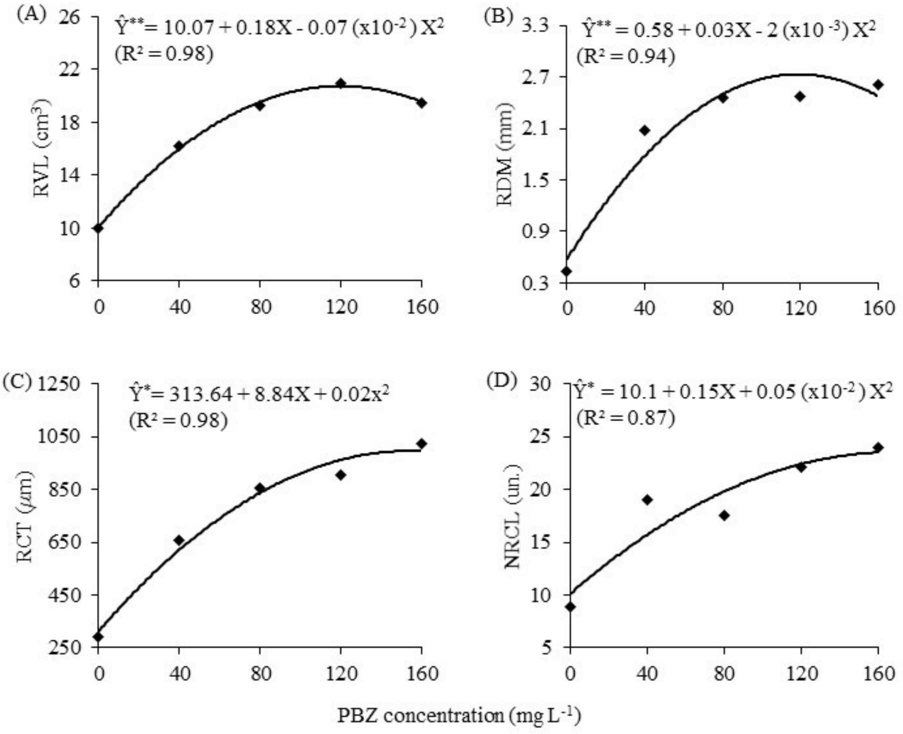

Figure 4: Root morphological and anatomical responses in yellow passion fruit seedlings (*Passiflora edulis*) to substrate PBZ treatment. (A) root volume (RVL); (B) root diameter (RDM); (C) root cortex thickness (RCT); (D) number of root cortex layers (NRCL). * Significant at 5% probability and **significant at 1% probability by F test.

The relationship between number of root cortex layers (NRCL) and PBZ concentrations was fitted to an increasing quadratic model showing an increase of 166% when compared to the control, from 9 to 24 cell layers (Figure 4D). This PBZ effect has also been found in studying roots of chrysanthemum (Burrows; Boag; Stewart, 1992) and soybeans (Barnes; Walser; Davis, 1989).

PBZ can generate an increase in cytokinin levels (Burondkar et al., 2016) maximizing cell division and, consequently increasing number of root cortex layers (NRCL) and root cortex thickness (RCT), root diameter (RDM) and root volume (RVL) (Figure 4C, 4B and 4A).

Changes in root diameter and root volume have been reported for other species treated with inhibitor of gibberellin biosynthesis (Nepomuceno et al., 2007; Te-chato; Nujeen; Muangsorn, 2009). Cell division still occurs when gibberellin biosynthesis is inhibited by PBZ, but the elongation of new cells is reduced (Nepomuceno et al., 2007); as a consequence, there is a stimulus to cell elongation and cell division, with increased root diameter and root volume.

PBZ can alter the source-sink relationship by increasing the flow of photosynthates to roots to the detriment of the shoot (Rademacher, 2015). This transport of photosynthates to the roots would be associated with a higher tolerance to biotic and abiotic stresses (Sankar; Karthishwaran; Somasundaram, 2013; Seleguini et al., 2013).

Calcium oxalate crystals (druses) were observed in stem and root tissues of PBZ-treated seedlings. In stem, the druses were observed at internal region of cortex and phloem and, in root it was verified only in phloem.

For the histochemical test, a positive reaction for phenolic compounds (brown) and lignin (pink color) was verified in PBZ-treated seedlings, as observed for the treatment at 80 mg L^{-1} PBZ (Figure 5). Idiobalsts containing phenolic compounds were observed in parenchyma of internal region of cortex and, mainly, associated to phloem

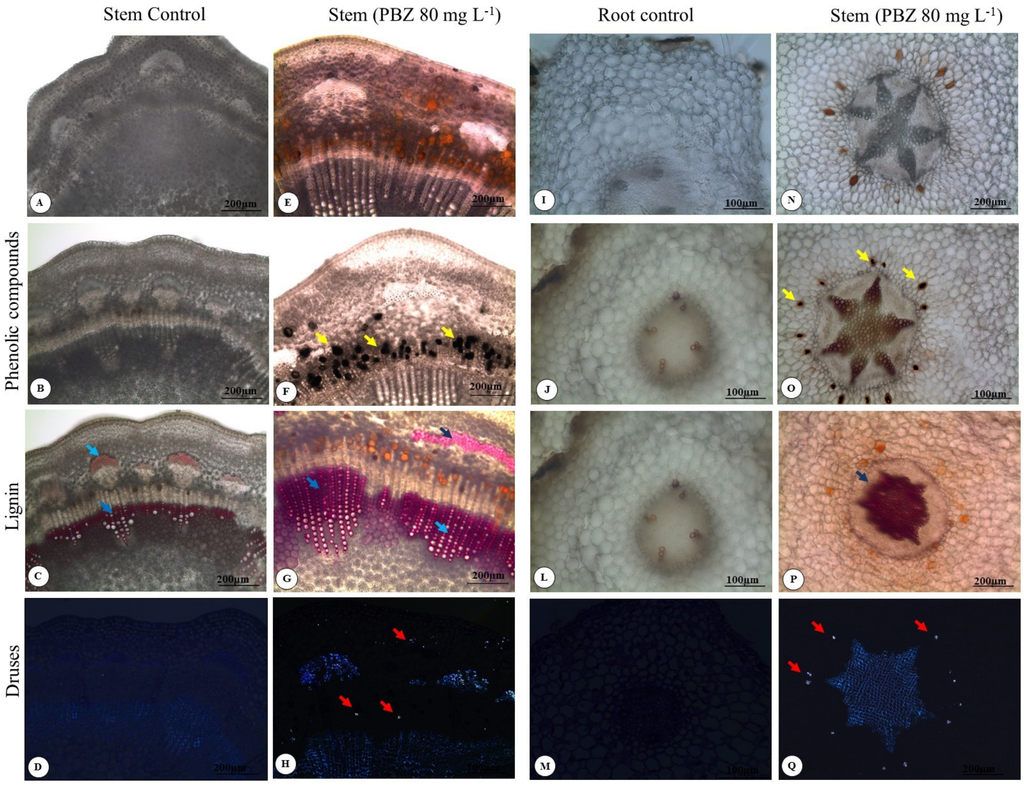

Figure 5: Photomicrographs of cross sections of stems and roots of yellow passion fruit (*Passiflora edulis*) seedlings. Stem in control: (A) unstained section; (B) ferric chloride; (C) phloroglucinol; (D) polarizing filter. Stem in PBZ-treated at 80 mg L^{-1}: (E) unstained section; (F) ferric chloride; (G) phloroglucinol; (H) polarizing filter. Root in control: (I) unstained section; (J) ferric chloride; (L) phloroglucinol; (M) polarizing filter. Root in PBZ-treated seedlings at 80 mg L^{-1}: (N) unstained section; (O) ferric chloride; (P) phloroglucinol; (Q) polarizing filter. Arrows indicate positive reaction: phenolic compounds (yellow arrow), lignin (blue arrow), and druses (red arrow).

cells of stem. To roots, this idioblasts occurred in internal region of cortex, next to endoderm. Lignin was evidenced by a higher intensity of red color reaction, presented in xylem cell walls (fibers and vessel elments) and at fibers associated to stem phloem. In roots, the pith region and xylem, it was verified a intensification of color reaction that was associated to greater lignification level. Druses were verified in stem, associated to parenchyma of internal cortex and to phloem, and in roots, only associated to phloem.

Calcium oxalate crystals occur naturally in many species. Functions of calcium oxalate include calcium regulation/sequestration, ion balance regulation, detoxification of high levels of heavy metals and oxalic acid, enhancement of absorption/reflection of incident radiation, protection from herbivory, and release of carbon when stomatal conductance is low (Li et al., 2019). When stomatal conductance is low, the number of druses was found to increase in the phloem of *Passiflora edulis*, *Passiflora setacea*, *Passiflora gibertii* and *Passiflora cincinatta* (Souza et al., 2018).

In studying the mitigation of temperature stress using PBZ (100 mg L^{-1}) in wheat, an increase in the number of druses in the stem tissue was observed when compared to the control (Mohamed; Allah; Rrazik, 2013). PBZ indirectly impacts ascorbic acid biosynthesis, thereby increasing the formation of calcium oxalate crystals (druses) in stem tissues (Soumya; Kumar; Pal, 2017). Ascorbic acid is thought to be the main precursor of calcium oxalate in idioblasts (Li et al., 2019).

The increase in synthesis of phenolic compounds in PBZ treated seedlings results in increasing antioxidant effect of defense mechanisms. Higher phenolic compounds contents were reported in studies on reducing gibberellin biosynthesis by applying PBZ to stevia (*Stevia rebaudiana)* (Hajhashemi, 2018) and pomegranate (*Punica granatum*) (Moradi et al., 2017).

CONCLUSIONS

Triazole-induced anatomical alterations in stems, leaves and roots cause morphological traits and intensify the specific reagent staining of phenolic compounds and lignin in yellow passion fruit (*Passiflora edulis*) seedlings. Individual leaf area of yellow passion fruit seedlings is related to effect of PBZ in reduce the epidermis elongation. The leaf thickness increasing in yellow passion fruit seedlings submitted to PBZ is associated to a higher width of spongy mesophyll. The higher stem diameter induced by PBZ is related to a higher xylem vessels number. The greater volume of roots induced by PBZ is associated with a major thickness and number of cortex layers. Idioblasts containing phenolic compounds and druses were more evident in plants treated with PBZ in comparison to control ones. There are a increasing of lignification of xylem cells area, histochemical analysis of shoot and roots as well as to fibers associated to phloem. The biosynthesis of lignin is higher in yellow passion fruit seedlings submitted to 87.6 mg L^{-1} PBZ.

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