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Production of Tomato Seedlings Submitted to Treatments with Foliar Application of Paclobutrazol

Vivyan J. Conceição[1,a], Simone C. Mello[1,b], Mayara Rodrigues[1,c], Durval Dourado Neto[1,d], Marcelle Michelotti Bettoni[2,e], Juliana Lorenz Mandro Campiol[3,f], Tefide Kızıldeniz[4,g,*]

[1]*Crop Science Department, Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo (Esalq/USP), Piracicaba, SP 13418-900, Brazil*
[2]*Indepent consultant of Agriculture. Curitiba, SP, Brazil*
[3]*Hugot Sugar Technology Laboratory, Luiz de Queiroz College of Agriculture, University of São Paulo. 13418-900, Piracicaba, SP, Brazil*
[4]*Department of Biosystems Engineering, Faculty of Agricultural Sciences and Technologies, Niğde Ömer Halisdemir University, 51200, Niğde Türkiye*
[*]*Corresponding author*

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ABSTRACT

Paclobutrazol (PBZ) is a growth regulator that widely used in horticulture and in the tomato seedling growth to compact the shoots, increase the stem diameter and, root biomass, allowing more tolerance of the seedlings against adverse weather conditions. The objective of this work was to evaluate the rates of paclobutrazol (0, 4, 7, 10 and 13 mg L^{-1}) applied 15 days after sowing by foliar spray on the growth, chemical composition and xylem vessel number of tomato seedlings cultivated in two periods. The PBZ regardless of the application rate reduced the height of tomato seedlings in both growth periods. The basal stem diameter and leaf area were increased with 13 mg L^{-1} of PBZ. The lignin percentage also increased with 10 and 13 mg L^{-1} of PBZ as compared to control for both periods. The number of xylem vessel was not affected by PBZ application on the seedlings in the first period. PBZ application at rates of 7 and 10 mg L^{-1} increased the xylem vessel number in the second period. In general, the application of 13 mg L^{-1} of PBZ generated seedling more robust to overcome climate adversities. These findings contribute to science by providing insights into how much dosage of Paclobutrazol can be utilized to modify plant morphology and enhance seedling resilience, offering potential applications in agriculture for improving crop yield and sustainability, particularly in challenging environmental conditions.

[a] *vivyan.conceicao@usp.br* *https://orcid.org/0000-0002-6582-732X*
[b] *scmello@usp.br* *https://orcid.org/0000-0002-1652-8171*
[c] *mayara.rodrigues@usp.br* *https://orcid.org/0000-0001-5050-3069*
[d] *ddourado@usp.br* *https://orcid.org/0000-0002-8452-8288*
[e] *m2bettoni@gmail.com* *https://orcid.org/0000-0003-2493-5890*
[f] *julianalorenz.m@usp.br* *https://orcid.org/0000-0003-3539-8162*
[g] *tkizildeniz@ohu.edu.tr* *https://orcid.org/0000-0002-5627-1307*

Introduction

Tomato (*Solanum lycopersicum* L.) is among the most important vegetable produced in the world, which production in 2018 was approximately 182 million of tons in an area of 47.6 million of ha (FAO, 2020). The tomato's high productivity depends on the seedling quality, among other factors characterized by compact shoots and vigorous root systems that allow more tolerance against adverse weather conditions as high temperatures and excessive rainfalls (Oliveira et al., 2022). Seedling growth is controlled for genetic, environmental conditions, and applied agricultural practices.

Agricultural practices such as growth regulator applications have been used to compact the plants and increase stem diameter (Desta and Amare, 2021). They are synthetic compounds that reduce the stem elongation by decrease of cell elongation and division rate.Among growth regulators, paclobutrazol (PBZ) [(2RS, 3RS)-1-(4-chlorophenyl)-4,4- dimethyl-2-(1,2,4-triazol-1-yl) pentan-3-ol] is a compound of the chemical group of triazoles that acts as an inhibitor of gibberellic acid biosynthesis by suppressing the oxidation of ent-kaurene to ent-kaurenoic acid through deactivating cytochrome P450-dependent oxygenase (Graebe, 1987). The inhibition of gibberellins biosynthesis causes alterations in plant hormonal balance and promotes prominent reduction of stem elongation (Rademacher, 2000). The application of PBZ is responsible to compact seedlings without damaging the leaf area and to allow an increase of approximately 37% in the dry matter of roots (Seleguini et al., 2013). Then In addition to this, PBZ has been used to reduce the height and increase the stem thickness of tomato seedling in the nurseries. PBZ might enhance the chloroplast differentiation and chlorophyll biosynthesis and prevents chlorophyll degradation attributed to the increase in endogenous cytokinin content (Banon et al., 2002).

Paclobutrazol also can be related with increase of compound amounts such as lignin, hemicellulose and cellulose, and xylem vessel number of the plants, resulting in high seedling quality (Chen et al. 2011). The chemical composition of stem has an impact on it is the mechanical strength and rigidity. Lignin is an integral structural carbohydrate of secondary cell walls, giving mechanical strength to the plants (Chen et al. 2011). In addition, triazoles as PBZ can induce anatomical modifications mediated by changing the hormonal balance of plant. In potato crop, PBZ increased root diameter by increasing the width of cortex and by enhancing the formation of more secondary xylem vessels (Tsegaw et al., 2005).

This study aimed to obtain ideal tomato seedlings, seedlings with more compact and thicker stems perform better during tomato development and to evaluate the effects of paclobutrazol application on the morphology, anatomy and development of tomato seedlings.

Materials and Methods

Experimental Site

The experiment was carried out in a commercial nursery located in the city of Mogi Guaçu (SP), Brazil, located at 22º22' S, 46º56' W at 617 m altitude in the greenhouse (10 x 25 m of height and wide), covered with diffusor plastic. The water or nutrient solution was applied with a mobile irrigation bar.

Tomato Seedlings and Treatments

Tomato cultivar of Serato F1 hybrid was used as it is well known as persimmon tomato is very vigorous, precocious and produces large and heavy fruits (average of 250 g), and it has resistance to nematodes and TSWV (headworm).

The tomato seeds were sown in polyethylene trays of 128 seeds filled with a substrate composed by coconut fiber. The soil substrate characteristics were as N = 0.76%, P_2O_5 = 0.34%, K_2O = 1.22%, Ca = 0.69%, Mg = 0.25%, S = 0.41%, organic matter = 86.00% and carbon = 47.80% in dry matter; Na = 500 mg kg^{-1}, Cu = 100 mg kg^{-1}, Fe = 2600 mg kg^{-1}, Mn = 266 mg kg^{-1}, Zn = 148 mg kg^{-1}, C / N = 63/1 and pH = 4.50.

The experimental design was completely randomized with five rates of paclobutrazol (0, 4, 7, 10 and 13 mg L^{-1}) and four replications. The tomato seedlings were sprayed with paclobutrazol at 15 days after sowing (DAS).

Evaluated Characteristics

At 35 DAS, the following characteristics were determined: plant height, leaf area, upper stem diameter of the seedlings, lower stem diameter of the seedlings, dry weight of the shoot, root dry weight, root length, surface diameter of roots and branches, xylem vessel number and cellulose, hemicellulose and lignin contents of the stems.

Growth Parameters

Plant height was measured from the base of the hypocotyl to the apex of the seedling with a digital caliper. The top and medium diameters of the stem were measured with a digital caliper. The shoots and roots of five seedlings per replication were dried at 65°C for 48 hours. The dry matter of each part was weighed on a digital scale. The leaf area was obtained trough of Image J software, and it was expressed in cm^2.

Five root systems of each replication were submerged in containers with distilled water for 30 minutes. This procedure facilities the root washing process to remove substrate particles. For the morphological analysis, a WinRHIZO Pro 2007a system (Régent Instr. Inc.), coupled to an Epson XL 10000 professional scanner equipped with additional light unit (TPU) was used. A definition of 400 (dpi) was used for measurements of root morphology, as described by Flores et al., (2018) and Jabir et al., (2017). The roots were laid in an acrylic vat 20 cm wide by 30 cm long containing water. The use of this accessory allowed the capture of images in three dimensions, also avoiding the overlapping of the roots. The readings were taken individually to avoid image overlap. The evaluated characteristics were root length, root system density and root ramifications.

Xylem Vessel Count

Portions of the tomato stem were fixed in a Karnovsky system (Karnovsky, 1965), and sectioned in a rotating 40 micrometer microtome. Sections were clarified with sodium hypochlorite and double stained with Astra Blue (0.5%) and Safranin (0.5%)and mounted on a slide with 50% glycerin. The images were captured on a Leica DM LB trinocular microscope coupled to the Leica DC 300 F camcorder and xylem vessel counts were performed using Image-Pro Plus software (Media Cybernetics, Inc., Bethesda, MD, USA) (Figure 1).

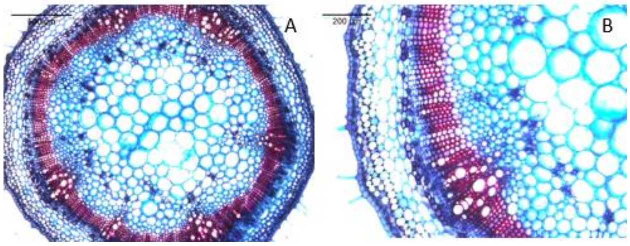

Figure 1. Overview of a cross section of fresh tomato seedling stem (A), and the xylem vessels measured in the analysis (B)

Cellulose, Hemicellulose and Lignin Concentration

The samples of 5 g of the stem were placed on a sachet prepared with filter paper. The solvent used for extraction was 95% ethanol. The procedure was performed on a Soxhlet apparatus for 6 hours under reflux. After the extraction, the samples were withdrawn and the cooling time of the samples was maintained at room temperature. Then, 300 mg were placed in crucible and dried until constant dry weight to calculate the moisture content.

The samples of 300 mg of the extracted stem were weighed into the test tubes, to which 3 mL of 72% (v/v) sulfuric acid (d = 1.84) were added and placed in a water bath at 30°C, submitted to shaking at 75 rpm for one hour. The material was diluted to a concentration of 3% (v/v) acid by the addition of 79 mL of distilled water and heated at 121 °C for an additional hour.

Soluble and insoluble lignin: The resulting hydrolysate material was vacuum filtered and placed on a porous crucible and dried to a constant mass value at 105 °C. The determination of the insoluble lignin was made by gravimetry and the soluble lignin at 205 nm by UV-visible Shimadzu Mini1240.

The liquid fraction from the hydrolysate was used for the determination of carbohydrates and organic acids by high performance liquid chromatography (HPLC), which vials with this liquid fraction were prepared. During the vial preparation the material was previously filtered through a 0.45 µm cellulose nitrate membrane (Sartorius Co.). Carbohydrates were analyzed on an Aminex HPX-87H column (300×0.8 mm) in a Shimadzu chromatograph LC-20AT. The mobile phase used was ultra-pure water (18 mΩ) at 0.6 mL min^{-1} flow rate and 45 °C. ELSD Shimadzu LT-11 at 80 °C, 350 kPa pressure and gain equal 7 was used as detector. Acetic acid determination was performed on the HPX-87H column, with mobile phase of H_2SO_4 at 0.5 mL.min^{-1} and 40 °C. The employed detector was DAD (Shimadzu SPD-M20A), operating at the wavelength of 210 nm. The conversion of fructose into 5-hydroxymethylfurfural (HMF) and furfural were analyzed through C-18 column (Shim-pack XR-ODS 3) and the mobile phase used was acetonitrile/acidic water with 1% acetic acid in the proportion 1:8. The detector used was DAD (Shimadzu SPD-M20A) with flow of 0.8 mL min^{-1} and 25 °C. The wavelength for HMF analysis was 285 nm and for furfural was 275 nm.

Results and Discussion

In this research, the PBZ reduced the height of tomato seedlings in both growth periods. The applications of 10 and 13 mg L^{-1} of PBZ reduced, on average, 36.2% and 35.4% the seedling height as compared to control, in the first and second growth period, respectively (Table 1). The reduction of plant height by PBZ application on the shoots is related to inhibition of ent-kurene conversion to ent-kaurenoic acid, in the gibberellins biosynthesis, which results in reduced levels of gibberellic acids all types, causing a decrease in elongation rate and cell division (March et al., 2013; Desta and Amare, 2021; Syahputra et al., 2018). Velázquez et al. (2018) also observed that PBZ reduced 45% the size of tomato seedlings and found retarded growth of tomato plants with PBZ applied by foliar sprays.

In contrast to the height, basal stem part diameter (D1) increased 21.3% with application of PBZ at rate of 13 mg L^{-1} during the first period. In the second period, the applications of PBZ at rates of 10 and 13 mg L^{-1} increased 17.4% and 15.5% the D1, respectively (Table 1). Thus, the lower H/D1 ratio with PBZ than control allowed a balanced growth relationship between seedling height and diameter, promoting seedlings more robust.

The seedlings treated with PBZ had smaller leaf area than control, which rate of 13 mg L^{-1} reduced 35% this characteristic, for both growth periods. According to Tsegaw and Hammes (2005) and Desta and Amare (2021), triazoles as PBZ might cause morphological changes on the leaves as reduced leaf area and increased leaf thickness. This reduction of foliar area did not generate abnormal leaves in the seedlings treated with foliar sprays of PBZ (Tsegaw and Hammes, 2005).

The dry biomass of shoots (SDM) and roots (RDM) were not affected by PBZ foliar application. Thus, the decrease of seedling size and foliar area were not sufficient to enhance the biomass of roots. These results contradict the role of PBZ in reduction of the leaf expansion rate with reduction of the sink strength of the leaves and increase of the carbon flux to the roots as verified by Berova and Zlatev (2000). The authors found decrease in plant height and increase in root development of tomato seedlings with foliar application of 25 mg L^{-1} of PBZ.

The root quality did not increase with PBZ applied by foliar spray in the seedlings, for the first period. However, for second period, the length, area and forks number of roots increased with PBZ foliar spray at rate of 10 mg L^{-1} (Table 2).

Table 1. Height (H), basal stem part diameter (D1), upper stem part diameter (D2), leaf area (LA), shoot dry mass (SDM) and root dry mass (RDM) of tomato seedlings under rates of paclobutrazol (PBZ) during the growth periods. Means with the same letter within a column were not different by LSD test (P<0.05).

First growth period						
Rates (mg L^{-1})	H (cm)	D1 (cm)	D2 (cm)	LA (cm^2 pl^{-1})	SDM (g pl^{-1})	RDM (g pl^{-1})
0	19.719a	2.30b	2.24a	21.12a	0.73a	0.114a
4	15.465b	2.28b	2.34a	17.2b	0.59a	0.106a
7	13.500c	2.21b	2.23a	16.65c	0.64a	0.103a
10	12.810c	2.32b	2.33a	16.53c	0.67a	0.108a
13	12.350c	2.79a	2.35a	13.67d	0.58a	0.105a
LSD	1.95	0.25	0.25	3.50	0.17	0.02
Second growth period						
Rates (mg L^{-1})	H (cm)	D1 (cm)	D2 (cm)	LA (cm^2 pl^{-1})	SDM (g pl^{-1})	RDM (g pl^{-1})
0	20.77a	1.74c	1.90b	23.14a	1.350a	0.211a
4	15.22b	1.96ab	2.10a	18.88b	1.275a	0.228a
7	13.56c	1.87bc	1.97ab	18.25bc	1.33a	0.213a
10	13.83c	2.04a	2.10a	16.54c	1.37a	0.223a
13	13.01c	2.01ab	2.05ab	14.89d	1.30a	0.234a
LSD	1.04	0.14	0.15	3.25	0.29	0.07

Table 2. Root length (L, mm), root area (RA, cm^2) and forks number (FN) of tomato seedlings under rates of paclobutrazol (PBZ) during the growth periods. Means with the same letter within a column were not different by LSD test (P<0.05).

First growth period			
Rates (mg L^{-1})	L (mm)	RA (cm^2)	FN
0	222.12a	7.94a	1459.20a
4	262.61a	8.35a	1472.00a
7	250.44a	7.53a	1269.30a
10	246.08a	7.77a	1403.25a
13	231.90a	8.23a	1501.05a
LSD	56.54	1.76	356.97
Second growth period			
Rates (mg L^{-1})	L (mm)	RA (cm^2)	FN
0	68.58bc	2.64c	476.40bc
4	70.48bc	3.88b	614.15b
7	103.87b	6.24a	881.65a
10	154.96a	6.23a	969.30a
13	39.82c	2.07c	342.70c
LSD	27.97	0.83	235.21

Table 3. Amounts of hemicellulose, cellulose and lignin in the stem of tomato seedlings. Means with the same letter within a column were not different by LSD test (P<0.05).

First period			
Level (mg L^{-1})	%Cel	%Hem	%Lig
0	30.68e	17.72a	14.67c
4	43.38d	17.69b	09.33d
7	45.07bc	12.98e	07.56e
10	49.59b	15.30c	19.03b
13	51.93a	13.17d	29.69a
LSD	0.48	0.18	0.18
Second period			
Level (mg L^{-1})	%Cel	%Hem	%Lig
0	28.33d	15.69bc	14.03d
4	29.68c	14.27e	12.56e
7	35.64b	26.69a	15.70c
10	35.06b	14.52d	28.09b
13	44.14a	15.96b	33.34a
LSD	0.03	0.02	0.02

PBZ might generate an increase in cytokinin levels (Burondkar et al., 2016; Desta and Amare, 2021; Soumya et al., 2017) leading an intensification of cell division and, consequently an increase of area and forks number of roots.

The chemical composition of the seedlings was investigated in order to understand if there are structural changes resulting from the application of PBZ. For both crop cycles, the percentage of cellulose increased in the stems of the seedlings with increase of PBZ rates (Table 3). The lignin percentage also increased with 10 and 13 mg L^{-1} of PBZ as compared to control in the first period. For second period, similar results were obtained with 7, 10 and 13 mg L^{-1}. These results are in line with other researches in the literature (Ayvaci et al., 2023; Si et al., 2023).

The lignification process as well as the formation of other polymers are dynamic and are activated by the regulation of several genes that control this phenomenon as function of stress (Wang et al., 2013). According to Srivastava et al. (2017), abiotic stress as PBZ application in the seedlings can also induce lignification in the walls of cells that do not normally lignify under non-stress. Lignin is an integral structural carbohydrate of secondary cell walls, generating mechanical strength to plants (Chen et al. 2011). Therefore, an increase of lignin content results in rigidity of stem (Peng et al., 2014).

However, hemicellulose content decreased with PBZ rates applied by foliar spray in the first period. For second period, the PBZ rates of 4, 7 and 10 mg L^{-1} also reduced this compound content as compared to control. According to Xu et al. (2017), the increase of lignin and hemicellulose contents together increased the stem mechanical strength. Therefore, our results suggest that the lignin and cellulose accumulations are associated with lodging resistant of tomato seedlings.

Another variable analyzed was the count of xylem vessel number in order to verify if the compaction of tomato seedlings was associated by an increase of the vessel number of xylems. The number of xylem vessel was not affected by PBZ application on the seedlings in the first period (Figure 2). For the second period, the PBZ application at rate of 7 and 10 mg L^{-1} increased the xylem vessel number (Figure 3), corroborating with studies obtained by Tsegaw et al. (2005) in potato crop, where PBZ induced shorter and thicker stem associated with more secondary xylem vessels than control.

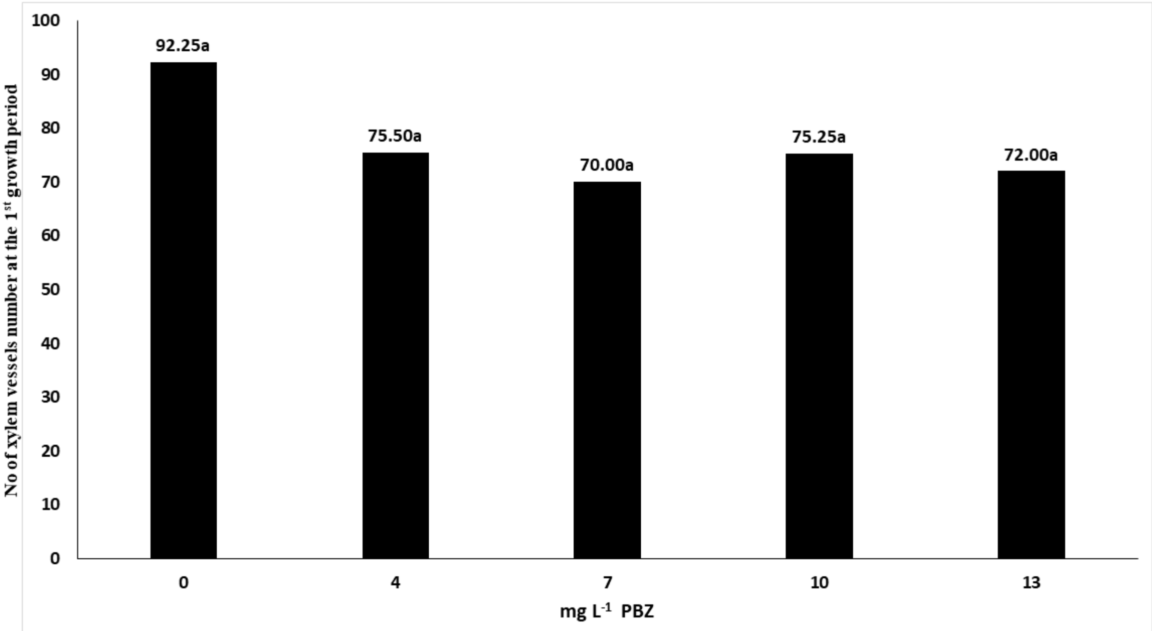

Figure 2. Xylem vessels number under rates of paclobutrazol (PBZ) during the first growth period.

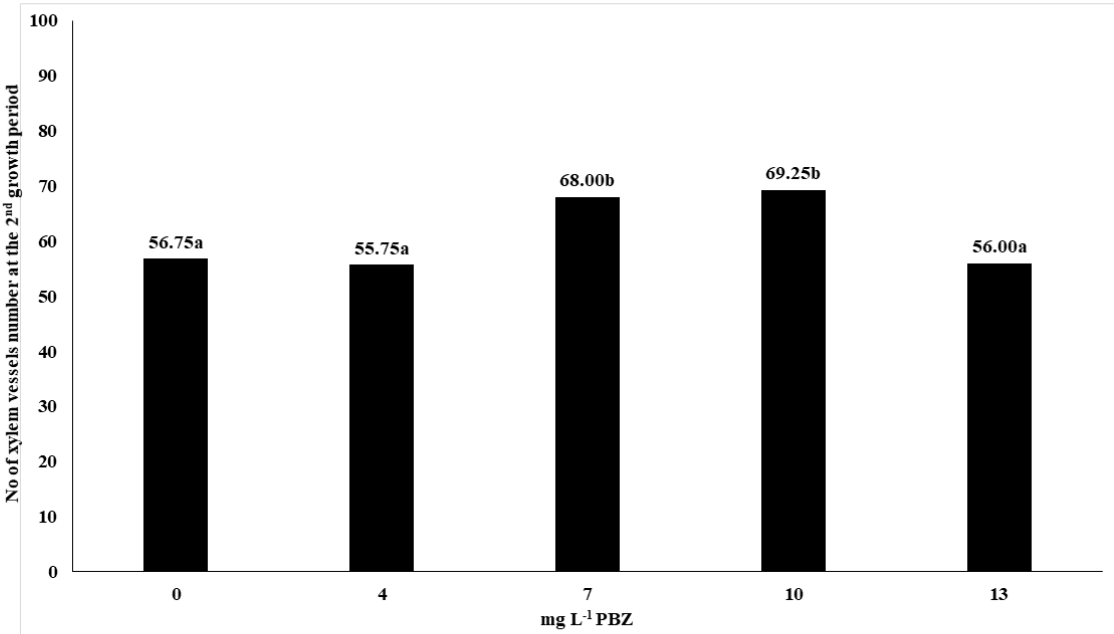

Figure 3. Xylem vessels number under rates of paclobutrazol (PBZ) during the second growth period.

In the study reported by Mohammadi et al. (2017), PBZ application at rate of 50 mg L^{-1} increased the xylem cell number and vessel cell size of juvenile plants of radish. In yellow passion seedlings, the higher stem diameter induced by PBZ also was related to a higher xylem vessels number (Teixeira et al., 2019).

In Chrysanthemum cultivars Jaguar Red, Snow White, and Fiji White, the utilization of PBZ at various concentrations caused in distinct influences on the plant anatomy (Lailaty and Nugroho, 2021). Notably, Jaguar Red exhibited greater stem thickness and stomatal density compared to Snow White and Fiji White (Lailaty and Nugroho, 2021). Moreover, the use of 150 ppm PBZ raised leaf thickness and stem diameter, while 100 ppm PBZ enhanced the size of guard cells and tissue dimensions (Lailaty and Nugroho, 2021). Ultimately, the optimal concentration for potted Chrysanthemum was determined to be 150 ppm (Lailaty and Nugroho, 2021).

Conclusions

Paclobutrazol modified the morphology of tomato seedlings. It induced anatomical changes such as reducing height and foliar area of seedlings and increasing diameter of stems. PBZ also improved the cellulose and lignin accumulation in the stems, thus increasing mechanical strength of seedlings. Therefore, these findings are valuable by using PBZ as a management practice for producing seedlings more robust to overcome the adverse weather conditions.

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Authors' Contribution: V.J.C.: conceptualization, methodology, validation, resources, writing, reviewing, editing, visualization, supervision. conceptualization, methodology, validation, and formal analysis. S.C.M: investigation, formal analysis, and writing. M.R.B.: investigation, formal analysis, and writing. D.D.N.: investigation, formal analysis, and writing. J.L.M.C.: conceptualization, methodology, validation, writing, reviewing, editing, visualization, supervision. conceptualization, methodology, validation, and formal analysis. M.M.B.: conceptualization, methodology, validation, writing, reviewing, editing, visualization, supervision. conceptualization, methodology, validation, and formal analysis. T.K.: conceptualization, methodology, validation, writing, reviewing and editing. All the authors approved the paper for publication.

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