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Original Article

Paclobutrazol reduces growth and increases chlorophyll indices and gas exchanges of basil (*Ocimum basilicum*)

Paclobutrazol reduz o crescimento e aumenta os índices de clorofila e trocas gasosas de manjericão (*Ocimum basilicum*)

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Abstract

Basil (*Ocimum basilicum*) is a medicinal, ornamental and aromatic plant, however, its size can be an obstacle to its commercialization as a potted ornamental plant. Paclobutrazol (PBZ) is a substance that can retard plant growth by inhibiting the synthesis of gibberellins. The objective of this work was to evaluate the effect of paclobutrazol on growth regulation and gas exchange of basil (var. Cinnamon). The experiment was carried out in a completely randomized design with five treatments (PBZ doses: 0, 2.5, 5, 7.5 and 10 mg L^{-1}), with eight replicates. Growth (plant height, number of leaves, stem diameter, leaf dry mass, stem dry mass, inflorescence dry mass, and total), growth rates (leaf mass ratio, stem mass ratio, inflorescence mass ratio, and robustness quotient), chlorophyll indices, gas exchange (*gs*, *A*, *E*, *Ci*, WUE, iWUE and iCE) were evaluated. Paclobutrazol reduced the growth of basil plants and increased the chlorophyll indices, *A*, *gs*, and WUE. Paclobutrazol can be used to regulate plant growth of basil plants var. Cinnamon, without altering its physiological and ornamental characteristics.

Keywords: growth regulation, ornamental plant, photosynthesis.

Resumo

Manjericão (*Ocimum basilicum*) é uma planta medicinal, ornamental e aromática, contudo, seu porte pode ser um entrave para a sua comercialização como planta ornamental de vaso. O paclobutrazol (PBZ) é uma substância que pode retardar o crescimento de plantas, através da inibição da síntese de giberelinas. O objetivo deste trabalho foi avaliar o efeito do paclobutrazol na regulação do crescimento e trocas gasosas no manjericão (*Ocimum basilicum* var. Cinnamon). O experimento foi realizado em delineamento inteiramente casualizado com 5 tratamentos (doses de PBZ: 0; 2,5; 5; 7,5 e 10 mg L^{-1}), com oito repetições. O crescimento (altura de planta, número de folhas, diâmetro de caule, massa seca da folha, do caule, da inflorescência e total), taxas de crescimento (razão de massa da folha, do caule e da inflorescência e quociente de robustez), índices de clorofilas a e b, trocas gasosas (*gs*, *A*, *E*, *Ci*, WUE, iWUE e iCE) foram avaliados. Paclobutrazol reduziu o crescimento de plantas de manjericão e aumentou o índice de clorofilas, *A*, *gs* e WUE. Paclobutrazol pode ser usado para regular o crescimento de plantas de manjericão var. Cinnamon, sem alterar suas características fisiológicas e ornamentais.

Palavras-chave: regulador de crescimento, planta ornamental, fotossíntese.

1. Introduction

Basil (*Ocimum basilicum* L. – Lamiaceae) is a medicinal, aromatic and ornamental plant, rich in essential oils (Rastogi et al., 2020), with linalool as the major component (Dias et al., 2018). Essential oils are volatile secondary metabolites that can be used as medicine and in the composition of perfumes, flavorings, insect repellents and other products (Chambre et al., 2020).

The cultivation of basil in Brazil extends throughout the territory, especially in areas of small producers and family farming (Favorito et al., 2011). The vegetative cycle depends on each variety or hybrid, and can be up to 80 days. Its size depends on the genotype, but it usually exceeds 1 m (Blank et al., 2012). This plant is easy to propagate and has a very attractive inflorescence and aroma and a short period for harvesting, being widely used as ornamentals. Basil var. Cinnamon has purplish-green leaves, with inflorescences that vary from pink to purple, having a very attractive character to be used as an ornamental plant in pots. However, its large size can be an obstacle to its marketing as such.

The cultivation of ornamental plants is expanding in Brazil, generating jobs and income. In recent years, a trend to produce plants at home has been growing worldwide. Growing plants indoors (Indoor Gardening) contributes

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to personal well-being and the home environment (Solis-Toapanta and Gómez, 2019). Plant growth regulators are chemical products commercially used in agriculture, mainly to adapt crops to commercial standards since they suppress growth without harming visual quality, do not promote phytotoxicity or thinning (Gazola et al., 2019).

Paclobutrazol (PBZ) is a triazole that acts mainly by reducing plant growth rates, increasing protection against diseases and increasing tolerance to water stress (Mohammadi et al., 2017). PBZ inhibits the synthesis of gibberellic acid, inducing metabolic changes in abscisic acid, cytokinins and ethylene, resulting in the decreased shoot growth and, consequently, in the total transpiration area (Thomas and Hedden, 2018). Furthermore, PBZ alters the source-sink ratio, directing more photoassimilates to the root system, increasing the number of adventitious roots. The effect of reduced transpiration area and more roots absorbing water results in greater plant vigor (Miller, 2016). PBZ can be applied via soil or foliar, however, it is more efficient via soil, as it is translocated almost exclusively by the xylem, where it is absorbed by the roots and taken to the growth points (Xia et al., 2018; Cregg and Ellison-Smith, 2020; Lima et al., 2020; Guimarães et al., 2021).

PBZ alters the photosynthetic potential and, consequently, the plant's gas exchange, this is because gibberellins are part of the biogenesis of chloroplasts (Rodrigues et al., 2016). PBZ also affects the carbon use efficiency in leaves, which depends on the balance between photosynthesis and respiration. An improvement caused by paclobutrazol in carbon use results in better photosynthesis and less plant respiration (Xu et al., 2020).

Researches about growth control was performed with others varieties of *Ocimum basilicum* (Hazzoumi et al., 2014; Koca and Karaman, 2015; Saha et al., 2016), however, there are no studies about the effects of PBZ application on growth and physiology of basil var. Cinnamon. The objective of this work was to evaluate the effect of paclobutrazol on growth regulation and gas exchange in basil (*Ocimum basilicum* var. Cinnamon).

2. Material and Methods

The experiment was performed in a greenhouse at the Research and Extension Teaching Unit – Floriculture, at the Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil. The average temperature during the period of the experiment was 21.9 °C (INMET, 2022).

The treatments were distributed in a completely randomized design, with five treatments (0, 2.5, 5, 7.5 and 10 mg L^{-1} of paclobutrazol), with eight replicates. Basil seedlings var. Cinnamon were produced in 128-cell Styrofoam trays with commercial substrate (Topstrato®). Three seeds were placed in each cell of the tray, leaving only the most vigorous after emergence. The seedlings were transplanted into plastic pots n. 15 containing a mixture of soil (Red-Yellow Latosol) and sand (2:1, v/v). A soil analysis was carried out: pH (H_2O): 5.6; P (mg dm^{-3}): 3.25; K^+ (mg dm^{-3}): 105.00; Na^+ (cmol dm^{-3}): 0.00; H^++Al^{+3} (cmol dm^{-3}): 5.61; Al^{+3} (cmol dm^{-3}): 0.00; Ca^{+2} (cmol dm^{-3}): 5.27; Mg^{+2} (cmol dm^{-3}): 1.51; SB (cmol dm^{-3}): 7.05; CEC (cmol dm^{-3}): 12.66; V (%): 55.70.

Paclobutrazol was applied seven days after transplanting, being 100 mL of PBZ doses corresponding to each treatment was applied directly to the pots. The plants were irrigated daily with distilled water. 100 mL of NPK + micronutrients (Peters 20-20-20 - 4 g L^{-1}) was applied weekly to each pot.

Plant height (measured with a ruler from root insertion to its last leaves - expressed in cm), stem diameter (measured with a digital caliper - expressed in mm) and number of leaves were evaluated at 44 days after PBZ application. The inflorescences, leaves and stem were dried in a drying oven with forced air circulation at 65 ºC for 72 hours. Each part of the plant was weighed separately on an analytical balance (0.001 g). The leaf mass ratio (LMR), stem mass ratio (SMR), inflorescence mass ratio (IMR) and robustness quotient (RQ) were calculated. LMR, SMR and IMR were calculated using the following equations: LMR = leaf dry mass (LDM)/total dry mass (TDM), IMR = inflorescence dry mass (IDM)/TDM, SMR = stem dry mass (SDM)/TDM. The RQ was calculated using the equation: QR = plant height (PH)/stem diameter (SD).

The determinations of the chlorophyll a, b and total indices were carried out by the non-destructive method, using a portable electronic chlorophyllometer (ClorofiLOG® - model CFL 1030, Porto Alegre, RS), and the values were dimensioned in Falker chlorophyll index (FCI). An infrared gas analyzer (IRGA – model LCPro, ADC BioScientific Ltd.) was used to assess gas exchange, and measurements were taken between 8 and 10 am on two leaves per plant. The photosynthetically active radiation (PAR) was 1000 µmol m^{-2} s^{-1} and the chamber temperature was 25 ºC. Net photosynthesis (A= µmol CO_2 m^{-2} s^{-1}), stomatal conductance (gs= mol H_2O m^{-2} s^{-1}), internal carbon concentration (Ci= µmol CO_2 mol air^{-1}), transpiration (E= mmol H_2O m^{-2} s^{-1}), instantaneous water use efficiency (WUE= A/E), instantaneous carboxylation efficiency (iCE= A/Ci) and intrinsic water use efficiency (iWUE= A/gs) were evaluated.

The means were compared by the Tukey's test ($p \leq 0.05$) using the ExpDes statistical package (Ferreira et al., 2018). Canonical variables analysis with confidence ellipses ($p \leq 0.01$) were performed to study the interrelationship between variables and factors using the candisc package (Friendly and Fox, 2017). Pearson's correlation analysis was performed using the package corrplot (Wei and Simko, 2017). The statistical program R (R Development Core Team, 2021) was used for statistical analyses.

3. Results

The application of paclobutrazol reduced the growth of basil plants (Figure 1).

The application of paclobutrazol reduced the height of basil plants (Figure 2). The lowest plant height (28.14 cm) was observed in the highest dose of PBZ (10 mg L^{-1}). The application of PBZ reduced the stem diameter, with the smallest diameter (2.81 mm) being observed at the dose of 9.48 mg L^{-1} of PBZ. The number of leaves, leaf dry mass, stem dry mass and total dry mass were reduced

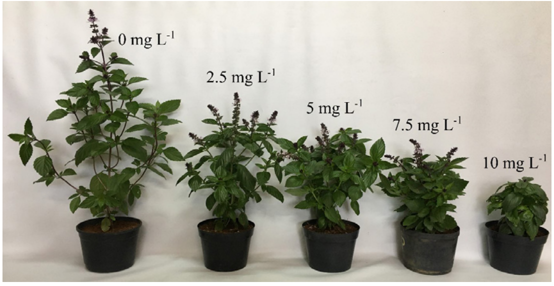

Figure 1. Effect of paclobutrazol application on the growth of basil plants (*Ocimum basilicum* var. Cinnamon).

with increasing PBZ dose. The smallest number of leaves (129), smaller leaf dry mass (3.05 g) and total dry mass (5.16 g) were observed in the dose 9.93 and 10 mg L^{-1} PBZ, respectively, and the lowest stem dry mass (1.70 g) was observed at a dose of 9.32 mg L^{-1} of PBZ. The application of PBZ increased the inflorescence dry mass (0.89 g) up to a dose of 3.31 mg L^{-1} of PBZ.

Leaf mass ratio was increased with increasing doses of PBZ (Figure 3). The highest leaf mass ratio (0.58 g g^{-1}) was observed in the highest dose of PBZ (10 mg L^{-1}). Stem mass ratio and robustness quotient were reduced with increasing PBZ doses. The lowest stem mass ratio (0.32 g g^{-1})

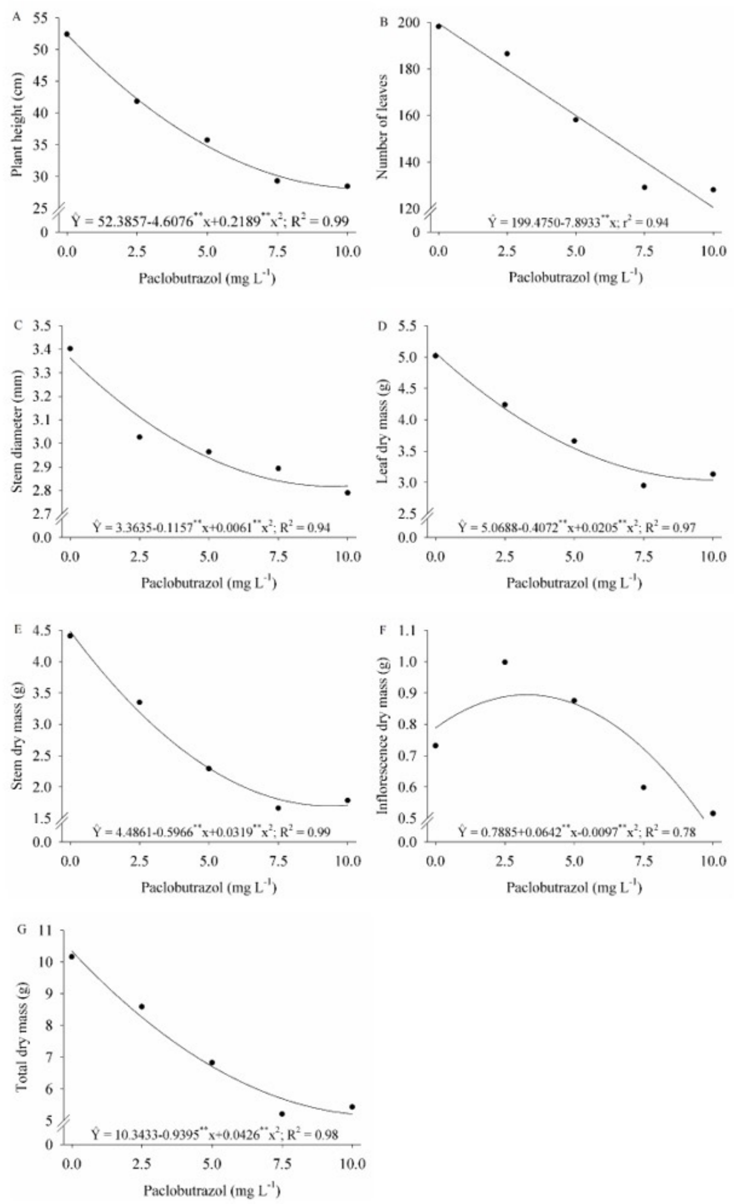

Figure 2. Plant height (A), number of leaves (B), stem diameter (C), leaf dry mass (D), stem dry mass (E), inflorescence dry mass (F) and total dry mass (G) of basil plants (*Ocimum basilicum* var. Cinnamon) under paclobutrazol application.

and robustness quotient (9.62) were observed in the dose 8.26 and 10 mg L^{-1} PBZ, respectively. The application of PBZ increased the inflorescence mass ratio (0.13 g g^{-1}) up to a dose of 5.39 mg L^{-1}.

The application of PBZ increased chlorophyll a (44.59 FCI) up to the dose of 5.56 mg L^{-1} of PBZ (Figure 4). There was also an increase in chlorophyll b (20.49 FCI) up to a dose of 6.85 mg L^{-1} of PBZ and in total chlorophyll (65.03 FCI) up to a dose of 6.31 mg L^{-1} of PBZ. The application of PBZ reduced the chlorophyll a/b ratio, withlowest chlorophyll a/b ratio (2.17) observed at the dose of 6.93 mg L^{-1}.

Stomatal conductance, net photosynthesis, internal carbon concentration and instantaneous water use efficiency were increased with increasing PBZ doses (Figure 5). The highest stomatal conductance (0.39 mol H_2O m^{-2} s^{-1}), net photosynthesis

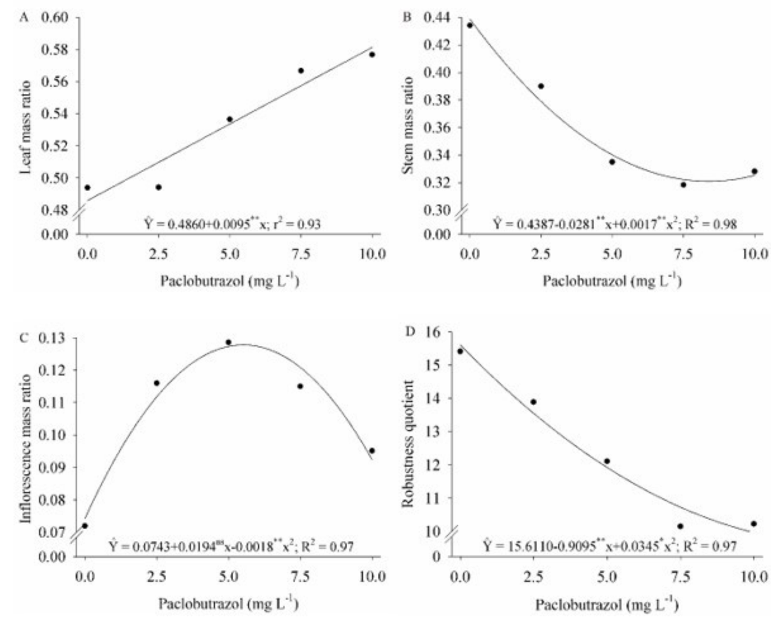

Figure 3. Leaf mass ratio (A), stem mass ratio (B), inflorescence mass ratio (C) and robustness quotient (D) of basil plants (*Ocimum basilicum* var. Cinnamon) under paclobutrazol application.

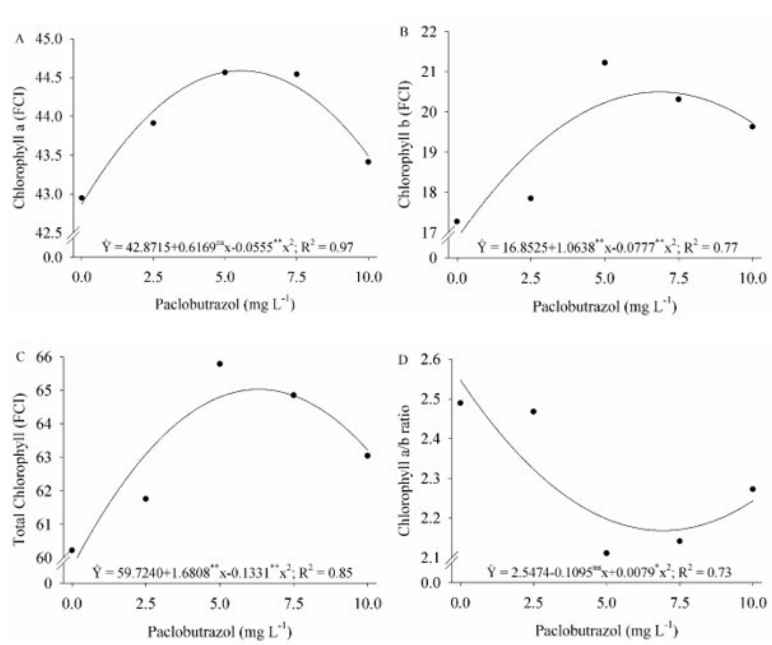

Figure 4. Chlorophyll a (A), chlorophyll b (B), total chlorophyll (C) and chlorophyll a/b ratio (D) of basil plants (*Ocimum basilicum* var. Cinnamon) under paclobutrazol application.

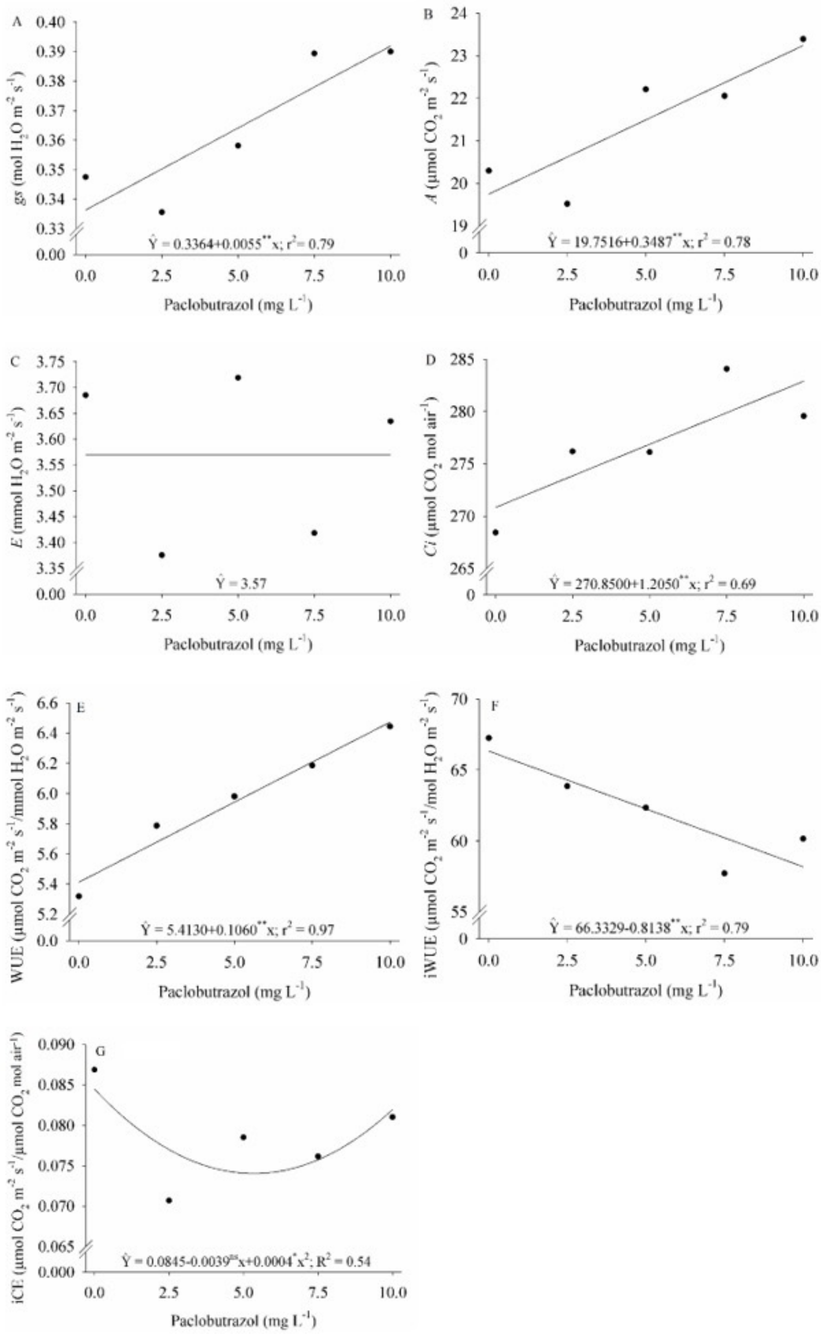

Figure 5. Stomatal conductance (A), net photosynthesis (B), transpiration (C), internal carbon concentration (D), instantaneous water use efficiency (E), intrinsic water use efficiency (F) and instantaneous carboxylation efficiency (G) of basil plants (*Ocimum basilicum* var. Cinnamon) under paclobutrazol application.

(23.39 μmol CO_2 m^{-2} s^{-1}), internal carbon concentration (279.56 μmol CO_2 mol air^{-1}), and water use efficiency (6.14 μmol CO_2 m^{-2} s^{-1}/mmol H_2O m^{-2} s^{-1}) were observed at the highest dose of PBZ (10 mg L^{-1}). The application of PBZ decreased the intrinsic water use efficiency and instantaneous carboxylation efficiency, with the lowest intrinsic water use efficiency (60.15 μmol CO_2 m^{-2} s^{-1}/mol H_2O m^{-2} s^{-1}) being observed in the highest dose of PBZ (10 mg L^{-1}) and the lowest instantaneous carboxylation efficiency (0.07 μmol CO_2 m^{-2} s^{-1}/ μmol CO_2 mol air^{-1}) observed at the dose of 4.88 mg L^{-1} of PBZ. The application of PBZ did not interfere on transpiration of basil plants.

Net photosynthesis had a high positive correlation with WUE (r^2= 0.86) and iCE (r^2= 0.95) (Figure 6). The total chlorophyll index was negatively correlated with plant height (r^2= -0.63).

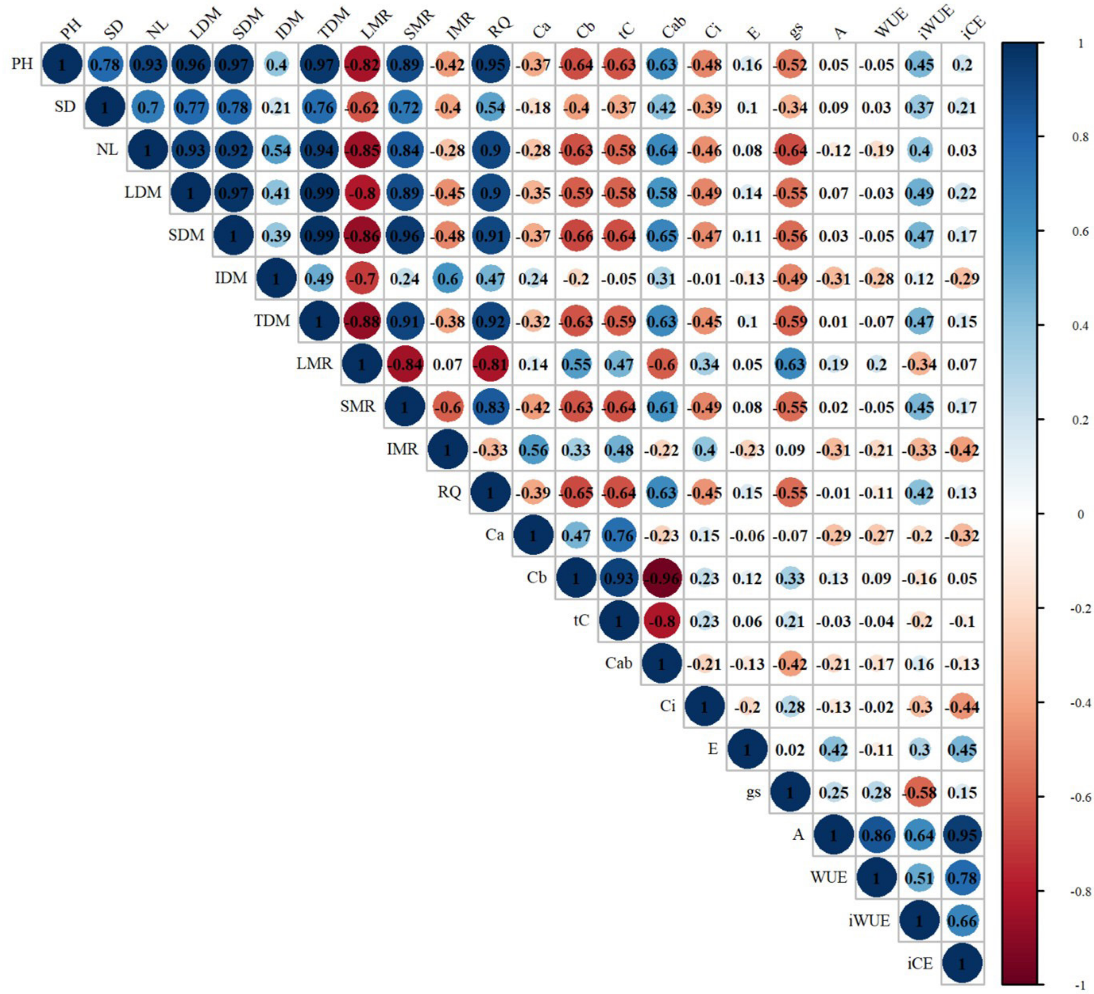

Figure 6. Pearson's correlation between the analyzed variables. PH= plant height; SD=diameter; NL= number of leaves; LDM= leaf dry mass; SDM= stem dry mass; IDM= inflorescence dry mass; IMR= inflorescence mass ratio; SMR= stem mass ratio; LMR= leaf mass ratio; RQ= robustness quotient; Ca= chlorophyll a; Cb= chlorophyll b; tC= total chlorophyll; Cab= chlorophyll a/b ratio.

A canonical variables analysis with confidence ellipses was performed to understand the interrelationships between factors and analyzed variables. The number of leaves (NL), leaf dry mass (LDM), stem dry mass (SDM), plant height (PH) and stem diameter (SD) had a lower relationship with the application of 7.5 and 10 mg L^{-1} of PBZ. These treatments were not different from each other (Figure 7). Stem mass ratio (SMR) and robustness quotient (RQ) were more related to the control and leaf mass ratio (LMR) with the application of 7.5 and 10 mg L^{-1} of PBZ. *A*, *gs* and WUE had a greater relationship with the application of 10 mg L^{-1} of PBZ. Chlorophyll a and b indices were more related to the application of 7.5 and 5 mg L^{-1} of PBZ, respectively.

4. Discussion

The growth of basil plants was reduced by the application of paclobutrazol because this compound alters the isoprenoid pathway and the levels of plant hormones by inhibiting the synthesis of gibberellin, reducing the evolution of ethylene and increasing the level of cytokinins (Soumya et al., 2017). When the synthesis of gibberellins is inhibited, more precursors in the terpenoid pathway accumulate, resulting in the production of abscisic acid, which leads to less growth and development of the shoot plant (Soumya et al., 2017). The application of PBZ also reduced the height and diameter of rose (*Rosa hybrida*) (Carvalho-Zanão et al., 2018) and platycodon (*Platycodon grandiflorus*) (Sabino et al., 2021). Inflorescence dry mass was increased to 0.89 g at the 3.31 mg L^{-1} dose of PBZ, possibly due to greater vascular development of reproductive structures caused by increased levels of cytokinin and inhibition of gibberellin. PBZ also increased the number of flower of mango (*Mangifera indica*) (Oliveira et al., 2020). The number of leaves decreased with increasing PBZ dose due to inhibition of the gibberellins route. This is related to the effect of this phytohormone in increasing internodal length, promoting cell division

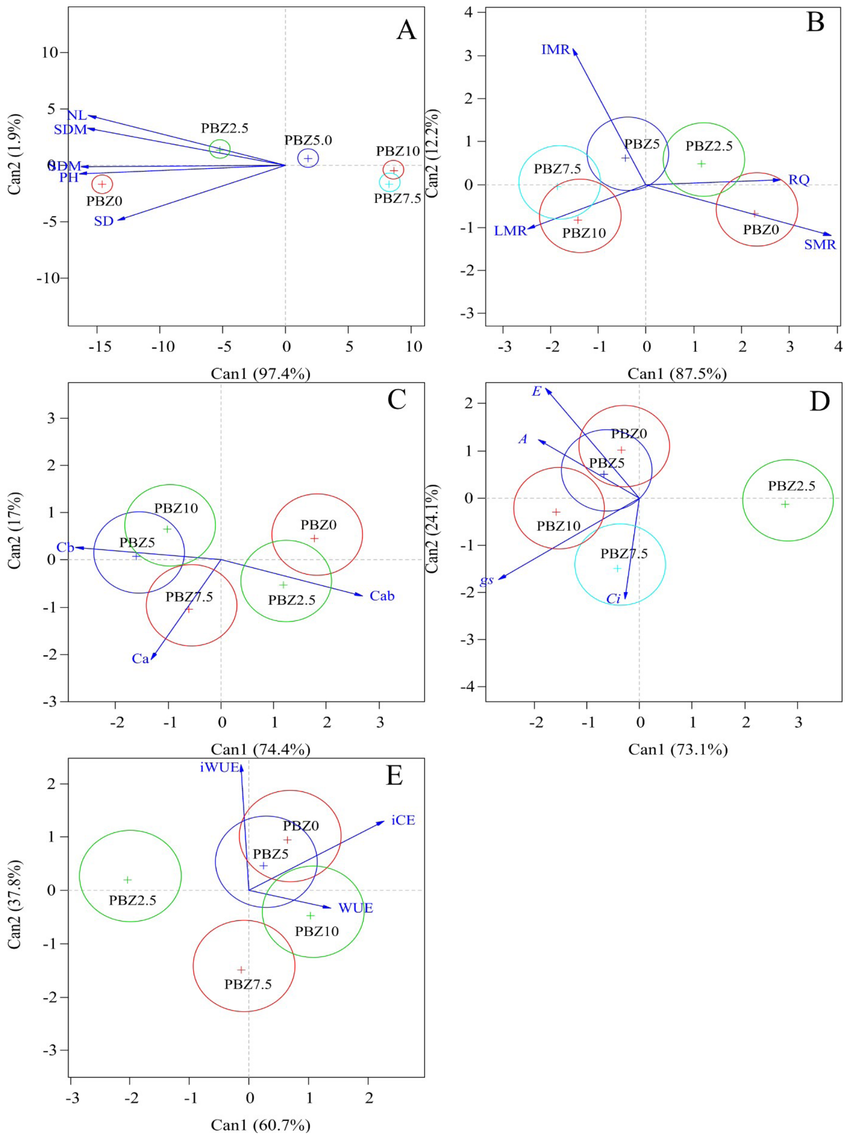

Figure 7. Canonical variables analysis with confidence ellipses for growth variables (A and B), chlorophyll indices (C) and gas exchange (D and E). PBZ= paclobutrazol; NL= number of leaves; LDM= leaf dry mass; SDM= stem dry mass; PH= plant height; SD= stem diameter; IMR= inflorescence mass ratio; SMR= stem mass ratio; LMR= leaf mass ratio; RQ= robustness quotient; Ca= chlorophyll a; Cb=chlorophyll b; Ca/b= chlorophyll a/b ratio.

and stimulating apical dominance (Munikrishnappa and Chandrashekar, 2014).

The leaf mass ratio increased due to the PBZ affecting the apical dominance and, consequently, increasing the number of lateral branches, increasing the number of shoots and leaves. In addition, the accumulation of biomass in leaves is due to PBZ increasing photosynthetic rates, resulting in greater growth (Mohan et al., 2020). The reduction in the stem mass ratio occurred due to the inhibition of the biosynthesis of gibberellic acid by PBZ, causing a reduction in cell division and resulting in compact plants (Parladore et al., 2019). The inflorescence mass ratio is associated with the restriction of cell expansion and the accumulation of phenolic compounds in the stem caused by the inhibition of the routes of gibberellins, which negatively regulate floral induction and promote cell expansion (Oliveira et al., 2020). High levels of phenolic compounds in the plant can act as growth inhibitors due to their negative effects on cell division and elongation (Teixeira et al., 2019). Plants that received PBZ had lower robustness quotients, which indicates the balance between height/stem diameter, where the smaller the quotient value, the more robust the plants are (Parladore et al., 2019). The use of PBZ to increase robustness is widely practiced in hybrid eucalyptus (*Eucalyptus urophylla*) (Santos et al., 2018).

The increase in chlorophyll indices with the application of paclobutrazol was due to the capacity of this growth regulator to increase cytokinin levels and, thus, increase chloroplast differentiation, chlorophyll biosynthesis and prevent its degradation (Carvalho-Zanão et al., 2018; Huang et al., 2019). The application of PBZ also increased chlorophyll levels in cucumber (*Cucumis sativus*) (Baninasab and Ghobadi, 2011) and stevia (*Stevia rebaudiana*) (Hajihashemi and Ehsanpour, 2013).

The application of PBZ increased stomatal conductance and net photosynthesis because this regulator increases Rubisco activity that increases carboxylation (Xu et al., 2020). The stomatal conductance indirectly influences photosynthesis through the increase or decrease of transpiration rates and CO_2 inflow into the mesophyll (Saykhul et al., 2013). Furthermore, photosynthetic rates are also influenced by increased carboxylation. The application of PBZ also increased the stomatal conductance of peony (*Paeonia lactiflora*) (Xia et al., 2018) and *Euonymus japonicus* (Xu et al., 2020). Improved water use efficiency after PBZ application was due to increased abscisic acid content, which decreases stomatal opening, promotes smaller leaf surface area for transpiration and increases the number of roots for water absorption (Soumya et al., 2017).

Vegetables absorb water in large amounts due to the direct competition of CO_2 absorption for photosynthesis. The water use efficiency indicates the plant's effectiveness in fixing carbon while transpiring, having a close relationship with carboxylation. The greater the gas exchange, the greater the carbon assimilation and, consequently, more photosynthesis (Ferraz et al., 2012). The negative correlation between plant height and total chlorophyll may have been caused by the effect of PBZ on cell size reduction, having a low concentration of chlorophyll and a reduction in the intracellular space, causing the chloroplasts to become more dense (Carvalho-Zanão et al., 2018).

5. Conclusions

Paclobutrazol (PBZ) reduces the growth of basil plants (*Ocimum basilicum* var. Cinnamon). The effect of paclobutrzaol also increases the chlorophyll indices, net photosynthesis, stomatal conductance and instantaneous water use efficiency. The application of 5 mg L^{-1} of PBZ is more suitable for use in regulating the growth of basil plants var. Cinnamon without changing its physiology, being indicated for ornamental use of this plant.

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