FEDERAL UNIVERSITY OF VIÇOSA

JOÃO HENRIQUE FERREIRA SABINO

GROWTH CONTROL OF *Platycodon grandiflorus* (Jacq.) A. DC. FOR PRODUCTION AS POTTED FLOWER

VIÇOSA - MINAS GERAIS

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Dissertation submitted to the Federal University of Viçosa, as part of the requirements of the Graduate Program in Plant Sciences, to obtain the title of *Magister Scientiae*.

Advisor: José Antonio Saraiva Grossi

Co-advisors: José Geraldo Barbosa
Sebastião Martins Filho

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Assent:

João Henrique Ferreira Sabino
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For my *mãinha*.

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ABSTRACT

SABINO, João Henrique Ferreira, M.Sc., Universidade Federal de Viçosa, August, 2020. **Growth control of *Platycodon grandiflorus* (Jacq.) A. DC. for production as potted flower.** Advisor: José Antonio Saraiva Grossi. Co-advisors: José Geraldo Barbosa and Sebastião Martins Filho.

Platycodon (*Platycodon grandiflorus*) is an ornamental species that needs growth control to satisfy the quality standards of the potted flower market. The appropriate management of growth regulators and plant density are fundamental factors to obtain harmony with the pot size. This study aimed to analyze the effects of the paclobutrazol application and plant density on the growth parameters of four varieties of platycodon 'Astra Semi-Double', namely Blue, Pink, Lavender and White. Five concentrations of paclobutrazol (0; 1.25; 2.5; 3.75 and 5.0 mg pot^{-1}) were investigated in an experiment with two plants per pot, while four plant densities (one, two, three and four plants per pot) were evaluated in another experiment. Both experiments were conducted in greenhouse, from October 2019 to March 2020, with randomized block design and four replications. In addition to growth parameters, chlorophyll contents were also measured in the second experiment. Shoot height, number of branches, leaf area and dry mass of the plant parts decreased with higher concentration of paclobutrazol or with larger number of plants per pot. Flower diameter was not influenced by the paclobutrazol application or plant density. Chlorophyll α and total chlorophyll contents increased with greater plant density. There was varietal effect on the growth parameters and chlorophyll contents of platycodon 'Astra Semi-Double'. Pink is an early-flowering variety, while Lavender is a late-flowering variety. The concentration of 3.75 mg pot^{-1} was the most efficient in controlling growth and improving the visual quality of the platycodon varieties for production as potted flower. The cultivation of four plants per pot occasioned the production of flowering stems with a better quality.

Keywords: Balloon flower. Paclobutrazol. Plant density.

RESUMO

SABINO, João Henrique Ferreira, M.Sc., Universidade Federal de Viçosa, agosto de 2020. **Controle do crescimento de *Platycodon grandiflorus* (Jacq.) A. DC. para a produção como flor de vaso.** Orientador: José Antonio Saraiva Grossi. Coorientadores: José Geraldo Barbosa e Sebastião Martins Filho.

Platycodon (*Platycodon grandiflorus*) é uma espécie ornamental que necessita de controle do crescimento para atender aos padrões de qualidade do mercado de flores de vaso. O manejo adequado de reguladores de crescimento e da densidade de plantas são fatores fundamentais para obter harmonia com o tamanho do vaso. Esse estudo teve como objetivo analisar os efeitos da aplicação de paclobutrazol e da densidade de plantas nos parâmetros de crescimento de quatro variedades de platycodon 'Astra Semi-Double', a saber, Blue, Pink, Lavender e White. Cinco concentrações de paclobutrazol (0; 1,25; 2,5; 3,75 e 5,0 mg vaso^{-1}) foram investigadas em um experimento com duas plantas por vaso, enquanto quatro densidades de plantas (uma, duas, três e quatro plantas por vaso) foram avaliadas em outro experimento. Ambos os experimentos foram conduzidos em casa de vegetação, de outubro de 2019 a março de 2020, com delineamento em blocos ao acaso e quatro repetições. Além dos parâmetros de crescimento, os teores de clorofila também foram mensurados no segundo experimento. A altura da parte aérea, número de ramos, área foliar e massa seca das partes da planta diminuíram com maior concentração de paclobutrazol ou com maior número de plantas por vaso. O diâmetro da flor não foi afetado pela aplicação de paclobutrazol ou pela densidade de plantas. Os teores de clorofila α e clorofila total aumentaram com a maior densidade de plantas. Houve efeito varietal nos parâmetros de crescimento e nos teores de clorofila de platycodon 'Astra Semi-Double'. Pink é uma variedade precoce, enquanto Lavender é uma variedade tardia. A concentração de 3,75 mg vaso^{-1} foi a mais eficiente em controlar o crescimento e melhorar a qualidade visual das variedades de platycodon para produção como flor de vaso. O cultivo de quatro plantas por vaso possibilitou a produção de ramos florais de melhor qualidade.

Palavras-chave: Flor-balão. Paclobutrazol. Densidade de plantas.

SUMMARY

1 GENERAL INTRODUCTION

Platycodon grandiflorus (Jacq.) A. DC. is the only recognized botanical species of the genus Platycodon, belonging to the family Campanulaceae, the same botanical family as the potted bellflower (*Campanula medium* L.) (POWO, 2020; TROPICOS, 2020). Different from *C. medium*, which is of French and Italian origin, *P. grandiflorus* is native to three Asian countries, namely China, Japan and Korea, as well as the far east of Russia (DEYUAN *et al.*, 2011; WCSP, 2019).

In these Asian countries, *P. grandiflorus* is often grown as a tuberous vegetable species, since its roots have a high medicinal value (KIM & LIU, 1999; LIU *et al.*, 2014). The roots of this species are typically used in salads and soups in Korea, while they are employed as an expectorant and antitussive in the treatment of tonsillitis, chest congestion, sore throat, cold and cough in Chinese culture (NYAKUDYA *et al.*, 2014). In addition to its nutritional and therapeutic importance, *P. grandiflorus* is also widely explored as an ornamental plant, mainly as a garden or bedding plant (HALEVY *et al.*, 2002).

Morphologically, an outstanding feature of this species is the shape of its flower buds, which resemble air balloons (KIM & LIU, 1999). As a result, this species became known internationally as balloon flower (HALEVY *et al.*, 2002), but it was known only as platycodon in Brazil. Beyond its flower buds, platycodon exhibits solitary flowers, in blue, white, pink or purple colors, with petals that remain partially attached to each other at the base, acquiring the form of a star (DEYUAN *et al.*, 2011; SAKATA, 2020).

Physiologically, platycodon is a day-neutral plant, blossoming with the same magnitude regardless of the photoperiod (IVERSEN & WEILER, 1994). Although it is considered a day-neutral plant, Halevy *et al.* (2002) report that a greater formation of flowering branches occurs when platycodon is grown under long days. For Starman *et al.* (1995), *P. grandiflorus* is an unconventional cut flower that is highly profitable in the United States of America, because of its high productivity combined with the lack of phytosanitary problems. Furthermore, this species has a long flowering period and a long vase life (EVENSEN & BEATTIE, 1986).

Despite the fact that platycodon has agronomic, morphological and physiological characteristics with great potential for potted flower, its utilization remained for a long time restricted to the composition of gardens or, more recently, as a cut flower. These limitations occurred in platycodon due to its tuberous roots and its considerable growth in height, which can reach around 1.20 m (DEYUAN *et al.*, 2011), making it impossible to cultivate this crop as potted flower.

In the last years, there was the development of platycodon cultivars for production as potted flower (SAKATA, 2020). Nevertheless, it is still necessary to apply growth control techniques, so that these cultivars can acquire an erect and rigid architecture, that is, without bent flowering stems.

Among the different methods to control plant growth, the application of growth regulators that inhibit the synthesis of gibberellins, such as chlormequat, paclobutrazol and uniconazole, stands out in floriculture (WANDERLEY *et al.*, 2014). According to Lee *et al.* (2015), growth regulators are employed to obtain the desired shape and size of potted flowers, since excessive branch elongation is avoided in this floriculture product. As gibberellins are the hormones responsible for stem elongation in plants (TAIZ *et al.*, 2017), inhibitors of their biosynthesis are used to produce potted flowers with a more compact formation.

In Brazil, only paclobutrazol has commercial products registered by the Ministry of Agriculture, Livestock and Food Supply for national commercialization (AGROFIT, 2020). This regulator, belonging to the triazole chemical class, prevents the gibberellin synthesis by inhibiting the catalytic activity of the enzyme *ent*-kaurene oxidase, which, in turn, acts on the biosynthetic pathway of this plant hormone (MATSOUKIS *et al.*, 2014; BISHOP *et al.*, 2015).

Paclobutrazol is identified as the main plant growth regulator by producers of annual crops, on account of its high chemical activity in almost all of these species and its cost-benefit ratio (GROSSI *et al.*, 2009; WHIPKER, 2017). As stated by Tinoco *et al.* (2011), this triazole reduces plant height in very low concentrations, around 1% in relation to chlormequat and daminozide.

Several studies have already described the possibility of applying paclobutrazol to reduce the growth of various ornamental species, such as geranium (TINOCO *et al.*, 2011), black iris (AL-KHASSAWNEH *et al.*, 2006), kalanchoe (HWANG *et al.*, 2008), narcissus (DEMIR & ÇELİKELE, 2019), physalis (BOSCH *et al.*, 2016), red firespike (REZAZADEH *et al.*, 2016), rose (CARVALHO-ZANÃO *et al.*, 2018), sunflower (WANDERLEY *et al.*, 2014) and star of bethlehem (LEE *et al.*, 2015), for production as potted flower.

Despite these researches, other reports have indicated that the paclobutrazol application suppressed the growth of ornamental crops, but it was not enough to satisfy the quality standards, such as in gladiolus (MILANDRI *et al.*, 2008), Thai tulip (PINTO *et al.*, 2006) and zinnia (PINTO *et al.*, 2005). In pepper ‘Biquinho Vermelha’, in turn, França *et al.* (2018) related that the drench application of paclobutrazol caused phytotoxicity symptoms and reduced plant height excessively, making it impossible to obtain plants with characteristics suitable for the floriculture market. Therefore, it is essential to study the viability of the paclobutrazol application in the potted platycodon production to achieve the quality requirements of the market, in addition to determine the concentration that promotes the best results without causing adverse symptoms.

Although the application of growth regulators is the most common method for producing more compact plants (REZAZADEH & HARKESS, 2015), chemical control must be used in conjunction with other cultural practices to reach better results, such as, for example, with practices that restrict the root system by decreasing the volume of the pot or increasing the number of plants per pot (LATIMER, 2001).

Plant density is a resource that makes it possible to regulate the availability of water and nutrients and the absorption of light by plant species (VIANA *et al.*, 2008; KIM *et al.*, 2018), allowing to modify the balance between different plant parts (MORANO *et al.*, 2017). In ornamental crops, this cultural practice influences the quality and quantity of flowers and the incidence of diseases and pests (BHOSALE *et al.*, 2012), as well as performing an important role in plant growth (CHOPDE *et al.*, 2015).

According to Luz *et al.* (2018), the desired plant size and final product are directly associated with the plant density employed. In fact, Bohme and Pinker (2014) declared that plant density interferes with the stem length of various crops. Therefore, it becomes fundamental to investigate the possibility of controlling growth and improving the quality of flowers and ornamental plants by manipulating the number of plants per pot.

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2 MANUSCRIPT I[1]

POTTED PLATYCODON PRODUCTION UNDER INFLUENCE OF THE PACLOBUTRAZOL APPLICATION

PRODUÇÃO DE PLATYCODON EM VASO SOB INFLUÊNCIA DA APLICAÇÃO DE PACLOBUTRAZOL

ABSTRACT

Platycodon grandiflorus is an ornamental species that can be employed as potted flower, but it has weak and bent flowering stems. Plant growth regulators, such as paclobutrazol, are compounds that reduce stem elongation, enabling the production of plants with more compact formation. The current study aimed to investigate the growth of platycodon depending on the drench application of paclobutrazol. The experiment was performed with four varieties of platycodon 'Astra Semi-Double' (Blue, Lavender, Pink and White) and five concentrations of paclobutrazol (0; 1.25; 2.5; 3.75 and 5.0 mg $vaso^{-1}$). Stem diameter, number of leaves, anthesis, number of flowers or flower buds and flower diameter were not affected by the paclobutrazol application. Shoot height, number of branches, leaf area and dry mass of the shoot parts were reduced with higher concentration of this regulator. Lavender exhibited the highest values of shoot height, number of leaves, leaf area and dry mass of the shoot parts. In relation to anthesis, Pink was the earliest variety, followed by Blue and White, and Lavender was the latest. The concentration of 3.75 mg $vaso^{-1}$ was the most efficient in controlling growth and improving the visual quality of platycodon varieties.

Index Terms: *Platycodon grandiflorus*, balloon flower, plant growth regulator, substrate drench, Floriculture.

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[1] Scientific article written according to the editorial standards of Ciência e Agrotecnologia (Science and Agrotechnology) journal.

RESUMO

Platycodon grandiflorus é uma espécie ornamental que pode ser comercializada como flor de vaso, porém apresenta ramos florais frágeis e pendentes. Reguladores de crescimento, como o paclobutrazol, são compostos que reduzem o alongamento do caule, possibilitando a produção de plantas com formação mais compacta. Com presente trabalho buscou-se investigar o crescimento de platycodon em função da aplicação de paclobutrazol no substrato. O experimento foi conduzido com quatro variedades de platycodon 'Astra Semi-Double' (Blue, Lavender, Pink e White) e cinco concentrações de paclobutrazol (0; 1,25; 2,5; 3,75 e 5,0 mg vaso^{-1}). O diâmetro do caule, número de folhas, antese, número de flores ou botões florais e diâmetro da flor não foram afetados pela aplicação de paclobutrazol. A altura da parte aérea, número de ramos, área foliar e massa seca das partes aéreas da planta foram reduzidas com maior concentração desse regulador. Lavender apresentou os maiores valores de altura, número de folhas, área foliar e massa seca das partes aéreas da planta. Em relação a antese, Pink foi a variedade mais precoce, seguida da Blue e White, e Lavender foi a mais tardia. A concentração de 3,75 mg vaso^{-1} foi a mais eficiente em controlar o crescimento e melhorar a qualidade visual das variedades de platycodon.

Termos para Indexação: *Platycodon grandiflorus*, flor-balão, regulador de crescimento vegetal, embebição do substrato, Floricultura.

INTRODUCTION

The floriculture sector is constantly searching for new and differentiated products to satisfy the demands of producers and consumers (Junqueira; Peetz, 2018). Platycodon or balloon flower (*Platycodon grandiflorus* (Jacq.) A. DC., Campanulaceae) is one of those products that has been explored, in recent years, as a potted flower in the national market. This species of Asian origin is widely used as a garden and bedding plant, but it can also be grown as cut or potted flower (Iversen; Weiler, 1994; Park; Oliveira; Pearson, 1998; Halevy; Shlomo; Ziv, 2002).

Platycodon has several desirable ornamental characteristics, such as: attractive flower buds, which resemble air balloons; captivating flowers, which keep their petals partially attached to each other acquiring the configuration of stars; low susceptibility to post-harvest problems; long flowering period and vase life (Evensen; Beattie, 1986; Starman; Cerny; Mackenzie, 1995; Song et al., 2012; Liu et al., 2014).

However, ornamental species, which are grown both as cut flower and as potted flower, need plant growth control in the vase to reach a product with adequate height composition and stem stiffness (Backes et al., 2005). Moreover, platycodon produces weak and bent flowering branches (Iversen; Weiler, 1994), especially when it is grown under high night temperatures (Halevy; Shlomo; Ziv, 2002). To avoid the requirement for staking and the loss of product quality, it is fundamental to perform techniques to reduce stem elongation in platycodon, such as the application of growth regulators.

Growth regulators are commonly employed in floriculture to control plant height and, consequently, produce plants with a more appropriate formation (Hawkins; Ruter; Robacker, 2015; Rezazadeh; Harkess, 2015). Paclobutrazol, an inhibitor of gibberellin biosynthesis that belongs to the class of triazoles, is the most widely used growth regulator (Binotti et al., 2018; Latimer, 2018). This triazole has high chemical activity in many species of ornamental plants and it can reduce the stem elongation with low concentrations (Grossi; Barbosa; Rodrigues, 2009; Tinoco et al., 2011; Carvalho-Zanão et al., 2018).

Several studies have already indicated the effectiveness of the paclobutrazol application in reducing the growth of various species for potted flower production (Al-Khassawneh; Karam; Shibli, 2006; Hwang et al., 2008; Wanderley; Faria; Rezende, 2014; Lee; Gomez; Miller, 2015; Bosch et al., 2016; Rezazadeh; Harkess; Bi, 2016; Demir; Çelikele, 2019), but there are no reports in the literature regarding the possibility of using this regulator to control the height of platycodon. For that reason, the current study aimed to investigate the effects of the drench application of different paclobutrazol doses on the growth of varieties of platycodon ‘Astra Semi-Double’.

MATERIAL AND METHODS

The experiment was conducted at the Floriculture Sector of the Agronomy Department of the Federal University of Viçosa, in Viçosa, Minas Gerais State, Brazil (20º45’36.0” S, 42º51’47.4” W, 685 m of altitude). It was performed between October 28, 2019 and February 25, 2020, in a greenhouse under natural photoperiod, from commercial seeds of *Platycodon grandiflorus* ‘Astra Semi-Double’ (Sakata®). The maximum and minimum temperature values were recorded daily by a digital thermo-hygrometer (J.Prolab®), which was installed inside the greenhouse at 1.80 m above the ground surface, during the experiment (Figure 1).

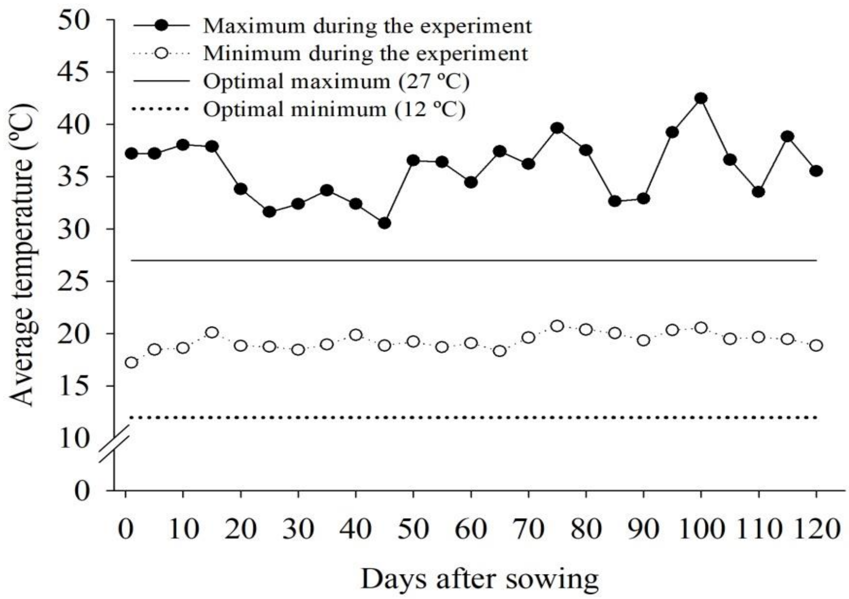

Figure 1: Maximum and minimum average temperatures during the experiment, compared to the optimal maximum and minimum temperatures of platycodon cultivation, proposed by Halevy, Shlomo and Ziv (2002).

Firstly, seedlings were produced in expanded polystyrene trays (28 mL cell^{-1}) with commercial substrate (Tropstrato®). In these trays, it was placed one seed for each cell, 0.5 cm deep. At 38 days after sowing, the seedlings were transplanted to plastic pots of No. 14 (9.8 cm high, 13.0 cm wide at the top, 1.0 L volume), allocating two of them per pot. Each container was previously filled with 350 g of substrate and 2.0 g of a homogeneous mixture of single superphosphate and potassium chloride (4:1) to avoid calcium deficiency. Thereafter, all the pots were arranged on a bench with 10.0 × 8.0 cm spacing between them.

The irrigation regime was on alternate days, applying 150 mL pot^{-1}, and fertilization via fertigation was interspersed with the water supply, providing a solution of NPK 20:20:20 and micronutrients (Peters®). Until 30 days after transplanting (DAT), a solution with 1.0 g L^{-1} of this soluble fertilizer was employed. Subsequently, it was used a nutrient solution with twice the concentration of the first. Regarding the cultivation practices, only the pinching (removal of shoot tip) was performed at 12 DAT.

The experimental design chosen was randomized complete blocks with four replications and in a 4 × 5 factorial scheme, combining four varieties of platycodon 'Astra Semi-Double', namely Blue, Lavender, Pink and White, with five doses of paclobutrazol: 0, 1.25, 2.5, 3.75 and 5.0 mg pot^{-1}. Each experimental unit was composed of a specific pot.

The active ingredient paclobutrazol was acquired by the commercial product Cultar 250 SC (250 g L^{-1}). Through this product, five solutions of paclobutrazol (0, 25, 50, 75 and 100 mg L^{-1}) were prepared, from which 50 mL aliquots were taken to constitute the respective treatments (0, 1.25, 2.5, 3.75 and 5.0 mg pot^{-1}). Each aliquot was evenly distributed on the substrate of each replication. This procedure was executed two more times, totaling three drench applications, at 37, 47 and 57 DAT. It should be noted that all applications were accomplished between 8:00 a.m. and 9:30 a.m., and with the substrate previously moistened.

The growth evaluation was performed at 82 DAT, when each pot had at least one open flower. The following variables were measured in one plant for each replication: shoot height; stem diameter on the substrate surface; number of branches, larger than 1.0 cm; number of leaves; anthesis, which it means the number of days between transplanting and opening the first flower (Figure 2); number of flowers or flower buds; flower diameter; leaf area and dry mass of shoot parts (leaves, stem and flowers or flower buds). The leaf area was determined with the aid of a bench-top area meter (LI-COR®), while the dry mass was obtained by individually packing the plant parts in a drying oven with forced air circulation at 70 ºC for 72 hours.

Figure 2: Anthesis of platycodon 'Astra Semi-Double', variety Pink: complete opening of the inner and outer petals of the first flower.

In the end, the data were subjected to variance analysis and regression equations were adjusted to verify the effects of paclobutrazol concentrations. The models were defined based on the coefficient of determination and the significance of the regression coefficients by the Student's t-test, at 0.1% or 5% probability. The averages of varieties were compared using the

Tukey's test, at 5% probability. All analyses were performed by ExpDes.pt package (Ferreira; Cavalcanti; Nogueira, 2018) in the R 3.6.3 statistical software (R Core Team, 2020).

RESULTS AND DISCUSSION

First of all, there was no significant interaction between varieties and concentrations of paclobutrazol (PBZ) for the variables evaluated, except for the dry mass of flowers or flower buds (Table 1 and 2). This result indicates that the varieties have similar growth responses in relation to the increase in PBZ applied to the substrate. Therefore, the commercial production of platycodon 'Astra Semi-Double' as a potted flower becomes even more viable, since the producer will be able to adopt the same concentration of this regulator for the four varieties.

Table 1: Shoot height (SH), stem diameter (SD), number of branches (NB), number of leaves (NL), anthesis (A), number of flowers or flower buds (NF) and flower diameter (FD) of four varieties (VAR) of platycodon 'Astra Semi-Double' treated with different concentrations of paclobutrazol (PBZ, mg pot^{-1}).

Sources of variation	SH (cm)	SD (mm)	NB -	NL[(1)] -	A (days)	NF -	FD (cm)
VAR (F_c)	36.90^{**}	6.30^{**}	6.69^{**}	33.38^{**}	81.94^{**}	3.94^{*}	9.77^{**}
Blue	17.92 b	6.05 b	11.90 b	9.53 b	70.65 b	20.95 b	7.00 a
Lavender	21.11 a	5.98 b	16.25 a	12.21 a	75.90 a	26.05 a	7.07 a
Pink	16.75 b	6.90 a	16.95 a	9.59 b	63.85 c	21.70 b	6.43 b
White	17.17 b	6.61 ab	14.05 ab	9.48 b	69.05 b	23.05 ab	6.10 b
PBZ (F_c)	13.71^{**}	1.22^{ns}	2.84^{*}	0.36^{ns}	2.46^{ns}	0.52^{ns}	0.88^{ns}
0	-	6.03	-	10.26	68.18	23.00	6.67
1.25	-	6.38	-	10.39	70.00	23.25	6.68
2.5	-	6.62	-	10.24	70.50	23.75	6.83
3.75	-	6.43	-	10.14	70.12	23.31	6.40
5.0	-	6.46	-	9.97	70.50	21.37	6.66
VAR × PBZ (F_c)	0.71^{ns}	1.21^{ns}	0.67^{ns}	0.76^{ns}	1.64^{ns}	1.11^{ns}	0.90^{ns}
C.V. (%)	7.98	12.31	26.74	10.14	3.51	22.08	10.02

"F_c" = calculated F value of variance analysis: ^{ns} p-value > 0.05; * p-value < 0.05; ** p-value < 0.001. "C.V." = coefficient of variation. Averages differ between themselves by Tukey's test, at 5% probability, when they are followed by distinct letters in the columns.

[(1)] Data transformed into their square roots ($y = \sqrt{x}$) to satisfy the assumptions of the variance analysis.

Table 2: Leaf area (LA), stem dry mass (SDM), leaf dry mass (LDM), dry mass of flowers or flower buds (DMF) and total dry mass (TDM) of four varieties (VAR) of platycodon 'Astra Semi-Double' treated with different concentrations of paclobutrazol (PBZ, mg pot^{-1}).

Sources of Variation	LA[(1)] (cm^2)	SDM (g)	LDM (g)	DMF (g)	TDM[(2)] (g)
VAR (F_c)	20.96**	14.76**	23.06**	-	7.26**
Blue	21.36 b	0.81 b	1.71 b	-	3.94 b
Lavender	24.69 a	1.13 a	2.20 a	-	4.63 a
Pink	20.09 b	0.83 b	1.52 b	-	3.86 b
White	20.02 b	0.81 b	1.50 b	-	3.75 b
PBZ (F_c)	7.06**	3.40*	4.05*	-	4.20*
VAR × PBZ (F_c)	1.23^{ns}	0.67^{ns}	0.88^{ns}	2.44*	1.15^{ns}
C.V. (%)	9.91	20.53	17.55	19.16	16.38

"F_c" = calculated F value of variance analysis: ^{ns} p-value > 0.05; * p-value < 0.05; ** p-value < 0.001. "C.V." = coefficient of variation. Averages differ between themselves by Tukey's test, at 5% probability, when they are followed by distinct letters in the columns.

(1) Data transformed into their square roots ($y = \sqrt{x}$) to satisfy the assumptions of the variance analysis.

(2) Excluding roots.

On the other hand, Francescangeli and Zagabria (2008) found that varieties of petunia 'Bravo F1' (Blue, Red and White) require different management in terms of the concentration and timing of application of PBZ to reduce the plant height without harming the number of flowers. For the potted production of hybrid dianthus, Lenzi et al. (2015) found that there was no interaction between the varieties (Pink, Red, Violet and White) and the concentrations of PBZ for growth variables evaluated in the autumn, but there was dependence between these factors in another similar experiment conducted in the summer.

Although the chemical activity of this triazole can be influenced by genetic attributes of different varieties, Carvalho-Zanão et al. (2018) indicated that the effects, in growth parameters, of this regulator do not depend on the genotype of different varieties of roses 'Terrazza'. Thus, it is essential to study the performance of different varieties, within the same cultivar, to confirm whether or not there are different responses regarding the application of PBZ.

Among the varieties, Lavender had the highest average values of shoot height, number of leaves (Table 1), leaf area, stem dry matter, leaf dry matter and total dry matter, differing from the others (Table 2). These statistical differences reveal that Lavender invested more in vegetative growth than the other varieties.

As a result of this investment, Lavender took more time to open the first flower, around 76 days, while Pink blossomed earlier with, approximately, 64 days (Table 1), in order that both differed statistically between each other and from the other two varieties in relation to the anthesis. Despite the fact that Lavender delayed its anthesis more, this variety produced a larger number of flowers and flower buds (Table 1), as well as bigger flowers, being statistically similar only to White in terms of this number and to Blue in terms of flower diameter.

Regarding the stem diameter, Pink obtained the highest value, but it was statistically similar to White's average. Furthermore, Blue was the least branched variety, but its average number of branches did not differ from White. In general, the variety most susceptible to stem bending was Lavender, because it simultaneously had a high shoot height with a thin stem diameter and large flowers, which facilitate the bent neck.

These results show that the growth and flowering of platycodon is greatly influenced by genetic factors associated with each variety. In addition to this factor, other studies indicate that temperature is another factor that affects the production of this crop. According to Kwon et al. (2019), the varieties 'Green' and 'Duplex' reduced the height and the number of branches when they grew at a constant temperature of 30 ºC, compared to colder temperatures (15, 20 or 25 ºC). For Park, Oliveira and Pearson (1998), *P. grandiflorus* is a species very sensitive to temperature, since the shoot height, number of branches, number of flower buds, leaf area and shoot dry mass of the variety 'Astra Blue' decreased as the temperature increased, in the range of 19 to 29 ºC. Moreover, these authors also identified a linear decrease in the number of days to flowering with the increase in temperature.

In view of the above, it appears that high temperatures provide a reduction in vegetative growth and the anticipation of anthesis, which, in turn, favor potted platycodon production. However, night temperatures above 14 ºC, as those in the current study (Figure 1), promote rapid growth in platycodon and the development of thin and fragile flowering stems (Halevy; Shlomo; Ziv, 2002). These stems, in turn, are unable to support the weight of their flowers, making it necessary to stake them in Brazilian climatic conditions.

Comparing the different concentrations of PBZ, there was no statistical difference by the F test for the following variables: stem diameter, number of leaves, anthesis, number of flowers or flower buds and flower diameter (Table 1). The other variables, except leaf area, adjusted better to the simple linear regression model, decreasing with the increase in concentration (Figure 3). The quadratic regression model was the most appropriate for the leaf area, showing a reduction of this variable with the increase in concentration until 3.75 mg pot^{-1} (Figure 3C).

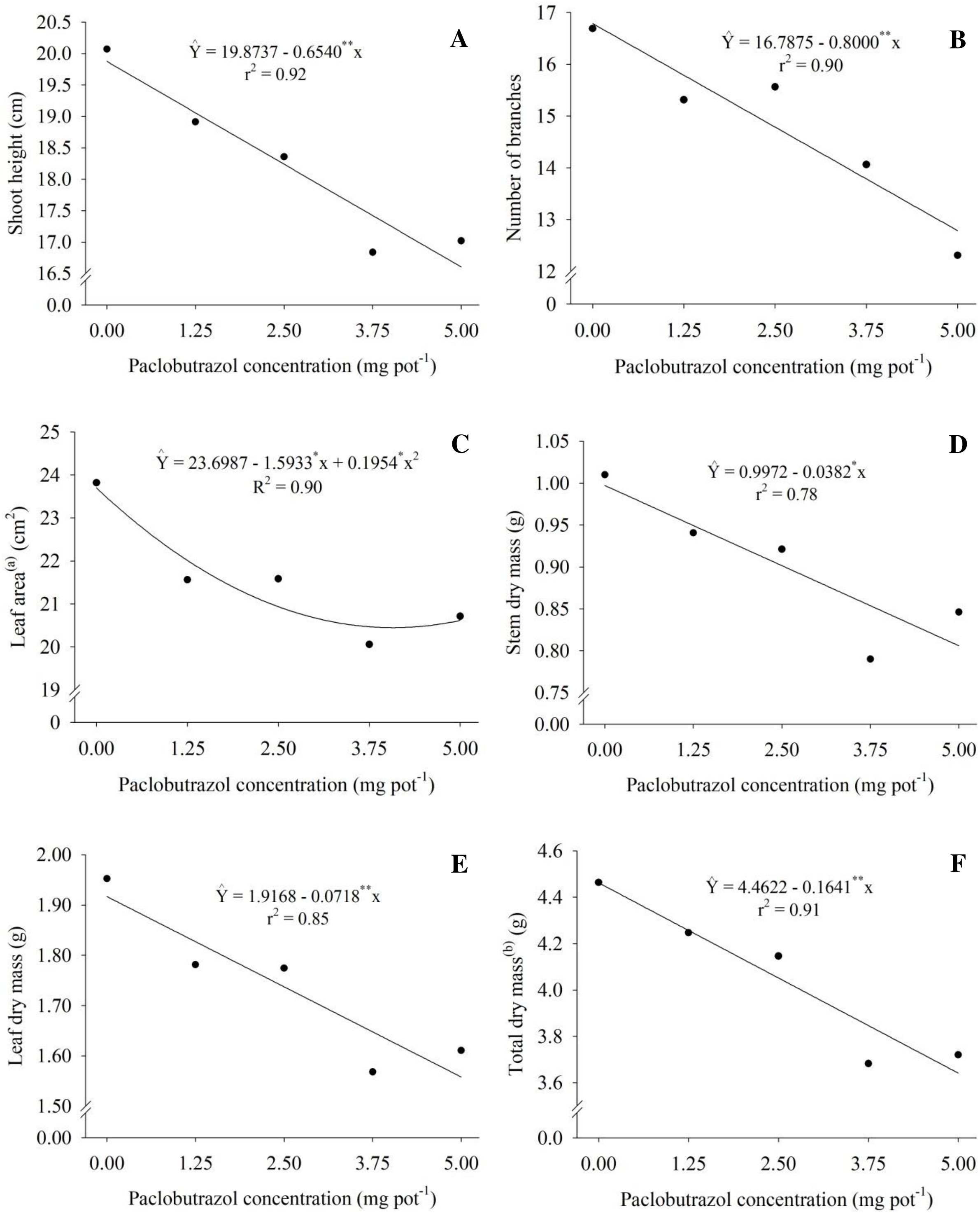

Figure 3: Shoot height (A), number of branches (B), leaf area (C), stem dry mass (D), leaf dry mass (E) and total dry mass (F) of varieties of platycodon 'Astra Semi-Double' treated with different concentrations of paclobutrazol. * p-value < 0.05; ** p-value < 0.001.

[(a)] Data transformed into their square roots ($y = \sqrt{x}$) to satisfy the assumptions of the variance analysis.

[(b)] Excluding roots.

Thereby, shoot height, leaf area and dry masses were reduced with the increase in the dose of PBZ, while the number of leaves was not altered. Larcher et al. (2011) found similar results to those obtained in the current work with the application of PBZ in camellia cultivars. These authors noted that the application of this regulator reduced the height and dry mass of stem and leaves without affecting the number of leaves in potted camellia. However, Carver et al. (2014) noticed a reduction not only in the height and shoot dry mass, but also in the number of leaves, with the increase in concentration of PBZ in sea marigold.

This triazole reduces plant height because it inhibits the enzymatic activity of *ent*-kaurene oxidase, which catalyzes the conversion of *ent*-Kaurene to GA_{12} on the gibberellin biosynthesis pathway (Bishop et al., 2015). Therefore, the application of PBZ interrupts the gibberellin synthesis and promotes the reduction in plant height, since the process of stem elongation is regulated by this hormone (Taiz et al., 2017).

Regarding the reduction in dry masses of plant parts, Bosch, Cuquel and Tognon (2016) explain that this effect is a consequence of the stem internode reduction caused by PBZ, since more compact plants have an architecture that does not favor the light interception by the lower leaves and, consequently, these plants will produce and assimilate a smaller amount of photoassimilates.

In addition, Takane et al. (2019) also noticed that the shoot height, dry mass of leaves and shoot part of desert rose (*Adenium obesum*) decreased with the increase in concentration of PBZ, but there was no statistical difference for the leaf area, diverging from the results of leaf area in platycodon. Nevertheless, Matsoukis, Gasparatos and Chronopoulou-Sereli (2014) and Carvalho-Zanão et al. (2017) found that there was a reduction in the leaf area with the elevation in the dose of PBZ in lantana and rose, respectively. According to Leyser and Day (2015), this reduction in leaf area can be attributed to a decrease in leaf expansion caused by the inhibition of gibberellin synthesis by this triazole.

Although the application of PBZ promoted reductions in height, number of branches, leaf area and dry masses, this regulator did not modify the flower diameter, the number of flowers and flower buds and the anthesis of the analyzed varieties. In potted narcissus (Demir; Çelikel, 2019) and zinnia (Ahmad; Whipker; Dole, 2015) production, the application of PBZ also reduced the shoot height, but this regulator decreased the number of narcissus flowers and the diameter of the zinnia flowers, as well as delayed the anthesis of both, contrasting with the results of platycodon. Furthermore, it was observed that the PBZ administered in potted red firespike reduced not only the height, the leaf area and the total dry mass, but also the number and height of the inflorescences, as well as it increased the number of days to open the first flower of this flowering potted plant (Rezazadeh; Harkess; Bi, 2016).

Nevertheless, Tinoco et al. (2011) found no statistical differences for anthesis and for the number and diameter of geranium inflorescences between the doses of PBZ. Based on this discussion, it is evident that the application of this triazole can negatively affect the number and size of the reproductive organs and extend the number of days to reach flower maturation. On the other hand, the drench applications of PBZ up to 5.0 mg pot^{-1} allowed reducing the growth of platycodon varieties without causing these negative effects.

In kalanchoe (Hwang et al., 2008), it was detected that the application of PBZ reduced the stem diameter, whereas this variable increased with the elevation in the dose of this regulator in lupine (Karaguzel et al., 2004). However, the stem diameter was not significantly affected by the application of PBZ in the current study, as well as in sunflower (Koutroubas; Damalas, 2015) and Thai tulip (Pinto et al., 2006). All previous comparisons confirm not only the efficiency of this triazole in controlling the vegetative growth of several flowers, including platycodon, but also that distinct species have different responses to the application of PBZ.

In the present work, the control treatment (0 mg pot^{-1}) was the only concentration that obtained a height (20.07 cm) (Figure 3A) outside the range recommended by Barbosa, Grossi and Borém (2019) for the marketing of potted flowers, which is 1.5 to 2.0 times the pot height. Although the other doses produced plants with heights appropriate for the pot size used, only the concentrations of 3.75 and 5.0 mg pot^{-1} provided, through visual analysis, more rigid and erect flowering stems, in other words, flowering stems of better quality. These doses reduced, respectively, 16.1% and 15.2% of the height, in relation to the control, and without causing phytotoxicity symptoms. As a further matter, no deficiency symptoms were observed throughout the experiment.

Latimer (2018) suggests that the concentration of 4.0 mg pot^{-1} be employed, in a single drench application of PBZ, to control growth and improve the toning of platycodon in the northern region of the United States of America. However, the current work recommends the dose of 3.75 mg pot^{-1}, to be applied three times during the cycle in intervals of 10 days, in Brazil, since it was the lowest dose among the concentrations of PBZ that provided the best results of growth control and quality of the final product. This management of paclobutrazol in platycodon allows obtaining flowering stems that support the weight of their own flowers, eliminating the need for staking.

As stated by Grossi, Barbosa and Rodrigues (2009), this discrepancy in the number of applications occurs because the plants grow faster under temperatures above their ideal range of cultivation (Figure 1), and, consequently, they will require higher concentrations or more frequent applications of the growth regulator. Additionally, these same authors emphasize that several applications with a low concentration of growth regulator provide better results and a lower chance of causing phytotoxicity, than a single application with a high concentration. In ornamental peppers, for example, a single drench application of 10 or 15 mg pot^{-1} of PBZ promoted stunted growth with wrinkled and brittle leaves in the variety ‘Biquinho Vermelha’ and accession 2334PB, then this type of management makes both genotypes inappropriate for the potted production (França et al., 2018).

As previously mentioned, there was a significant interaction between the evaluated factors for the dry mass of flowers or flower buds (Table 2). Comparing the varieties for each concentration (Table 3), White had the lowest dry mass for the dose of 3.75 mg pot^{-1}, but its average value did not differ from Blue. For 5.0 mg pot^{-1}, Lavender had the lowest average value, differing only from Pink and White. The other concentrations did not reveal differences between the varieties by the F test. There was no appropriate adjustment of polynomial equations to analyze the effects of concentrations within each variety for dry mass of flowers or flower buds.

Table 3: Dry mass of flowers or flower buds (g) of four varieties of platycodon 'Astra Semi-Double' treated with different concentrations of paclobutrazol (PBZ).

PBZ (mg pot^{-1})	Blue	Lavender	Pink	White
0	1.48 A	1.42 A	1.49 A	1.59 A
1.25	1.43 A	1.36 A	1.70 A	1.60 A
2.5	1.64 A	1.22 A	1.34 A	1.59 A
3.75	1.36 AB	1.52 A	1.49 A	0.92 B
5.0	1.16 AB	0.93 B	1.48 A	1.47 A

Averages differ between themselves by Tukey's test, at 5% probability, when they are followed by distinct letters in the rows.

CONCLUSION

Drench applications of paclobutrazol should be performed with doses of 3.75 mg pot^{-1} for the commercial production of potted platycodon in Brazil.

Lavender has a more vigorous vegetative growth than the other varieties. Pink is an early-flowering variety, while Lavender is a late-flowering variety.

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3 MANUSCRIPT II[2]

Plant density for the potted cultivation of *Platycodon grandiflorus* (Jacq.) A. DC.

Densidade de plantas para o cultivo em vaso de *Platycodon grandiflorus* (Jacq.) A. DC.

Running Title: Plant density for potted platycodon production

ABSTRACT

The current study aimed to identify the effects of plant density on the growth and chlorophyll contents of platycodon varieties grown in pots. The experiment was conducted in randomized block design, with four replications, combining four varieties of platycodon 'Astra Semi-Double' (Blue, Lavender, Pink or White) with four plant densities (one, two, three or four plants per pot). The variables stem diameter, shoot height, number of branches, number of leaves, anthesis, number of flowers or flower buds, flower diameter, leaf area, shoot dry mass, root dry mass and chlorophyll contents were measured. Pink and White were the earliest varieties to blossom, followed by Blue, while Lavender was the latest. All variables decreased with larger number of plants per pot, except the flower diameter and chlorophyll contents. Chlorophyll α and total chlorophyll indices increased with greater number of plants per pot. The density of four plants per pot promoted the formation of more compact plants, as well as the acquisition of a better quality product to satisfy the requirements of the market.

KEYWORDS: Balloon flower; growth analysis; chlorophyll indices; Floriculture.

RESUMO

O presente estudo objetivou identificar os efeitos da densidade de plantas no crescimento e nos teores de clorofila de variedades de platycodon cultivadas em vaso. O experimento foi conduzido em delineamento de blocos ao acaso, com quatro repetições, combinando-se quatro variedades de platycodon 'Astra Semi-Double' (Blue, Lavender, Pink ou White) com quatro densidades de plantas (uma, duas, três ou quatro plantas por vaso). Foram mensuradas as variáveis diâmetro do caule, altura da parte aérea, número de ramos, número de folhas, antese, número de flores ou botões florais, diâmetro da flor, área foliar, massa seca da parte aérea, massa seca das raízes e teores de clorofila. Pink e White foram as variedades mais precoces

[2] Scientific article written according to the editorial standards of Acta Scientiarum (Agronomy) journal.

para florescer, seguidas da Blue, enquanto Lavender foi a mais tardia. Todas as variáveis diminuíram com maior número de plantas por vaso, exceto o diâmetro da flor e os teores de clorofila. Os índices de clorofila α e clorofila total aumentaram com maior número de plantas por vaso. A densidade de quatro plantas por vaso possibilitou a formação de plantas mais compactas, além da aquisição de um produto de melhor qualidade para atender às exigências do mercado.

PALAVRAS-CHAVE: Flor-balão; análise de crescimento; índices de clorofila; Floricultura.

INTRODUCTION

Platycodon grandiflorus (Jacq.) A. DC. is a plant species that occurs naturally in China, Japan, Korea and the far east of Russia (Urbańska, Giebułtowicz, Olszowska, & Szypuła, 2014). The roots of this species have traditionally been used both as food and as medicine in East Asian countries for many years (Liu et al., 2014; Ji et al., 2020). According to Nyakudya, Jeong, Lee, and Jeong (2014), *P. grandiflorus* is employed as an expectorant and antitussive in Chinese culture and as a vegetable in Korean cuisine.

Moreover, *P. grandiflorus* is an ornamental crop widely cultivated as garden or bedding plant, but it can also be marketed as cut or potted flower (Halevy, Shlomo, & Ziv, 2002; Kim, Roh, Roh, & Yoo, 2017). This species is known mainly as balloon flower, because of the configuration of its flower buds, which resemble air balloons (Kim & Liu, 1999). However, this crop is commercially identified only as platycodon in the Brazilian floriculture market. In the last years, platycodon has started to be utilized as potted flower in Brazil, but there is still a lack of information regarding the best way to manage this flowering plant in pots.

For the production of potted flowers, one of the main requirements of the market is the plant harmony with the size of the vase (Backes, Barbosa, Backes, Ribeiro, & Morita, 2005). This exigency can be achieved through the height control of ornamental species (Rezazadeh & Harkess, 2015). The height of the potted flowers, in turn, can be influenced or not by the number of plants per pot (Asghari, 2014; Menegaes, Backes, Bellé, & Peres, 2015; Sultanpuri et al., 2018), since this factor changes the balance between the different plant parts (Morano et al., 2017).

Plant density is an important tool for the production of flowers and ornamental plants, because it influences light absorption, water and nutrient consumption and incidence of pests and diseases (Kim, Mascarini, Lorenzo, & González, 2018). In addition, plant density is also related to the plant size and final product (Luz, Sobrinho, & Tavares, 2018). Therefore, the proper management of plant density allows obtaining the desired growth and quality in flower crops (Chopde, Jadhav, & Bhande, 2015; Mladenović et al., 2020). Based on this context, the current work aimed to investigate the growth and chlorophyll indices of platycodon varieties grown with different numbers of plants per pot.

MATERIAL AND METHODS

This study was conducted in the Floriculture Sector of the Department of Agronomy of the Federal University of Viçosa, in city of Viçosa, Minas Gerais State, Brazil (20º45'36.0"S, 42º51'47.4"W, with 685 m of altitude). The experiment was performed between October 28, 2019 and February 17, 2020, in a greenhouse, under natural photoperiod, from commercial seeds of *P. grandiflorus* (Sakata®). Throughout the experiment, the maximum and minimum values of temperature (ºC) were registered daily by a digital thermo-hygrometer. The average maximum temperature was 35.6 ºC (± 4.23), while the average minimum temperature was 19.4 ºC (± 1.31).

Initially, seedlings were produced in 128-cell expanded polystyrene trays (68.0 × 34.0 × 5.5 cm, 28 mL cell^{-1}), with commercial substrate (Tropstrato®). In these containers, one seed per cell was laid at a depth of 0.5 cm. At 38 days after sowing, the seedlings were transferred to plastic pots of No. 14 (9.8 cm high, 13.0 cm wide at the top and 1.0 L volume), according to the treatments. Each pot was previously filled with 350 g of substrate (Tropstrato®) and 2 g of a homogeneous mixture of single superphosphate and potassium chloride (4:1). Afterward, all the pots were placed on a bench with 12.0 × 10.0 cm spacing between them.

The irrigation regime was on alternate days, applying 150 mL pot^{-1}, and the fertilization via fertigation was interspersed with the water supply, providing a nutrient solution of NPK 20:20:20 and micronutrients (Peters®). Until 30 days after transplanting, a solution of 1.0 g L^{-1} of this soluble fertilizer was used. After that period, another solution was applied with twice the concentration of the first.

The randomized complete block design was selected, with four replications and in a 4 × 4 factorial scheme, combining four plant densities, namely one, two, three or four plants per pot, with four varieties of platycodon 'Astra Semi-Double': Blue, Lavender, Pink and White. Each experimental unit was represented by a specific pot.

The growth analysis was accomplished at 74 days after transplanting of seedlings, when each replication had at least one open flower. The following variables were measured in one plant of each pot: stem diameter at the substrate surface; shoot height; number of branches, larger than 1.0 cm; number of leaves; anthesis, which refers to the number of days between transplanting and opening the first flower; number of flowers or flower buds; flower diameter; leaf area and dry mass of the shoot and root parts.

The leaf area was determined by scanning the fresh leaves on a flatbed scanner, model L365 (Epson®). Posteriorly, this variable was quantified in the ImageJ program, version 1.52a (Schneider, Rasband, & Eliceiri, 2012). The shoot and root parts were kept separately in a drying oven with forced air circulation at 70 ºC until these parts reached constant weight in a precision analytical balance, to obtain the dry mass. Chlorophyll contents (α, β and total) were measured on three intact leaves from the middle third of each replication with a chlorophyll meter (Falker®), between 7:30 a.m. and 8:45 a.m., at 60 days after transplanting.

In the end, the data were subjected to variance analysis and regression equations were adjusted to verify the effects of the number of plants per pot. The models were chosen based on the coefficient of determination and the significance of the regression coefficients by the Student's t-test at 0.1% or 5% probability. The varieties were compared using the Tukey's test, at 5% probability. All statistical analyzes were performed by the ExpDes.pt package (Ferreira, Cavalcanti, & Nogueira, 2014) in the R 3.6.3 statistical software (R Core Team, 2020).

RESULTS AND DISCUSSION

None of the measured variables, except the root dry mass, demonstrated a significant interaction between the sources of variation (Table 1 and Table 2). Analyzing the effect of the varieties, White had the largest stem diameter, but it did not differ from Pink (Table 1). Regarding the shoot height and the number of leaves, Lavender obtained the highest averages, differing from the other varieties. Furthermore, Blue had the lowest number of branches and number of flowers or flower buds, but it was statistically similar to Lavender in relation to this first variable and to Pink and White in regard to this second variable. Relating to the anthesis, that is, the number of days for the opening of the first flower, Pink and White were the earliest varieties, followed by Blue, while Lavender was the latest. In addition, Blue had the largest flower diameter, but it did not differ from Lavender.

Table 1. Stem diameter (SD), shoot height (SH), number of branches (NB), number of leaves (NL), anthesis (A), number of flowers or flower buds (NF) and flower diameter (FD) of four varieties (VAR) of platycodon ‘Astra Semi-Double’ according to the number of plants per pot (NPP).

Sources of Variation	SD	SH	NB(1)	NL	A	NF	FD
	(mm)	(cm)	-	-	(days)	-	(cm)

VAR (F_c)	9.82**	40.54**	5.11*	12.06**	61.87**	4.41*	4.10*
Blue	4.75 b	17.36 b	0.99 b	66.00 b	59.06 b	15.12 b	7.48 a
Lavender	4.85 b	21.23 a	1.04 ab	100.37 a	64.50 a	21.31 a	7.17 ab
Pink	5.78 a	16.43 b	1.09 a	66.56 b	54.87 c	18.43 ab	6.78 b
White	5.81 a	16.68 b	1.13 a	82.56 b	55.43 c	18.93 ab	6.76 b
NPP (F_c)	17.41**	9.57**	40.56**	31.28**	3.71*	60.22**	0.77ns
1	-	-	-	-	-	-	7.08
2	-	-	-	-	-	-	6.94
3	-	-	-	-	-	-	7.25
4	-	-	-	-	-	-	6.93
VAR × NPP (F_c)	0.86ns	1.93ns	1.43ns	0.52ns	1.91ns	0.16ns	0.83ns
C.V. (%)	13.92	7.84	9.67	23.74	3.85	26.3	9.63

"F_c" = calculated F value of variance analysis: ns p-value > 0.05; * p-value < 0.05; ** p-value < 0.001. "C.V." = coefficient of variation. Averages differ between themselves by Tukey's test, at 5% probability, when they are followed by distinct letters in the columns.

(1) Data transformed into their logarithm ($y = \log_{10} x$) to satisfy the assumptions of the variance analysis.

Table 2. Leaf area (LA), shoot dry mass (SDM), root dry mass (RDM), chlorophyll α (Chl α), chlorophyll β (Chl β) and total chlorophyll (TChl) of four varieties (VAR) of platycodon 'Astra Semi-Double' according to the number of plants per pot (NPP).

Sources of variation	LA (cm^2)	SDM (g $plant^{-1}$)	RDM (g $plant^{-1}$)	Chl α (FCI)	Chl β (FCI)	TChl (FCI)
VAR (F_c)	18.76**	3.67*	-	9.81**	17.83**	13.28**
Blue	397.81 b	3.15 b	-	34.48 a	9.58 b	44.07 b
Lavender	574.44 a	3.79 a	-	31.55 b	7.97 c	39.53 c
Pink	372.91 b	3.24 ab	-	37.07 a	11.63 a	48.71 a
White	422.55 b	3.32 ab	-	34.94 a	10.08 b	45.03 ab
NPP (F_c)	79.05**	107.06**	-	4.34*	1.83ns	3.71*
1	-	-	-	-	9.55	-
2	-	-	-	-	9.29	-
3	-	-	-	-	10.09	-
4	-	-	-	-	10.34	-
VAR × NPP (F_c)	1.81ns	0.35ns	2.15*	1.63ns	1.54ns	1.64ns
C.V. (%)	18.94	17.58	20.79	8.41	14.55	9.35

"F_c" = calculated F value of variance analysis: ns p-value > 0.05; * p-value < 0.05; ** p-value < 0.001. "C.V." = coefficient of variation. Averages differ between themselves by Tukey's test, at 5% probability, when they are followed by distinct letters in the columns. "FCI" = Falker chlorophyll index.

In a similar way, Kapczyńska (2013) noticed the influence of the genotype of lachenalia cultivars on the anthesis and number of flowers. This same author revealed that the cultivars Namakwa and Ronina blossomed earlier than Rosabeth and Rupert, and that Rupert was the cultivar that produces the most flowers. Bhosale, Deshmukh, and Takte (2012), in turn, also mentioned that the flower diameter depends on the gerbera variety, indicating that the variety Diablo had the largest flower diameter.

Concerning the influence of varieties, Lavender showed not only the highest shoot height and the largest number of leaves, but also the largest leaf area (Table 2). Similar to the number of flowers and flower buds, Blue exhibited the lowest shoot dry mass and it did not differ from Pink and White. Regarding the chlorophyll contents, Lavender had the lowest average values for the three indices. In fact, it is noteworthy that Pink had higher indices of chlorophyll β and total chlorophyll, but its total chlorophyll content did not differ from White.

Kim et al. (2018) also indicated differences between the varieties of ornamental cabbage in terms of leaf area. According to these authors, the varieties Bicolor and Red reached higher values of leaf area compared to the varieties Pink and Rose. Similarly, Bredmose and Nielsen (2004) recorded that the cultivar Red Velvet manifested the largest leaf area, followed by Texas, in relation to other cultivars of cut rose, namely Lambada and Sonia.

Comparing the numbers of plants per pot, it was determined that these treatments did not promote significant alterations in the flower diameter (Table 1). Nevertheless, all other variables measured, with the exception of chlorophyll contents, suffered reductions with the increase in plant density. The stem diameter, shoot height and anthesis decreased linearly with larger number of plants, while the number of branches, leaves and flowers or flower buds decreased following a parabolic curve (Figure 1).

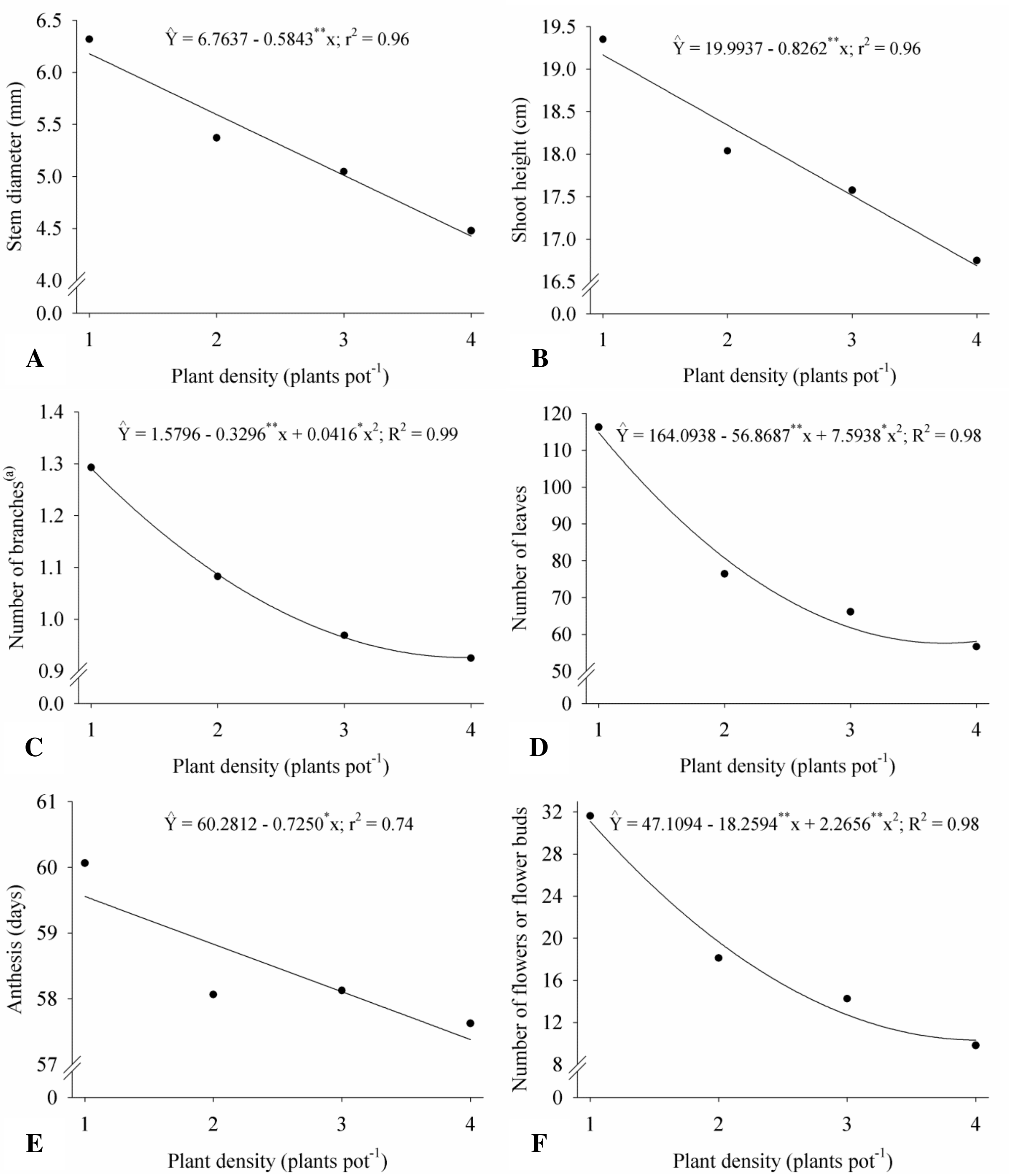

Figure 1. Stem diameter (A), shoot height (B), number of branches (C), number of leaves (D), anthesis (E) and number of flowers or flower buds (F) of four varieties of platycodon 'Astra Semi-Double' according to the number of plants per pot. * p-value < 0.05; ** p-value < 0.001.

(a) Data transformed into their logarithm ($y = \log_{10} x$) to satisfy the assumptions of the variance analysis.

Therefore, there was a reduction in the shoot height and the number of branches of the platycodon varieties with the increase in the number of plants per pot. A smaller number of branches with higher plant density in chrysanthemum (Sun et al., 2019) and in zinnia (Yagi, Gafar, & Taha, 2015) have also been reported. In spite of that, plant density did not promote statistical differences for the height in African bush daisy (Menegaes et al., 2015) and the number of branches in costus (Luz et al., 2018).

Similar to the results of the current study, Abdullah, Ahmad, and Dole (2020) noticed that two snapdragon cultivars also had a higher shoot height, stem diameter, number of leaves and number of flowers with lower plant density. However, these authors described a smaller flower diameter for these cultivars with higher plant density. Other works have also reported a smaller flower diameter in calendula (Chopde et al., 2015) and carnation (Asghari, 2014) with higher plant density.

Furthermore, it was identified that lisianthus (Backes et al., 2005) exposed a behavior similar to platycodon. Both ornamental crops reduced the shoot height, number of branches, number of leaves and number of flowers with greater number of plants per pot, but without changing the diameter of the flower bud, in the case of lisianthus, or of the flower, in the case of platycodon.

The reductions of the variables mentioned above in platycodon with the increase in the number of plants per pot can be explained because of the decrease in the amount of light that penetrated the canopy of each plant with higher density and, consequently, by the lower production of photoassimilates (Kandil, Sharief, & Odam, 2017; Abdullah et al., 2020). For the flower diameter, it is supposed that this attribute is mainly controlled by the genotype of the platycodon varieties, being little influenced by the cultural practices performed. Therefore, it is possible to control the growth of potted platycodon, without compromising the size of the flowers, by manipulating the plant density.

Although the flower diameter in platycodon was not affected by the number of plants per pot, the anthesis was anticipated with higher plant density (Figure 1E). Sultanpuri et al. (2018) also noticed that the flower diameter in chrysanthemum did not differ in relation to the number of cuttings per pot, but a higher plant density delayed the flowering of this ornamental crop. Notwithstanding, Kapczyńska (2013) described that the plant density did not promote differences in the number of days for the flowering of lachenalia.

In Asiatic lily, Ahmad, Naeem, and Abdullah (2019) recorded results similar to those obtained in the present work for the anthesis, that is, a decrease in the number of days for the first flower bud to open with higher plant density. In accordance with this discussion, it is demonstrated that different species of ornamental plants have distinct responses, concerning the time required for opening the flowers, with greater or lesser intraspecific competition.

More competitive conditions, in other words, conditions with greater number of plants per pot, caused an anticipation of the anthesis in platycodon, probably as a strategy to guarantee the perpetuation of its own species.

The leaf area and shoot dry mass were adjusted better to the quadratic regression model, in which they were reduced with the increase in plant density (Figure 2). In addition, the chlorophyll α and total chlorophyll contents increased linearly with the increase in the number of plants per pot. On the other hand, the chlorophyll β index showed no statistical difference for this source of variation (Table 2).

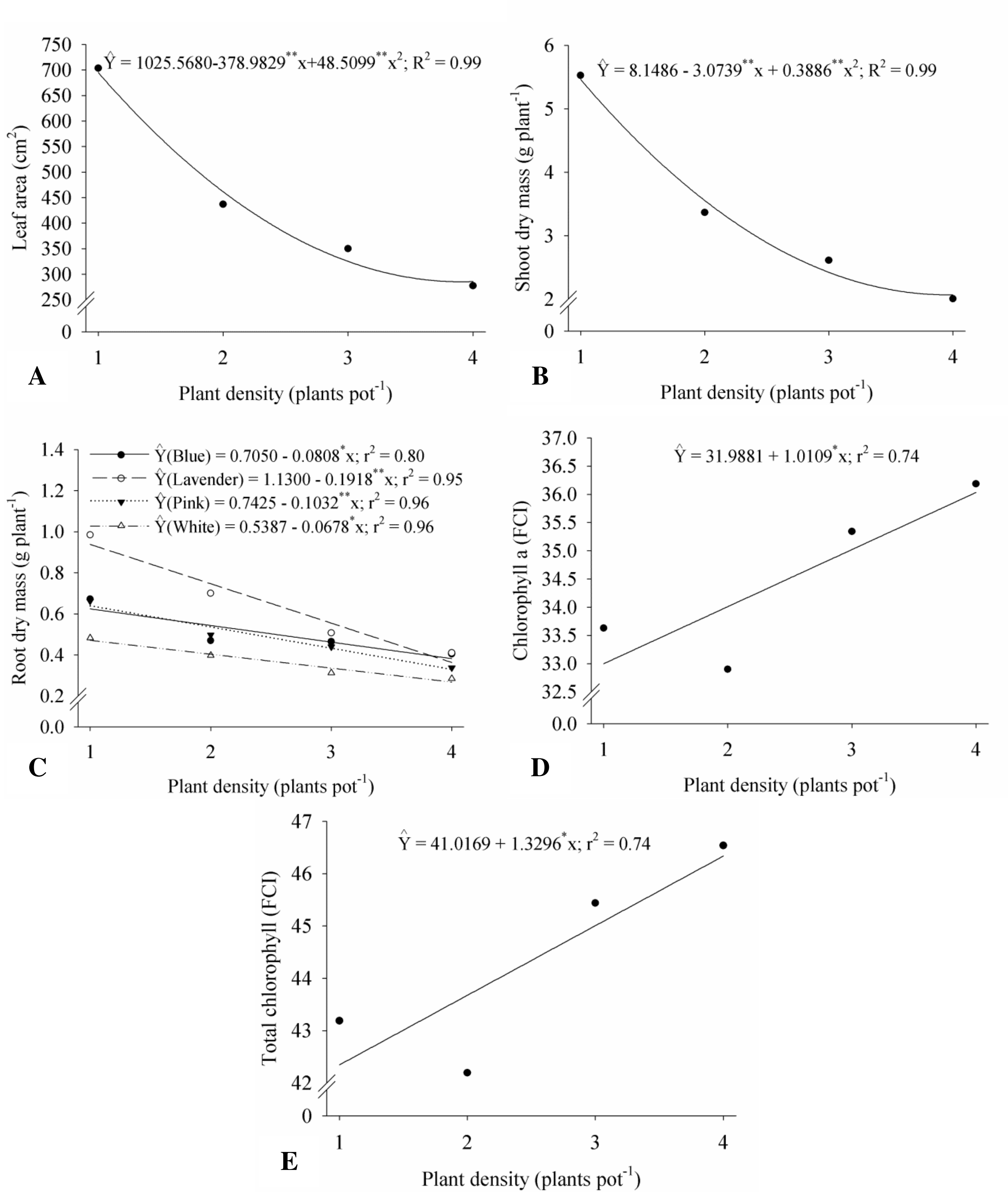

Figure 2. Leaf area (A), shoot dry mass (B), root dry mass (C), chlorophyll α (D) and total chlorophyll (E) of four varieties of platycodon ‘Astra Semi-Double’ according to the number of plants per pot. * p-value < 0.05; ** p-value < 0.001.

As stated initially, the root dry mass was the only variable that indicated dependence between the factors. Analyzing the plant densities within each variety (Figure 2C), Lavender had a more pronounced reduction in root dry mass than the other varieties with the increase in the number of plants per pot. Comparing the effect of the varieties within each density (Table

3), Lavender achieved a greater root dry mass, in relation to the other varieties, with one or two plants per pot. There were no significant differences between varieties for densities of three and four plants per pot.

Table 3. Root dry mass of four varieties of platycodon 'Astra Semi-Double' according to the number of plants per pot.

Number of plants per pot	Blue	Lavender	Pink	White
1	0.67 B	0.98 A	0.66 B	0.48 B
2	0.47 B	0.70 A	0.49 B	0.39 B
3	0.46 A	0.50 A	0.44 A	0.31 A
4	0.40 A	0.41 A	0.33 A	0.28 A

Averages differ between themselves by Tukey's test, at 5% probability, when they are followed by distinct letters in the rows.

In summary, the increase in the number of plants per pot in the current work caused a decrease in both leaf area and dry mass of the different plant parts. In a similar way, Kim et al. (2018) also reported reductions in the leaf area and dry weight of the shoot and root parts with a high plant density in ornamental cabbage.

Kandil et al. (2017) and Nain, Beniwal, Dalal, and Sheoran (2017) also found a smaller leaf area in sunflower and a lower shoot dry mass in African marigold, respectively, with higher plant density. However, the leaf area of cut roses (Bredmose & Nielsen, 2004) and lily (Amjad & Ahmad, 2012) were not influenced by plant density. According to Morano et al. (2017), the increase in the number of plants per pot reduces the water and nutrient absorption efficiency, inducing reductions in plant growth, such as in the leaf area and accumulation of dry matter.

Different from the other variables evaluated in platycodon, the chlorophyll α and total chlorophyll increased with greater number of plants per pot. Other studies have described an opposite effect on sunflower (Abido & Abo-El-Kheer, 2020) and on tuberose (Ahmad et al., 2019), that is, higher total chlorophyll with lower plant density.

These increases in chlorophyll indexes occurred because of the decrease in the size of platycodon leaves with higher plant density, which, consequently, promoted a higher content of chlorophyll per unit of leaf area. Furthermore, a greater number of plants per pot reduce the quality and quantity of light that is intercepted by each plant, because there is an increase in shading. As a consequence of the increase in shading, the plants produced more chlorophyll to increase the efficiency of light interception process (Souza et al., 2016). These increases in the chlorophyll contents are beneficial in the commercial production of potted flowering plants, since greener leaves enhance the color of the flowers, improving the visual quality of the final product (Barbosa et al., 2009).

Concerning the commercialization pattern of potted flowers, Pivetta, Mattiuz, Barbosa, Moraes, and Grossi (2019) recommend that the plant height remain between 1.5 and 2.0 times the pot height, that is, the plants must have a height between 14.7 and 19.6 cm for pots of No. 14 (9.8 cm high). In view of this, Lavender was the only variety that had an average height above the suggested range (Table 1). In relation to the number of plants per pot, all densities had an adequate height for this proposed pattern (Figure 1B), but the density of four plants per pot was the only treatment that produced more compact plants and without bent flowering stems. Finally, the current study proposes that other growth control practices be applied when one, two or three plants per pot are employed in the commercial production of platycodon, such as the application of growth regulators.

CONCLUSION

The cultivation of four plants per pot occasioned greater reduction in the growth of platycodon, as well as the production of flowering stems with better commercial quality. The other plant densities can be used for potted platycodon production, but they need additional growth control techniques.

Lavender was the variety that invested the most in vegetative growth. Pink and White were the earliest varieties to bloom, while Lavender was the latest.

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4 GENERAL CONCLUSIONS

When two plants are cultivated per pot, it is suggested applications of paclobutrazol with doses of 3.75 mg pot^{-1}.

The growth of platycodon varieties does not need to be controlled to accomplish the quality standards when the density of four plants per pot is used.

There was varietal effect on the growth parameters and chlorophyll indices. Pink is an early-flowering variety, while Lavender is a late-flowering variety.