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Scientific Article

Gibberellin and polyamines effects in growth and flowering of New Guinea impatiens

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Abstract

To meet the high demand of the consumer market for ornamental plants, various techniques are used to increase production and flowers quality, through growth regulators. Despite all the benefits arising from the use of regulators, it is essential to establish concentrations that meet the purpose of their use. The aim of the study was to evaluate the growing and flowering characteristics of *Impatiens hawkeri*, after the exogenous application of different dosages of spermine, spermidine and gibberellic acid. Two pulverizations were made separated by 15 days, with polyamines (500/2,000 and 1,000 µM), or gibberellic acid (50 and 100 µM), and for the control, water was used. The evaluated parameters were the number of leaves, plant height, number of flower buds, dry and fresh weights of the root system and the aerial parts, and also volume of the root system and the chlorophyll content (SPAD). The treatments with gibberellin caused higher averages in all measurements, except for SPAD. Based on the results obtained, the use of gibberellin with the dosage of 100 µM as a regulator is the most appropriate to meet the needs of the *Impatiens hawkeri* market with more vigorous plants and with a greater number of flowers.

Keywords: *Impatiens hawkeri*, floral induction, gibberellic acid, spermidine, spermine.

Resumo

Efeito de giberelina e poliaminas no crescimento e florescimento do Beijo-pintado

Para atender a alta demanda do mercado consumidor de plantas ornamentais, variadas técnicas são empregadas para incremento produtivo e na qualidade das flores, por meio de reguladores de crescimento. Apesar de todos os benefícios oriundos do uso dos reguladores, é imprescindível estabelecer dosagens e concentrações que atendam a finalidade do seu uso. O objetivo do presente trabalho foi avaliar crescimento e o florescimento de *Impatiens hawkeri*, após a aplicação de diferentes dosagens de espermina, espermidina e ácido giberélico. Foram feitas duas pulverizações, com poliaminas (500/2000 e 1000 µM), ou ácido giberélico (50 e 100 µM), e para o controle foi utilizado a água. Os parâmetros avaliados foram o número de folhas, altura da planta, número de botões florais, massa seca e fresca do sistema radicular e da parte aérea, e ainda volume do sistema radicular e o teor de clorofila (SPAD). A análise dos dados foi por meio de modelos mistos com as principais matrizes de covariância e estudo qualitativo por médias. Os tratamentos com giberelina obtiveram maiores médias em todas as medições, com exceção do SPAD. Com base nos resultados obtidos, o uso da giberelina como regulador a 100 µM é o mais adequado para atender as necessidades do mercado de *Impatiens hawkeri* com plantas mais vigorosas e com maior número de flores.

Palavras-chave: *Impatiens hawkeri*, indução floral, *ácido giberélico,* espermidina, espermina.

Introduction

The ornamental plant market is an economically promising industry, since the products have high added value and constant consumption (Finger et al., 2016). In 2017, in Brazil, sales were over 7.3 billion (reais) considering the stages of production up to the final consumer (IBRAFLOR, 2018). The great edaphoclimatic diversity of the Brazilian territory, allows the extensive cultivation of pot and cut flowers, with varied native and exotic species (Harri, 2015; Junqueira and Peetz, 2017; Souza et al., 2020). A species widely cultivated in the country for ease of handling and abundant flowering is *Impatiens hawkeri* W. Bull., popularly known as "beijo-pintado". It is a species belonging to the Balsaminaceae family and the genus *Impatiens*, which covers more than 1,000 species, being considered one of the largest genera of angiosperm plants (Yu et al., 2016).

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There is a great demand for high quality and variability of flowers, in addition to being durable, with low cost and wide availability in the market (Finger et al., 2016; Junqueira and Peetz, 2018). In order to serve the consumer market, optimization and resource planning are necessary in order to use all production technologies for greater profit margin (Junqueira and Peetz, 2017). Various techniques are employed for floral induction through growth regulators, which allows for a productive increase and prolongs the flowering period. A groups of exogenous regulators with great potential in the production process of commercial flowers are polyamines and gibberellins (Amorim et al., 2017).

Polyamines are polycationic molecules divided into putrescine, spermine and spermidine, which act in various physiological processes such as cell division and elongation, flower formation, leaf senescence delay, fruit ripening and pollen grains (Liu et al., 2015). Gibberellins, on the other hand, are an extensive family of diterpenic acids that perform functions similar to polyamines, however regulation occurs through different biosynthetic routes (Hedden, 2020). These regulators combined with different phenological stages of the plants, perform different functions, according to the volume, concentration, and kind of application used (Harri, 2015). The exogenous application of the both results in positive responses with increment in flower quality and number, associated with more vigorous plants (Tatte et al., 2016; Chen et al., 2019; Lv et al., 2019).

Despite all the productive potential of the regulators, they may not have significant effects and even cause damage to the plants when mismanaged. Polyamines and gibberellins participate in several other biosynthetic / catabolic pathways, with specific distribution patterns in the tissues (Chen et al., 2019). The relation between gibberellin and polyamines reflects in flower induction (Qin et al., 2019; Nambeesan et al., 2019; Zhu et al., 2020).

In addition, there are strong correlations between cultivar, growth regulator and the environment, which must be taken into account to establish the time and optimum dosage to be applied. In consideration of all these interrelationships, studies that establish safe doses according to the needs of each species for flower production are essential (Liu et al., 2015; Ahmeda et al., 2017; Amorim et al., 2017;).

The aim of the present work was to evaluate the expression of phenotypic and flowering characteristics of *Impatiens hawkeri* in the field, after the exogenous application of different spermine, spermidine and gibberellic acid (GA_3) dosages.

Material and Methods

From the nodal segments cutting of four matrix plants, 70 plantlets of *Impatiens hawkeri* were produced, planted in plastic containers (2L) containing commercial plant substrate in 50% shade nursery on August, 2019.

The experiment was performed as completely randomized design with 7 treatments and 10 replications (each plant per container). The growth regulators used as treatment were spermine (Spm), spermidine (Spd), gibberellin (GA_3) and the control with water only. Two foliar sprays with 100 mL per plant were carried out with the growth regulators, using manual spray equipment, spraying the entire plant. The first spray was 44 days after the plantlet's establishment, and the second spray did 15 days after that first. The treatments were: 1) 500 μM Spm with a second application at 2,000 μM; 2) 1,000 μM Spm; 3) 500 μM Spd with a second application at 2,000 μM; 4) 1,000 μM Spd; 5) 50 μM GA_3; 6) 100 μM GA_3; 7) water. During the cycle, the plants were kept in a 50% shade nursery, with micro-sprinkler irrigation and without supplementary fertilization. During application, the plants were removed in groups according to the respective treatment for the external area, an average of 10 minutes was waited until the leaves dried before returning to the nursery.

In the first spray, Spm and Spd were used in two concentrations (500 and 1,000 μM), and GA_3 in concentrations of 50 and 100 μM. In the second spray the 500 μM dosage of the polyamines Spm and Spd was replaced by a 2,000 μM dosage. (Figure 1).

Figure 1. Different growth regulators at different phenological stages. A) Plantlets right after planting; B) Plants after first spraying; C) Plants after second spraying; D) Plants during last measurement.

The characteristics of leaf numbers, plant heights and number of flower buds were measured, by means of four measurements at different physiological stages, in order to evaluate the effect of concentrations of growth regulators through repeated measures over time. These characteristics were evaluated due to the direct correlation with the floral productivity of the plants.

After the plants were in full blooms (89 days), 5 plants per treatments were randomly taken to measure the weigh fresh and dry weights of shoots and roots, total root volume, and chlorophyll content via SPAD. To determine the SPAD index, the 4th leaf of the main branch was used. The root volume was determined by the difference in the volume of water displaced in the cylinder.

The data were analyzed based on normality of the residues (Shapiro-Wilk, $p \leq 0.05$) and homogeneity of variance (Neill and Mathews, $p \leq 0.05$), and analysis of variance. Theanalyzes were performed with statistical software R version 3.5.6 (R CORE TEAM, 2019) and RBio version 122 (Bhering, 2017). As the measurements were over time, there is no normality and homogeneity of the values, so an analysis was made via mixed models with the main types of covariance matrices.

The structures of covariance matrices evaluated were: component of variance (different variances between measurements and independent observations), composite symmetry (homogeneous variances and covariance), heterogeneous composite symmetry (uneven variances), auto regressive (homogeneous variances and covariance decreasing exponentially with increasing time interval); heterogeneous autoregressive (heterogeneous variances and covariance decreasing exponentially over time), unstructured (all variances and covariance are unequal).

Results

Data from repeated measures over time can be analyzed via univariate models as if it were an experiment in subdivided plots, or by univariate and multivariate analysis that transforms linear data into regression curves, or even mixed model methodology (Cruz et al., 2014). The use of mixed models allows to analyze the experiments in fact as repetitions in time, without making transformations and much more accurate, through special parametric structures that generate matrices of residual covariance.

The residual covariance matrices that showed the lowest Akaikeinformation criteria (AIC) and Bayesianinformation criteria (BIC) values for number of leaves, plant height and number of flower buds were those of Heterogeneous Composite Symmetry, Composite and Unstructured Symmetry, respectively

There are highly significant differences between the seven treatments and between the four measurements, evidenced by the results of F test (significant at 1% and 5%) and p-value ($p < 0.05$) for number of leaves, plant height and number of flower buds, in their matrices of lower AIC and BIC values. These results prove that different concentrations of Spm (500/2,000 μM and 1,000 μM), Spd (500/2,000 μM and 1,000 μM) and GA_3 (50 μM and 100 μM) influence different behaviors in *Impatiens hawkeri* plants.

The matrices show that there is no interaction between treatments and measurements for any of the three characteristics evaluated in the field (F and P tests> 0.05, not significant). This implies that the differential behavior of growth regulators in plants is not conditioned to different measurements, but to the growth regulator itself. And yet, it can only be said that the same growth regulator will be good at all stages of the plant's development through a means test.

Throughout all measurements of the number of leaves, treatment with 50 µM GA_3 obtained the highest averages while Spd 500/2,000 µM was the lowest, with discrepancies between them being 18.49%, 29.87%, 24.43% and 27.46% in measurements 1, 2, 3 and 4 respectively (Figure 2). The increase in Spm from 500 to 2,000 µM in the second spray did not contribute to a significant increase in the number of leaves. The second highest average was 100 µM GA_3, which had an average growth of 54.51% from the first to the second measurement, 35.13% from the second to the third and in the last 23.71%.

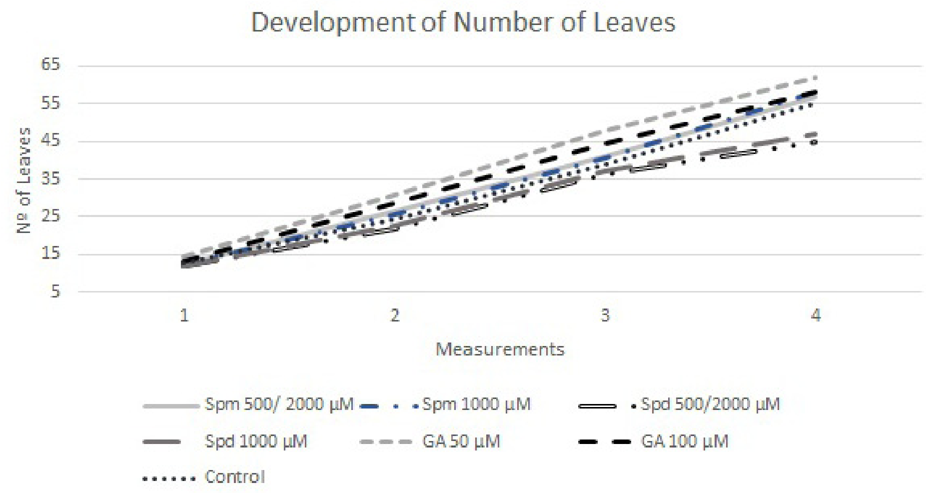

Figure 2. Number of leaves in treatments with growth regulators throughout the four measurements in *Impatiens hawkeri*. Spermine (Spm), Spermidine (Spd), Gibberellin (GA_3).

Two treatments with GA_3 resulted in the highest plant heights, and in the final cycle, 100 µM GA_3 causes an average of 13.55 cm, and 50 µM GA_3 was 13 cm (Figure 3). In measurement 4, treatments with 1,000 µM Spm, 500/2,000 µM Spd and 1,000 µM Spm were approximately 5% lower than the control (12.95 cm). The 500/2,000 µM Spm provided an average increase of 6.36% in height compared to 1,000 µM Spm.

The flower buds were evaluated from the second measurement. The general averages were 1.74, 5.59 and 14.8 flower buds in measurements 2, 3 and 4, respectively (Figure 4). The 100 µM GA_3 induced greater responsiveness compared to the average of all treatments, with a superiority of 51.07%, 32.03% and 27.66%, in measurements 2, 3 and 4. In the last measurement, the highest flower bud was obtained with 100 µM GA_3, followed by 1,000 µM Spm and 1,000 µM Spd. The Spm and Spd treatments with initial application of 500 µM, and later 2,000 µM, did not increase the number of flower buds compared to the same growth regulators at a constant dose of 1,000 µM.

The root volume of plants treated with 50 µM GA_3 were 10.5% higher and the control 16.97% lower than the general average of treatments (Table 1). The chlorophyll content measured via SPAD had an overall average of 34.17, with the control having the highest average and the 500/2,000 µM Spd the lowest, reporting a discrepancy of more than 20% between them.

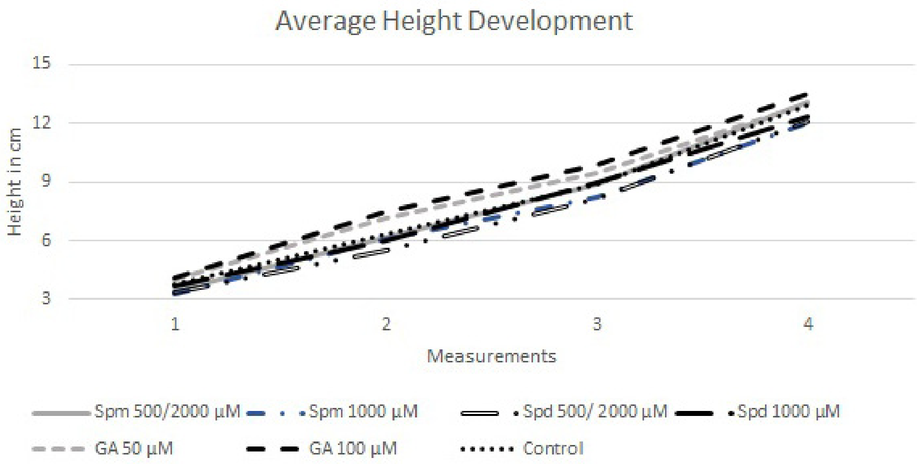

Figure 3. *Impatiens hawkeri* plants with average height (cm) under treatments with growth regulators over the four measurements. Spermine (Spm), Spermidine (Spd), Gibberellin (GA_3).

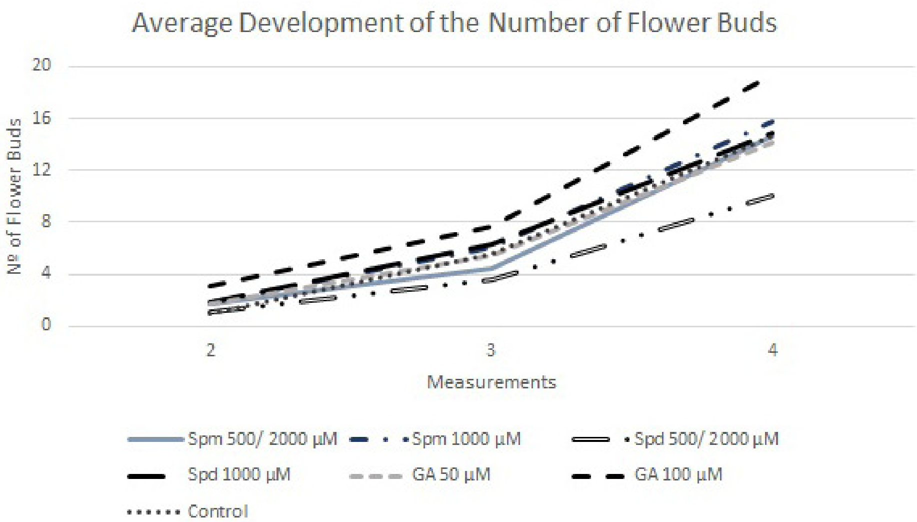

Figure 4. Average number of flower buds on Impatiens hawkeri plants under treatments with growth regulators over the four measurements. Spermine (Spm), Spermidine (Spd), Gibberellin (GA_3).

Table 1. Average of root volume, SPAD, fresh and dry weights of the shoots and root in the treatments of *Impatiens hawkeri*.

Treatments	Root volume (mL)	SPAD	Fresh weight (g)		Dry weight (g)	
			Shoot	Root	Shoot	Root
Spm 500/2,000 µM	24	35.04	48.12	25.86	30.94	11.74
Spm 1,000 µM	26	36.18	48.51	27.39	26.99	10.78
Spd 500/2,000 µM	25	28.8	43.93	24.76	29.17	11.07
Spd 1,000 µM	26.6	33.84	49.09	25.24	28.79	9.05
GA_3 50 µM	28	36.1	43.92	26.75	30.33	12.23
GA_3 100 µM	25	32.34	52.96	26.2	33.89	11.68
Water (control)	20.8	36.9	44.82	21.95	28.92	9.1

Spermine (Spm), Spermidine (Spd), Gibberellin (GA_3).

In the fresh weigh of shoots, the best result was obtained from 100 µM GA_3 application, however the lowest average was observed in 50 µM GA_3, with a difference of 17.07% between them (Table 1). Although 50 µM GA_3 caused the lowest average in fresh weight, this was the treatment that caused the least of dry weight (reduction of 30.94%). It was observed in treatment with 1,000 µM Spm the most difference between dry and fresh weights (44.36%).

Spm 1,000 µM caused greater fresh weight of roots and also the least dry weights, with a reduction of 60.64% in weight. The second largest fresh weight was observed with 50 µM GA_3, followed by 100 µM GA_3 and 500/2,000 µM Spm compared to 1,000 µM Spm as 2.34%, 4.34% and 5.58%, respectively. The control showed the lowest root weight in fresh weight with 15.71% less than the average of the other treatments, but it shows the smallest difference between dry and fresh weight (reduction of 58.54%).

Discussion

Gibberellin promotes high cell division rates mainly in meristems (Amorim et al., 2017), a fact evidenced in this experiment in the treatments with GA_3 in the 50 and 100 µM concentrations that presented more vigorous shoots, resulting in higher averages in height and number of flower buds.

The GA_3 application for floral induction in this work corroborates the results obtained by other authors who also had high averages in ornamental species, as Carvalho-Zanão et al. (2016) studying the GA_3 effects on gladiolus determined that the concentration of 2888 µM increased floral development, but concentrations higher than this can impair flowering. According to Cardoso et al. (2012), the best response regarding flowering induction in *Phalaenopsis* orchid was found at a concentration of 1587 µM, in two leaf sprays. Lakshmaiah et al. (2019) obtained higher flowering quality, prolonged pot time, delay in the yellowing of the leaves, in lisianthus (*Eustoma grandiflorum*) by the GA_3 application. It is important to note that the other researchers used the GA_3 concentrations much higher than those used in this work.

The GA application is almost always related to the increase in height in plants, and much research has been done with GA inhibitors for ornamental purposes (Currey et al., 2016). However, in this work, GA_3 did not affect substantially the height, but have a great effect in flower buds, and this response should be related with the concentration employed.

Although GA treatments have the highest averages for the parameters, plants treated with Spm showed good averages, especially to flowering, which does not happen with Spd. Zhu et al. (2020) working with mutants indicated that Spd and Spm are mainly involved in the vegetative growth and early flowering stages, respectively. Anwar et al. (2015) suggest that ratio Spd/Spm could affect the GA activity by conjugation. Maybe in this experiment the high Spm concentration, by exogenous application, reduce the conversion of Spd, increasing their endogenous levels and keeping the high levels of GA free.

There is a specific pattern of gibberellins concentrations and polyamines that induce the differentiation of vegetative buds into floral buds being that the crosstalk between polyamines and gibberellins can affect floral differentiation (Anwar et al., 2015; Qin et al., 2019; Kamiab et al., 2020). Nambeesan et al. (2019) observed that polyamines, especially spermidine, interfere in the floral identity and development of tomato fruits affecting the GA metabolism and signaling. High concentrations of putrescine can inhibit the GA synthesis, inducing dwarfism and delayed flowering (Ahmed et al., 2017). In general, the perturbation of polyamines homeostasis should be affect the plant development by a impacts in GA biosynthesis (Nambeesan et al., 2019; Alharbi et al., 2020).

Conclusions

The treatment with gibberellin promotes better development in height, leaf numbers and flower buds, and the concentration of 100 µM induced the highest averages of fresh and dry weights of the shoots compared to other growth regulators. Based on these results, the use of GA_3 at 100 µM is the most suitable based on market needs for *Impatiens hawkeri* with more vigorous plants and a greater number of flowers.

Author Contribution

LGV: conceived of the presented idea, carried out the experiment, and wrote the manuscript in consultation with BLM and LLCD. **BLM:** contributed to the design and implementation of the research, processed the experimental data and performed the statistical analysis. **LLCD:** encouraged LGV to investigate and supervised the findings of this work. All authors discussed the results and contributed to the final manuscript.

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