SCIENTIFIC ARTICLE

Involvement of auxins in *Impatiens walleriana* plants grown in different plug tray systems during nursery

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Abstract

The key role of auxins as integrators of environmental signals has become well-known in recent years, and emerging evidence indicates that auxin biosynthesis is an essential component of the overall mechanisms of plant tolerance to stress. However, although the knowledge of the involvement auxins in the responses related to the generation of roots and accumulation of biomass, studies in ornamental species concerning the commercial propagation in plug cell trays are scarce. Thus, the aim of this work was to evaluate the accumulation of pre-transplant biomass in *Impatiens walleriana* plants growing in two propagation systems (soilless medium-based plug cell trays and a floating system) with trays of different cell sizes and sprayed either with a single dose of the auxin indole acetic acid (IAA) or with two inhibitors of endogenous auxins, N-1-naphthylphthalamic acid (NPA) and 2,3,5-triiodobenzoic acid (TIBA). Our results demonstrate that plants from both floating system and those sprayed with IAA present both the highest leaf area and fresh and dry weight at the end of the experiment. Conversely, as a negative control, the use of NPA and TIBA as antagonists of the synthesis and translocation of auxins caused an opposite effect in such variables as compared to control plants. However, before making commercial recommendations, it would be necessary to evaluate the potential post-transplant effects of these treatments on the generation of *I. walleriana* yield.

Keywords: floating system, medium-based plug tray, ornamental annual pot plant, plug cell volume.

Resumo

Envolvimento de auxinas no crescimento inicial de mudas de *Impatiens walleriana* cultivadas em bandejas

O papel chave das auxinas como integradores de sinais ambientais tornou-se bem conhecido nos últimos anos e evidências emergentes indicam que a biossíntese de auxinas é um componente essencial dos mecanismos gerais de tolerância das plantas ao estresse. No entanto, apesar do conhecimento do envolvimento das auxinas nas respostas relacionadas à geração de raízes e acúmulo de biomassa, estudos em espécies ornamentais sobre a propagação comercial em bandejas multicelulares são escassos. Assim, o objetivo deste trabalho foi avaliar o acúmulo de biomassa pré-transplante em plantas de *Impatiens walleriana* cultivadas em dois sistemas (bandejas multicelulares com substrato para plantas sem solo solo e sistema *floating*) com bandejas de diferentes tamanhos de células e pulverizadas com dose única da auxina ácido indol acético (AIA) ou com dois inibidores de auxinas endógenas, ácido-1-N-naftilftalâmico (NPA) e ácido 2,3,5-triiodobenzóico (TIBA). Nossos resultados demonstram que as plantas no sistema *floating* e as pulverizadas com AIA apresentam a maior área foliar e massa fresca e seca ao final do experimento. Por outro lado o uso de NPA e TIBA, como antagonistas da síntese e translocação de auxinas, causou efeito oposto nessas variáveis em relação às plantas controle. No entanto, antes de fazer recomendações comerciais, seria necessário avaliar os potenciais efeitos pós-transplante desses tratamentos na geração de produtividade de *I. walleriana*.

Palavras-chave: floating, planta ornamental anual em vaso, substrato para bandejas, tamanho do recipiente.

Introduction

Although the restriction to root growth due to a small cell volume in trays is a known fact, it has only recently been highlighted that this restriction can define the profitability of an ornamental commercial crop (Di Benedetto et al., 2020). In traditional plug tray propagation systems, the root restriction generated by the base of the cell-trays affects the direction of growth of this organ, thereby reducing both the expansion rate and root branching. Because of

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this, the 'floating tray' technology (which determines a drastic reduction of the root restriction associated with the volume of the cell in the plug tray) has been used to increase the efficiency of the plant propagation stage. Our previous work has shown that the use of a floating tray system allows reversing the main negative effects of root restriction (Salinas et al., 2022).

In general, the effects of auxins and cytokinins on the growth of the root system are opposite (Kurepa et al., 2019), cytokinins inhibit root growth through their effect on the division and differentiation of the root apex and on the pericycle of the main root (Gonin et al., 2019), whereas auxins increase the root mass through the differentiation of branches (Gomes and Scortecci, 2021).

During the last years, the emerging evidence has shown that the exogenous application of cytokinins (such as 6-benzylaminopurine, BAP) in different ornamental species (including *Impatiens walleriana*) can mitigate the negative effect of limited cell sizes (Di Benedetto et al., 2020). However, recent studies on *Impatiens hawkeri* have suggested that auxins do not inhibit cytokinin synthesis in the root tips, indicating that both hormones could be involved in the same sequence of events associated with the development of new lateral roots (Molinari et al., 2020). Since all root apices are regions of cytokinin synthesis, the promoting action of auxins could imply an increase in cytokinin synthesis (Jing and Strader, 2019).

Since auxins are inexpensive and easy to acquire, if they could replace cytokinins in promoting growth, their use could be of productive interest. On the other hand, the development of lateral roots can be blocked by using inhibitors of the transport of auxins (which are synthesized in the vegetative apex of the stem and move basipetally by polar transport), such as naphthylphthalamic acid (NPA), which blocks the first transverse divisions (Singh et al., 2020; Seo et al., 2021) or tri-iodobenzoic acid (TIBA), which disturbs the movement of vesicles containing auxin transporters to and from cell membranes (Soeno et al., 2021).

Therefore, whether the growth-promoting effect of exogenous auxins is mediated by their translocation to roots, it is possible to hypothesize that the joint application of any of these auxin inhibitors should at least partially block growth promotion. Based on this hypothesis, the aim of this work was to evaluate the accumulation of pre-transplant biomass in *Impatiens walleriana* plants growing in two propagation systems (soilless medium-based plug cell trays and a floating system) with trays of different cell sizes and sprayed with a single of dose of the exogenous auxin indole acetic acid (IAA) or with two inhibitors of endogenous auxins (NPA or TIBA).

Materials and Methods

The experiment was carried out in a greenhouse of the campus of the Faculty of Agronomy of the University of Buenos Aires, Argentina (34°35' 59''S, 58°22'23''W) between December 3rd 2019 and January 9th 2020.

To achieve the proposed goals, *Impatiens walleriana* 'Xtreme White' seeds (Goldsmith Inc., NY, USA) were grown in 50- (55.7 cm^3 $cell^{-1}$), 128- (17.37 cm^3 $cell^{-1}$), 288- (6.18 cm^3 $cell^{-1}$) and 512- (2.50 cm^3 $cell^{-1}$) cell plug trays in a Klasmann411® medium (Klasmann-Deilmann, GmbH, Germany) for 37 days. Plug trays were placed on wooden platforms (medium-based treatment) or in a floating tray system.

Plants were sprayed with distilled water (control), indole acetic acid (IAA, 100 mg L^{-1}) (SIGMA EC 201-748-2), N-1-naphthylphthalamic acid (NPA, 50 mg L^{-1}) and 2,3,5-triiodobenzoic acid (TIBA, 5 mg L^{-1}) 7 days after transplanting. Doses were chosen from the results of preliminary experiments not included in this work. All leaves were sprayed to run-off at sunset when plants showed the first pair of true expanded leaves (approximately 2.5 cm^3 $plant^{-1}$).

After transplant, plants were grown in 3-L pots filled with *Sphagnum maguellanicum*-river waste-perlite (2-2-1 v/v/v) medium. The pots were arranged at a density of six plants m^{-2} to avoid mutual shading.

Plants were irrigated as needed with high quality tap water (pH: 6.64 and electrical conductivity: 0.486 dS m^{-1}), using intermittent overhead mist to compensate evapotranspiration losses. The fertilizer solution used in the floating system was composed of 143.0, 62.9 and 118.7 mg L^{-1} of N, P and K respectively. Plants in the soilless medium-based system were fertilized with an equivalent fertilizer solution through the overhead water irrigation.

Daily mean temperatures (21.05 to 25.80 °C) and daily photosynthetic active radiation (10.16 to 11.77 mole photons m^{-2} day^{-1}) during the experiment were recorded with a HOBO sensor (H08-004-02) (Onset Computer Corporation, MA, USA) connected to a HOBO H8 data logger.

Plants were harvested at the transplant stage (37 days from transplanting). Roots were washed, and root, stem and leaf fresh weights (FW) were recorded. Dry weights (DW) were obtained after drying roots, stems and leaves to constant weight at 80 °C for 96 hours. The number of leaves was recorded, and each leaf area was determined using the ImageJ® software (Image Processing and Analysis in Java).

The relative rate of leaf area expansion (RLAE), the rate of leaf appearance (RLA), the relative growth rate (RGR), the mean net assimilation rate (NAR) and the specific leaf area (SLA) on a FW basis were calculated as previously (Di Benedetto and Tognetti, 2016).

We used a completely randomized design. Data were subjected to three-way analysis of variance and means were separated by Tukey's test ($p < 0.05$); STATISTICA 8 software (StatSoft) was used. Least significant differences (LSD) values were calculated.

The slopes from the straight-line regressions of RLAE, RLA, RGR and NAR were tested using the SMATR package.

Results and Discussion

Our previous results in *Impatiens walleriana* have shown that total leaf area at the transplant stage increases in all hormonal treatments according to cell volume, in agreement with previous reports in this bedding plant and other ornamentals (Di Benedetto et al., 2020). However, in the present study, the highest values of total leaf area were recorded in the plants from the floating system, in agreement with that previously reported (Salinas et al., 2022). In both propagation systems (medium-based and floating system), IAA application increased leaf area, whereas TIBA and NPA decreased it (Figure 1), although significant differences were found in the response between propagation systems. In agreement, Hanaa and Safaa (2019) observed that the application of 100 mg L^{-1} of IAA (the same IAA amount used in our experiment) led to maximum flag leaf area in wheat plants.
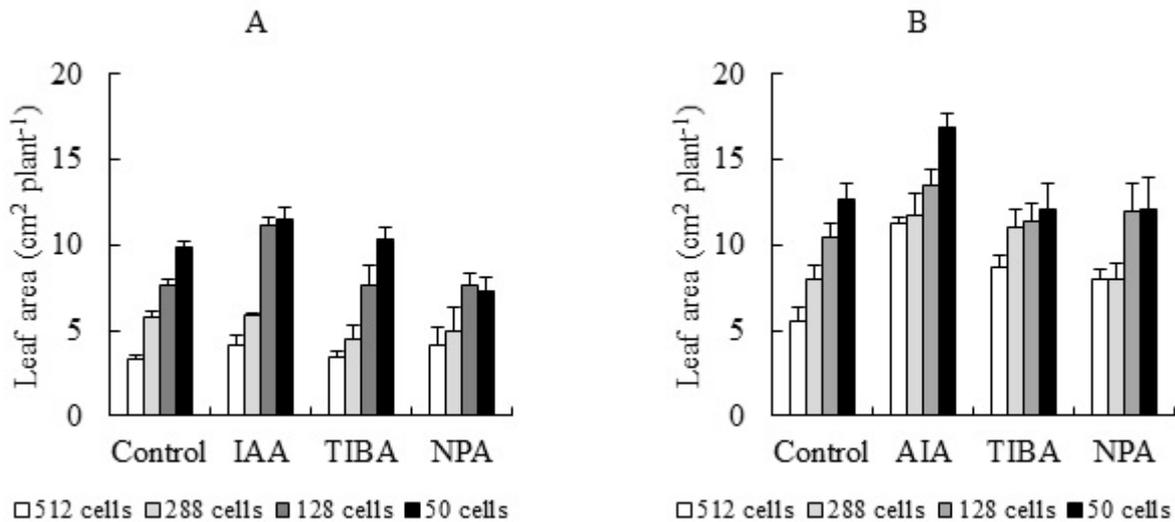

Figure 1. The effect of plug cell volume and the propagation system on leaf area at the transplant stage from two propagation systems. A. Media-based system; B: Floating system. Vertical lines indicate standard errors. $LSD_{media-base} = 1.922$ cm² plant⁻¹, $LSD_{florating system} = 3.525$ cm² plant⁻¹.

On the other hand, the spatial patterning of auxin accumulation at the tissue level is generated by a combination of localized auxin biosynthesis and both non-polar (in areas of high auxin concentration) and polar transport across cells. TIBA and NPA potentially inhibit the directional movement of auxin and block the plant responses mediated by auxin transport (Dhillon et al., 2018; Fukui and Hayashi, 2018).

Differences in total leaf area at the end of the experiment could be explained by the RLA and RLAE, which were significantly higher in trays with higher plug cell volume, in the floating system and in IAA-sprayed plants (Table 1).

Table 1. The effect of plug cell volume, the propagation system and exogenous applications of auxins (IAA) or endogenous auxin-inhibitors (TIBA and NPA) on the rate of leaf appearance (RLA) and the relative leaf area expansion (RLAE) during nursery. Different lower case letters indicate significant differences ($p < 0.05$) between plug cell trays. Different capital letters indicate significant differences ($p < 0.05$) between nursery growth systems.

	RLA (leaves day^{-1})		RLAE (cm^2 cm^{-2} day^{-1})	
	Medium-based	Floating system	Medium-based	Floating system
Control				
512	0.124dB	0.138cA	0.113dB	0.136cA
288	0.130cb	0.168bA	0.136cA	0.137cA
128	0.157bB	0.184aA	0.139bB	0.144bA
50	0.162aB	0.184aA	0.142aB	0.149aA
IAA				
512	0.129dB	0.181cA	0.119cB	0.131dA
288	0.146cB	0.188cA	0.135bB	0.146cA
128	0.200bB	0.232bA	0.145aB	0.151bA
50	0.222aB	0.270dA	0.145aB	0.159bA
TIBA				
512	0.132dB	0.184dA	0.111dB	0.145bA
288	0.141cB	0.194cA	0.134cB	0.146bA
128	0.146bB	0.205dA	0.143bA	0.146bA
50	0.189aB	0.243cA	0.149aB	0.151aA
NPA				
512	0.135cB	0.178dA	0.115bB	0.136cA
288	0.141bB	0.185cA	0.133aB	0.145bA
128	0.141bB	0.211bA	0.135aB	0.148bA
50	0.146aB	0.260aA	0.135aB	0.153aA

Studies over the past two decades have shown that auxins play a crucial role in leaf initiation. In tomato plants, for example, Xiong and Jiao (2019) found that vegetative shoot apices treated with the auxin transport inhibitor NPA failed to form leaf primordia, whereas the meristem continued to generate stem tissue, leading to the formation of a pin-like structure lacking leaves.

Leaves initiate on the flanks of the shoot meristem, with successive leaves arising in the position of an auxin maximum. In turn, the localized maxima are positioned by the auxin efflux carrier PIN1, which directs auxin towards initiation sites according to a feedback loop between auxin signaling and PIN1 polarity (Heisler and Byrne, 2020). The increase in RLA and RLAE in *Impatiens walleriana*

in IAA-sprayed plants could be explained by the changes in endogenous auxin levels at the shoot apical meristem.

In the same way as leaf area, both FW and DW showed a similar response pattern. The highest values were found in IAA-sprayed plants grown in the smallest plug cell trays (Figure 2A), and the floating system significantly increased the differences between treatments (Figure 2B). Again, plants sprayed with TIBA and NPA decreased FW even compared to untreated controls although to a different extent between the two growth regulators.

When results were compared on a DW basis, the differences between controls and IAA-sprayed plants were reduced but the negative effect of TIBA and NPA on plants remained highly significant (Figure 2 C and D).
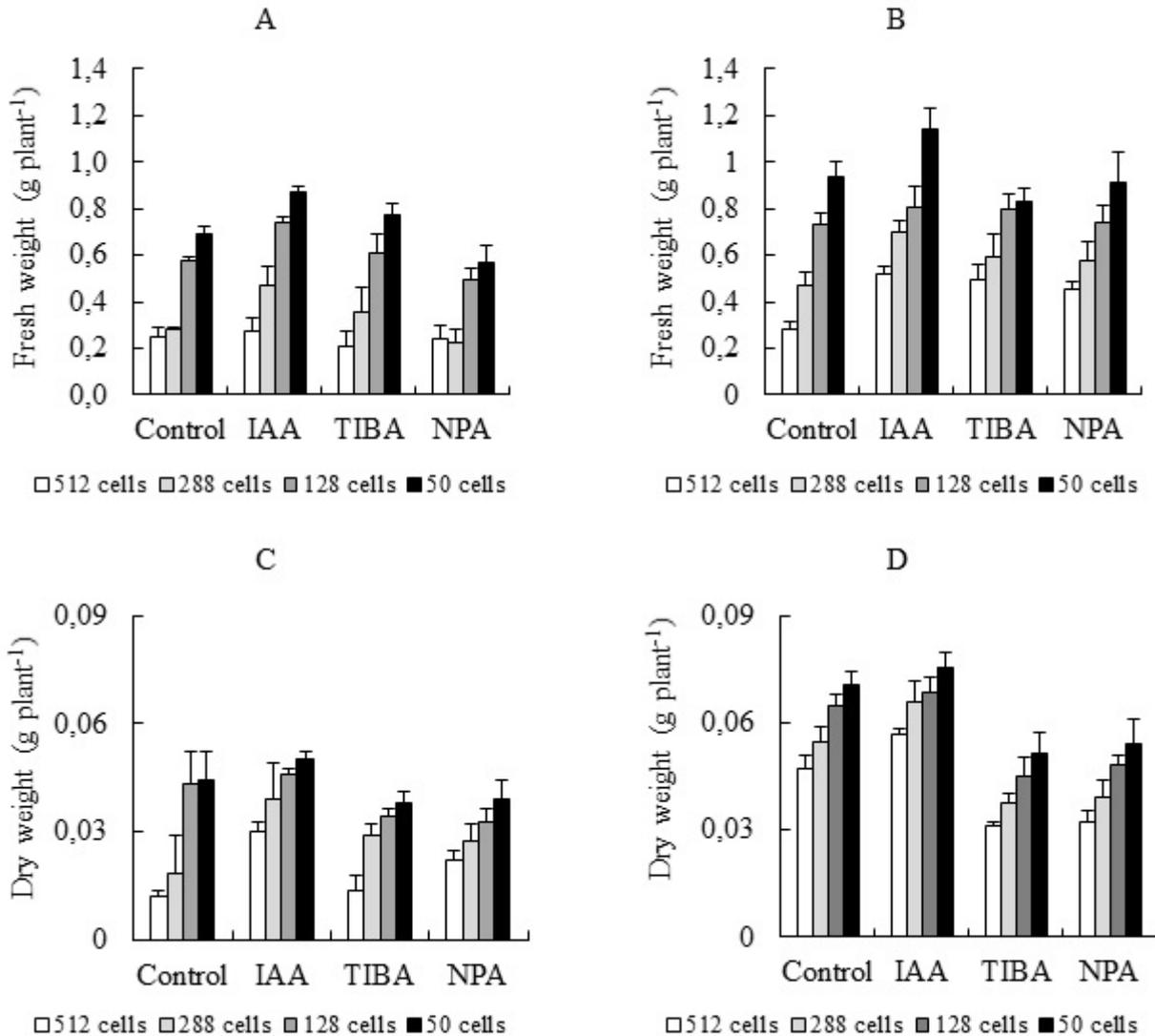
A

B

C

D

Figure 2. The effect of plug cell volume and the propagation system (A-C: media-based system; B-D: floating system) on fresh weight (A-B) and dry weight (C-D) at the transplant stage in *Impatiens walleriana*. $LSD_{fresh\ media-base}$ = 0.141 cm^2 plant^{-1}, $LSD_{fresh\ floating\ system}$ = 0.246 cm^2 plant^{-1}, $LSD_{dry\ media-base}$ = 0.014 cm^2 plant^{-1}, $LSD_{dry\ floating\ system}$ = 0.018 cm^2 plant^{-1}.

As described by Demmig-Adams et al. (2018), high plant productivity in any given environment depends on high activities of three key functions: (i) the photosynthetic capacity of source leaves; (ii) the capacities of the leaf sugar-exporting and water-importing vascular pipelines and (iii) the activity of plant sinks. The modulation of foliar vascular organization thus has the potential to serve in co-optimizing plant productivity and abiotic and biotic stress tolerance (Demmig-Adams et al., 2018). In this way, auxin has been directly related to the development of the vascular system (Burian et al., 2021).

In the present study, biomass accumulation estimated through the RGR showed a positive response to an increase in cell volume, with the floating system with higher significant differences with respect to the traditional medium-based system. Again, a single IAA spray increased RGR, whereas single TIBA and NPA sprays decreased it. The physiological mechanism responsible for this result would seem to be the photosynthetic capacity of the plant. The data of the NAR showed changes coordinated with the increases and decreases in RGR. In the same way, the SLA on a FW basis, indicated as an estimator of leaf thickness, would be correlated with RGR (Table 2).

Table 2. The effect of plug cell volume, the propagation system and exogenous applications of auxins (IAA) or endogenous auxin-inhibitors (TIBA and NPA) on the relative growth rate (RGR) and the net assimilation rate (NAR) during nursery and the specific leaf area (SLA) at the end of the experiment. Different lower case letters indicate significant differences ($p < 0.05$) between plug cell trays. Different capital letters indicate significant differences ($p < 0.05$) between nursery growth systems.

	RGR (g g⁻¹ day⁻¹)		NAR (g cm⁻² day⁻¹) x 10⁻⁵		SLA (cm² g⁻¹)	
	Medium-based	**Floating system**	**Medium-based**	**Floating system**	**Medium-based**	**Floating system**
Control						
512	0.0701cB	0.1023dA	4.36bB	7.75cA	36.16aA	34.36aB
288	0.0887bB	0.1136cA	4.60bB	10.49bA	35.19aA	28.66bB
128	0.1066aB	0.1226bA	7.77aB	11.39aA	33.06bA	25.03cB
50	0.1082aB	0.1257aA	7.87aB	11.65aA	33.32bA	25.71cB
IAA						
512	0.0867cA	0.1033cA	8.12dA	8.31cA	34.36aA	34.20aA
288	0.0970bB	0.1069bA	10.88cB	16.68bA	28.66bA	28.12bA
128	0.1042aB	0.1089aA	13.88bB	21.46aA	25.03cA	23.69cB
50	0.1052aB	0.1213aA	17.18aB	23.53aA	25.71acA	22.68cB
TIBA						
512	0.0751dB	0.1028cA	5.22dB	7.14dA	43.44aA	35.99aB
288	0.0965cB	0.1053cA	10.74cB	14.22cA	38.55bA	32.38bB
128	0.1039bB	0.1087bA	13.61bB	15.82bA	32.63cA	30.68bcB
50	0.1112aA	0.1115aA	14.10aA	17.26aA	31.53cA	28.87cB
NPA						
512	0.0880cA	0.0887cA	7.78cA	7.35cA	39.13aA	39.34aA
288	0.0969bB	0.1104bA	10.91bB	14.96bA	31.92bA	30.36bA
128	0.1029aB	0.1186aA	13.74aB	15.92bA	30.34bA	25.44cB
50	0.1052aB	0.1187aA	14.18aB	17.30aA	25.54cA	24.16cA

The close negative relationship between NAR and SLA would support this hypothesis as well (Figure 3). In agreement, Li et al. (2019) showed that exogenous IAA could improve the net photosynthetic rate in *Zizania latifolia* and that TIBA could decrease the contents of sucrose, glucose, fructose and total soluble sugar, while Mir et al. (2021) suggested that a foliar spray of IAA to *Brassica juncea* plants modulates photosynthesis. To explain these results, McAdam et al. (2017) linked auxin biosynthesis with maximum photosynthetic rate through leaf venation.
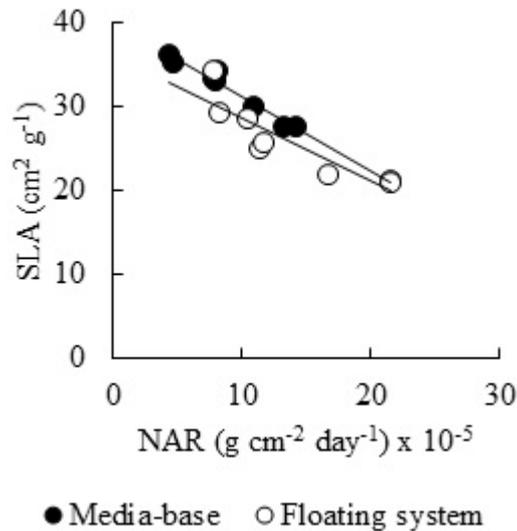
Figure 3. Relationships between the net assimilation rate (NAR) and the leaf specific leaf area on a fresh basis (SLA) at the transplant stage in *Impatiens walleriana* plants propagated in media-base or floating systems in trays with different plug size and sprayed or not with IAA, TIBA and NPA. The straight-line regressions were $SLA_{Media-based} = 0.889 NAR + 40.06$ ($r^2 = 0.960$); $SLA_{Florating system} = 0.756 NAR + 36.16$ ($r^2 = 0.792$).

Although auxins and cytokinins interact in the control of plant growth (Bielach et al., 2017), with auxins playing a central role in the crosstalk between plant hormones (Hussain et al., 2021), data on combined exogenous applications are scarce (Di Benedetto et al., 2015, 2018; Molinari et al., 2020). In any case, our results with inhibitors of the synthesis and transport of auxins indicate the clear participation of endogenous auxins in the physiological functioning of *I. walleriana* during nursery.

Conclusions

Although the root restriction associated with the size of the cell in a plug tray is critical during the propagation of ornamental and horticultural plants, it is not an abiotic stress usually addressed in the literature. Experiments performed at our laboratory have provided significant information regarding the participation of endogenous hormones and the effect of exogenous spraying with synthetic ones. The results of this work showed the participation of endogenous auxins and the potential use of synthetic auxins to overcome this initial root restriction. However, before making commercial recommendations, it would be necessary to evaluate the potential post-transplant effects of these treatments on the generation of *I. walleriana* yield.

Author contribution

MS, GH, EG: provided the structure and conditions to develop the experiments and conducted its. **ADB, EG, JDL:** wrote the manuscript, carried out the statistical analysis and contributed to the discussion of results. **All authors:** read and approved the final version of the paper.

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