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SCIENTIFIC ARTICLE

Involvement of cytokinins in biomass accumulation of *Limonium sinuatum* under root restriction during nursery

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Abstract

It has been suggested that the response of the specialty cut flower *Limonium sinuatum* to different abiotic stress situations related to the plug tray cell size during nursery could be associated with the synthesis and translocation of endogenous cytokinins produced in the root tips. To validate this hypothesis, the aim of this work was to evaluate the participation of cytokinins in the process of biomass accumulation in *L. sinuatum* plants through foliar spraying with a synthetic cytokinin (benzyl aminopurine, BAP) and an inhibitor of the synthesis of endogenous cytokinins (dopamine). Our results indicated that spraying *L. sinuatum* plants with BAP increased leaf area initiation and expansion, biomass accumulation through increased plant photosynthetic capacity, and differential partitioning towards the shoot apical meristem by a positive feedback mechanism that has a threshold of optimal response close to 100 mg L^{-1} BAP. Dopamine spraying appeared to affect the synthesis of endogenous cytokinins, reducing the previously mentioned responses. Responses were dose-dependent, with an optimum of 100 mg L^{-1} BAP and 200 mg L^{-1} dopamine. Our results allow concluding that the level of endogenous cytokinins during the abiotic stress such as root restriction during nursery is a determining variable of the biomass accumulation process in this species.
Keywords: abiotic stress, cut flower, dopamine.

Resumo

Citocininas no acúmulo de biomassa de *Limonium sinuatum* sob restrição radicular durante a fase de viveiro

A bibliografia aponta que existe resposta de *Limonium sinuatum* a diferentes situações de estresse abiótico relacionadas ao tamanho das células durante o cultivo no viveiro e que este pode estar associada à síntese e translocação de citocininas. Para testar essa hipótese, o objetivo deste trabalho foi avaliar a participação das citocininas no processo de acúmulo de biomassa em plantas de *L. sinuatum* por meio de pulverização foliar com uma citocinina sintética (benzilaminopurina, BAP) e um inibidor da síntese de citocininas endógenas (dopamina). Nossos resultados indicaram que a pulverização com BAP aumentou a iniciação e expansão da área foliar, acúmulo de biomassa através do aumento da capacidade fotossintética da planta e partição diferencial em direção ao meristema apical da parte aérea com um ponto de máxima próximo a 100 mg L^{-1} BAP. A pulverização de dopamina pareceu afetar a síntese de citocininas endógenas, reduzindo as respostas mencionadas anteriormente. As respostas foram dose-dependentes, com um ótimo de 100 mg L^{-1} de BAP e 200 mg L^{-1} de dopamina. Nossos resultados permitem concluir que o nível de citocininas endógenas durante o estresse abiótico como a restrição radicular durante a fase de viveiro é uma variável determinante do processo de acúmulo de biomassa nesta espécie.
Palavras-chave: estresse abiótico, flor de corte, dopamina.

Introduction

The specialty cut flower *Limonium sinuatum* is produced from fast germinating seeds in plug cell trays. This species has a deep main root of woody consistency, with many secondary roots, mainly in the upper 10 cm of soil (Shillo and Zamski, 2019).

The main abiotic stress of plants propagated in plug trays is related to the “root restriction syndrome” that results from the limited plug cell size. In most ornamental plants, including *L. sinuatum*, the negative effects (lower leaf area, lower photosynthetic rate and lower biomass accumulation) is quite amplified after transplant (Gandolfo et al., 2022).

It has been suggested that the response of *L. sinuatum* plants to different abiotic stress situations related to the plug cell size and the quality of the growth medium in the plug tray during nursery could be associated with the synthesis

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and translocation of endogenous cytokinins produced in the root tips. This situation, has traditionally been addressed through an increase in the level of cytokinins, which reaches the shoot apical meristem (SAM), by exogenously spraying plants (both ornamental and horticultural) with synthetic cytokinins (such as benzyl aminopurine, BAP) (Di Benedetto et al., 2020a, b).

Testing the participation of cytokinins in the response to different abiotic stresses in ornamental species presents the difficulty related to the lack of cytokinin-depressed transgenic mutants. One possibility is to use endogenous inhibitors of cytokinin synthesis such as dopamine (Van Staden et al., 2008). Although there is little information on the effects of the exogenous spraying of plants with dopamine, most studies mention that dopamine works as a stimulant rather than as an inhibitor of metabolism, to compensate for situations of hydric, saline, thermal and nutrient stress (Jiao et al., 2019, 2021; Gao et al., 2020; Lan, et al., 2020; 2021; Liu et al., 2020a, b; Miglioli et al., 2020; Wang et al., 2021).

The effect of dopamine spraying on the abiotic stress generated by cell size in the propagation tray during nursery has only been evaluated in Brussels sprouts (Miglioli et al., 2020). On the other hand, Liu et al. (2020a) indicated that the ability of dopamine to promote plant growth is determined by plant-specific interactions with growth hormones and that, since the endogenous dopamine content varies significantly among different plant species, dopamine function may also vary from plant to plant.

Based on the above, the aim of this work was to evaluate the participation of cytokinins in the process of biomass accumulation in the specialty cut flower *Limonium sinuatum* through foliar spraying with a synthetic cytokinin (benzyl aminopurine, BAP) and an inhibitor of the synthesis of endogenous cytokinins (dopamine).

Materials and Methods

The experiment was carried out inside a greenhouse in the campus of the Faculty of Agronomy, University of Buenos Aires, Argentina (34°35’ 59”S, 58°22’23”W).

To reach the objectives proposed, *Limonium sinuatum* ‘Iceberg white’ seeds (Hem Zaden BV, Amsterdam, the Netherlands) were grown in 50 plug trays (55.7 cm^3 $cell^{-1}$) in Klasmann411® medium (Klasmann-Deilmann, GmbH, Germany) (K). At the beginning of the experiments, total porosity (%), air-filled porosity (%), container capacity (%) and bulk density (g cm^{-3}) were 60.00, 12.93, 36.89 and 0.21 respectively. After transplant, plants were grown in 3-L pots filled with the same K medium. Pots were arranged at a density of five plants m^{-2}, which avoided mutual shading. Leaves were sprayed at sunset with different BAP or dopamine solutions (0, 5, 50, 100 and 200 mg L^{-1}) when the first true leaf pair was developed. Plants were irrigated as needed with high-quality tap water (pH: 6.64 and electrical conductivity of 0.486 dS m^{-1}) using intermittent overhead mist to compensate evapotranspiration losses, and weekly fertilized with nitric acid, phosphorus acid, potassium nitrate, and calcium nitrate (Agroquímica Larocca S.R.L., Buenos Aires, Argentina) (Stage 2: 50 mg L^{-1} N; Stage 3-4: 100 mg L^{-1} N).

Daily mean temperatures (21.80 to 36.08°C) and the daily photosynthetic active radiation inside the greenhouse (14.80 to 20.33 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 50 days from transplanting (when seedlings had 8-9 fully expanded true leaves, the roots occupied most of the cell and the root ball remained intact when they were removed from the plug cell tray) and at monthly intervals. Roots were washed and root, stem, leaf and flower fresh weights (FW) recorded. Dry weights (DW) were obtained after drying roots, stems, leaves and flowers to constant weight at 80°C for 96 hours. The number of leaves was also recorded, and each leaf area was determined using the ImageJ® software (Image Processing and Analysis in Java).

The relative rate of leaf area expansion (RLAER), the rate of leaf appearance (RLA), the relative growth rate (RGR), the mean net assimilation rate (NAR), the leaf area ratio (LAR), the root:shoot allometries and the leaf:stem allometries were calculated as previously (Di Benedetto and Tognetti, 2016).

A completely aleatory design was used. Data were subjected to two-way analysis of variance and means were separated by Tukey’s test ($p < 0.05$). The STATISTICA 8 software (StatSoft) was used. Least significant differences (LSD) values were calculated. Slopes from straight-line regressions of RLAE, RLAER, RGR and allometric values were tested using the SMATR package.

Results and Discussion

Spraying with increasing doses of BAP increased total leaf area at the end of the experiment up to concentrations of 100 mg L^{-1}; higher doses (200 mg L^{-1}) showed significantly lower values than the controls. On the other hand, the plants sprayed with increasing concentrations of dopamine expanded an increasingly smaller total leaf area than the controls without treatment. The differences in total leaf area between treatments at the end of the experiment were mainly associated with changes in RLA rather than with changes in RLAER (Table 1).

Table 1. Total leaf area at the end of the experiment and leaf appearance rate (RLA) and relative leaf area expansion rate (RLAER) during the experiment for *Limonium sinuatum* plants sprayed with four BAP doses (5, 50, 100 and 200 mg L^{-1}) or four dopamine doses (5, 50, 100 and 200 mg L^{-1}). Different lower case letters indicate significant differences ($P < 0.05$) between treatments.

	Leaf area (cm^2 $plant^{-1}$)	**RLA (leaf day^{-1}) x 10^{-5}**	**RLAER (cm^2 cm^{-2} day^{-1})**
Control	608.63c	0.311d	0.047b
BAP-5	630.27b	0.378a	0.052a
BAP-50	635.84b	0.354b	0.049b
BAP-100	665.24a	0.350b	0.048b
BAP-200	578.24d	0.329c	0.049b
Dopamine-5	529.79e	0.310d	0.046b
Dopamine-50	492.84f	0.304d	0.044b
Dopamine-100	473.92f	0.303d	0.043b
Dopamine-200	408.58g	0.267e	0.043b

The stimulation of the leaf area in *L. sinuatum* plants sprayed with BAP is a response similar to that found in other ornamental and horticultural species (Di Benedetto et al., 2020a, b). However, the decrease in the expanded leaf area with respect to controls without treatment in plants subjected to this type of abiotic stress during nursery against exogenous spraying with dopamine is a response not previously reported.

The data also show that of the two main processes that determine the establishment of a photosynthetically functional canopy, leaf initiation (estimated through RLA) is much more important than the expansion of initiated leaves (estimated through RLAER). Given that the initiation process has traditionally been linked to the action of endogenous cytokinins (Banasiak et al., 2019; Di Benedetto et al., 2020a, Gao, 2020) and that the expansion of initiated leaves has been related to the action of endogenous auxins (Du et al., 2020), the results shown in Table 1 highlight the participation of cytokinins in the development of the leaf area in *L. sinuatum* plants and the participation of dopamine as a potential inhibitor of this development.

In general, DW accumulation was similar to that observed for leaf area accumulation, i.e., it increased in plants sprayed with BAP and decreased in plants sprayed with dopamine. The quantification of the total DW partitioned by organ showed that the differences between the controls and the rest of the treatments were directly related to each specific treatment (BAP or dopamine). The differences were amplified as the application dose increased. The greatest variations in DW were associated with the root component (Figure 1).

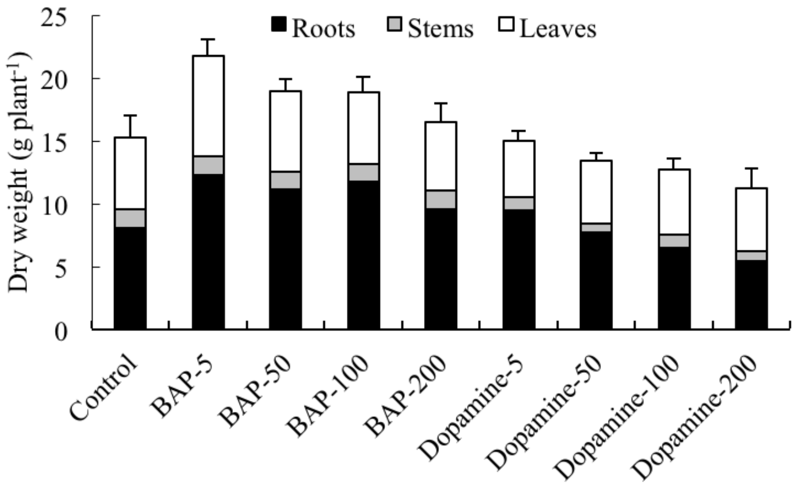

Figure 1. Dry weight accumulated in different plant organs at the end of the experiment for *Limonium sinuatum* plants sprayed with four BAP doses (5, 50, 100 and 200 mg L^{-1}) or four dopamine doses (5, 50, 100 and 200 mg L^{-1}). The bars indicate standard errors for total dry weight.

This is consistent with previous reports regarding the action of cytokinins in ornamentals and vegetables (Di Benedetto et al., 2020a, b; Molinari et al., 2020) but completely different from that documented for dopamine (Liu et al., 2020a).

The traditional growth analysis (Di Benedetto and Tognetti, 2016) allowed identifying the mechanisms involved. The RGR values developed throughout the experiment explained the different accumulation of total biomass in response to spraying with BAP or dopamine. When RGR was disaggregated into the physiological component NAR and the morphological component LAR, differences in RGR between treatments were found to be primarily associated with changes in NAR, which is an estimate of the average photosynthetic rate during the experiment (Table 2). The stimulation of the photosynthetic rate is a common response in plants sprayed with cytokinins (Di Benedetto et al., 2020a, b). However, previous information indicated a stimulation of the photosynthetic rate associated with exogenous spraying with dopamine (Liang et al., 2018; Jiao et al., 2021), an inverse response to that indicated in Table 2 for *L. sinuatum* plants under a plug cell volume abiotic stress during nursery.

Table 2. Changes in the relative growth rate (RGR), net assimilation rate (NAR) and leaf area ratio (LAR) during the experiment for *Limonium sinuatum* plants sprayed with four BAP doses (5, 50, 100 and 200 mg L^{-1}) or four dopamine doses (5, 50, 100 and 200 mg L^{-1}). Different lower case letters indicate significant differences ($P < 0.05$) between treatments.

	RGR ($g\ g^{-1}\ day^{-1}$)	NAR ($g\ cm^{-2}\ day^{-1}$) x 10^{-5}	LAR ($cm^2\ g^{-1}$)
Control	0.0566d	10.72b	54.00c
BAP-5	0.0643a	12.36a	52.02c
BAP-50	0.0586c	11.48a	53.13c
BAP-100	0.0579c	10.91b	51.90c
BAP-200	0.0578c	10.83b	54.11c
Dopamine-5	0.0610b	9.86c	58.62b
Dopamine-50	0.0564d	9.35d	58.31b
Dopamine-100	0.0505e	9.10d	55.51c
Dopamine-200	0.0545d	8.80e	64.10a

The root:shoot allometries showed a decrease in the β coefficient with respect to the controls in the plants sprayed with 5, 50 or 100 mg L^{-1} BAP and a significant increase with all the dopamine concentrations used in the experiment. A decrease in the β coefficient implies greater partitioning of photo assimilates towards the aerial part, while an increase in it implies an inverse partitioning (Figure 2A). A change in the partitioning of photo assimilates towards the aerial part of the plant in relation to that towards roots is a usual response in plants sprayed with BAP (Di Benedetto et al., 2020a, b). The fact that plants sprayed with dopamine increased the partitioning towards the roots is an additional element to identify the control of the concentration of endogenous cytokinins by exogenous spraying with dopamine.

In apple plants under salt stress transformed with dopamine synthesis genes, the results obtained by Gao et al. (2020) indicated that carbohydrate levels changed, showing that dopamine is linked to sugar metabolism. These authors showed that exogenous dopamine increased the content of glucose and fructose by increasing the expression of sucrose phosphate synthase, cell wall invertase and neutral invertase. However, previous data in Brussels sprouts indicated that dopamine spraying did not modify the root:shoot ratios (Miglioli et al., 2020).

In the present study, the leaf:stem allometries showed a significant change in the partitioning of photoassimilates towards the stems in plants sprayed with BAP or dopamine. However, spraying with dopamine reduced this effect although the values were still below those of the controls (Figure 2B).

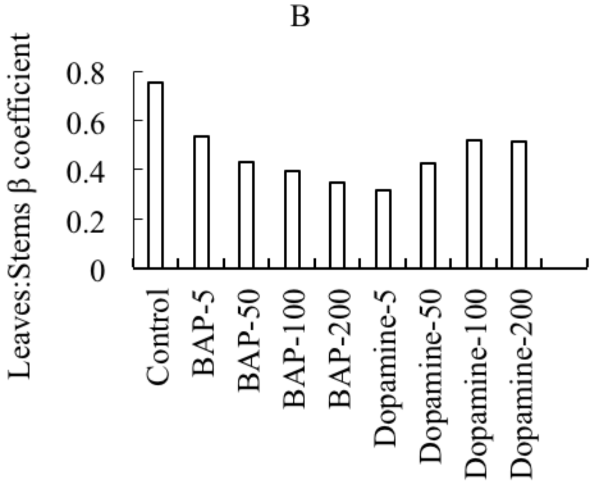

Figure 2. Root:shoot (A) and leaves:stems (B) allometries during the experiment (130 days after transplanting) for *Limonium sinuatum* plants sprayed with four BAP doses (5, 50, 100 and 200 mg L^{-1}) or four dopamine doses (5, 50, 100 and 200 mg L^{-1}).

These results imply a greater flux of photo assimilates towards the SAM in plants sprayed with BAP and a reverse partitioning in those sprayed with dopamine, which explains the differences in RLA and in expanded leaf area (Table 1).

Previous research suggests that the response to spraying with bio-stimulant substances such as hormones (cytokinins) and metabolism activators (dopamine) in situations of abiotic stress is a complex process that depends on the type and degree of stress, the species and the physiological and metabolic status of the plant. As an example, Miglioli (2020) has recently shown that the response to spraying with increasing doses of dopamine depends on the time of year in which the experiment is carried out.

The results obtained in *L. sinuatum* plants could be related to the initial endogenous content of dopamine and to the dynamics of synthesis under situations of root restriction abiotic stress; however, this is a hypothesis that must be validated in future experiments.

Conclusions

The results of the present study indicate that spraying *L. sinuatum* plants with BAP increases leaf area initiation and expansion, biomass accumulation through increased plant photosynthetic capacity, and differential partitioning towards the SAM, within a positive feedback mechanism that has a threshold of optimal response close to 100 mg L^{-1} BAP.

Dopamine spraying appears to affect the synthesis of endogenous cytokinins, reducing the previously mentioned responses.

These results would allow validating the hypothesis that root restriction during nursery as an abiotic stress limits post-transplant biomass accumulation in *L. sinuatum* due to an insufficient supply of endogenous cytokinins to the SAM, while synthetic cytokinins (BAP) sprayed exogenously could reduce the negative effects of root restriction.

Author Contribution

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

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