Ornamental Horticulture
ISSN 2447-536X | HTTPS://ORNAMENTALHORTICULTURE.EMNUVENS.COM.BR/RBHO
SBFPO Sociedade Brasileira de Floricultura e Plantas Ornamentais

SCIENTIFIC ARTICLE

Amino acid solutions on the growth of the ornamental plant *Impatiens walleriana* grown under root restriction stress

Guido Hakim[1], Esteban Gandolfo[1], Máximo Salinas[1],
Ernesto Giardina[1], Adalberto Di Benedetto[1, 2*]

[1] Universidad de Buenos Aires, Facultad de Agronomía, Buenos Aires, Argentina.
[2] Universidad Nacional de Mar del Plata, Facultad de Ciencias Agrarias, Balcarce, Provincia de Buenos Aires, Argentina.

Abstract
The use of amino acid solutions as bio-fertilizers was introduced in the food and ornamental plant industry about two decades ago. Sprays containing amino acids either alone or combined have been proven effective to increase plant growth. However, many of the new commercial solutions including amino acids as bio-fertilizers have no published data available on bedding plants. Thus, the aims of this study were to determine the changes in fresh weight accumulation in the bedding pot plant *Impatiens walleriana* and the physiological mechanism involved in plants sprayed with different amino acid solutions and to characterize the responses to root restrictions under nursery with non-limited nitrogen supply. The experiment was carried out inside a greenhouse in the campus of the Faculty of Agronomy of the University of Buenos Aires, Argentina. To reach the objectives, *Impatiens walleriana* 'Xtreme White' seeds were grown in 50-cell (55.7 cm^3 $cell^{-1}$) and 288-cell (6.18 cm^3 $cell^{-1}$) plug trays and then transplanted and grown at 3-L pots. Eleven solutions (100 mg L^{-1}) containing an equal amount of each of the following amino acids: alanine, cysteine, aspartic acid, glutamic acid, glycine, leucine, lysine, methionine, threonine, tryptophan and valine, a control without amino acids, and a solution containing all amino acids (Mix) (9.1 mg L^{-1} of each amino acid) were sprayed when the first true leaf pair was developed. Our results showed that the different synthetic amino acids tested, alone or combined, had additive and antagonistic effects on the leaf area and fresh-dry weight in *Impatiens walleriana* plants.
Keywords: abiotic stress, bio-fertilizers, bio-stimulants, ornamental bedding plants.

Resumo
Solução de aminoácidos no crescimento da planta ornamental *Impatiens walleriana* sob estresse de restrição de desenvolvimento radicular
O uso de soluções de aminoácidos como biofertilizantes foi introduzido na indústria de alimentos e plantas ornamentais há cerca de duas décadas. Pulverizações contendo aminoácidos, sozinhos ou combinados, têm se mostrado eficazes para aumentar o crescimento das plantas. No entanto, muitas das novas soluções comerciais que incluem aminoácidos como biofertilizantes não têm dados publicados disponíveis sobre plantas de forração. Assim, os objetivos deste estudo foram determinar as mudanças no acúmulo de massa fresca de *Impatiens walleriana* e o mecanismo fisiológico envolvido em plantas pulverizadas com diferentes soluções de aminoácidos e caracterizar as respostas às restrições radiculares em viveiro com fornecimento de nitrogênio. O experimento foi realizado em casa de vegetação no campus da Faculdade de Agronomia da Universidade de Buenos Aires, Argentina. Para atingir os objetivos, sementes de *Impatiens walleriana* 'Xtreme White' foram cultivadas em bandejas de 50 células (55,7 cm^3 $célula^{-1}$) e 288 células (6,18 cm^3 $célula^{-1}$) e depois transplantadas e cultivadas em vasos de 3 L. Onze soluções (100 mg L^{-1}) contendo uma quantidade igual de cada um dos seguintes aminoácidos: alanina, cisteína, ácido aspártico, ácido glutâmico, glicina, leucina, lisina, metionina, treonina, triptofano e valina, um controle sem aminoácidos , e uma solução contendo todos os aminoácidos (Mix) (9,1 mg L^{-1} de cada aminoácido) foi pulverizada quando o primeiro par de folhas verdadeiras foi desenvolvido. Os resultados mostraram que os diferentes aminoácidos sintéticos testados, isolados ou combinados, tiveram efeitos aditivos e antagônicos sobre a área foliar e peso fresco-seco em plantas de *Impatiens walleriana*.
Palavras-chave: estresse abiótico, bioestimulante, biofertilizante, plantas de forração.

*Corresponding author: dibenede@agro.uba.ar

https://doi.org/10.1590/2447-536X.v28i2.2439
Received Oct 11, 2021 | Accepted Jan 24, 2022 | Available online Feb 14, 2022

Area Editor: Gilmar Schafer

Introduction

Amino acids are involved in plant growth and development through the synthesis of proteins, phyto-hormone precursors and other organic compounds such as enzymes, amines, alkaloids and vitamins (Dinkeloo et al., 2018; Batista-Silva et al., 2019). Previous studies on ornamental cut flowers such as *Gerbera jamesonii* (Afifipour and Khosh-Khui, 2015), *Polianthes tuberosa* (Abd-Elkader et al., 2020) and *Eustoma grandiflorum* (Mondal et al., 2015) have shown that foliar applications with some amino acids increase the fresh and dry weights of leaves and roots, total leaf area, number of leaves plant^{-1}, chlorophyll (a, b and total), total carbohydrates, and leaf N, P and K contents.

Although the quality of a bedding plant is related to its aesthetic appearance, the accumulated biomass and the expanded leaf area during nursery define their post-transplant growth (Di Benedetto et al., 2020a).

Since substances or microorganisms exogenously supplied to plants, which increase growth, environmental stress tolerance or traits related to their commercial quality are considered bio-stimulants (Du Jardin, 2015), amino acids may be considered as such. Available data on the use of amino acids are abundant on vegetables (Haghighi and Barzegar, 2017; Moreira and Moraes, 2017; Wang et al., 2017; Khan et al., 2019), but scarce on cut flowers (Afifipour and Khosh-Khui, 2015; Geshnizjani and Khosh-Khui, 2016) and ornamental pot plants (Oliva et al., 2015, Abd-Elkader et al., 2020). Based on these data, active bio-formulations of amino acid mixtures from animal and plant constituents as bio-fertilizers have been developed and marketed during the last twenty years to improve horticultural plant growth, especially under abiotic-growth stresses (Hildebrandt, 2018; Batista-Silva et al., 2019; Salinas et al., 2019).

Plant responses to abiotic stress include various modifications in amino acid metabolism. Batista-Silva et al. (2019) have recently reported results, which support a tight relationship between amino acid metabolism and stress responses. 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 (Di Benedetto et al., 2020a). In pot ornamental plants, the negative effects (lower leaf area, lower photosynthetic rate and lower biomass accumulation) is quite amplified after transplant to pots (De Lojo et al., 2017, 2019a, b) and field soils (Piotti et al., 2018; De Lojo et al., 2021).

In lettuce plants under a nursery abiotic stress related to plug cell size, Salinas et al. (2019) showed significant positive and negative differences in fresh-dry weight, total leaf area, and photo-assimilate partitioning after foliar sprays with different amino acids either alone or combined. However, the physiological processes involved in these responses are not yet known.

Amino acids stimulate physiological and biochemical processes and participate in protein and carbohydrate synthesis. Those usually included in commercial formulations are: alanine (affect plant growth velocity and active chlorophyll formation), arginine (increase tolerance to the hard conditions such as heat, frost, drought, and salinity; it has a role in chlorophyll formation and enhancing root formation as well as cell division and poly amid formation), cysteine (increase vital processes and regulating them within plants and increasing the disease resistance), glycine (active photosynthesis and raise its efficiency as it enhances chlorophyll formation and encourages vegetative growth as well as it has a role in pollination and fruitfulness), glutamic acid (increase the shoot system, growth, and early yield), leucine (increase the shoot system, growth, and early yield), lysine (increase the shoot system, growth, and early yield), methionine (speed out the fruit ripening as it enters the cycle of ethylene formation and it has a role in the root activation), serine (increase plant tolerance to diseases, activating chlorophyll, and it has a role in hormone balance inside plant), threonine (increase plant tolerance to diseases), tryptophan (help to the formation of active auxins indole acetic acid necessary for plant growth and has a role in the early yield), tyrosine (increase plant tolerance to diseases) and valine (affect the velocity of growth, root formation, and seed production) (Baqir et al., 2019).

Impatiens walleriana (Hook.f.), also known as busy Lizzie (United Kingdom), balsam, sultan or simply impatiens, is a commercially important year-round garden crop for landscape, and the first best-selling bedding plants in both developed and undeveloped countries.

Thus, the aims of this study were to determine the changes in fresh weight accumulation of the bedding pot plant *Impatiens walleriana* and the physiological mechanism involved in plants sprayed with different amino acid solutions usually suggested as bio-fertilizers and bio-stimulants and to characterize the responses to root restrictions under nursery with non-limited nitrogen supply.

Materials and Methods

The experiment was carried out inside a greenhouse at the campus of the Faculty of Agronomy of the University of Buenos Aires, Argentina (34°35' 59''S, 58°22'23''W) between November 4th 2018 and February 14th 2019.

To reach the objectives, *Impatiens walleriana* 'Xtreme White' seeds (Goldsmith Inc., NY, USA) were grown in 50-cell (55.7 cm^3 cell^{-1}) and 288-cell (6.18 cm^3 cell^{-1}) plug trays in Klasmann411® medium (Klasmann-Deilmann, GmbH, Germany) for 35 days. When seedlings reached the transplant stage (when the roots fully occupy the cell and it is possible to extract the plug without dismantling it), they were transplanted into 3-L pots filled with *Sphagnum maguellanicum*-river waste-perlite (2-2-1 v/v/v) medium.

At the beginning of the experiments, total porosity (%), air-filled porosity (%), container capacity (%) and bulk density (g cm^{-3}) were 63.50, 17.06, 10.06 and 0.35 respectively. Weeds were manually removed.

Eleven solutions (100 mg L^{-1}) containing an equal amount of each of the following amino acids: alanine, cysteine, aspartic acid, glutamic acid, glycine, leucine, lysine, methionine, threonine, tryptophan and valine, a control without amino acids, and a solution containing all amino acids (Mix) (9.1 mg L^{-1} of each amino acid) were tested. Leaves were sprayed at sunset when the first true leaf pair was developed. Plants were irrigated with high-quality tap water (pH: 6.64 and electrical conductivity: 0.486 dS m^{-1}) as needed, using intermittent overhead mist to compensate evapotranspiration losses. The growing medium was weekly fertilized with 1:1:1:1 (v/v/v/v) N: P: K: Ca (Stage 2: 50 mg L^{-1} N; Stage 3-4: 100 mg L^{-1} N; pot: 200 mg L^{-1} N) through the overhead water irrigation.

Daily mean temperatures (20.15 to 24.08 °C) and daily photosynthetic active radiation (9.61 to 10.99 mole photons m^{-2} day^{-1}) for the experiment were recorded with a HOBO sensor (H08-004-02) (Onset Computer Corporation, MA, USA) connected to a HOBO H8 data logger. The pots were arranged at a density of six plants m^{-2}, which prevented mutual shading.

Ten plants were harvested at transplant stage and at 40, 70, 90 and 110 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 allometric coefficients between both roots/shoots and leaves/stems were calculated according to Di Benedetto and Tognetti (2016).

We used a complete aleatory design. Data were subjected to one-way analysis of variance and means were separated by Tukey's test (p < 0.05). The STATISTICA 8 software (StatSoft) was used. Slopes from straight-line regressions of RLAE, RLAE, RGR and allometric value were tested using the SMATR package.

Results and Discussion

Plants germinated and grown in 50-cell plug trays showed higher fresh weight (FW) accumulation than those grown in 288-cell plug trays (Figure 1).

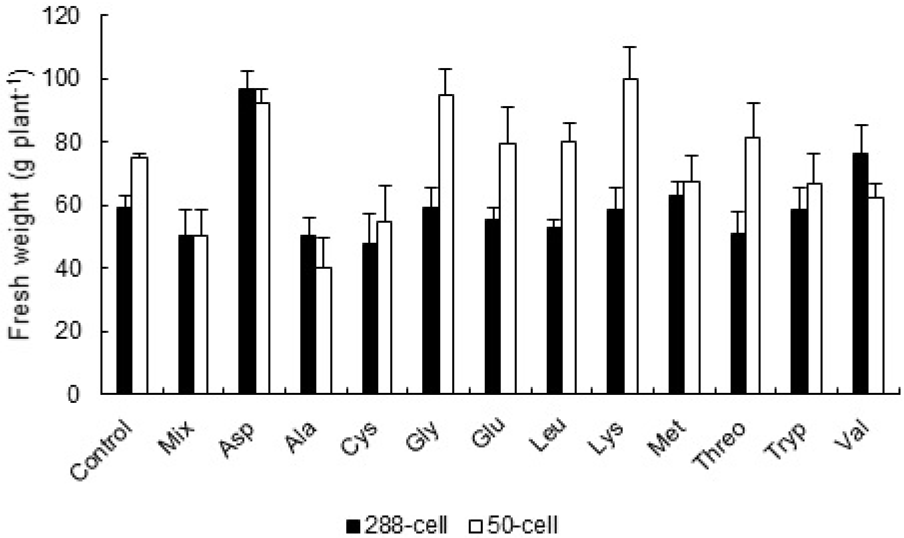

Figure 1. Total fresh weight at the end of the experiment (110 days from transplanting) for *Impatiens wallerana* plants grown in 288-or 50-cell plug trays and sprayed at the pre-transplant stage with a control without amino acids, a solution containing all amino acids (Mix), or solutions (100 mg L^{-1}) containing one of each of the following amino acids: Asp= Aspartic acid; Ala= Alanine; Cys= Cysteine; Gly= Glycine; Glu= Glutamic acid; Leu= Leucine; Lys= Lysine; Met= Methionine; Threo= Threonine; Tryp= Trypsin; or Val=Valine. Vertical lines indicate standard errors (n = 10).

Differences in total FW related to plug cell volume is a common response in vegetables (Rattin et al., 2017; Geraci et al., 2018; Salinas et al., 2019; Di Benedetto et al., 2020b) and ornamentals (De Lojo et al., 2017, 2019a, b, 2021; Di Benedetto et al., 2020a) as a result of the "root restriction" abiotic stress.

Pre-transplant amino acid sprays containing aspartic acid increased FW over controls in plants from both 50- and 288-cell plug trays, although the stimulus associated with other amino acids depends on the cell size used during nursery. An increase in the fresh weight in relation to the controls without treatment was found in the plants grown in trays with 50 cells and sprayed with glycine, glutamic acid, leucine and lysine. However, only the plants grown in trays with 288 cells and sprayed with valine exceeded the fresh weight of the controls (Figure 1).

Some studies on the accumulation of amino acids in plants in response to various abiotic stresses revealed that endogenous amino acids are not enough to regulate various metabolic activities in plants. However, their exogenous supply was found to be effective for proper cellular metabolic activities and induction of drought stress as well as physiological processes (Ali et al., 2019). They stimulate physiological and biochemical processes and participate in protein and carbohydrate synthesis. It also believed that amino acids are accountable for cell division and producing some natural growth hormones such as auxin and gibberellin consequently increasing the yield (Baqir et al., 2019).

Differently, the solution containing all the amino acids (Mix) led to lower FW in plants grown in both 288- and 50-cell plug trays. When the plants were sprayed with each amino acid, the lowest fresh weight was found when the amino acids alanine and cysteine were used (Figure 1). Although antagonistic effects by single and mixed amino acid solutions has been published in *Eustoma grandiflora* (Mondal et al., 2015) and lettuce (Salinas et al., 2019), our negative results are the first reported in bedding plants.

At plant level, biomass accumulation in *Impatiens walleriana* on a fresh-dry weight basis is related to total leaf area expanded and photo-assimilate fixation and partitioning (De Lojo et al., 2017, 2019a, b, 2021). In the present study, total (Figure 2A) and individual (Figure 2B) leaf areas of control plants from both 50- and 288-cell plug trays showed the same pattern as total FW and are in agreement with our previous reports. Plants sprayed with the mixed amino acid solution showed decreased total leaf area but increased individual leaf area. The response to different amino acid sprays at the pre-transplant stage on both total and individual leaf area was quite different as well. The total leaf area increased in the plants coming from trays with 50 cells and sprayed with alanine, cysteine, leucine, tryptophan and valine. On the other hand, the foliar spray with glycine, leucine, lysine, threonine, tryptophan and valine increased the total leaf area in relation to the controls in plants from trays with 288 cells (Figure 2A).

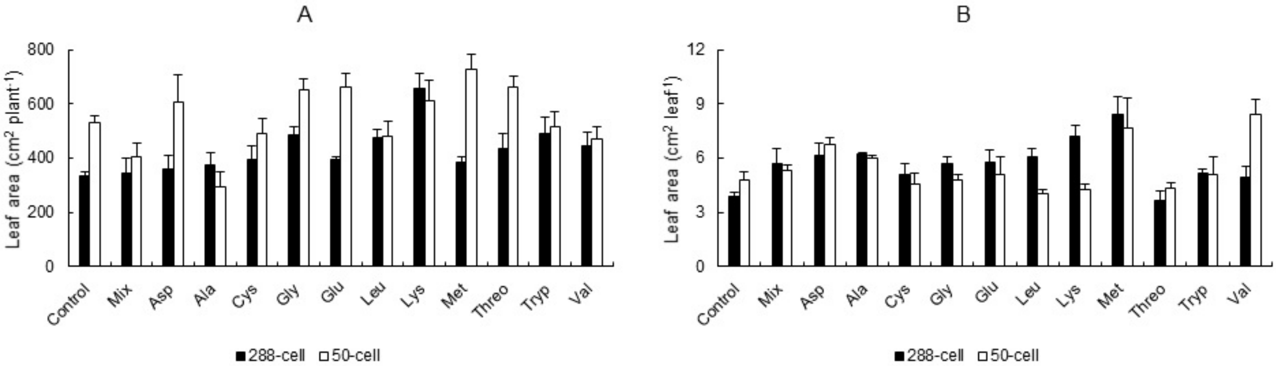

Figure 2. Total (A) and individual (B) leaf area at the end of the experiment (110 days from transplanting) for *Impatiens wallerana* plants grown in 288- or 50-cell plug trays and sprayed at the pre-transplant stage with a control without amino acids, a solution containing all amino acids (Mix), or solutions (100 mg L^{-1}) containing one of each of the following amino acids: Asp= Aspartic acid; Ala= Alanine; Cys= Cysteine; Gly= Glycine; Glu= Glutamic acid; Leu= Leucine; Lys= Lysine; Met= Methionine; Threo= Threonine; Tryp= Trypsin; or Val=Valine. Vertical lines indicate standard errors (n = 10).

When a seedling grows in an environment without limitations, it increases its leaf area and photosynthetic machinery to generate the most positive carbon balance possible. The largest leaf area developed in plants where the root restriction has been partially or totally eliminated by the addition of bio-stimulants is a response to this previously mentioned growth pattern (Di Benedetto et al., 2020a).

Total leaf area is the result of leaf initiation and leaf expansion, estimated through the rate of leaf appearance (RLA) and the relative leaf area expansion (RLAE) respectively. Although there are no previous reports on the effects of amino acid sprays on total leaf area in bedding plants, the RLA and RLAE values found in the present study (Table 1) are in agreement with previous reports from our laboratory in lettuce (Salinas et al., 2019) and allow explaining the differences here observed in total leaf area of *Impatiens walleriana* plants. These results show that the higher the plug cell volume, the higher the RLA and RLAE in agreement with *Impatiens walleriana* and other ornamental pot plants to the root restriction (Di Benedetto et al., 2020a; Molinari et al., 2020). Although some amino acid sprays increased RLA (cysteine, glycine, lysine, methionine, threonine, trypsin and mix solution in plants from 288-cell trays and cysteine, glycine, glutamic acid, leucine, lysine, methionine, threonine and valine in those from 50-cell trays), others decreased it (aspartic acid, alanine, cysteine, glycine, glutamic acid and leucine in plants from 288-cell trays and alanine, aspartic acid, trypsin and the mix solution in those from 50-cell trays). RLAE showed no difference between control plants and those sprayed with different amino acids alone or combined, independently of the plug cell volume used (Table 1).

Table 1. Changes in the rate of leaf appearance (RLA) and the relative rate of leaf area expansion (RLAE) during the experiment for *Impatiens wallerana* plants grown in 288-or 50-cell plug trays and sprayed at the pre-transplant stage with a control without amino acids, a solution containing all amino acids (Mix), or solutions (100 mg L^{-1}) containing Aspartic acid, Alanine, Cysteine, Glycine, Glutamic acid, Leucine, Lysine, Methionine, Threonine, Trypsin, or Valine. Different lower case letters indicate significant differences ($p < 0.05$) between control and amino acid-sprayed treatments. Different capital letters indicate significant differences ($p < 0.05$) between plants from different cell plug size trays. (n = 50).

	RLA (leaves day^{-1})		**RLAE (cm^2 cm^{-2} day^{-1})**	
	288-cell plug trays	**50-cell plug trays**	**288-cell plug trays**	**50-cell plug trays**
Control	0.756dB	0.933dA	0.056aB	0.083aA
Mix	0.532fB	0.647gA	0.059aB	0.078aA
Aspartic acid	0.491gB	0.869eA	0.058aB	0.069aA
Alanine	0.483gB	0.717fA	0.060aB	0.071aA
Cysteine	0.703eB	0.945dA	0.059aB	0.095aA
Glycine	0.758dB	1.176bA	0.057aB	0.072aA
Glutamic acid	0.568fB	1.148bA	0.061aB	0.072aA
Leucine	0.680eB	1.019cA	0.058aB	0.069aA
Lysine	0.840cB	1.234aA	0.066aB	0.079aA
Methionine	0.968aB	1.176bA	0.064aB	0.083aA
Threonine	0.915bB	1.226aA	0.061aB	0.089aA
Trypsin	0.984aA	0.899eB	0.053aB	0.091aA
Valine	0.922bB	0.975dA	0.057aB	0.068aA

The higher production of leaves per unit of time is associated with a decrease in the duration of the plastochron, which is determined by the capacity of the vegetative apex to increase its size (Zhang et al., 2021). Several hormones, such as cytokinin and auxin are essential regulators of shoot apical meristem activities (Shi and Vernoux, 2022).

The relative growth rate (RGR) and the net assimilation rate (NAR) were higher in plants grown in 50-cell plug trays than in those grown in 288-cell plug trays. These results are in agreement with previous reports from our laboratory (Di Benedetto et al., 2020a). On the other hand, significant differences in RGR and NAR were found between plants sprayed with different amino acid solutions (alone or combined) (Table 2).

Table 2. Changes in the relative growth rate (RGR) and the mean net assimilation rate (NAR) during the experiment for *Impatiens walleriana* plants grown in 288- or 50-cell plug trays and sprayed at the pre-transplant stage with a control without amino acids, a solution containing all amino acids (Mix), or solutions (100 mg L^{-1}) containing Aspartic acid, Alanine, Cysteine, Glycine, Glutamic acid, Leucine, Lysine, Methionine, Threonine, Trypsin, or Valine. Different lower case letters indicate significant differences ($p < 0.05$) between control and amino acid-sprayed treatments. Different capital letters indicate significant differences ($p < 0.05$) between plants from different cell plug size trays. (n = 50).

	RGR ($g\ g^{-1}\ day^{-1}$)		**NAR ($g\ cm^{-2}\ day^{-1}$) x 10^{-5}**	
	288-cell plug trays	**50-cell plug trays**	**288-cell plug trays**	**50-cell plug trays**
Control	0.058aB	0.074bA	25.93eB	70.20bA
Mix	0.054aB	0.059cA	30.66dB	48.43cA
Aspartic acid	0.058aB	0.066bA	29.87dB	43.56dA
Alanine	0.052bB	0.064bA	30.01dB	47.31cA
Cysteine	0.045bB	0.067bA	44.65aB	86.65aA
Glycine	0.058aB	0.063bA	34.58cB	42.78cA
Glutamic acid	0.056aB	0.064bA	29.92dB	48.90cA
Leucine	0.061aA	0.057cA	40.12bA	45.40dA
Lysine	0.064aA	0.067bA	40.69bB	49.05cA
Methionine	0.056aB	0.069bA	29.64dB	45.78dA
Threonine	0.056aB	0.071bA	33.92cB	72.43bA
Trypsin	0.054aB	0.069bA	33.73cB	70.97bA
Valine	0.059aB	0.082aA	34.98cB	67.17bA

In *Gerbera jamesonii* plants, Geshnizjani and Khosh-Khui (2016) found that commercially available amino acid mixtures were able to enhance the assimilation of fertilizer, facilitate the uptake of water and nutrients, and improve photosynthesis. The higher NAR values here observed as an estimator for the photosynthetic capacity of *Impatiens walleriana* plants are in agreement with this previous report, although the magnitude of the response is related to the specific amino acid exogenously applied.

It has been indicated that an effect of some of the amino acids used in our experiment would be related to an increase in the photosynthetic rate and its efficiency, as well as in the formation of chlorophyll (Baqir et al., 2019). Although the precise mechanisms remain unclear, this type of response is similar to that found when hormones (cytokinins and auxins) are applied exogenously (Molinari et al., 2020).

The root/shoot allometries shown in Table 3 indicate a higher photo-assimilate partitioning to shoots (lower β coefficient) in plants grown in 50-cell plug trays and in those sprayed with some amino acids (mainly in plants grown in 50-cell plug trays). Similarly, leave/stem allometries suggest a higher photo-assimilate partitioning to stems for the same treatments. Similar results have been found in ornamentals and vegetables under a root restriction and sprayed with other bio-stimulants (Di Benedetto et al., 2020a, b).

Table 3. Changes in allometric relationships between roots/shoots and leaves/stems for *Impatiens walleriana* plants grown in 288- or 50-cell plug trays and sprayed at the pre-transplant stage with a control without amino acids, a solution containing all amino acids (Mix), or solutions (100 mg L^{-1}) containing Aspartic acid, Alanine, Cysteine, Glycine, Glutamic acid, Leucine, Lysine, Methionine, Threonine, Trypsin, or Valine. Different lower case letters indicate significant differences ($p < 0.05$) between control and amino acids-sprayed treatments. Different capital letters indicate significant differences ($p < 0.05$) between plants from different cell plug trays (n = 50).

	β coefficient **(root/shoot)**		β coefficient **(leaves/stems)**	
	288-cell plug trays	**50-cell plug trays**	**288-cell plug trays**	**50-cell plug trays**
Control	0.787dA	0.687dB	0.828dA	0.719aB
Mix	0.933aA	0.843aB	0.719fA	0.640cB
Aspartic acid	0.822cA	0.744cB	0.760eA	0.629cB
Alanine	0.855bA	0.714cB	0.642hA	0.570dB
Cysteine	0.862bA	0.672dB	0.903bA	0.220fB
Glycine	0.797dA	0.772bB	0.862bA	0.660bB
Glutamic acid	0.756dA	0.717cB	0.674gA	0.673bA
Leucine	0.811cA	0.593eB	0.701fA	0.669bB
Lysine	0.777dA	0.723cB	0.836cA	0.730aB
Methionine	0.786dA	0.496fB	0.871bA	0.646cB
Threonine	0.742eB	0.797bA	0.846cA	0.726aB
Trypsin	0.813cA	0.675dB	0.854cA	0.642cB
Valine	0.619fB	0.800bA	0.966aA	0.544eB

The effects observed in growth parameters that describe biomass accumulation (RGR, NAR) and photo-assimilate translocation (β coefficients) resemble those observed in *Impatiens walleriana* plants treated with synthetic cytokinin (De Lojo et al., 2017, 2019a, b, 2021) or synthetic auxin (Molinari et al., 2020). In the present experiments, amino acids were applied in only one opportunity at the pre-transplant stage, for which it is reasonable to assume that they acted as metabolism stimulants.

In the present study, when RLA (Figure 3A), RLAE (Figure 3B) and NAR (Figure 3C) were plotted as a function of RGR, positive relationships were found, whereas a negative β coefficient from root/shoot allometries was found (Figure 3D). These responses involved a direct effect of the increased metabolism related to biomass accumulation (estimated through RGR) on leaf expansion (estimated through RLA and RLAE), photosynthetic CO_2 fixation capacity (estimated through NAR) and photo-assimilate partitioning to the apical shoot meristem (estimated through β coefficients).

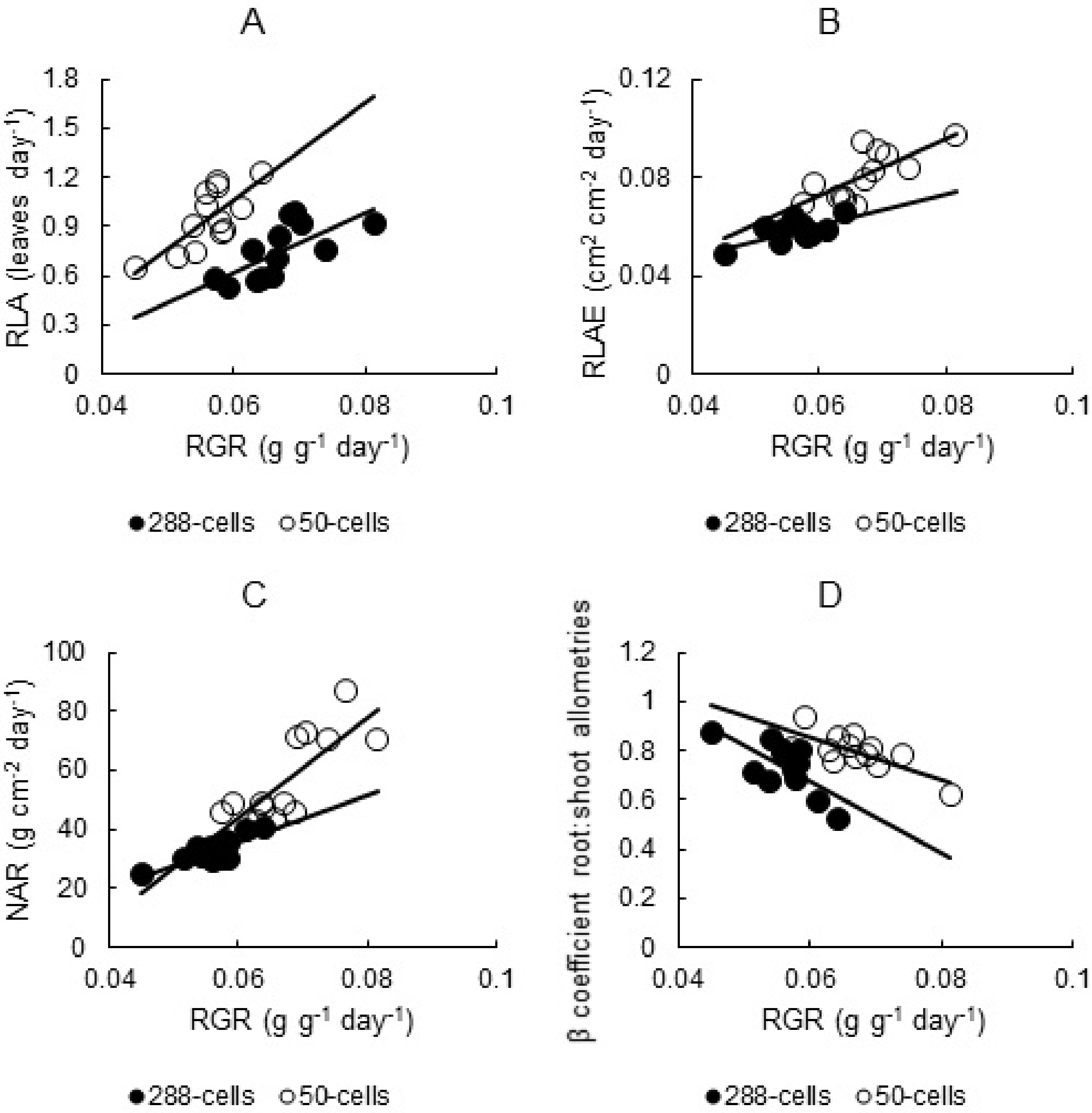

Figure 3. RLA (A), RLAE (B), NAR (C), and the β coefficient from root/shoot allometries (D) related to RGR for *Impatiens walleriana* plants grown in 50- or 288-cell plug trays and sprayed at the pre-transplant stage with a control without amino acids, a solution containing all amino acids (Mix), or solutions (100 mg L^{-1}) containing Aspartic acid, Alanine, Cysteine, Glycine, Glutamic acid, Leucine, Lysine, Methionine, Threonine, Trypsin, or Valine. The straight-line regressions were RLA_{50} = 29.60 RGR - 0.72 (r^2 = 0.560; $p < 0.05$); RLA_{288} = 18.36 RGR - 0.48 (r^2 = 0.475; $p < 0.05$); $RLAE_{50}$ = 1.16 RGR + 0.003 (r^2 = 0.540; $p < 0.05$); $RLAE_{288}$ = 0.62 RGR + 0.023 (r^2 = 0.439; $p < 0.05$); NAR_{50} = 1698.10 RGR - 58.06 (r^2 = 0.614; $p < 0.05$); NAR_{288} = 799.365 RGR - 12.09 (r^2 = 0.694; $p < 0.05$); β_{50} = -8.73 RGR + 1.38 (r^2 = 0.554; $p < 0.05$); β_{288} = -14.48 RGR + 1.55 (r^2 = 0.476; $p < 0.05$) (n = 50).

Results from Salinas et al. (2019) showed that different single amino acids can change different plant traits, such as, fresh weight accumulation, dry weight accumulation, leaf area expansion and photo assimilates partitioning in agreement with our present results. When the amino acid synthesis processes in plants are analyzed, it is common to observe the participation of other amino acids, so changes in the endogenous concentration of some can generate significant modifications in many others that can, in turn, participate in the formation of different organic molecules, including phyto-hormones (Ali et al., 2019). As an example, tryptophan serves as a common precursor for the endogenous indole acetic acid biosynthesis in plants (Casanova-Sáez and Voβ, 2021) while methionine is associated with the biosynthesis of cytokinins and auxins (Khan et al., 2019).

Another issue to point out is the specific effect of each individual amino acid on plant growth, considering that most commercial formulations usually include many of them. From a theoretical point of view, it is extremely difficult to predict which specific amino acid will affect some of the many plant physiological mechanisms (Yang et al., 2020). Baroccio et al. (2017) indicated that the mechanisms of action of amino acids are multiple and often work synergistically, but results from Salinas et al. (2019) and data from this study suggest antagonistic effects of some of them in agreement with our results.

Lea and Miflin (2010) indicated that the response to an exogenous spray with bio-stimulants, such as amino acids, depends on the species, plant genotype, environment, formula concentration and application time. On the other hand, Afifipour and Khosh-Khui (2015) reported that the effects of amino acids on two *Polianthes tuberosa* cultivars were not of the same magnitude. Our present results are in agreement with this previous report.

The hormonal balance between auxins and cytokinin allows a correct functioning of the plant, which is modified in plants under stress (Bielach et al., 2017). Although the accumulation of amino acids in response to different stress situations has been documented (Batista-Silva et al., 2019; Khan et al., 2020), the information related to radical restriction is extremely limited (Salinas et al., 2019).

There are only 20 amino acids involved in protein building, but there are 250 more that are known to be involved in different physiological plant mechanisms (Halpern et al., 2015). Since the synthesis of auxins and cytokinins are affected by the relative concentration of different amino acids and that there is a significant interaction between different amino acids, the exogenous addition of pure or combined solutions of them would generate an imbalance in the relative concentrations of both hormones. The overproduction of any of them would affect metabolic processes and the accumulation of biomass (Wu et al., 2021). However, the experimental design used in our experiments does not allow us to validate the preceding hypothesis.

Conclusions

In summary, a single amino acid spray early at nursery would be a tool to improve the growth of the bedding plant *Impatiens walleriana* in the presence of an abiotic stress related to plug cell volume. However, since additive and antagonistic effects between different synthetic amino acids would be involved, a conclusive result to use this technological alternative needs future experiments able to include different amino acid combinations, synthetic phyto-hormones and different plant species.

Author contribution

GH, **EG and MS:** provided the structure and conditions to develop the experiments and conducted its. **ADB and 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.

Acknowledgements

This work was supported by the University of Buenos Aires Science Program 2017-2021 (Grant Nº 145BA).

References

ABD-ELKADER, H.H.; MASSOUD, H.Y.; EL-BAZ, T.T.; EL-ERIAN, M.A. Effect of amino acids spray on growth, flowering and keeping quality of *Gerbera jamesonii* L. as a pot plant. **Journal of Plant Production**, v.11, n.2, p.201-206, 2020. ttps://doi.org/10.21608/jpp.2020.79110

AFIFIPOUR, Z.; KHOSH, K.M. Efficacy of spraying a mixture of amino acids on the physiological and morphological characteristics of tuberose (*Polianthes tuberosa* L.). **International Journal of Horticultural Science and Technology**, v.2, n.2, p.199-204, 2015.

ALI, Q.; ATHAR, H.; HAIDER, M.Z.; SHAHID, S.; ASLAM, N.; SHEHZAD, F.; NASEEM, J.; ASHRAF, R.; ALI, A.; HUSSAIN, S.M. **Role of amino acids in improving abiotic stress tolerance to plants**. Plant Tolerance to Environmental Stress. Boca Raton: CRC Press, 2019.

BAQIR, H.A.; ZEBOON, N.H.; AL-BEHADILI, A.A.J. The role and importance of amino acids within plants: a review. **Plant Archives**, v.19, n.2, p.1402-1410, 2019.

BAROCCIO, F.; BARILARO, N.; TOLOMEI, P.; MASCINI, M. Classification of biostimulants origin using amino acids composition of hydrolyzed proteins. **Journal of Horticultural Science and Research**, v.1, n.2, p.30-35, 2017. https://doi.org/10.36959/745/395

BATISTA SILVA, W.; HEINEMANN, B.; RUGEN, N.; NUNES NESI, A.; ARAÚJO, W.L.; BRAUN, H.P.; HILDEBRANDT, T.M. The role of amino acid metabolism during abiotic stress release. **Plant, Cell & Environment**, v.42, n.5, p.1630-1644, 2019. https://doi.org/10.1111/pce.13518

BIELACH, A.; HRTYAN, M.; TOGNETTI, V.B. Plants under stress: involvement of auxin and cytokinin. **International Journal of Molecular Sciences**, v.18, n.7, p.1427, 2017. https://doi.org/10.3390/ijms18071427

CASANOVA-SÁEZ, R.; MATEO-BONMATÍ, E.; LJUNG, K. Auxin metabolism in plants. **Cold Spring Harbor Perspectives in Biology**, v.13, n.3, a039867, 2021. https://doi: 10.1101/cshperspect.a039867

DE LOJO, J.; GANDOLFO, E.; GÓMEZ, D.; FEURING, V.; MONTI, S.; GIARDINA, E.; BOSCHI, C.; DI BENEDETTO, A. Root restriction effects on the bedding pot plant *Impatiens walleriana*. **Journal of Experimental Agriculture International**, v.15, n.4, p.1-16, 2017. https://doi.org/10.9734/JEAI/2017/31997

DE LOJO, J.; GANDOLFO, E.; GIARDINA, E.; BOSCHI, C.; DI BENEDETTO, A. Growing media quality and plug cell volume would be interactive abiotic stresses for *Impatiens walleriana* pot yield. **Asian Journal of Agricultural and Horticultural Research**, v.4, n.1, p.1-14, 2019a. https://doi.org/10.9734/AJAHR/2019/v4i130008

DE LOJO, J.; GANDOLFO, E.; GIARDINA, E.; BOSCHI, C.; DI BENEDETTO, A. Pot cell volume, growing media quality and benzyl aminopurine (BAP) spray effects for nursery growth of *Impatiens walleriana*. **International Journal of Plant Soil Science**, v.27, n.6, p.1-13, 2019b. https://doi.org/10.9734/IJPSS/2019/v27i630091

DE LOJO, J. M.; GANDOLFO, E.; FEURING, V.; GIARDINA, E. B.; BOSCHI, C. L.; DI BENEDETTO, A. Garden post-transplant effects of pre-transplant plug cell volume and growing medium quality (as abiotic stresses) in *Impatiens walleriana*. **Ornamental Horticulture**, v.27, n.3, p.320-333, 2021. https://doi.org/10.1590/2447-536X.v27i3.2295

DI BENEDETTO, A.; TOGNETTI, J. Técnicas de análisis de crecimiento de plantas: su aplicación a cultivos intensivos. **RIA**, v.42, p.258-282, 2016.

DI BENEDETTO, A.; GIARDINA, E.; DE LOJO, J.; GANDOLFO, E.; HAKIM, G. Exogenous benzyl aminopurine (BAP) applications for the ornamental pot industry. In: KORTESMÄKI, S. **Cytokinins: Biosynthesis and Uses**. New York: Nova Science Publishers, 2020a. p.1-56.

DI BENEDETTO, A.; RATTIN, J.; CARNELOS, D.; LOZANO-MIGLIOLI, J.; GIARDINA, E.; ARAKI, A.; CORO, M.; PICO-ESTRADA, O.; TERUEL, J.; DI MATTEO, J.; GERASI, J.; BARRERA, L.; ALONSO, E.; GRIGOLI, L. Technological uses of exogenous cytokinins in vegetables. In: KORTESMÄKI, S. **Cytokinins:** Biosynthesis and Uses. New York: Nova Science Publishers, 2020b. p.107-155.

DINKELOO, K.; BOYD, S.; PILOT, G. Update on amino acid transporter functions and on possible amino acid sensing mechanisms in plants. **Seminars in Cell & Developmental Biology**, v.74, p.105-113, 2018. https://doi.org/10.1016/j.semcdb.2017.07.010

DU JARDIN, P. Plant biostimulants: definition, concept, main categories and regulation. **Scientia Horticulturae**, v.196, p.3-14, 2015. http://doi.org/10.1016/j.scienta.2015.09.021

GERACI, J.; DI MATTEO, J.; FEURING, V.; GIARDINA, E.; DI BENEDETTO, A. Exogenous BAP spray applications against to abiotic stress related by root restrictions in spinach. **Journal of Experimental Agriculture International**, v.25, n.6, p.1-17, 2018. https://doi.org/10.9734/JEAI/2018/43753

GESHNIZJANI, N.; KHOSH-KHUI, M. Promoted growth and improved quality of *Gerbera jamesonni* L. flowers using exogenous application of amino acids. **International Journal of Horticultural Science and Technology**, v.3, n.2, p.155-166, 2016. https://doi.org/10.22059/ijhst.2016.62915

HAGHIGHI, M.; BARZEGAR, M.R. Effect of amino acid and mycorrhiza inoculation on sweet pepper growth under greenhouse conditions. **Iran Agricultural Research**, v.36, n.2, p.47-54, 2017.

HALPERN, M.; BAR-TAL, A.; OFEK, M.; MINZ, D.; MULLER, T.; YERMIYAHU, U. The use of biostimulants for enhancing nutrient uptake. **Advances in Agronomy**, v.130, p.141-174, 2015. https://doi.org/10.1016/bs.agron.2014.10.001

HILDEBRANDT, T.M. Synthesis versus degradation: directions of amino acid metabolism during Arabidopsis abiotic stress response. **Plant Molecular Biology**, v.98, n.1, p.121-135, 2018. https://doi.org/10.1007/s11103-018-0767-0

KHAN, S.; YU, H.; LI, Q.; GAO, Y.; SALLAM, B. N.; WANG, H.; LIU, P.; JIANG, W. Exogenous application of amino acids improves the growth and yield of lettuce by enhancing photosynthetic assimilation and nutrient availability. **Agronomy**, v.9, n.5, p.266, 2019. https://doi.org/10.3390/agronomy9050266

KHAN, N.; ALI, S.; ZANDI, P.; MEHMOOD, A.; ULLAH, S.; IKRAM, M.; SHADID, M.A.; BABAR, M.A. Role of sugars, amino acids and organic acids in improving plant abiotic stress tolerance. **Pakistan Journal of Botany**, v.52, n.2, p.355-363, 2020. http://dx.doi.org/10.30848

LEA, P.J.; MIFLIN, B.J. Nitrogen assimilation and its relevance to crop improvement. **Annual Plant Reviews**, v.42, p.1-40, 2018. https://doi.org/10.1002/9781444328608.ch1

MOLINARI, J.; PAGANI, A.; BUYATTI, M.; GIARDINA, E.; DI BENEDETTO, A. Effects of exogenous cytokinin application on the nursery of ornamental plants, mainly 'New Guinea' Impatiens (*Impatiens hawkeri* Bull) and on their pre- and post-transplant biomass accumulation. In: KORTESMÄKI, S. **Cytokinins: Biosynthesis and Uses**. New York: Nova Science Publishers, 2020. p.57-106.

MONDAL, M.F.; ASADUZZAMAN, M.; TANAKA, H.; ASAO, T. Effects of amino acids on the growth and flowering of *Eustoma grandiflorum* under autotoxicity in closed hydroponic culture. **Scientia Horticulturae**, v.192, p.453-459, 2015. http://doi.org/10.1016/j.scienta.2015.05.024

MOREIRA, A.; MORAES, L.A.C. Yield, nutritional status and soil fertility cultivated with common bean in response to amino-acids foliar application. **Journal of Plant Nutrition**, v.40, n.3, p.344-351, 2017. https://doi.org/10.1080/01904167.2016.1240194

OLIVA, M.; OVADIA, R., PERL, A.; BAR, E.; LEWINSOHN, E.; GALILI, G., OREN SHAMIR, M. Enhanced formation of aromatic amino acids increases fragrance without affecting flower longevity or pigmentation in *Petunia* × *hybrida*. **Plant Biotechnology Journal**, v.13, n.1, p.125-136, 2015. https://doi.org/10.1111/pbi.12253

PIOTTI, M.; MOLINARI, J.; PAGANI, A.; GIARDINA, E.; DI BENEDETTO, A. Root restriction effects on *Zantedeschia aethiopica*. **International Journal of Plant & Soil Science**, v.23, n.2, p.1-15, 2018. https://doi.org/10.9734/IJPSS/2018/42140

RATTIN, J.; PICO ESTRADA, O.; GIARDINA, E.; DI BENEDETTO, A. Nursery pre- and pos-transplant effects on tomato (*Solanum lycopersicum* L.) growth and yield. **Journal of Experimental Agriculture International**, v.18, n.5, p.1-14, 2017. https://doi.org/10.9734/JEAI/2017/37515

SALINAS, M.; GANDOLFO, E.; HAKIM, G.; GIARDINA, E.; DI BENEDETTO, A. Foliar amino acids sprays on lettuce (*Lactuca sativa* L.) biomass accumulation. **International Journal of Current Microbiology Applied Science**, v.8, n.1, p.2183-2196, 2019. https://doi.org/10.20546/ijcmas.2019.801.228

SHI, B.; VERNOUX, T. Hormonal control of cell identity and growth in the shoot apical meristem. **Current Opinion in Plant Biology**, v.65, 102111. https://doi.org/10.1016/j.pbi.2021.102111

WANG, G.; XU, M.; WANG, W.; GALILI, G. Fortifying horticultural crops with essential amino acids: a review. **International Journal of Molecular Sciences**, v.18, n.6, p.1306, 2017. https://doi.org/10.3390/ijms18061306

WU, W.; DU, K.; KANG, X.; WEI, H. The diverse roles of cytokinins in regulating leaf development. **Horticulture Research**, v.8, n.1, p.1-13, 2021. https://doi.org/10.1038/s41438-021-00558-3

YANG, Q.; ZHAO, D.; LIU, Q. Connections between amino acid metabolisms in plants: lysine as an example. **Frontiers in Plant Science**, v.11, p.928, 2020. https://doi.org/10.3389/fpls.2020.00928

ZHANG, T.Q.; CHEN, Y.; WANG, J.W. A single-cell analysis of the *Arabidopsis* vegetative shoot apex. **Developmental Cell**, v.56, n7, 1056-1074, 2021. https://doi.org/10.1016/j.devcel.2021.02.021