Effect of cell size and growing medium quality on the commercial productivity of *Limonium sinuatum* plants

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Abstract

The plug cell volume and the quality of the growing medium during nursery stand out as the most important factors affecting the success of ornamental plants, because they significantly affect biomass accumulation and post-transplant yield. These two technological inputs may also be a source of potential abiotic stress capable of modifying the grower’s profit. The aim of this work was to evaluate the physiological mechanisms involved in the growth of *Limonium sinuatum* plants in three different pre-transplant plug cell volumes and two different growing media as well as in their transplant to pots or to an amended soil. The hypothesis tested was that the negative effects of these combined abiotic stress sources could limit *L. sinuatum* growth and yield. Our results showed that, in response to limiting situations of root growth, *L. sinuatum* plants modified the expanded leaf area, the accumulation of fresh-dry weight and the partitioning of photo-assimilates. The physiological processes identified include the capacity for leaf initiation and expansion, the photosynthetic capacity, the growth rate per unit area and time, and the partitioning of photo-assimilates between different plant organs. Since these responses to different types and degrees of abiotic stress are similar to those found in plants depressed in endogenous cytokinins, we speculate that these hormones may be involved in the results obtained in this work.

Keywords: growth parameters, pot plant, biomass accumulation, speciality cut flower, technological abiotic stress.

Resumo

Efeito do volume do recipiente e do substrato na produtividade comercial de *Limonium sinuatum*

O volume do recipiente e o substrato de cultivo na produção das mudas destacam-se como os fatores mais importantes que afetam o sucesso das plantas ornamentais, pois influenciam significativamente no acúmulo de biomassa e na produtividade pós-transplante. Esses dois insumos tecnológicos também podem ser uma fonte potencial de estresse abiótico capaz de modificar o lucro do produtor. O objetivo deste trabalho foi avaliar os mecanismos fisiológicos envolvidos no crescimento de plantas de *Limonium sinuatum* en três diferentes volumes de recepientes de bandejas multicelulares e dois substratos, na produção das mudas e a sua influência no posterior desenvolvimento das plantas cultivadas em vasos ou no solo em ambiente protegido. A hipótese testada foi que os efeitos negativos dessas fontes combinadas de estresse abiótico poderiam limitar o posterior crescimento e a produtividade de *L. sinuatum*. Os resultados mostraram que, em resposta a situações limitantes de crescimento radicular, plantas de *L. sinuatum* modificaram a área foliar, o acúmulo de massa fresca e seca e a partição de fotoassimilados. Os processos fisiológicos identificados incluem a capacidade de iniciação e expansão foliar, a capacidade fotosintética, a taxa de crescimento por unidade de área e tempo e a partição de fotoassimilados entre diferentes órgãos vegetais. Uma vez que essas respostas a diferentes tipos e graus de estresse abiótico são semelhantes às encontradas em plantas com baixa concentração de citocininas endógenas, especulamos que esses hormônios possam estar envolvidos nos resultados obtidos neste trabalho.

Palavras-chave: acúmulo de biomassa, crescimento, flor de corte, planta de vaso, stress abiótico.

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Introduction

*Limonium sinuatum* is a perennial, herbaceous plant, which is marketed both as a potted plant and as a specialty cut flower (Shillo and Zamski, 2019). Currently, almost all seed-propagated ornamental species are germinated in plug cell trays and transplanted into a pot or a soil modified by the addition of organic amendments. However, the grower’s choice of plug cell size during nursery constitutes an abiotic stress that has been extensively documented by our laboratory in various other ornamental species (Di Benedetto et al., 2020a).

Although it is a known fact that the cell size cannot be chosen without taking into account the quality of the growth medium, it is also true that the growth medium cannot be chosen without taking into account the volume of the plug cell tray. However, the effect of the growing medium quality on ornamental plant growth in plug cell trays has received little attention (Gandolfo et al., 2016; Williams et al., 2016; Hakim et al., 2017). Previous results have also shown an additive interaction of both factors, which results in a significant decrease in post-transplant biomass accumulation in plants that end their useful life in a pot (De Lojo et al., 2017, 2019a, b). However, much less information is available on the effect of these pre-transplant stress situations when seedlings are transplanted into amended soils (Piotti et al., 2018; De Lojo et al., 2021).

The aim of this work was to evaluate the physiological mechanisms involved in the growth of *L. sinuatum* plants grown in three different pre-transplant cell volumes and two different growing media during nursery as well as in their transplant to pots or to amended soil. The hypothesis tested was that the negative effects of these combined abiotic stress sources could limit the growth and yield of this ornamental plant.

Materials and Methods

The experiment was carried out in a greenhouse inside 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- (55.7 cm$^3$), 200- (13.90 cm$^3$ cell$^{-1}$) and 288- (6.18 cm$^3$ cell$^{-1}$) plug trays in the following two growing media for 37 days:

a) 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.

b) *Sphagnum magellanicum*-river waste-perlite (40-40-20, v/v/v) medium (S). At the beginning of the experiments, total porosity (%), air-filled porosity (%), container capacity (%) and bulk density (g cm$^{-3}$) were 20.17, 4.33, 15.83 and 0.84 respectively.

At the transplant stage, half of the plants were grown in 3-L pots filled with S medium (2-2-1 v/v/v) or K medium on countertops at floor level. A second set of plants were transplanted to the greenhouse-amended soil on raised beds that kept them at the same level as the potted lot.

At the beginning of the experiment, the analysis of the greenhouse soil indicated a total porosity (%), air-filled porosity (%), container capacity (%) and bulk density (g cm$^{-3}$) of 25.02, 5.60, 31.56 and 0.90 respectively. Organic matter (%), pH and electrical conductivity (dS m$^{-1}$) were 2.73, 7.83 and 1.17 respectively. Both the pots and field-soil plants were arranged at a density of five plants m$^{-2}$, which avoided mutual shading.

Plants were irrigated as needed with high quality tap water by using intermittent overhead mist to compensate evapotranspiration losses, and weekly fertilization with nitric acid, phosphorus acid, potassium nitrate, and calcium nitrate (Agroquímica Larocca S.R.L., Buenos Aires, Argentina) was applied as follows: Stage 2: 50 mg L$^{-1}$ N; Stage 3-4: 100 mg L$^{-1}$ N; and pot-field soil: 150 mg L$^{-1}$ N (Styer and Koransky, 1997).

During the experiment, the daily mean temperatures (19.40 to 35.09 °C) and the daily photosynthetic active radiation (5.00 to 9.44 mole photons m$^{-2}$ day$^{-1}$) inside the greenhouse 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 sowing) and at monthly intervals. Roots were washed and root, stem, leaf and flower fresh weights (FW) were 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 recorded, and the area of each leaf was determined using the ImageJ® software (Image Processing and Analysis in Java).

The relative leaf area expansion rate (RLAER), the rate of leaf appearance (RLA), the relative growth rate (RGR), the net assimilation rate (NAR), and the root: shoot and leaf: stem allometries were calculated as previously (Di Benedetto and Tognetti, 2016). Samples of young, fully expanded leaves and adventitious roots were collected to examine leaf thickness, root diameter and root vascular system on the final harvest date (135 days from sowing). Tissues from the middle region of each lamina and adventitious root were fixed in a mixture of 70% (v v$^{-1}$) ethanol, 5% (v v$^{-1}$) formalin, 5% (v v$^{-1}$) glacial acetic acid, and 20% (v v$^{-1}$) distilled water prior to dehydration in an ethanol and tert-butyl alcohol series. Fixed tissues were sectioned (10-20 μm thick) on a rotary microtome and stained with safranin-crystal violet-fast green. The data presented are the means of three leaves or roots per treatment, using ten cross-sections per leaf. Quantitative anatomical data were obtained using Image Pro Express Version 6.0 (Media Cybernetics, Rockville, MD, USA).

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

Regarding the cell volume, the results showed that as the plug cell volume used during nursery decreased, the expanded leaf area at the end of the experiment decreased (Figure 1A). In addition, regarding the quality of the growing medium, in the pot set, the use of a higher growing medium quality in terms of physical properties, such as the K medium, allowed reaching the highest values of expanded leaf area. Finally, the comparison between plants transplanted to pots versus those transplanted to an organic soil modified by the addition of amendments (greenhouse soil) showed that the expanded leaf area was greater when pots were used (Figure 1A). However, leaf thickness showed less significant differences than total leaf area (Figure 1B).

The ability of plants to initiate and expand leaves can be estimated through the RLA (Figure 1C) and the RLAER (Figure 1D), respectively. In both growth parameters, the highest values were found in plants germinated in 50-cell trays and with a high-quality growing medium (K). The response to the quality of the crop site reproduces that found for total leaf area when the RLAER values were analyzed; however, RLA presented differences related to the other variables (cell size and substrate quality during nursery).

Figure 1. Total leaf area (A) and leaf thickness (B) at the end of the experiment (135 days after sowing), and RLA (C) and RLAER (D) during the experiment for Limonium sinuatum plants grown in three plug cell volumes (50-, 200- and 288-cell tray\(^{-1}\)) and two growing media during nursery and then grown either in pots or in a greenhouse soil after transplant. The bars indicate standard errors.

It has been indicated that the higher the total leaf area and RLA, the higher the shoot apical meristem (SAM) (Shi and Vernoux, 2019). A decrease in the plastochron (i.e. the time between successive leaf initiation events) needs an increase in the SAM (Kitagawa and Jackson, 2019), the presence of non-limiting sugar availability (Saddhe et al., 2021) and the regulation of relative assimilate allocation (Keller et al., 2021).

Cabal et al. (2020) have recently concluded that plants can sense the volume of the rooting space available, and a limited number of studies on individual roots have shown that plant roots may sense the identity of neighboring...
roots and respond accordingly (Novoplansky, 2019). This seems to be achieved by means of cytokinins, which are root-synthesized molecules transported via the xylem to the shoot (Kieber and Schaller, 2018). Some authors have shown that, in transgenic plants with reduced cytokinin levels, the plastochron seems to be altered (Strable, 2021).

The initial mechanism of foliar initiation and expansion is a prerequisite for an efficient uptake of photosynthetically active radiation and the accumulation of photo-assimilates. The positive feedback between the photosynthetic capacity and the growth of the SAM is clearly reduced by the presence of different situations and degrees of root abiotic stress. Root restriction during nursery affects biomass accumulation in all plant organs post-transplantation. In agreement with results in other ornamental plants (Di Benedetto et al., 2020a), as the size of the cell decreased, the size of the root system decreased (Figure 2). In addition, significant differences were observed when different growth media with differences in their physical properties were used in pre-transplantation. However, the most important differences in the distribution of photo-assimilates within the plants were observed when comparing plants grown in pots with those grown in greenhouse soil.

Figure 2. Fresh weight at the end of the experiment (135 days after sowing) for Limonium sinuatum plants grown in three plug cell volumes (50-, 200- and 288-cell tray\(^{-1}\)) and two growing media during nursery and then grown either in pots (A) or in a greenhouse soil (B) after transplant. The bars indicate standard errors.

Yin et al. (2019) showed that for an optimal development of the plant as a whole, both the root and shoot biomass allocation must be balanced. In agreement with previous reports in other ornamental plants (Gandolfo et al., 2016; Williams et al., 2016; Hakim et al., 2017; De Lojo et al., 2017, 2019a, b, 2021; Piotti et al., 2018), our results indicate that, under different situations of abiotic stress, the plants modify the growth of different organs and the interactions that regulate the final total FW by adjusting the partitioning of photo-assimilates.

The increase in the accumulated total DW as a function of time showed a traditional sigmoid evolution. However, while in the plants grown in 3-L pots (Figure 3A), the differences between treatments became significant after 120 days from sowing, plants transplanted to greenhouse soil only showed differences 135 days after sowing (Figure 3B).
Figure 3. Changes in total dry weight during the 135 days of the experiment for *Limonium sinuatum* plants grown in three plug cell volumes (50-, 200- and 288-cell tray$^{-1}$) and two growing media during nursery and then grown either in pots (A) or in a greenhouse soil (B) after transplant.

The differences in DW between the different treatments observed in Figure 3 can be explained with the RGR data shown in Table 1. The highest RGR values were found in situations with less root restriction. On the other hand, when RGR was decomposed into its physiological component NAR and its morphological component leaf area ratio (LAR), a direct relationship between NAR and RGR and an inverse relationship between LAR and RGR were observed (data not shown) in agreement with our previous reports (Di Benedetto et al., 2020a).

Table 1. Changes in the relative growth rate (RGR), the net assimilation rate (NAR) and leaf area ratio (LAR) during the experiment for *Limonium sinuatum* plants grown in three plug cell volumes (50-, 200- and 288-cell tray$^{-1}$) and two growing media during nursery and then grown either in pots or in a greenhouse soil after transplant. Different lower case letters indicate significant differences ($P < 0.05$) between treatments. Different capital letters indicate significant differences ($P < 0.05$) between plants grown in pots or in a greenhouse soil.

<table>
<thead>
<tr>
<th></th>
<th>RGR (g g$^{-1}$ day$^{-1}$)</th>
<th>NAR (g cm$^{-2}$ day$^{-1}$) x 10$^{-5}$</th>
<th>LAR (cm$^2$ g$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pot</td>
<td>Greenhouse soil</td>
<td>Pot</td>
</tr>
<tr>
<td>K-50</td>
<td>0.0585aA</td>
<td>0.0558aB</td>
<td>11.27aA</td>
</tr>
<tr>
<td>K-200</td>
<td>0.0576bA</td>
<td>0.0539cB</td>
<td>6.39cA</td>
</tr>
<tr>
<td>K-288</td>
<td>0.0532cA</td>
<td>0.0531cA</td>
<td>3.42dA</td>
</tr>
<tr>
<td>S-50</td>
<td>0.0580bA</td>
<td>0.0583bA</td>
<td>7.46bA</td>
</tr>
<tr>
<td>S-200</td>
<td>0.0570bA</td>
<td>0.0549dA</td>
<td>4.36dA</td>
</tr>
<tr>
<td>S-288</td>
<td>0.0479dA</td>
<td>0.0475eA</td>
<td>1.64eA</td>
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</table>

An increase in the total DW of plants has been closely related to the photosynthetic capacity of aerial organs (Silva et al., 2018). In general, NAR is the best general predictor of the variation in RGR, as indicated in previous reports from our laboratory in other ornamental plants (Di Benedetto et al., 2020a).

According to the classical growth analysis, the partitioning of photo-assimilates between different plant organs can be estimated from the allometries between them. When the root: shoot (Figure 4A) or leaf: stem (Figure 4B) allometries were calculated, as the root restriction increased, the partition coefficient $\beta$ decreased in both. This implies that the lower the value of $\beta$, the higher the proportion of photo-assimilates that is redistributed towards the aerial part of the plant and within the aerial biomass to the stems.

The data in Figure 4 also show differences in photo-assimilate partitioning influenced by pre-transplantation cell size, pre-transplantation substrate quality and final growth site. Sink organs can potentially stimulate sugar supply by activating their consumption rate, thereby increasing their sink strength; the relative carbon allocation to a particular organ must be regarded as a function of the source and sink activities of all parts of the plant (Bechtold and Field, 2018).
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The different physical properties of the substrates used during nursery or post-transplantation would determine a different resistance to the growth of the root system. An easily visible aspect of this restriction to root growth is manifested in the shortening and thickening of the roots, as seen in Figure 5. Figure 5A shows that the length of the root system is significantly modified in the presence of abiotic stress of different degrees depending on the treatment considered. On the other hand, the lower the root restriction, the greater the thickness of the vascular system (Figure 5B). These last results could be associated with the fact that the partitioning of photo-assimilates implies their load to the source or storage level, movement through the vascular system, and discharge to the sink (McCubbin and Braun, 2021).

In Limonium sinuatum plants, commercial yield is related to the number and length of the flowers produced. In the present study, final yield decreased as pre-transplant cell size decreased, although in some treatments the number of flowers was greater in plants grown in greenhouse soil than in pots (Figure 6A). On the other hand, the size of the pre-transplantation plug cell would seem to have little effect on the length of the flower stem, being it related to the cropping site. Regarding this aesthetic attribute, the plants grown in the greenhouse soil showed significantly higher values than those grown in the pots (Figure 6B). In this regards, it has been indicated that the quality of the growing medium in terms of its physical properties can reduce root elongation and limit crop productivity (Jacobsen et al., 2021) and flower quality (Karagüzel, 2020).
Another result of the present study was the positive relationship found between yield and total DW (Figure 7A), yield and RGR (Figure 7B), yield and NAR (Figure 7C) and yield and the thickness of the vascular system (Figure 7D). Only small differences were found in relation to the cropping site.

Figure 6. Yield (A) and flower stem length (B) (135 days after sowing) for Limonium sinuatum plants grown in three plug cell volumes (50-, 200- and 288-cell tray\(^{-1}\)) and two growing media during nursery and then grown either in pots or in a greenhouse soil after transplant. The bars indicate standard errors.

Figure 7. Relationships between yield and total dry weight (A), yield and RGR (B), yield and NAR (C), yield and vascular system for Limonium sinuatum plants grown in three plug cell volumes (50-, 200- and 288-cell tray\(^{-1}\)) and two growing media during nursery and then grown either in pots or in a greenhouse soil after transplant. The straight-line regressions were: Yield\(_{\text{pot}}\) = 0.44 DW + 12.97 ($r^2 = 0.939$); Yield\(_{\text{Greenhouse soil}}\) = 0.589 DW + 11.55 ($r^2 = 0.658$); Yield\(_{\text{pot}}\) = 792.50 RGR − 19.14 ($r^2 = 0.584$); Yield\(_{\text{Greenhouse soil}}\) = 1182.00 RGR − 37.42 ($r^2 = 0.594$); Yield\(_{\text{pot}}\) = 1.53 NAR + 14.60 ($r^2 = 0.936$); Yield\(_{\text{Greenhouse system}}\) = 1.65 NAR + 18.56 ($r^2 = 0.810$); Yield\(_{\text{pot}}\) = 0.05 Vascular system + 5.06 ($r^2 = 0.933$); Yield\(_{\text{Greenhouse system}}\) = 0.05 Vascular system + 0.60 ($r^2 = 0.624$).
A large amount of evidence, gathered from studies on a wide range of horticultural and ornamental species, indicates that the effects of different abiotic stresses during nursery such as small plug cell volumes or lower growing medium quality are associated with a reduced volume of the root system, which in turn affects the synthesis of endogenous cytokinins, and may thus be at least partially overcome by the exogenous supply of cytokinin (Di Benedetto et al., 2020a, b). Previous data from our laboratory indicate that all the variables contrasted in Figure 7 (yield, DW, RGR, NAR and thickness of the vascular system) are negatively influenced by the root restriction during nursery and positively stimulated by external spraying with cytokinins (De Lojo et al., 2017, 2019a, b, 2021; Piotti et al., 2018).

Conclusions

The higher photo-assimilate partitioning to aerial organs such as stems (presumably towards SAM), as well as the higher RLA, higher leaf area (as a feasible increase in SAM) and higher RGR found in response to the different growing media and plug cell volume used during nursery and at the post-transplant stage are in agreement with the results found in other ornamental plants with root restriction previously published by our laboratory. These previous results have been associated with changes in both the synthesis and translocation of endogenous cytokinins. To validate these conclusions and offer both adequate suggestions to growers and key traits for breeding research in the same way as when assessing the effect of volume root restriction on biomass accumulation. However, this investigation line needs additional experiments, which are already in progress.

Author Contribution

EG and GH provided the structure and conditions to develop the experiments and conducted them. 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.

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