Growth analysis of six lisianthus (*Eustoma grandiflorum***) cultivars in different growing seasons**

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Abstract

The objective of work was to evaluate the growth of six lisianthus cultivars at different times, in a protected environment, in the southern region of Brazil. The experimental design was randomized blocks, in split plots, with three replications. The plots were constituted by the lisianthus cultivars: 'White Excalibur', 'Blue Picotee', 'Blue Echo', 'Arena Red', 'Robella' and 'Echo Champagne'and the subplots by the dates of plant collections (evaluations), which were carried out at the time of transplantation (0) and 21, 42, 63 and 84 days after transplantation (DAT) for seasons 1(October 2017 to January 2018), 2 (November 2017 to February 2018) and 5 (August to December 2018); and at (0) and at 30, 60, 90, 120, 150 and 180 DAT at seasons 3 (April to November 2018), 4 (July to December 2018) and 6 (February to June 2019). All cultivars showed an increasing tendency to accumulate a dry mass of the plants until the harvest of the floral stems. The maximum dry mass value was obtained by the cultivar 'Robella' (20.3 g/plant-1) in season 3. For all cultivars, the absolute and relative growth rates of the crop increased with the advancement of the cultivation period. In seasons 1, 2 and 5 all cultivars showed higher growth velocities between 63 and 84 days after transplanting (DAT). In seasons 3 and 4 the cultivars showed higher growth from 90 to 150 DAT. In seasons 1 and 2 the cultivars showed a higher rate of plant growth, resulting in a shortening of the cycle. The cultivars conducted in seasons 3 and 4 had a longer cultivation time due to the autumn/winter period. The cultivar 'Arena Red' showed the lowest growth rates in all seasons.

Keywords: climatic variables, cut flowers, cycle, floriculture, lisianthus

Introduction

The Lisianthus [Eustoma grandiflorum (Raf.). Shinn.] is an annual cycle herbaceous ornamental plant, belonging to the *Gentianaceae* family, native to northern Mexico and the southern United States (kuronuma et al., 2020).

The species is one of the most popular ornamental plants in the world, due to its beauty, color diversity, and post-harvest durability, which have contributed to increased production in recent decades (Kawakatsu et al., 2018).

In Brazil, the culture is of great importance in the segment of cut flowers, being among the main species commercialized in the country. However, there is a lack of information regarding the adaptation of cultivars to different growing seasons in the southern region of Rio Grande do Sul, as it is a species that is still not widespread in the region of Pelotas.

Lisianthus growth is characterized as slow, from seedling planting to flower harvest, and it may take 22 to 28 weeks to reach the flowering stage Seydmohammadi et al. (2019), depending on the conditions and growing season. In this way, it is important to know the performance of cultivars through growth analysis throughout their cultivation under different conditions.

Growth analysis aims to quantify the performance of genotypes, making it possible to understand the different morpho physiological processes involved throughout plant development (Zuffo et al., 2016). The study of growth analysis translates the primary production and is considered an effective practice to evaluate the net production derived from the photosynthetic process (Benincasa, 2003). Several factors can cause changes in the plant growth process, and their effects can be studied from the growth rates (Da Silva et al., 2015). Through these analyzes, the causes of variations in plant development

can be estimated (DE Oliveira et al., 2018).

In this context, the study of the growth of cultivars as a function of the growing season is a tool that allows evaluating the behavior of plants for later choosing the material to be adopted for better quality production, considering that the use of more adapted cultivars is a fundamental factor in the production system. Thus, to obtain good quality lisianthus flowers throughout the year, it is necessary to know the growth of different cultivars under different growing seasons and conditions.

Given the above, the objective of this work was to evaluate the growth of six lisianthus cultivars produced at different growing seasons in the southern region of Rio Grande do Sul.

Material and Methods

The study was conducted during the 2017-2018 and 2018-2019 agricultural years on a rural property located in the municipality of Capão do Leão, RS, with approximate geographic coordinates: latitude 31°46'34'' S, longitude 52°21'34'' W and altitude of 13 m. The climate of this region is characterized as humid subtropical, with well-distributed rainfall and hot summer, classified as Cfa type according to (Köppen, 1936).

To carry out the experiments, a plastic greenhouse model "Arch Ceiling" was used, with a wooden structure, covered with a low-density polyethylene film (100 μm thick), with dimensions of 10.0 m x 24.0 m and 3.5 m (width x length x height), arranged in the L - W direction. The management of the environment was carried out by daily opening and closing the curtain sides of the greenhouse.

The cultivation system adopted was conventionally used for the species: in beds made directly in the soil. For each experiment (planting season), three beds measuring 6.00 m long by 1.00 m wide and 0.15 m high were used, with paths between them of 0.50 m, arranged parallel to the direction of the length. of the greenhouse. Each bed represented a block.

The planting of 40 plants per bed of each cultivar under study was carried out, totaling 720 plants in each experiment. These plants were arranged in six rows with a spacing of 0.10 m between rows and 0.10 m between plants. Considering a population of 240 plants per bed and a useful area of 6.0 m², the planting density in the bed, without considering the path, was 40 plants m². Each plot was divided into six plots of 1.0 m², corresponding to the six cultivars. The fertigation of the plants was done using drip hoses.

Simple soil samples were collected at several points before starting the experiments and after the work was finished. The collections were carried out according to the technical recommendations for soil collection for analysis and sent to the Laboratory of Chemical and Physical Analysis of Soils, Federal University of Pelotas.

The limestone requirement calculation was adjusted to a pH value of 5.5 to 6.5. Liming was carried out according to the initial chemical analysis of the soil, using dolomitic limestone, seeking to increase the base saturation (V%) to 75%. The base fertilization was carried out before the installation of the experiments according to the soil analysis. Fertigation was carried out considering the number of nutrients in the soil, being carried out once a week in the spring/summer seasons and once every 15 days in the autumn/winter seasons. The fertilizer formula used in the fertigation of the plants was NPK: 13-2-44 of Ouro Fértil®, used by the producer and also recommended for other species of flowers. Irrigation was performed on average once a day in spring/summer and once a week during autumn/winter, as needed.

The experimental design was randomized blocks, in split plots, with three replications. The plots were constituted by the lisianthus cultivars: 'White Excalibur', 'Blue Picotee', 'Blue Echo', 'Arena Red', 'Robella' and 'Echo Champagne'and the subplots by the dates of plant collections (evaluations), which were carried out at the time of transplantation (0) and 21, 42, 63 and 84 days after transplantation (DAT) for seasons 1(October 2017 to January 2018), 2 (November 2017 to February 2018) and 5 (August to December 2018); and at (0) and at 30, 60, 90, 120, 150 and 180 DAT at seasons 3 (April to November 2018), 4 (July to December 2018) and 6 (February to June 2019). The last evaluation date corresponded to the point of harvesting the floral stems (**Table 1**) provides detailed information on the six experiments performed.

During each growing season, the main characteristics related to the growth of the lisianthus crop were analyzed: plant height, leaf area, dry mass of plants e based on AF and DM, the leaf area ratio (RAF), the absolute growth rate (ACR), and the relative growth rate (RCR). Plant height (AP) was obtained with the aid of a graduated ruler, measuring from the base of the plant (next to the ground) to its highest point. This measurement was performed on six plants for each treatment, two in each repetition. For the leaf area (FA) evaluation, a LICOR® leaf area integrator, Model LI-3100, was used.

After the AP and AF measurements, the fresh mass of the plants was determined using a precision analytical balance (0.0001g) and then the stems were packed in paper bags and placed to dry in an oven with forced air circulation. with a temperature of 60 ºC, until reaching constant weight, to determine their dry mass

** Assessments at 0, 21, 42, 63, and 84 days after transplantation.

***Evaluations at 0, 30, 60, 90, 120, 150, and 180 days after transplantation.

Tº: temperature daily average, maximum, and minimum (ºC) RH: maximum and minimum relative humidity (%)

RS: global external solar radiation (MJ/m2/day-1)

Source: author herself.

(DM). Based on AF and DM, the leaf area ratio (RAF), the absolute growth rate (ACR), and the relative growth rate (RCR) were determined, according to Benincasa (2003):

 $RAF = AF/MS$ (cm2/g -1), where AF is the leaf area; and MS, the plant dry mass;

 $TCA = (P2 - P1)/(T2 - T1)$ (g day-1) absolute growth rate;

 $TCR = \ln(P2) - \ln(P1) / (T2 - T1)$ (g g-1 day-1) relative growth rate;

Where P2 and P1 correspond to the masses of two successive samplings and T2 and T1 represent the sampling times, in this case, this time difference was set at 21 and 30 days according to Table 1; ln(P2) and ln(P1) were the natural logarithms of the dry mass of two successive samplings.

The results obtained were submitted to analysis of variance, from which, the effects of cultivars, evaluation dates, and the interaction between cultivars and data were analyzed by the F test (p<0.05). Cultivar means were compared by Tukey's test at 5% probability. The effect of the sampling data was represented through regression analysis based on statistical significance, biological significance, and the value of the coefficient of determination (R2).

Results and Discussion

Analysis of variance showed that there was a significant interaction between cultivars and collection dates for the variables AP, MS, AF, in seasons 1 (October 2017 to January 2018) and 2 (November 2017 to February 2018), in seasons 3 (April to November 2018) and 4 (July to December 2018) for MS, AF and RAF and in season 6 (February to June 2019) for the AP and AF. For the other variables, no interaction and significant differences were found between cultivars and collection dates, as well as at season 5 (August to December 2018). The data of all variables: AP, MSAF, RAF, TCA, TCR showed a quadratic trend for all cultivars in the six periods studied (**Figures 1**, **2**, **3**, **4**, **5**, and **6**).

There were significant differences between the cultivars for the variables AP, MS, and AF at 63 and 84 DAT at 1(October 2017 to January 2018) and 2 (November 2017 to February 2018), and the cultivar 'Arena Red' was inferior to the others, which did not differ from each other. In season 2(November 2017 to February 2018), significant differences were observed between the cultivars at the beginning of the evaluations at 0 DAT for the RAF, in which the 'Blue Echo' and 'Arena Red' cultivars were superior to the others, which did not differ statistically. At the same time, the cultivars showed statistical differences for the RGR from 42 to 63 DAT, and 'Arena Red' was inferior to the others, which did not differ between them.

In season 3 (April to November 2018) statistical differences were observed between the cultivars from 150 to 180 DAT for the variables AP, MS, and TCA, with lower means for 'Arena Red', while the other cultivars did not show differences. At the same time, the cultivars showed differences for AF at 180 DAT, in which 'Robella', 'Blue Echo', and 'Echo Champanhe' were superior to 'White Excalibur' and 'Blue Picotee', while 'Arena Red' obtained lower average.

In season 6 (February to June 2019) there were statistical differences between the cultivars studied from 63 to 90 DAT for the parameters AP and AF, in which 'Arena Red' was lower, followed by 'Echo Champanhe' and 'Blue Picotee'. The other cultivars did not show significant differences between them at the same time.

In general, there was a slow increase in the variables analyzed in the first evaluation dates, with the same behavior trend at different times. The slow growth in the initial phase of lisianthus cultivation is a characteristic factor of the culture that, after planting, takes a considerable period to establish itself (Ahmad et al., 2017). At the beginning of the cycle, when plants are young, there is a low photosynthetic capacity, due to the small leaf area and, consequently, low production of dry matter, which results in reduced growth.

The results showed that the greatest gains in plant

Figure 1: Evolution of the height of plants(A), dry mass (B), leaf area (C), leaf area ratio (RAF) (D), absolute growth rate (ACR) (E), and relative growth rate (RCR) (F), of lisianthus (Eustoma Grandiflorum) cultivars 'Blue Echo' (BE), 'Robella' (RO), 'White Excalibur' (WE), 'Echo Champanhe' (EC), 'Blue Picotee' (BP) and 'Arena Red ' (AR) cultivated in season 1 (October 2017 to January 2018) in a protected environment. Capão do Leão – RS, 2020.

Figure 2: Evolution of the height of plants(A), dry mass (B), leaf area (C), leaf area ratio (RAF) (D), absolute growth rate (ACR) (E), and relative growth rate (RCR) (F), of the lisianthus (Eustoma Grandiflorum) cultivars 'Blue Echo' (BE), 'Robella' (RO), 'White Excalibur' (WE), 'Echo Champanhe' (EC), 'Blue Picotee' (BP) and 'Arena Red ' (AR) cultivated in season 2 (November 2017 to February 2018) in a protected environment. Capão do Leão – RS, 2020.

Figure 3: Evolution of the height of plants(A), dry mass (B), leaf area (C), leaf area ratio (RAF) (D), absolute growth rate (ACR) (E), and relative growth rate (RCR) (F), of lisianthus (Eustoma Grandiflorum) cultivars 'Blue Echo' (BE), 'Robella' (RO), 'White Excalibur'(WE), 'Echo Champanhe' (EC), 'Blue Picotee' (BP) and 'Arena Red' (AR) cultivated in season 3 (April to November 2018) in a protected environment. Capão do Leão – RS, 2020.

Figure 4: Evolution of the height of plants(A), dry mass (B), leaf area (C), leaf area ratio (RAF) (D), absolute growth rate (ACR) (E), and relative growth rate (RCR) (F), of lisianthus (Eustoma Grandiflorum) cultivars 'Blue Echo' (BE), 'Robella' (RO), 'White Excalibur'(WE), 'Echo Champanhe' (EC), 'Blue Picotee' (BP) and 'Arena Red' (AR) cultivated at season 4 (July to December 2018), in a protected environment. Capão do Leão – RS, 2020.

Figure 5: Evolution of the height of plants of the cultivars (A), dry mass (B), leaf area (C), leaf area ratio (RAF) (D), absolute growth rate (ACR) (E), relative growth rate (RCR) (F), of lisianthus (Eustoma Grandiflorum) cultivars 'Blue Echo' (BE), 'Robella' (RO), 'White Excalibur'(WE) and 'Echo Champanhe' (EC) 'Blue Picotee' (BP) and 'Arena Red' (AR) grow in season 5 (August to December 2018), in a protected environment. Capão do Leão – RS, 2020.

Figure 6: Evolution of the height of plants of the cultivars (A), dry mass (B), leaf area (C), leaf area ratio (RAF) (D), absolute growth rate (ACR) (E), relative growth rate (RCR) (F), of lisianthus (Eustoma Grandiflorum) cultivars 'Blue Echo' (BE), 'Robella' (RO), 'White Excalibur'(WE) and 'Echo Champanhe' (EC) 'Blue Picotee' (BP) and 'Arena Red' (AR) grow in season 6 (February to June 2019), in a protected environment. Capão do Leão – RS, 2020.

growth, represented mainly by height, MS, and AF, were observed from a certain stage of crop development, depending on the growing season, for all cultivars analyzed.

All cultivars showed more significant growth from 42 DAT, with greater intensity from 63 to 84 DAT, when cultivated in seasons 1(October 2017 to January 2018), 2 (November 2017 to February 2018), and 5 (August to December 2018), showing higher growth speed in the most advanced stages of cultivation (Figures 1, 2 and 5). This result is consistent with what was observed by CUNHA et al. (2015), who observed that the growth of lisianthus occurs up to 84 DAT, and from this stage on wards the process of plant senescence begins.

When the cultivars were conducted in seasons 3 (April to November 2018) and 4 (July to December 2018), it was detected that the greatest gain in growth occurred from 60 DAT and more intensely from 90 to 150 DAT (Figure 3 and 4), which shows a more accentuated growth, according to the advance of the cultivation stages also in these two seasons.

In general, cultivars when conducted in seasons 4 (July to December 2018) and 3 (April to November 2018) had the longest cultivation time, up to 150 and 180 DAT, in that order respectively, demonstrating that the crop cycle was longer, caused by winter conditions, such as low temperatures and lower solar radiation (Table 1).

Souza Neto (2017), evaluating lisianthus in a protected environment, obtained a variation of 2.2MJ/ m2/day-1of solar radiation, which resulted in a 50-day shortening of the second cycle (November to February), compared to the first (May to September). In the present study, in seasons 3 (April to November 2018) and 4 (July to December 2018), the lower solar radiation, associated with low temperatures, extended the period of cultivation of the cultivars when compared to the others.

In this context, it is worth noting that for the production of lisianthus in these times of climatic restrictions, it is essential to use climate-controlled greenhouses for the adequate control of environmental variables, especially in regions such as the South of Brazil, where there is a high amplitude of the values of the elements of the climate, mainly, of the temperature and the relative humidity of the air, in the greenhouses. Otherwise, there will be a delay in the cultivation cycle, compromising production planning.

As the growth parameters increased, as well as height and FA along the stages, MS also increased considerably (Figures 1B, 2B, 3B, 4B, 5B, and 6B). This phase was characterized by the formation of new structures in

plants, such as flower buds and flowers. This condition led to a substantial increase in plant DM, resulting in greater growth at the end of the lisianthus developmental stages.

(**Table 2** and **3**) show the evolution of the DM dynamics of plants of different cultivars throughout the cultivation cycle.

The maximum value of DM accumulation was obtained by the 'Blue Echo' cultivar, which reached an average of 8.1 g/plant⁻¹ at the end of the analyzed stages, while 'Arena Red' obtained a lower average (4.0 g/plant-1) in the same period (Table 2). The highest production of MS observed over the evaluation dates until the end was in seasons 1 (October 2017 to January 2018) and 2 (November 2017 to February 2018), while the lowest performance occurred in season 5 (August to December 2018) (Table 2). Seasons 1 and 2 were conducted in the spring period, which favored the conditions for cultivation at this stage.

When the cultivars were produced in the 3 seasons (April to November 2018) and 4 (July to December 2018) showed higher values for DM production at the end of the evaluations (Table 3). The highest DM accumulation observed at the end was for the cultivar 'Robella' (20.3 g/plant-1) and with lower means 'Arena Red' (11.4 g/ plant-1) (Table 3). However, it is observed that in seasons 3(April to November 2018) and 4 (July to December 2018), the cultivars had a longer cycle, accumulating more phytomass than in the other seasons as a function of time. The lowest DM production accumulated at the end of the experiment occurred in season 6 (February to June 2019), which, however, was of a shorter duration compared to season 3. (April to November 2018) and 4 (July to December 2018).

DM production increased progressively and was higher at the end of the evaluations in all cultivars, demonstrating the expected behavior that the dry matter of plants increases with leaf expansion, the increase in plant growth and age, as long as the evaluations are completed before the senescence process, as was the case in the present research. This is a usual behavior and several authors also report it for other cultures, such as aster (Zosiamliana et al., 2012), gerbera (Singh et al., 2017), and mini watermelon (Marques et al., 2016). The accumulation of MS stems from the increase in FA, which rises as the plant increases the number of leaves and its capacity for photosynthesis and, consequently, for the production of assimilates, leads to a greater accumulation of dry matter, which is responsible for plant growth.

Regarding the leaf area ratio (RAF) the cultivars showed similar behavior. The highest values found for this

Table 2. Dynamics of dry mass evolution of six lisianthus cultivars (*Eustoma Grandiflorum*) cultivated at different times, in a protected environment in southern Brazil. Capão do Leão – RS, 2020

* Represented by the total MS of plants.

Means in the column followed by the same letter, for each cultivar and evaluation period, do not differ from each other, using the Tukey test at 5% probability (ns) non-significant differences. Source: author herself.

Table 3. Dynamics of dry mass evolution of six lisianthus cultivars (Eustoma Grandiflorum) cultivated at different times, in a protected environment. Capão do Leão – RS, 2020

*Represented by the total DM of plants.

Means in the column followed by the same letter, for each variable and evaluation period, do not differ from each other, using the Tukey test at 5% probability. (ns) non-significant differences.

parameter occurred in the first evaluations (Figure 1D, 2D, 3D, 4D, 5D, and 6D). Throughout the stages of crop development, a significant decrease in RAF rates was observed in all treatments in seasons 1 (October 2017 to January 2018), 2 (November 2017 to February 2018), and 3 (April to November 2018) (Figure 1D, 2D, and 3D). For cultivars grown in seasons 4 (July to December 2018), 5 (August to December 2018), and 6 (February to June 2019), there was a small increase in RAF between 40 and 80 DAT, approximately, which then established and declined at the end (Figures 4D, 5D and 6D). According to Benincasa (2003), the RAF declines as the plant grows, due to the increase in the interference of upper leaves on the lower ones, as a consequence of self-shading with

the age of the plant.

In this study, the decline in RAF occurred in all cultivars, demonstrating that the availability of photoassimilates to the leaves reduced throughout the cycle, being more expressive at the end, probably due to the emergence of non-assimilatory structures such as floral buds and flowers.

The analysis of RAF allows detecting the translocation of photoassimilates to the leaves about the dry mass produced, being characterized as a morphophysiological component that expresses the leaf area useful for photosynthesis. (Abade, 2018). In this sense, the lisianthus cultivars behaved similarly throughout the evaluated period, indicating a gradual decline in the

distribution of assimilates to the leaves at the end of the evaluations. This behavior was also observed by other authors in other species, such as zinia (Kumar & Marwein, 2018) and the chrysanthemum (Dewan et al., 2016).

In general, the TCA of lisianthus plants followed similar dynamics for all cultivars, with continuous growth during cultivation in seasons 1 (October 2017 to January 2018), 2 (November 2017 to February 2018), 3 (April to November 2018), 4 (July to December 2018), 5 (August to December 2018) and 6 (February to June 2019). Lower TCA was found for all cultivars in seasons 5 and 6. The lower dry mass produced in this period resulted in lower TCA values. Among the cultivars, only the cultivar 'Arena Red' showed significantly lower values of TCA in all evaluated times, with no differences being detected between the others (Table 2 and 3).

The cultivars are grown in seasons 1 (October 2017 to January 2018) and 2 (November 2017 to February 2018) (Figures 1 and 2) showed faster growth rates and shorter cycle duration. Bearing in mind that these crops took place in spring when environmental conditions are more favorable.

However, the cultivation cycle of the same cultivars in seasons 3 (April to November 2018) and 4 (July to December 2018) significantly lengthened (Figures 3 and 4). A large part of the experimental period of these seasons coincided with autumn/winter. In season 3 (April to November 2018), the average daily solar radiation and temperatures were the lowest (Table 1) among the experiments, which in itself explains the longer cycle. For season 4 (July to December 2018), the average values (Table 1) of these climate variables increased, since this was a very long cycle that advanced during the spring. However, the post-planting stage and the first stages of plant development took place during the winter, whose conditions of light and temperature in the region are limited. Thus, it turns out that

As for the performance of cultivars regarding RCT, the data reveal a low growth between one evaluation and another, with a tendency to increase at the end of cultivation (Figures 1F, 2F, 3F, 4F, 5F and 6F). Initially, there was no relative growth of cultivars at all times, given that the RGR was negative at the beginning of the evaluations. This reveals that there was no real growth of the plants, which can be attributed to the low capacity of assimilation and conversion of light into phytomass, due to the reduced FA, characteristic of the species in the initial stages, and consequently, low accumulation of initial dry matter.

The subsequent increase in TCR values (Figures

1F, 2F, 3F, 4F, 5F, and 6F) evidence the fact that lisianthus plants express greater vigor in the more advanced stages when they increase the capacity to produce DM concerning the existing phytomass.

In the present work, the greatest growth of plants was observed at the end of the vegetative period and entry into the reproductive phase, when there was a greater expansion of AF and accumulation of dry mass (Figures 1, 2, 3, 4, 5 and 6), with the stems, then harvested, which prevented the entry into senescence and the final drop in growth rates.

Therefore, it can be inferred that the growth rates are favored by the advance of the cultivation period. However, it is worth noting that in seasons 1 (October 2017 to January 2018) and 2 (November 2017 to February 2018), the cultivars had satisfactory performance and the cycle was shortened, with a greater balance between growth and time of cultivation.

Conclusions

Lisianthus growth is influenced by seasons and crop growth rates increase as the growing season progresses. All the cultivars studied show an increasing tendency of DM accumulation in the plants, with continuous growth until the harvest of the stems in all evaluated times. The cultivar 'Arena Red' presents lower mass accumulation and growth, regardless of the growing season. The maximum dry mass value was obtained by the cultivar 'Robella' (20.3 g/plant⁻¹) in season 3 (April to November 2018). The cultivars Excalibur', 'Blue Picotee', 'Blue Echo', 'Robella' and 'Echo Champagne' present a similar growth pattern in all growing seasons. In seasons 1 (October 2017 to January 2018) and 2 (November 2017 to February 2018) all cultivars showed higher speed in plant growth rate, resulting in a shortening of the cycle. The cultivars conducted in seasons 3 (April to November 2018) and 4 (July to December 2018) showed longer cultivation time, due to the influence of climatic variables in the autumn/ winter period.

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