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MODEL TO ESTIMATE THE OPTIMAL BLOOMING AND FLOWERS HARVESTING INTERVAL IN *Lisianthus exaltatum* IN RELATION TO VEGETATION PERIOD

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Abstract

The study followed the flowering dynamics over a 84-days vegetation period, at four genotypes of the species Lisianthus exaltatum Salisb. The biological material was represented by the Twinkles Dark Blue (TDB), Arena Series Rose (ASRose), Arena Series Red (ASRed) and Heidi Salmon (HS) genotypes. In relation to the biology of the analyzed genotypes, the vegetation period (VP) under study was of 84 days during which, five flower-counting moments were delineated at 14-days intervals, VP28, VP42, VP56, VP70 and VP84. Based on the average number of flowers open at the time of determination, the highest number of flowers was found in the TDB genotype, followed by genotypes HS, ASRose and ASRed. On the basis of the univariate statistics analysis, the highest variance was found for genotypes TDB (10.6194) and ASRose (10.24407) and a lower variance in HS genotypes (5.60978) and ASRed (5.0022), respectively. The coefficient of variation (CV) had the highest value for the ASRose genotype (CV = 111.4428), followed by the ASRed genotype (CV = 86.0215), then TDB (CV = 66.5049) and HS (CV = 62.4178), respectively. The statistical regression analysis facilitated the development of a model of a grade 3 polynomial equation and smoothing spline models (for the ASRose, ASRed and HS genotypes), models that most accurately described the flowering dynamics in relation to the vegetation period. Thus, a model of a grade 3 polynomial equation facilitated the estimation of flowering over the study period to the TDB genotype under $R^2 = 0.996$, $F = 90.681$, $p = 0.0770$. In the other three genotypes smoothing spline models described the most accurate growth dynamics during the vegetation period under conditions of $\varepsilon_i = 0.2098$ at the ASRose genotype, $\varepsilon_i = 0.0593$ at the ASRed genotype and $\varepsilon_i = 0.0607$ in the HS hybrid. Clustering analysis has facilitated the classification and grouping of observational moments from the study period into two distinct, statistically safe clusters, Coph. corr = 0.899.

Key words*: flowers, Lisianthus, polynomial equation, smoothing spline model, vegetation period.*

INTRODUCTION

As a relatively new crop for cut flowers, *Lisianthus* ranked relatively fast in the top ten in the international market, especially due to its very good post-harvest time, then it's beautiful flowers, in the form of roses, of the colorful flowers colored in blue, but also a in wide range of floral designs such as sizes and colors available through improved genotypes (Harbaugh, 2007; Baris and Uslu, 2009; Uddin et al., 2013). With the highly diversified and improved genetic material (species, hybrids and varieties) grown predominantly for cut flowers, the importance of *Lisianthus* (*Eustoma grandiflorum* Salisb.) has increased greatly over the last decades, also associated with consumer sensitivity and preference for these flowers, being one of the important categories for cut flowers in the US market, then Europe, Asia and Australia (Harbaugh et al., 2000; Barba-Gonzalez et al., 2017).

Among the conditions of vegetation, a pronounced effect is represented by the high temperatures that cause the rosette and the continuous vegetative growth of *Eustoma grandiflorum*. As a result, many studies have compared the behavior of the biological material represented by varieties and hybrids of the genus *Lisianthus* in cultivation conditions characterized especially by high temperatures (Harbaugh et al., 1992; Ohkawa et al., 1991,

1994; Bradley et al., 2000; Zaccai and Edri, 2002). For floral induction, *Eustoma* requires passing through a period of lower temperatures, which in cultivation conditions becomes a mandatory treatment (Li et al., 2015).

Harbaugh et al. (1992) considered that a significant percentage of rosette plants that did not bloom in an acceptable period (≈140 days) are a very important factor limiting *Lisianthus* production with negative economic effects.

Due to the increasing demand for *Lisianthus* in the flower market, a series of studies and improvement programs have followed the floral induction by technological and biochemical means, through ample processes of improvement (Harbaugh, 2007; Barba-Gonzalez et al., 2017 a, b).

Using improvement programs, F1 seed hybrids were obtained with bloom uniformity throughout the year, eradication of rosettes, better heat tolerance, a larger variety of petals, flowers of varying sizes and shapes, flowers with double petals and high resistance to disease (Harbaugh, 2007). Various studies have evaluated the resistance of *Lisianthus* plants to *Fusarium* and *Botrytis cinerea*, the most common diseases (Harbaugh and McGovern, 2000; Wegulo and Vilchez, 2007).

Modern techniques of molecular biology have led to *Lisianthus* various colors and perfume of different flowers, but also variable flowering time. Molecular and reproductive *in vitro* technologies aim to increase plant post-trans-plant tolerance, heat tolerance, photo rejuvena-tion corrections through day neutrality, early flowering and longer flower life, increased resistance to *Fusarium* (Harbaugh, 2007; Barba-Gonzalez et al., 2017). Modern techno-logies based on biotechnology have many ad-vantages for inducing advantageous floral attri-butes in ornamental plants (Noman et al., 2017).

Through interspecific crossing between *Eustoma exaltatum* and *Eustoma grandiflorum*, followed by programmed and rigorous selections, valuable hybrid forms were obtained in the following generations: F1, BC1, S1 and S2. Thus, wide variations of color were obtained based on the colors of the parents used in the breeding program, while the forms with a better heat tolerance were selected (Barba-Gonzalez et al., 2017a, b). Also by biotechnology-based methods a pigmentation of petals and sepals in *Lisianthus* was obtained and induction of changes in different floral characteristics (Schwinn et al., 2014).

At the same time, some significant phenotypic alterations have been reported in Lisianthus transgenic plants in terms of reducing the number of flowers and the flowering time (Zuker et al., 2001; Casanova et al., 2004; Aranovich et al., 2007; Thiruvengadam and Yang, 2009; Ruokolainen et al., 2011).

Due to the dependence of *Lisianthus* plants on photoperiods, some studies evaluated the influence of two photoperiod regimens (long day and short day) on the floral transition to *Lisianthus* plants (*Eustoma grandiflora* (Raf) Shinn.) and highlighted the strong influence of this environmental factor on floral induction (Zaccai and Edri, 2002). The effect of the vernalization and post-vernalization process on flowering has also been studied at *Eustoma grandiflorum* (Nakano et al., 2011).

Studies of aspects of "gene expression" for floral induction at *Eustoma grandiflorum* have also been performed (Nakano et al., 2011; Li et al., 2015). Aspects of growth and physiology of *Lisianthus* plants have been studied in relation to growing media and growth stimulators (Crăciun and Băla, 2015 a, b, 2016 a, b).

The present study aimed to evaluate the flowering dynamics of some genotypes of *Lisianthus exaltatum* Salisb., in relation to the vegetation period.

MATERIALS AND METHODS

Biological material

Four genotypes belonging to *Lisianthus exaltatum* Salisb.: Twinkles Dark Blue (TDB), Arena Series Rose (ASRose), Arena Series Red (ASRed) and Heidi Salmon (HS) represent the studied biological material. Twinkles Dark Blue (TDB) is a genotype of dark blue flowers, with large and simple petals. It has a height of up to 100 cm, being recommended for curbs or cut flowers. Arena Series Red (ASRed) is a high-quality genotype with bright red double flowers, unique for *Lisianthus.* The flowers have a diameter of up to 6 cm and the height of the stem is 80-100 cm; blooming is late. Arena Series Rose (ASRose) is a genotype belonging to the *Lisianthus* Arena Series group. The ASRose hybrid has double pink-rose flowers.

Flower stems are strong and 80-100 cm long. Heidi Salmon (HS) is a simple, large, pink-salmon-type genotype. It has early flowering, and the height of the floral stems up to 100 cm.

Experimental conditions

Plants of the four genotypes were grown on a substrate of 2: 3: 1 soil, peat and sand, in nutrient pots with a volume of 10 dm^3. The experiments were conducted in greenhouse with controlled temperature and humidity, with the recommended germination temperature of 18-22ºC at TDB, ASRed and ASRose, respectively 22-24ºC at SH. The rising temperature was reduced to 16-22ºC.

Studied floral index

The number of flowers opened in dynamics during the vegetation period has been studied with a total time span of 84 days. Determinations were made at 14-days intervals, respectively at 28, 42, 56, 70 and 84 days.

Statistical analysis of experimental data

The distribution of the number of open flowers in relation to the vegetation period of the four *Lisianthus* genotypes was analyzed with the statistical method in the Excel 2007 application and the PAST software (Hammer et al., 1997). The variance analysis facilitated LSD in order to compare the differences between the variants and the significance attribution in relation to LSD framing. The overall data was analyzed based on variance and coefficient of variation (CV). The behavior of the experimental data according to the vegetation period was described by polynomial models, the splines model and the database and tabular calculations. For the smoothing spline model, predictive error testing was calculated using the relationship (1), looking for the mean error value to be as close to zero.

$$\bar{\varepsilon} = \left(\sum_{i=1}^{n} \varepsilon_i \right) / n = \left(\sum_{i=1}^{n} \left| \frac{ys_i - y_i}{y_i} \right| \right) / n \qquad (1)$$

The evolution of values for the number of open flowers was also described by the growth index from the fixed base $I_{i/1} = y_{si}/y_{s1}$. This index expressed the degree of multiplication and the evolution of the number of flowers at the moments of determination during the vegetation period compared to the initial value, considered as the fixed base. For the polynomial equation, statistical safety parameters of the identified relationship were the correlation coefficient (R^2), the statistical safety parameter p, the sample F.

RESULTS AND DISCUSSIONS

The experimental results on the number of opened flowers during the vegetation period for the four genotypes cultivated in similar conditions, revealed the differential behavior of each of them. The variation of the average number of flowers during the vegetation period, at the unit determinations, was between 0.88-8.66 for TDB genotype, 0.00-7.12 for ASRose genotype, 0.50-5.88 for ASRed genotype and 1.12-7.00 for HS genotype, detailed experimental data being shown in Tables 1-4.

Table 1. The effect of vegetation period on flowers number of Twinkles Dark Blue (TDB) genotype

Vegetation period (days)	Number of flowers		Differences / Significance
28-14	3.62	4.00	-0.38
42-14	8.62	4.00	4.62***
56-14	7.88	4.00	3.88***
70-14	3.50	4.00	-0.50
84-14	0.88	4.00	-3.12ººº
42-28	8.62	3.62	5.00***
56-28	7.88	3.62	4.26***
70-28	3.50	3.62	-0.12
84-28	0.88	3.62	-2.74ººº
56-42	7.88	8.62	-0.74
79-42	3.50	8.62	-5.12ººº
84-42	0.88	8.62	-7.74ººº
70-56	3.50	7.88	-4.38ººº
84-56	0.88	7.88	-7.00ººº
84-70	0.88	3.50	-2.62ººº
$LSD_{5\%} = 0.66$; $LSD_{0.1\%} = 0.87$; $LSD_{0.01\%} = 1.12$			

Table 2. The effect of vegetation period on flowers number of Arena Series Rose (ASRose) genotype

Vegetation period (days)	Number of flowers		Differences / Significance
28-14	0.00	0.00	0.00
42-14	5.37	0.00	5.37***
56-14	7.12	0.00	7.12***
70-14	1.62	0.00	1.62***
84-14	0.25	0.00	0.25
42-28	5.37	0.00	5.37***
56-28	7.12	0.00	7.12***
70-28	1.62	0.00	1.62***
84-28	0.25	0.00	0.25
56-42	7.12	5.37	1.75***
79-42	1.62	5.37	-3.75
84-42	0.25	5.37	-5.12ººº
70-56	1.62	7.12	-5.50ººº
84-56	0.25	7.12	-6.87ººº
84-70	0.25	1.62	-1.37ººº
$LSD_{5\%} = 0.66$; $LSD_{0.1\%} = 0.87$; $LSD_{0.01\%} = 1.12$			

Table 3. The effect of vegetation period on flowers number of Arena Series Rose (ASRose) genotype

Vegetation period (days)	Number of flowers		Differences / Significance
28-14	0.50	0.25	0.25
42-14	3.00	0.25	2.75***
56-14	5.88	0.25	5.63***
70-14	3.12	0.25	2.87***
84-14	0.50	0.25	0.25
42-28	3.00	0.50	2.50***
56-28	5.88	0.50	5.38***
70-28	3.12	0.50	2.62***
84-28	0.50	0.50	0.00
56-42	5.88	3.00	2.88***
79-42	3.12	3.00	0.12
84-42	0.50	3.00	-2.50ooo
70-56	3.12	5.88	-2.76ooo
84-56	0.50	5.88	-5.38ooo
84-70	0.50	3.12	-2.62ooo
$LSD_{5\%} = 0.66$; $LSD_{0.1\%} = 0.87$; $LSD_{0.01\%} = 1.12$			

The Univariate statistical analysis eased the identification of the genotype behavior based on the experimental data obtained in terms of bloom in relation to the vegetation period. A higher variance was recorded for genotypes TDB (10.6194) and ASRose (10.24407) and a lower variance for genotypes HS (5.60978), respectively ASRed (5.0022).

Table 4. The effect of vegetation period on flowers number of Heidi Salmon (HS) genotype

Vegetation period (days)	Number of flowers		Differences / Significance
28-14	3.12	1.88	1.24***
42-14	5.50	1.88	3.62***
56-14	7.00	1.88	5.12***
70-14	2.38	1.88	0.50
84-14	1.12	1.88	-0.76
42-28	5.50	3.12	2.38***
56-28	7.00	3.12	3.88***
70-28	2.38	3.12	-0.74
84-28	1.12	3.12	-2.00ooo
56-42	7.00	5.50	1.50***
79-42	2.38	5.50	-3.12ooo
84-42	1.12	5.50	-4.38ooo
70-56	2.38	7.00	-4.62ooo
84-56	1.12	7.00	-5.88ooo
84-70	1.12	2.38	-1.26ooo
$LSD_{5\%} = 0.66$; $LSD_{0.1\%} = 0.87$; $LSD_{0.01\%} = 1.12$			

The coefficient of variation, showing the non-uniformity of the analyzed parameter (flowering), had the highest value for the ASRose genotype (CV = 111.4428), followed by the ASRed genotype (CV = 86.0215), then TDB genotype (CV = 66.5049) and HS genotype (CV = 62.4178).

The specific statistical analysis evaluated the interdependence between the number of flowers and the vegetation period and eased the development of patterns of behavior for the studied genotypes in relation to time (in days). These models were of the polynomial equation and smoothing spline type.

In case of TDB genotype, a model of the type polynomial equation 3rd degree, equation (2), described most accurately the blooming behavior in relation to the vegetation period, under $R^2 = 0.996$, $F = 90.681$, $p = 0.0770$. The graphical distribution of the number of opened flowers during the vegetation period is shown in Figure 1.

$$y = 0.0002278x^3 - 0.04515x^2 + 2.686x - 41.24 \quad (2)$$

In case of ASRose genotype, the distribution of flowers in relation to the vegetation period was best described by a smoothing spline model, the values and terms of the equation being shown in Table 5, and the graphical distribution in Figure 2.

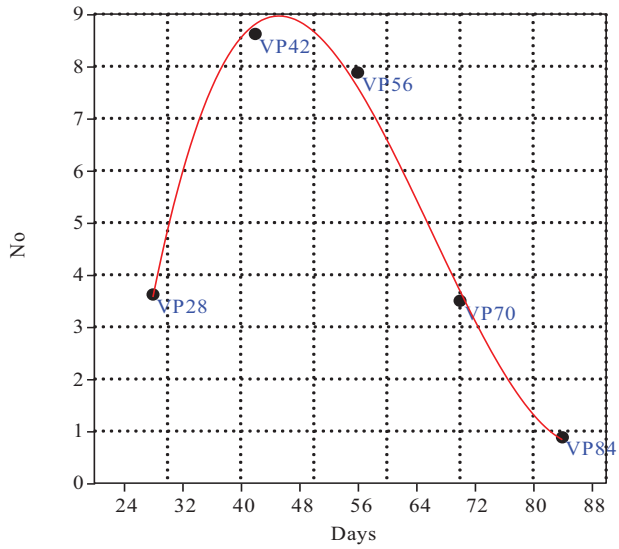

Figure 1. The distribution of the number of flowers for TDB genotype, smoothing spline model

Similarly, in case of ASRed and HS genotypes, the distribution of flowers during the vegetation period was best described by smoothing spline models, the values and terms of the equations being shown in Table 6 for ASRed and Table 7 for the HS genotype with the graphical representation in Figures 3 and 4, respectively.

Multivariate analysis has eased the obtaining of a clustering group of the moments during the vegetative period with statistical safety, Coph. Corr. = 0.899.

Table 5. Spline - statistics of given data point for describing the variation of ASRose flower values

No	x_i	ASRose			
		y_i	ys_i	ε_i	$I_{i/1}$
1	28	0.5	0.568	0.137	1.000
2	42	5.37	5.438	0.013	9.567
3	56	7.12	6.644	0.067	11.690
4	70	1.62	2.095	0.293	3.685
5	84	0.25	0.115	0.540	0.202
				$\varepsilon_i = 0.2098$	

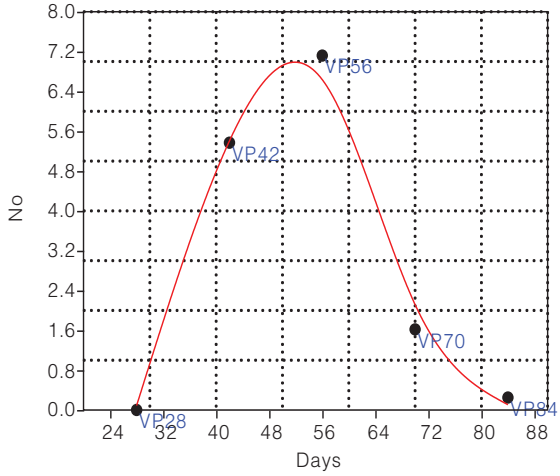

Figure 2. The distribution of the number of flowers for ASRose genotype, smoothing spline model

Table 6. Spline - statistics of given data point for describing the variation of ASRed flower values

No	x_i	ASRed			
		y_i	ys_i	ε_i	$I_{i/1}$
1	28	0.500	0.474	0.051	1.000
2	42	3.000	3.226	0.075	6.798
3	56	5.880	5.488	0.067	11.566
4	70	3.120	3.331	0.067	7.019
5	84	0.500	0.482	0.036	1.016
				$\varepsilon_i = 0.0593$	

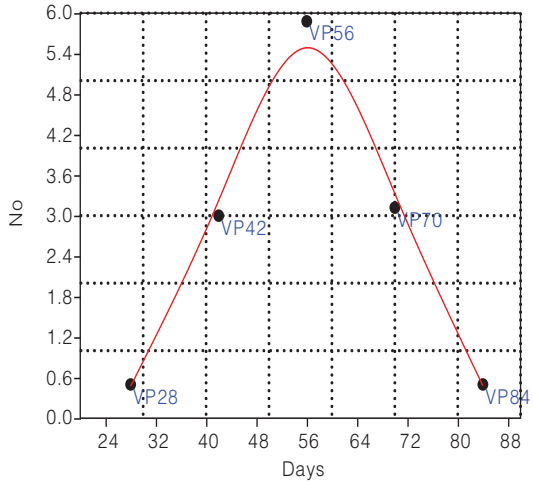

Figure 3. The distribution of the number of flowers for ASRed genotype, smoothing spline model

Table 7. Spline - statistics of given data point for describing the variation of HS flower values

No	x_i	ASRed			
		y_i	ys_i	ε_i	$I_{i/1}$
1	28	3.120	3.123	0.0008	1.000
2	42	5.500	5.641	0.0257	1.807
3	56	7.000	6.618	0.0546	2.119
4	70	2.380	2.712	0.1394	0.868
5	84	1.120	1.027	0.0829	0.329
				$\varepsilon_i = 0.0607$	

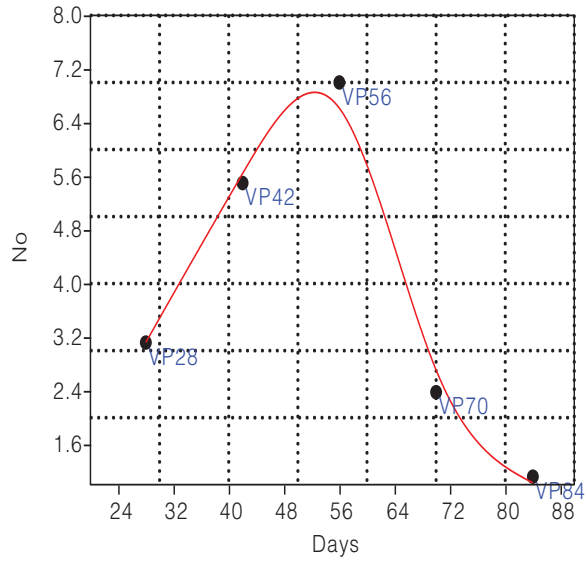

Figure 4. The distribution of the number of flowers for HS genotype, smoothing spline model

Two distinct clusters resulted, a C1 cluster comprising VP52 and VP56 variants of the vegetation period, with the best results on the number of open flowers and a cluster C2 with VP28 and VP70 variants and an independent position of VP84 with the smallest number of opened flowers. The graphical distribution is shown in Figure 5.

From the analysis of the dendrogram with the classification of the variants given by the number of flowers at the moment of determination, it was concluded that the most efficient harvesting periods for cut flowers were VP42 and VP56.

In VP28, TDB genotype with an average number of 3.62 flowers per floral stem and HS genotype with 3.12 flowers per floral stem were revealed. They are early hybrids, but with insufficient number of flowers opened at that time for profitability to be capitalized.

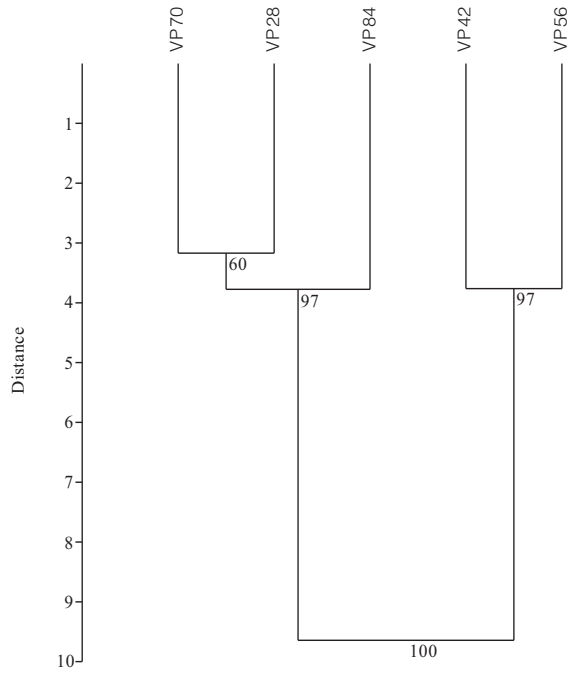

Figure 5. Clusterial grouping of variants based on Euclidean distances, in relation to the number of flowers during the vegetation period

In VP42, TDB genotype was revealed with an average of 8.66 flowers opened on the floral stem, followed by HS genotype with an average number of 5.50 flowers/stem and ASRose with an average number of 5.37 flowers/stem, while the ASRed genotype has registered 3.00 flowers/stem.

In VP56 there was the highest average number of flowers on the floral stem, 7.88 for TDB genotype, 7.12 for ASRose genotype, 7.00 for HS genotype and 5.88 for ASRed genotype. In VP70 with a higher average number of flowers there were observed TDB genotype with 3.50, ASRed genotype with 3.12, followed by HS genotype with 2.38 and ASRose genotype with 1.62.

In VP84 all genotypes showed a small number of flowers ranging from 0.25 for ASRose genotype and 0.12 for HS genotype.

Due to the particular vegetation requirements of *Lisianthus* species, as well as the increase in the interest of cultivating different genotypes, a series of studies evaluated the behavior of the biological material from germination to flowering, paying attention to the height of the plants (floral stems) to floral buds, duration to bloom, number of floral buds per plant, number of flowers per plant and flower duration to senescence (Uddin et al., 2013).

The size, color and pigmentation of petals have also been carried out in researches with particular emphasis on these cut flowers in general and especially for the *Lisianthus* genus (Uddin et al., 2002). The behavior of hybrids of *Lisianthus grandiflorum* Shinn has been studied under different conditions of nutrition without soil (Fascella et al., 2009) or the influence of *Lisianthus* inoculated mycorrhizals on floral indices of practical and economic interest (Meir et al., 2010).

The level of nutrition of plants generally affects their growth and development, the number of flowers and their persistence (Sala, 2011). In this context, it has been studied the influence of nutritional salts upon *Lisianthus* plants physiological indices such as foliar surface, chlorophyll content, stem diameter, floral buds (Hernández-Pérez et al., 2016).

New non-destructive methods, based on artificial intelligence, for the determination of plants' foliar surface and their relation to various pathogens have been developed, including application to *Lisianthus* (Sala et al., 2015; Anitha et al., 2016; Drienovsky et al., 2017 a, b).

In the present study, the uniform nutrition medium did not generate any differentiation of the flowering flow of *Lisianthus* plants, which was determined only by genotype in relation to the vegetation period.

CONCLUSIONS

The four studied *Lisianthus* genotypes showed a specific variation of flowering with a high average number of flowers opened at VP42 and V56, which also represents optimal harvesting and capitalization periods for cut flowers.

The variation coefficient (CV) values revealed a higher variation in ASRose hybrid bloom dynamics and a reduced variation in the HS genotype, the other two having intermediate values.

Bloom dynamics has been most accurately described by a polynomial 3rd grade model for TDB genotype and by smoothing spline patterns in ASRose, ASRed and HS genotypes, based on which it is possible to estimate the optimal moment of harvesting and capitalization as cut flowers.

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