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BIOLOGY

Lisianthus (*Eustoma grandiflorum)* leaf degradation analysis in the postharvest by VIS-NIR-SWIR reflectance spectroscopy

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ABSTRACT: It is known that floriculture is very important for the world economic scenario. Nevertheless, the vase life of cut flowers is determined by a short time span. This study evaluated the spectral behavior during leaf degradation of lisianthus (*Eustoma grandiflorum*) flower stems in the postharvest, in different preservative solutions, and estimating its leaf pigments by hyperspectral data. Lisianthus floral stems were subjected to preservative solutions with different concentrations of sucrose, glucose, phytohormones and deionized water. Leaves from these stems were collected every 4 days for further hyperspectral analysis. Spectra was determined in laboratory with a sensor collecting at the range of 350-2500 nm. In order to analyze the use of the spectra to detect the performance of the preservative solutions, statistical tests were used at each wavelength. Pigment prediction was assessed by the CAR/CLF ratio (Ratio between Carotenoids and Chlorophyll) from the spectral curves, using PLSR and RRMSE. The glucose-based solutions registered positive effects on the preservation of floral stems, especially at the dose of 180 g/L. The solutions based on sucrose and phytohormones registered similarities in the spectral curves among the doses, demonstrating equality in leaf preservation. The discriminant analyses demonstrated there was statistical difference in the spectral responses in the doses for each solution used. The CAR/CLF ratio had reasonable coefficients of 0.6 and RRMSE below 6.99%. The hyperspectral analyses presented a potential for the evaluation of leaf degradation in lisianthus floral stems in different pulsing solutions used in the postharvest.
Key words: spectroradiometry, pigments, senescence.

Análise da degradação foliar de Lisianthus (*Eustoma grandiflorum*) na pós-colheita por espectroscopia de reflectância VIS-NIR-SWIR

RESUMO: Sabe-se que a floricultura é muito importante para o cenário econômico mundial, porém a vida útil de vaso das flores de corte é determinada por um pequeno intervalo de tempo. O estudo procurou avaliar o comportamento espectral durante a degradação foliar de hastes florais de lisianthus (*Eustoma grandiflorum*) na pós-colheita, em diferentes soluções conservantes, e estimar pigmentos foliares a partir dos dados hiperespectrais. As hastes florais de lisianthus foram submetidas a soluções conservantes de diferentes concentrações de sacarose, glicose, distintos fitormônios e água deionizada. Foram coletadas folhas dessas hastes a cada quatro dias para posterior análise hiperespectral. A fim de analisar o uso dos espectros para detectar o desempenho das soluções conservantes, foi utilizado testes estatísticos em cada comprimento de onda entre estas soluções. Foi avaliado a predição de pigmentos através da razão CAR/CLF (Razão entre Carotenoides e Clorofila) a partir das curvas espectrais, tendo o PLSR e RRMSE como indicadores da eficiência dos resultados. As soluções a base de glicose registraram efeitos positivos na conservação das hastes florais principalmente na dose de 180 g/L. Já as soluções a base de sacarose e fitormônios registraram semelhanças nas curvas espectrais entre as doses, mostrando-se igualdade na conservação das folhas. As análises discriminantes demostraram que houve diferença estatística nas respostas espectrais nas doses para cada solução utilizada. A razão CAR/CLF teve coeficientes razoáveis de 0,6 e RRMSE abaixo de 6,99%. As análises hiperespectrais apresentaram potencial na avaliação da degradação foliar de hastes florais de lisianthus em diferentes soluções *pulsing* utilizadas na pós-colheita.
Palavras-chave: espectrorradiometria, pigmentos, senescência.

INTRODUCTION

Floriculture is an important activity in the world economic scenario. Brazil has, as a result, an annual growth of 6.26% in the last years, moving around US$ 750 million per year. In this scenario, this country exports for 40 destinations, with the Netherlands as the greatest purchaser, followed by the United States (SOUZA et al., 2020). Ornamental flowers and plants are commonly sold in vases or as cut flowers. *Eustoma grandiflorum* (or *E. russelianum*) is an ornamental plant of the family *Gentianaceae*, popularly known as lisianthus (LUGASSI-BENHAMO et al., 2010). Among the most cultivated in

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the world (FANG et al., 2021), they are native to the arid areas of the Southern United States and Northern Mexico (GÓMEZ-PÉREZ et al., 2014).

The vase life of cut flowers is determined as the time interval during which their ornamental value is maintained (SHIMIZU-YUMOTO, 2018). Therefore, studies on the physiology and postharvest technologies are important to assist in the supply of ornamental flowers with long vase life to consumers (SHIMIZU-YUMOTO & ICHIMURA, 2010). One of the techniques to extend vase life is the use of pulsing solutions, which are preservatives applied for a period of 12 to 24 hours (HALEVY & MAYAK, 1981). In this context, hormones such as abscisic acid (ABA), gibberellic acid (GA_3), and benzylaminopurine (BAP), as well as solutions based on sucrose and glucose, have been frequently used to retard plant senescence (WANG et al., 2022; YU et al., 2009; DIAS-TAGLIACOZZO et al., 2005; DAR et al., 2014; ZHANG et al., 2012).

Abscisic acid (ABA), in this case, is directly related to the process of stomatal opening, being the most important regulator in the water response of plants (DIAS-TAGLIACOZZO et al., 2005; DANQUAH et al., 2014). Plants such as the perennial herbaceous *Paris polyphylla* treated with GA_3 age at a relatively slower pace, and the reduction in pigments and total protein is significantly retarded by the treatment with GA_3 (YU et al., 2009). Hormones based on BAP (6-benzylaminopurine) have been demonstrated as efficient in retarding senescence, especially in leafy vegetables (WANG et al., 2022). Broccoli and Chinese cabbage, for instance, presented a significant reduction in both leaf senescence and chlorophyll degradation with the use of BAP solutions (WANG et al., 2022; LIU et al., 2013).

Sucrose-based solutions have also expanded postharvest life, retarding flower senescence. EASON et al. (1997) reported that, besides the delay in senescence, the flowers on sucrose-treated stems were larger, firmer, with greater amounts of carotenoids and carbohydrates, and with brighter colors than the flowers treated with only deionized water. Studies using sugars to regulate senescence in the flower of *Dianthus chinensis* L have demonstrated that sucrose was efficient in increasing flower longevity in five days, whereas glucose improved longevity in only three days (DAR et al., 2014). Nonetheless, flowers treated with glucose present a greater vase life, in addition to suppressing ethylene production at the initial stage of the plant (ZHANG et al., 2012).

Leaf degradation in the floral stems is related to the pigments of the leaf, which are mainly represented by chlorophyll and carotenoid. Besides being essential for plant development, photosynthetic pigments (chlorophylls a, b and carotenoids) are responsible for capturing the solar energy used in photosynthesis (TAIZ & ZEIGER, 2013). Therefore, strategies with spectroradiometry play an important role in functional links among physiological and chemical processes and spectral features, especially the information related to the chlorophyll content in the leaf (HOUBORG et al., 2015). Remote sensing, in this case, by optical reflectance sensors, tends to become an alternative in the identification of the flower senescence stages in the postharvest. Besides this technique being promising in nutritional and turgidity studies, it offers faster and non-destructive estimates in comparison with laboratory analyses (MAHAJAN et al., 2014).

Other studies reported that remote sensing by spectroradiometry presents a potential for pigment prediction in the postharvest of fruits and vegetables, such as the anthocyanin from the pericarp of lychees (YANG et al., 2015), peach chlorophyll (SUN et al., 2017), and the ripening of strawberries (ZHANG et al., 2016) and spinach (DIEZMA et al., 2013). Nevertheless, studies on spectroradiometry in the region of VIS-NIR directed to the segment of floriculture have not been identified, especially regarding flower maintenance by the use of preservative solutions.

Therefore, it is believed that it is possible to identify the spectral properties in the leaves of flower stems capable of indicating degradation in relation to pigment concentration, dehydration and the changes in the internal structure. Thus, this study evaluated the spectral behavior during leaf degradation of lisianthus floral stems in the postharvest, in different preservative solutions, and to estimate the leaf pigments from the hyperspectral data.

MATERIALS AND METHODS

The stems of lisianthus (*Eustoma grandiflorum*) cv. Flare Deep Rose were obtained from a commercial production in the municipality of Paranapanema, SP, Brazil (23º23'19" S and 48º43'22" W, altitude: 610 m). The study was conducted as three experiments where the stems were stored in a protected environment. The period of the experiments was three months, lasting 12 days each, with a spectral reading every four days, with a total of three reads for each experiment, on days 4, 8 and 12 after the first cut of the crop.

Data acquisition and processing

The stems were harvested when they presented from two to three open flowers, and they were transported to the Laboratory of Postharvest Physiology. They were standardized in 50 cm in length and the leaves of the first 15 cm of the inferior part of the stem were removed, and then they were randomized for the application of the treatments. Subsequently, they were placed in pots with pulsing solutions for 12 hours, according to the three experiments and their respective treatments, as presented in table 1. The solutions were based on sucrose (Experiment 1), glucose (Experiment 2) and hormones (Experiment 3). In the treatments where hormone bioregulators (70 µM BAP, 5 µM GA3 and 15 µM ABA) were applied, preliminary longevity experiments were performed testing doses of ABA, GA3 and BAP. Those with the best performances were used in the experiment.

After the application of the pulsing solutions, the floral stems remained in recipients containing solutions of distilled water and the germicide Startcolor® based on sodium dichloroisocyanurate (0.2%), which were substituted every four days. Every day of leaf harvest, recipients with three unique floral stems were used to compose each plot, totalizing 12 floral stems in each treatment.

The experiments were set in a completely randomized design, in a 4 x 3 factorial scheme, using four different preservative solutions (Table 1) and three dates of evaluation (4, 8 and 12 days after applying the solution). The plants were standardized with 50 cm in length and had their leaves removed, when they were located in the range of 15 cm of the inferior end of the stem. Three leaves from the region of the middle third of each of the floral stems of each plot were collected and stored in plastic bags identified and placed in a refrigerated thermal cooler box, to maintain the turgidity of the leaves (Figure 1).

To obtain the hyperspectral data, the spectroradiometer FieldSpec³ (ASD – Analytical Spectral Devices Inc., Boulder, CO, USA) was employed, using a computer with the software RS³ of the same company. The spectroradiometer collects data in the spectrum between the wavelengths of 350 and 2500 nm, thus covering the visible, near-infrared and short-wave infrared regions, with spectral resolution of 3 nm in the range of 350-1000 nm and of 10 nm between 1000 and 2500 nm (ASD, 2010).

The spectral curves were obtained in terms of reflectance using the software ViewSpec Pro (ASD – Analytical Spectral Devices Inc., Boulder, CO, USA) and exported to the software Microsoft Excel. A pretreatment of the spectral data was performed with the exclusion of responses caused by noise at the ends of the spectral curves, thus resulting in a spectral curve from 450 to 2450 nm. Furthermore, outliers were removed using the principal component analysis (PCA) in the software Unscrambler (version 9.7).

Pigment acquisition

After performing the spectral reads, the leaves collected from each experimental unit were involved in aluminum foil for further freezing in liquid nitrogen and maintenance in a freezer at -20 °C. Subsequently, the samples were freeze-dried in the freeze-dryer Liotop model L108 and macerated with a crucible. Following the methodology of HISCOX & ISRAELSTAM (1979) with modifications, 3.0 mg of freeze-dried leaf sample together with 5 mL of the solvent dimethylsulfoxide (DMSO) were placed in a test tube, and stored for 48 hours in a dark environment, for chlorophyll extraction.

Subsequently, the solution was collected and transferred to cuvettes in order to perform the reads in the spectrophotometer Biochron model libra S22. Chlorophylls a and b were analyzed at 665 nm and 649 nm; respectively, and carotenoids at 433 nm. The quantifications of the pigments were performed following WELLBURN (1994).

Table 1 - Components of the pulsing solutions applied in Lisianthus "Flare Deep", in each of the experiments, which are: I. Glucose; II. Sucrose; and III. Hormones based on BAP (6-benzylaminopurine), GA_3 (gibberellic acid) and ABA (abscisic acid).

Treatment	Experiments		
	I. Glucose	II. Sucrose	III. Hormones
1	Control (deionized water)	Control (deionized water)	Control (deionized water)
2	45 g/L glucose solution	20 g/L sucrose solution	70 µM BAP solution
3	90 g/L glucose solution	30 g/L sucrose solution	5 µM GA_3 solution
4	180 g/L glucose solution	40 g/L sucrose solution	15 µM ABA solution

Figure 1 - Process of experiment set up and the respective treatments (A), identification of the leaves of the middle third of the floral stems (B), collection of three leaves per plot (C) and storage of the leaves in thermal boxes (D).

Statistical analysis

The data were analyzed using the analysis of variance (ANOVA), and when significant, the post-hoc Tukey's test was applied at $P < 0.01$ of significance, to test each wavelength in pairs, as performed by CARVALHO et al. (2013). The analyses were performed as a function of the treatments in each experiment.

For the study of separation of the groups as a function of the preservative solutions, a linear discriminant analysis (LDA) was performed. This analysis obtains linear combinations of the independent variables of the previously defined groups, in order to obtain higher discrimination, which was reached by the maximization of the correlation between the distance among the groups and the distance inside the group (GARRIDO-NOVELL et al., 2012).

To evaluate the potential of separation of the groups obtained by LDA, the centroids of the groups were obtained and an ANOVA followed by the Tukey's test at $P < 0.05$ was applied to these values, the centroids being the mean values of the discriminant scores (DIAS et al., 2014). These statistical analyses were performed in the environment R (version 3.4.3).

The spectral and biochemical data obtained from experiments 1, 2 and 3 were used in the ratio of carotenoids and chlorophyll, CAR/CLF. This quantification was performed using partial least squares regression (PLSR) in the software Unscrambler (version 9.7). The model PLSR was used to find the hyperplanes of maximum variance between predictable and observable variables, and it develops a linear model. In the validation, the method *leave-one-out (LOOCV)* was used, which is based on the use of a single observation for the validation of the model, while the others are used as data for training, and this process is repeated using each of the observations as a validation set (NEVALAINEN et al., 2014).

To evaluate regression precision, the coefficient of determination (R^2) was employed, which is frequently used in the literature, and the relative root mean square error (RRMSE) that is not influenced by the dimensionality of the data and is less sensitive to outliers (RICHTER et al., 2012).

RESULTS

Effect of the different preservative solutions treatments on spectral curve

The spectral responses of each treatment in the three days of analysis are presented in figure 2, 3 and 4, of experiments 1, 2 and 3, respectively. Additionally, the post-hoc Tukey's test at $P < 0.01$ of probability is demonstrated for each wavelength, each point demonstrating the treatments which differed statistically.

On the 4th day of the first experiment (Figure 2A), the solution of 90 g/L presented the highest reflectance in the whole spectral curve. Thus, it can be inferred that the application of this solution led to a worse leaf preservation on the day analyzed. Nevertheless, the treatment which presented significantly lower reflectance values was the one of 180 g/L of glucose, thus indicating that this solution had leaves which were initially more conserved.

The control and the solution of 90 g/L, followed by 90 and 180 g/L, registered the highest significant differences, in virtually all wavelengths; on the other hand, the comparison between the pairs control and 45 g/L, followed by 90 and 180 g/L, did not differ in any point of the spectral curve (Figure 2A). Conversely, on the 8th day, the treatments presented virtually equal reflectances in almost all wavelengths of the spectrum, with an exception in

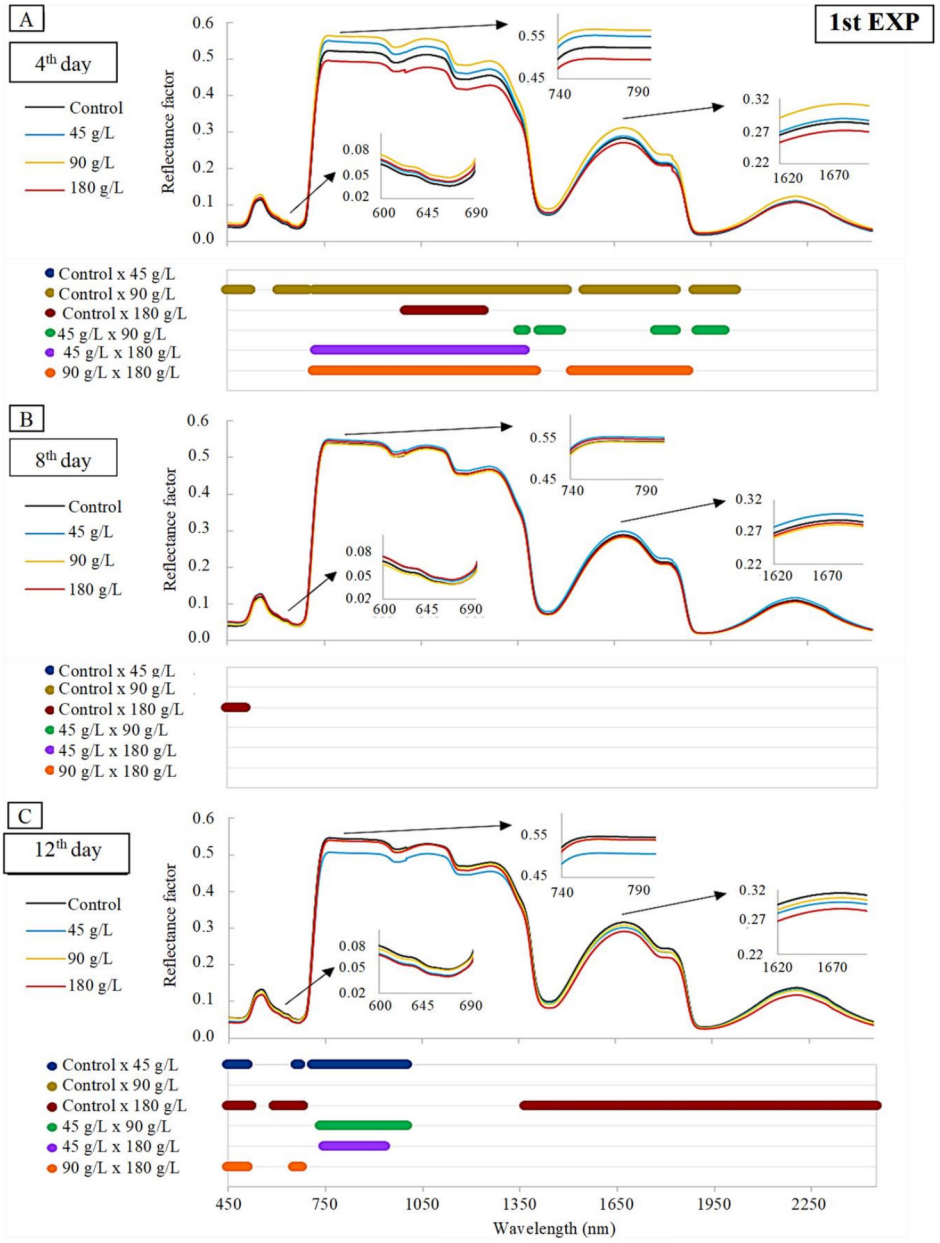


Figure 2 - Spectral curves of the treatments of the pulsing solutions with Glucose in Lisianthus Deep Flare, on the 4th (A), 8th (B) and 12th (C) days of analysis. The graph of the spectral curves represents the post-hoc Tukey's test at 1% of significance among all treatments analyzed two by two, each point representing that the pair of treatments analyzed differs statistically.

the region of blue (450 to 500 nm), which had the pulsing solution of 180 g/L of glucose and the control statistically different (Figure 2B).

On the last day of analysis of the first experiment, 12th day (Figure 2C), the treatment which presented the spectral curve with the lowest reflectance was the solution of 45 g/L of glucose, suggesting more preserved leaves, followed by the solution of 180 g/L. In the control solution, a worse leaf preservation was observed, since this treatment presented a statistically higher reflectance in the whole spectral curve. Furthermore, the control

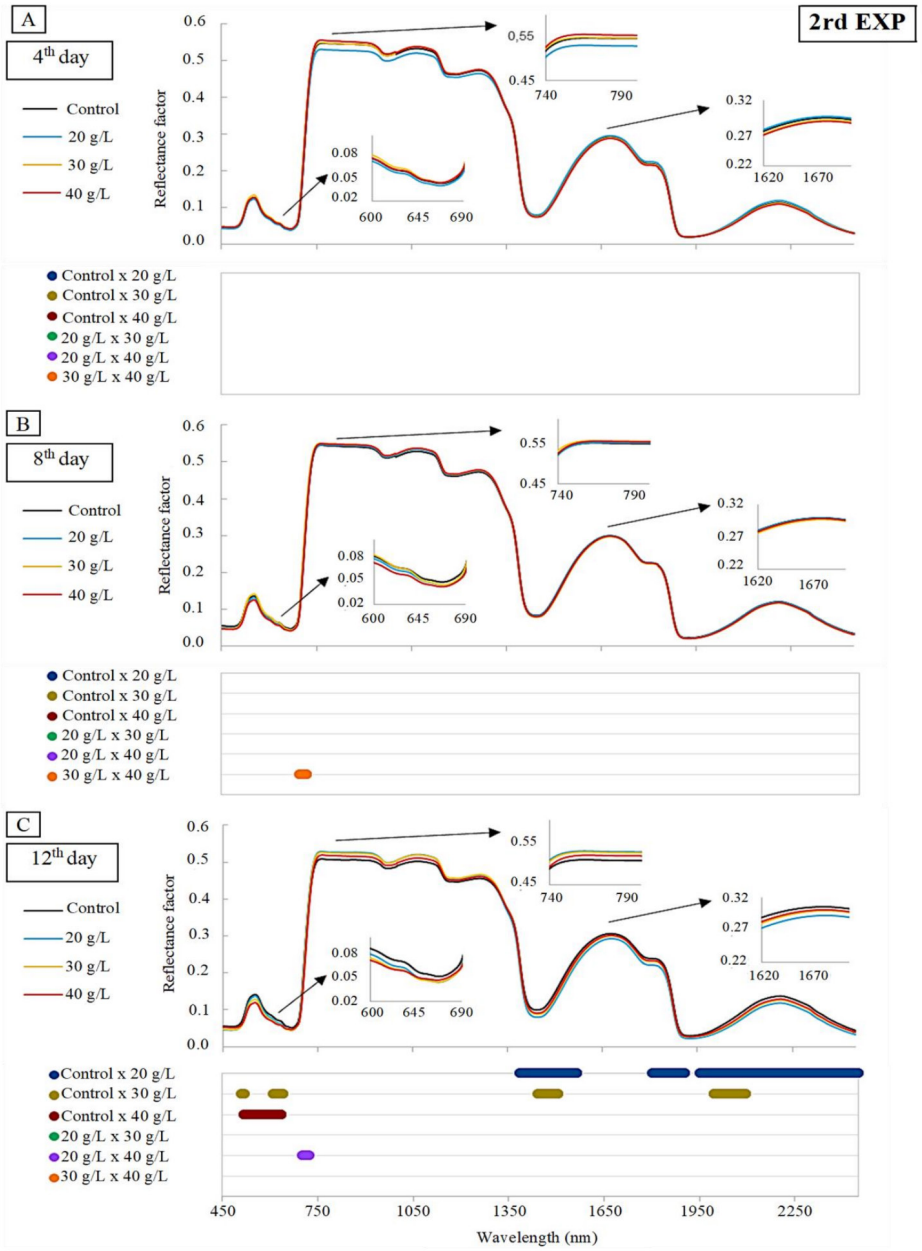


Figure 3 - Spectral curves of the treatments of the pulsing solutions with Sucrose in Lisianthus Deep Flare, on the 4th (A), 8th (B) and 12th (C) days of analysis. The graph of the spectral curves represents the post-hoc Tukey's test at 1% of significance among all treatments analyzed two by two, each point representing that the pair of treatments analyzed differs statistically.

with the solution of 180 g/L registered a significant difference, especially in the mid infrared region. The spectral response of the leaves reveals that the glucose-based solutions generated a longer flower vase life in comparison with the control (deionized water), and on the last day of analysis (12th day) the curves of the glucose doses registered a lower reflectance in the region of visible (450 to 680 nm) and SWIR (1450 nm), indicating more conserved and turgid leaves.

On the three days of analyses (4th, 8th and 12th), the mid infrared region, especially in the range

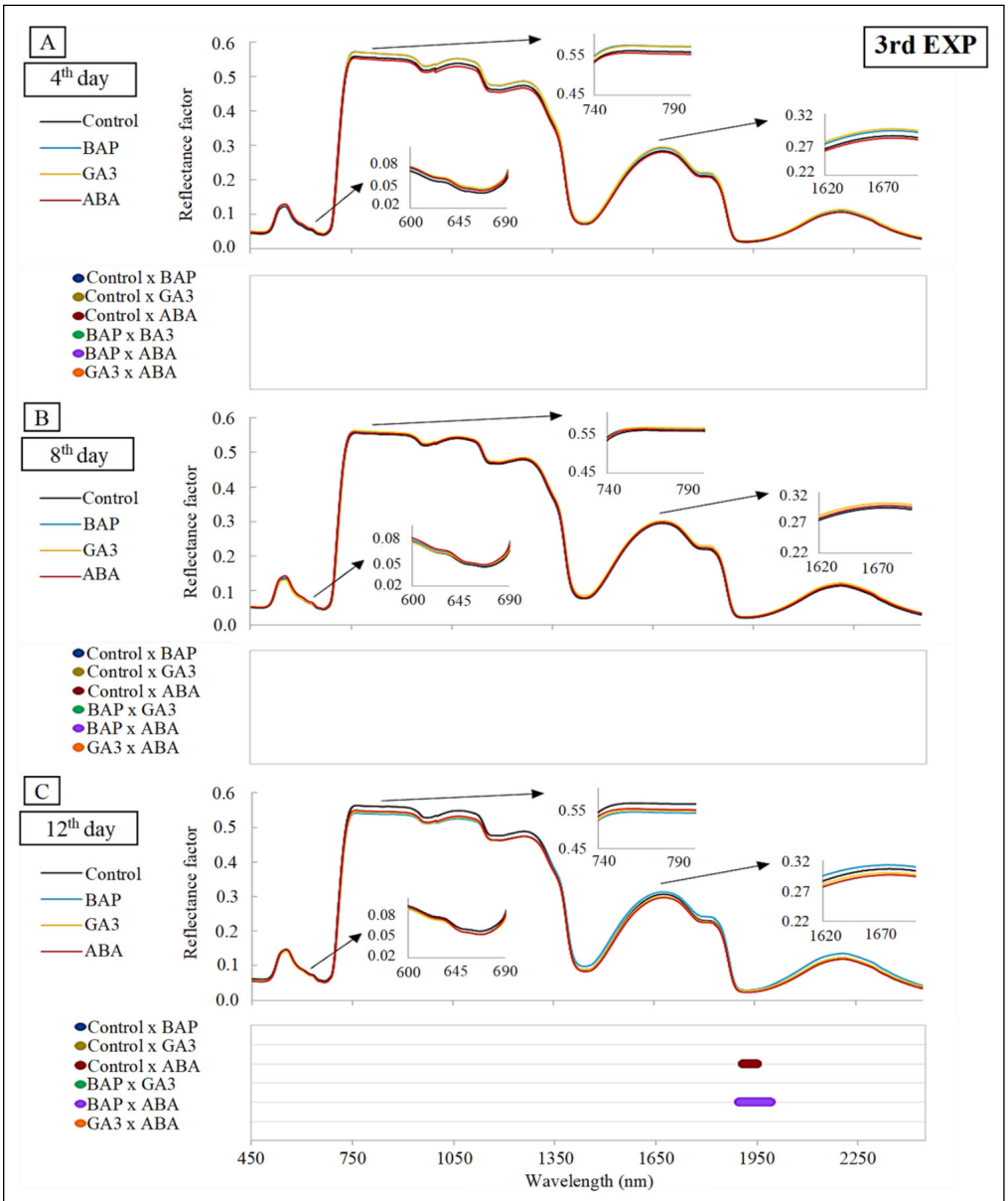


Figure 4 - Spectral curves of the treatments of the pulsing solutions with Phytohormones in Lisianthus Deep Flare, on the 4th (A), 8th (B) and 12th (C) days of analysis. The graph of the spectral curves represents the post-hoc Tukey's test at 1% of significance among all treatments analyzed two by two, each point representing that the pair of treatments analyzed differs statistically.

of 1400 nm, showed better preservation of water in the leaves using the solution of 180 g/L of glucose, an aspect which can be seen by the low reflectance factor in the range of 1400 nm, a region known by the high capacity of electromagnetic energy absorption by the water content in the leaf (Figure 2).

In the second experiment (Figure 3), the control solution and the different sucrose concentrations were statistically similar in almost all days of analysis, only presenting differentiation in parts of VIS and SWIR on the 12th day. On the 4th day of analysis of the second experiment (Figure 3A), it was observed that all treatments presented similar leaf preservation, since there was no statistical difference among the reflectances of any of the solutions applied. On the 8th day (Figure 3B), there was only significant

difference between the treatment of 30 g/L and the treatment of 40 g/L in the red-edge region, where the spectral curve of the solution of 40 g/L presented a higher shift to longer wavelengths.

On the 12th day of the second experiment, the spectral curves showed (Figure 3C) that the control had the highest degradation in relation to the maintenance of pigments and turgidity, since it presented a reflectance statistically higher in SWIR than the treatments of 20 and 30 g/L, and in VIS when compared to 30 and 40 g/L, which is an indicative of higher plant degradation when only deionized water is used. The sucrose solutions provided a similar preservation, since they presented, in general, significantly equal reflectances.

In the third experiment (Figure 4), the phytohormones and the control solution were used. The spectral responses of each of the treatments, in general, did not differ statistically in all days of analysis (Figure 4A and 4B). Nonetheless, on the 12th day (Figure 4C) there was a difference between the solutions of ABA with BAP and ABA with the control in the region of 1950 nm, in which ABA presented a lower reflectance. According to the spectral response, abscisic acid (ABA) influenced visually and significantly the region known by the high energy absorption by water (1450 and 1950 nm), according to figure 4.

Visually, the substances based on phytohormones presented greater energy absorption in the region of Vis and, more expressively, in NIR. In this case, hormone-based solutions may improve the conditions of floral stem preservation, especially some days after flower harvest.

Discriminant analysis

The graphs of the discriminant analysis of each day of analysis of the three experiments are presented in figure 5, 6 and 7, as well as the Tukey's test of the centroids of the first and second discriminant functions (LD1 and LD2, in this order), with their respective loadings. The variation of the data was explained by LD1 together with LD2 on days 4, 8 and 12, in 93.94%, 89.27% and 84.71% in the first experiment; 82.91%, 76.14% and 79.07% in the second experiment; and 74.11%, 83.46% and 82.55% in the third experiment.

In experiment 1 (Figure 5), the solution of 90 g/L of glucose only presented a statistically different spectral response on the 12th day of analysis, in the two discriminant equations. Conversely, the control treatment was differentiated from the glucose solutions in almost all discriminant equations of the three days of analysis, except in the LD2 of the 8th day, thus demonstrating that the spectral response of the control treatment was characteristic. The treatments of 45 and 180 g/L presented clustering on the last day of analysis, since the centroids of these treatments were significantly equal in the two discriminant equations.

In experiment 2 (Figure 6), treatments control and 40 g/L of sucrose were discriminated by the LD1 and LD2 and the 8th and 12th days of analysis, being significantly equal only on the 4th day. On the other hand, the solutions of 20 and 30 g/L presented opposite behaviors, all being statistically equal in almost all discriminant equations of the 8th and 12th days, totally differentiating only on the 4th day.

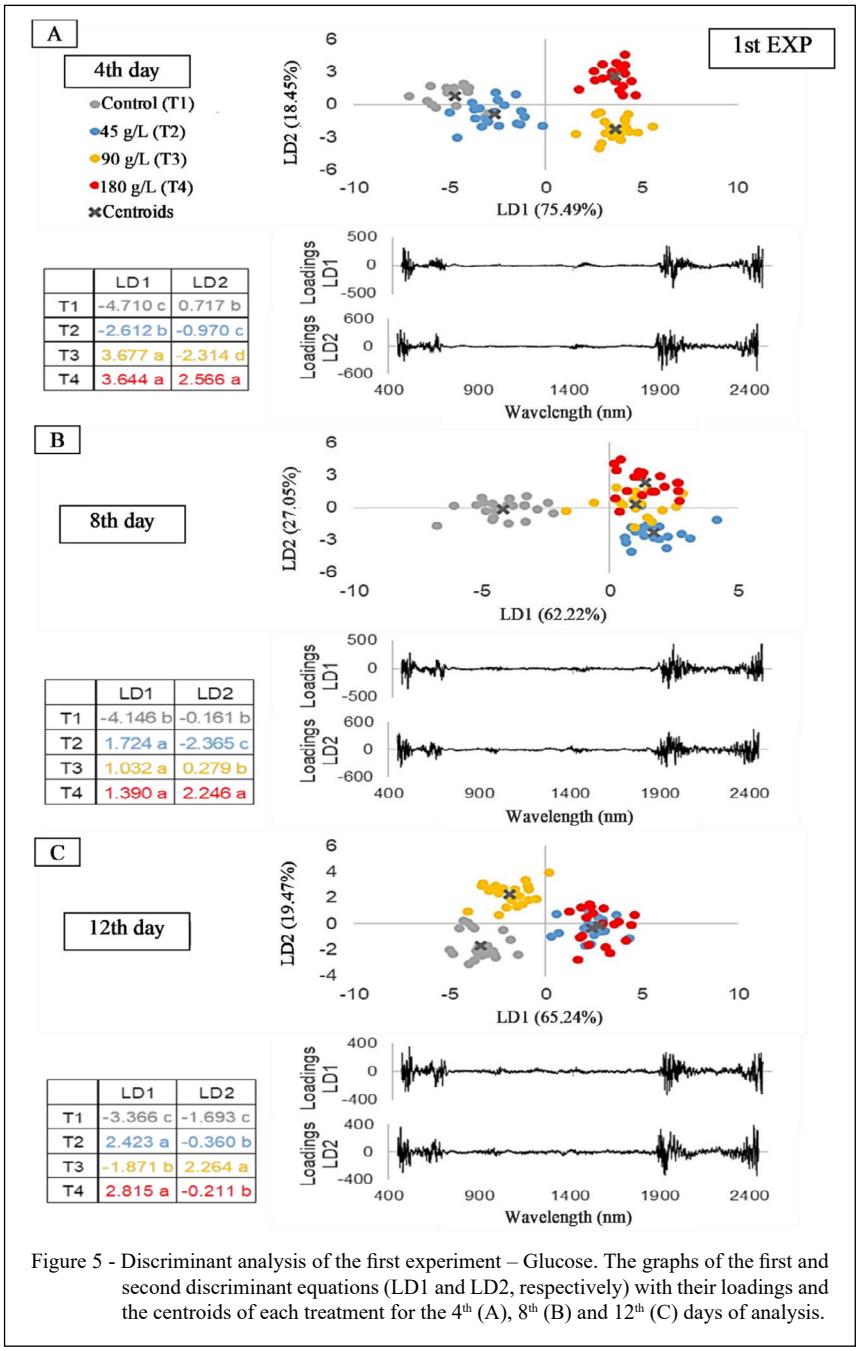
In experiment 3 (Figure 7), the control treatment was statistically different from all treatments in the whole experiment. The centroids of BAP and of GA_3 were significantly equal in the LD2 in all days of analysis. In the LD1 of the 4th and 8th days, the centroids of all treatments were statistically different; and on the 12th day, BAP presented similarity in LD1 and LD2 with the ABA and GA_3 solutions, respectively.

The loadings of all experiments presented higher values in the VIS regions, especially in blue and red, and in SWIR, especially around the wavelengths 1950 and 2450 nm.

Prediction of CAR/CLF

In the prediction of CAR/CLF, the values of R^2 were superior to 0.6 in the 3rd experiment and in general. Conversely, in the 1st and 2nd experiments, R^2 of 0.54 and 0.3 were obtained, respectively. Experiments 1, 3 and General (Figure 8A, 8C and 8D) presented high coefficient values in the region of green (around 530, 522 and 524 nm, respectively). In experiment 2, the contribution of the visible region in the equation of the multiple regression was in the region of yellow (between 560 and 580 nm) (Figure 8B). The region of the red edge presented an influence in all multiple regressions proposed, with experiments 1, 2, 3 and general presenting high coefficient values around the wavelengths 707, 708, 685 and 690 nm, respectively.

The NIR region also influenced in the regressions of experiments 2 and 3, since the first presented high coefficients from 770 to 900 nm and in experiment 3 the contribution was in the regions of 763 to 780 nm and 980 to 1000 nm. Thus, the influence of a great range of coefficients in NIR may have provided a lower value of coefficient of determination and the highest RRMSE in the 2nd experiment.



	LD1	LD2
T1	-4.710 c	0.717 b
T2	-2.612 b	-0.970 c
T3	3.677 a	-2.314 d
T4	3.644 a	2.566 a

	LD1	LD2
T1	-4.146 b	-0.161 b
T2	1.724 a	-2.365 c
T3	1.032 a	0.279 b
T4	1.390 a	2.246 a

	LD1	LD2
T1	-3.366 c	-1.693 c
T2	2.423 a	-0.360 b
T3	-1.871 b	2.264 a
T4	2.815 a	-0.211 b

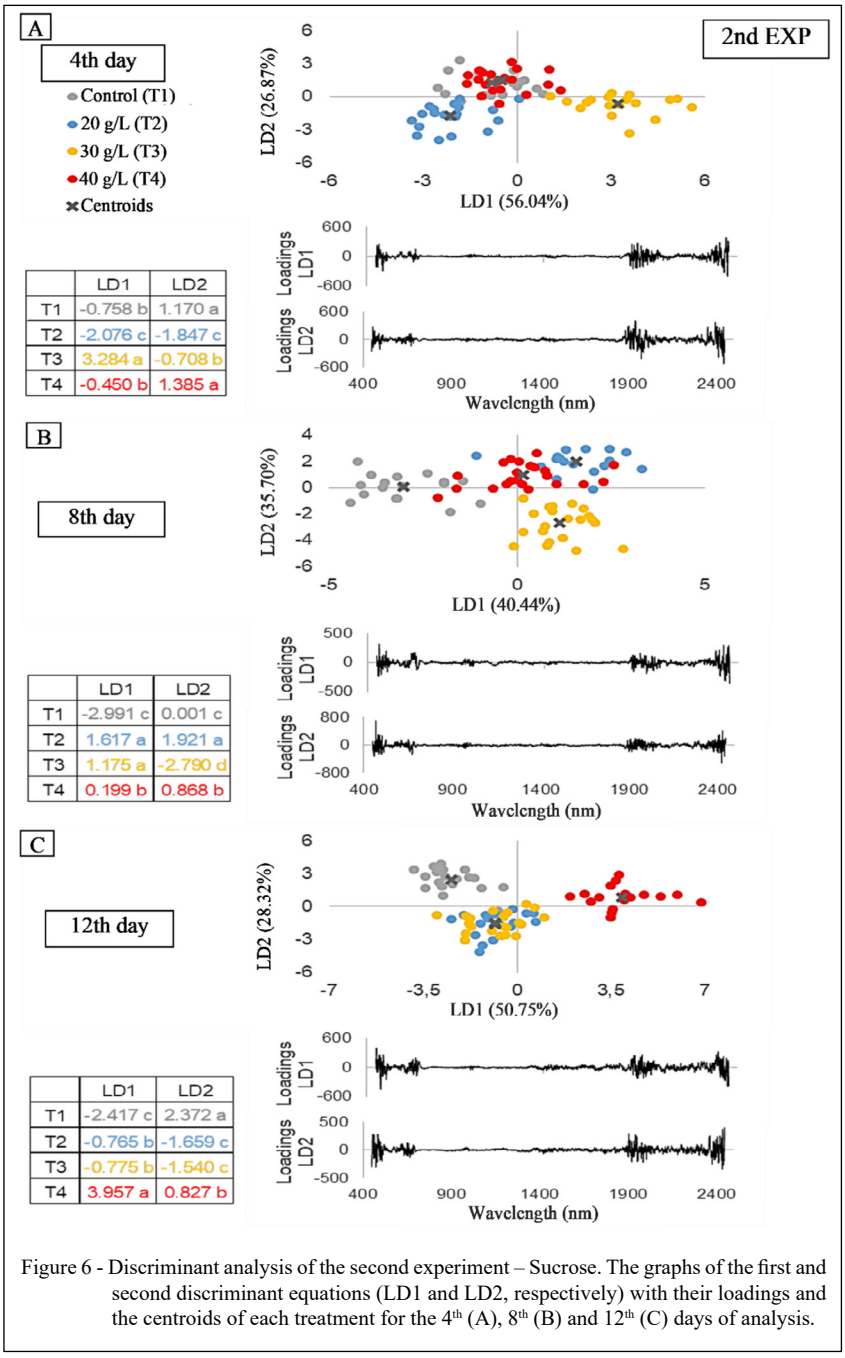
Figure 5 - Discriminant analysis of the first experiment – Glucose. The graphs of the first and second discriminant equations (LD1 and LD2, respectively) with their loadings and the centroids of each treatment for the 4th (A), 8th (B) and 12th (C) days of analysis.

DISCUSSION

Effects of pulsing solutions on spectral curves

Petal senescence in cut flowers is accelerated by ethylene production, because of a fast decrease in the sugar contents (KONDO et al., 2020). Therefore, there is a greater shelf life of the vase flowers with concentrations of glucose applications in comparison with flowers maintained continuously in distilled water (ZHANG et al., 2012).

The results according to reflectance suggested glucose-based solutions present the potential for the preservation of lisianthus flowers (Figure 2). In this study, the glucose concentrations of 45, 90 and 180 g/L registered the most preserved leaves, besides presenting significantly lower



	LD1	LD2
T1	-0.758 b	1.170 a
T2	-2.076 c	-1.847 c
T3	3.284 a	-0.708 b
T4	-0.450 b	1.385 a

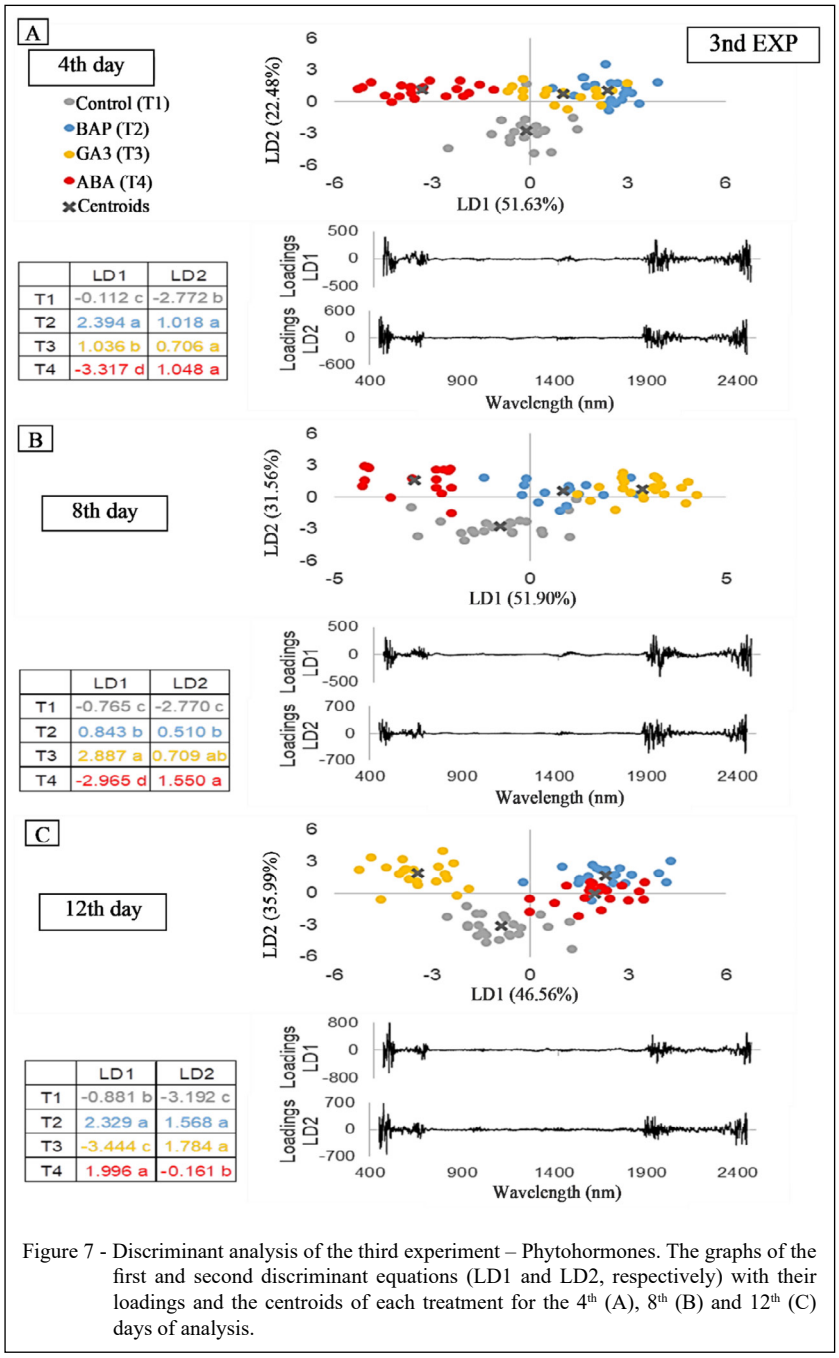
	LD1	LD2
T1	-2.991 c	0.001 c
T2	1.617 a	1.921 a
T3	1.175 a	-2.790 d
T4	0.199 b	0.868 b

	LD1	LD2
T1	-2.417 c	2.372 a
T2	-0.765 b	-1.659 c
T3	-0.775 b	-1.540 c
T4	3.957 a	0.827 b

Figure 6 - Discriminant analysis of the second experiment – Sucrose. The graphs of the first and second discriminant equations (LD1 and LD2, respectively) with their loadings and the centroids of each treatment for the 4th (A), 8th (B) and 12th (C) days of analysis.

reflectance values in the region of Vis, NIR or SWIR (Figure 2). In this case, by leaf spectra, it was possible to identify the flowers with the greatest shelf life, since reflectance in NIR increases during leaf senescence, because of the increase in the leaf intercellular space (SINCLAIR et al., 1971). On the other hand, the leaf dehydration that occurs in senescence promotes the rise in reflectance in SWIR, since the radiative property of water promotes the absorption of radiation; therefore, when water is lost from one leaf, absorption decreases and reflectance tends to increase in this spectral range (CARTER, 1991).

According to DIAS-TAGLIACOZZO et al. (2005), the fact these leaves are better preserved derives from the sugars provided in the form of sucrose or glucose, which contribute to expand the



	LD1	LD2
T1	-0.112 c	-2.772 b
T2	2.394 a	1.018 a
T3	1.036 b	0.706 a
T4	-3.317 d	1.048 a

	LD1	LD2
T1	-0.765 c	-2.770 c
T2	0.843 b	0.510 b
T3	2.887 a	0.709 ab
T4	-2.965 d	1.550 a

	LD1	LD2
T1	-0.881 b	-3.192 c
T2	2.329 a	1.568 a
T3	-3.444 c	1.784 a
T4	1.996 a	-0.161 b

Figure 7 - Discriminant analysis of the third experiment – Phytohormones. The graphs of the first and second discriminant equations (LD1 and LD2, respectively) with their loadings and the centroids of each treatment for the 4th (A), 8th (B) and 12th (C) days of analysis.

life of many vase flowers, applied either in the form of "pulsing" or continuously as a component of the solutions in the pots. It is widely accepted that sugars improve water relations, rising the levels of osmotic solutes, and they also assist in water loss by the petals during the senescence of cut flowers (VAN-DOORN & WOLTERING, 2008). This happens because petal withering and senescence are intimately related to water deficit, which is mainly caused by continuous reductions in water absorption (COSTA et al., 2021).

In the second experiment (Figure 3), which used sucrose concentrations, reflectance in thee three days of analyses (4th, 8th and 12th) did not differ statistically in almost all wavelengths. However, on the 12th day of analysis, the curves with higher doses of sucrose showed higher energy absorption

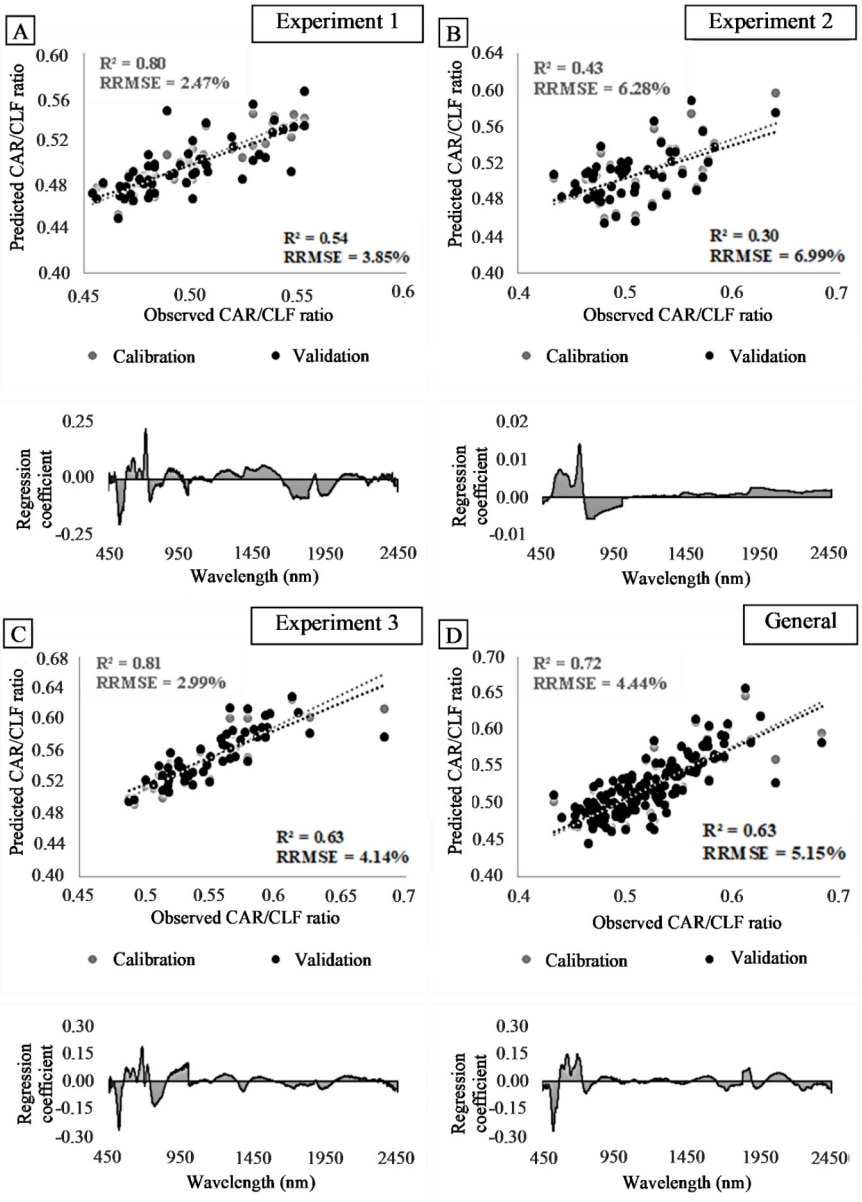


Figure 8 - Results of the prediction of the CAR/CLF (carotenoids/chlorophyll) ratio by PLSR (partial least squares regression) of experiments 1, 2 and 3 (A, B and C, respectively), and in all experiments together (D), with the appropriate regression coefficients. R^2: coefficient of determination, RRMSE: relative root mean square error.

in the visible range (400-680 nm), indicating more preserved plants and a higher photosynthetic rate. This is because sucrose-based solutions facilitate water absorption by cut flowers, resulting in better water balance, freshness, and reduced wilting; as a result, the vase life of cut flowers was increased (ASRAR, 2012). Therefore, it is believed that sucrose solutions can indeed be better for the preservation compared to water without any other solution in the mixture, regarding the water balance of the floral stem (ELHINDI, 2012), which is in accordance with what was obtained in the spectral curves.

The hormones abscisic acid (ABA), gibberellic acid (GA_3) and benzylaminopurine (BAP), on the 4th and 8th days of analysis, presented very close spectral curves, not differing from each other. Only on

the 12th day of the third experiment (Figure 4C) was there a significant difference ($P < 0.01$) between the solution ABA with BAP and the control in the region of 1950 nm. The solutions based on abscisic acid (ABA) presented a greater energy absorption in the region of visible (450 to 680 nm), and more markedly in NIR and SWIR (1450 and 1950 nm), suggesting a greater leaf turgidity (CARTER, 1991). This influence of abscisic acid (ABA) in the region of the spectrum of water absorption (1450 and 1950 nm) was already expected, since ABA, in this case, is directly related to the process of stomatal opening, being the most important regulator in the water response of plants (DIAS-TAGLIACOZZO et al., 2005; DANQUAH et al., 2014; WOLTERING & PAILLART, 2018).

Discriminant analysis

Each point of the graph of discriminant analysis corresponds to the projection of each spectral curve in the two-dimensional discriminative space encompassed by the discriminant vectors (DIEZMA et al., 2013).

In this case, the loadings from all experiments (Glucose, Sucrose and Homonyms) presented the greatest values in the regions of visible, especially in blue and red, whereas in SWIR, they were around the wavelengths 1950 and 2450 nm. This result suggested the differentiation of the days of analysis was mainly influenced by pigment concentration, since in the region of visible the two absorption bands (480 and 680 nm), because of the presence of carotenoids and related to the process of photosynthesis (ROSA, 2009) and leaf turgidity, are the most sensitive regions to the variation in the water content in the leaf. Water considerably absorbs the incident radiation, especially in the bands of 1450, 1950 and 2700 nm, which also correspond to the bands of atmospheric absorption (NOVO, 2010; PONZONI et al., 2012).

Therefore, the results reveal that the effects of the preservatives based on glucose, sucrose and hormones in lisianthus flowers may be better studied applying hyperspectral sensors, since they affect leaf spectra in the region of visible, near-infrared and SWIR, especially in the bands responsive to the pigments (chlorophyll and carotenoids) and leaf water content. Thus, they can be tools for new technologies of non-destructive analysis to evaluate different causes of stress in the plant.

Prediction of CAR/CLF

In the prediction of CAR/CLF, the R^2 values were reasonable (KALACSKA et al., 2015), superior to 0.6 in the 3rd experiment and in the general. GITELSON et al. (2017) obtained a coefficient of determination of 0.73 for leaves of tree species and 0.5 for agricultural crops, from the photochemical reflectance index (PRI), an index that was originally designed to evaluate the cycle of xanthophyll and LUE (Light Use Efficiency) in short periods. In this sense, the indexes reported in this study may have presented lower values because these analyses were performed in the postharvest conditions. Regarding the values of RRMSE, they can be classified as excellent for all proposed regressions, since they were lower than 10% (RICHTER et al., 2012).

Experiments 1, 3 and General (Figure 8A, 8C and 8D) presented high coefficient values in the region of green (around 530, 522 and 524 nm, respectively). In experiment 2, the contribution of the visible region in the equation of multiple regression was in the region of yellow (between 560 and 580 nm) (Figure 8B). The regions green and yellow are also used in the PRI index, which presents in its equation the wavelengths 531 and 570 nm (GAMON & PENUELAS, 1992). The region of the red edge presented influence on all multiple regressions proposed, with experiments 1, 2, 3 and general presenting high coefficient values around the wavelengths 707, 708, 685 and 690 nm, respectively. Different studies have also observed that the regions of visible and red-edge can be used in the prediction of chlorophyll and carotenoids (KIRA et al., 2015; YANG et al., 2010; LING et al., 2019; SONG & WANG, 2022; LIN et al., 2022).

Regarding the NIR region, it is usually influenced by the inner leaf structure, because of the spongy mesophiles, which are responsible for the spread of radiation. When the number of empty spaces increases, the factor reflectance increases in the near-infrared region (LIU, 2015). Therefore, the influence of a wide range of coefficients in NIR might have provided a lower value of coefficient of determination and the highest RRMSE in the 2nd experiment.

CONCLUSION

The reflectance spectra demonstrated a potential to detect the performance of the preservative solutions on leaf degradation during postharvest, which may be the way for new studies on the development of technologies directed to the postharvest preservation of flowers. Nonetheless, the discriminant analysis did not define well the different preservative solutions in each experiment. By the spectral curves, the glucose and sucrose-based solutions registered the highest reflectance intensity variations, with a statistical difference observed between the doses and the control

treatment. Conversely, the phytohormones registered a similarity in the spectral curves, showing equality in leaf preservation.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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