Scientific Article

Carbohydrate reserves on postharvest of lisianthus cut flowers [1]

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ABSTRACT

Floriculture industry demands for products with high quality and durability; however, there is a lack of studies related to the post-harvest physiology of cut flowers. We aimed to study phenolic contents of lisianthus (*Eustoma grandiflorum*) stems treated with ethylene inhibitors (1-Methylcyclopropene - 1-MCP and Salicylic Acid - SA) and different storage temperatures (room at 24 ± 2 °C and pre-exposure to the cold chamber at 9 ± 2 °C for 24 hours) during the post-harvest. Total soluble carbohydrate contents decreased during the experimentation, characterizing the consumption of the reserves during lisianthus post-harvest. The 1-MCP treatment slowed the decrease of total soluble carbohydrate contents. SA treatment had the lowest total soluble carbohydrate contents in both storage temperatures.

Keywords: *Eustoma grandiflorum*, ethylene inibhitors, sugar, salicilic acid.

RESUMO

Carboidratos de reserva na pós-colheita de flores cortadas de lisianthus

O Setor da Floricultura demanda grande quantidade de produtos de alta qualidade e durabilidade; entretanto, existe a carência de estudos relacionados à fisiologia pós-colheita de flores. O objetivo deste trabalho foi estudar os teores de fenóis na pós-colheita de hastes de lisianthus (*Eustoma grandiflorum*) submetidas ao tratamento com inibidores de etileno (1-Metilciclopropeno -1-MCP e Ácido Salicílico - SA) e diferentes temperaturas de armazenamentos (ambiente a 24 ± 2°C e pré-exposição à câmara fria a 9 ± 2°C por 24 horas). Os teores de carboidratos solúveis totais diminuíram durante a experimentação, caracterizando o consumo das reservas durante a pós-colheita de flor cortada de lisianthus. O tratamento com 1-MCP retardou a diminuição dos índices de carboidratos solúveis totais de lisianthus. O tratamento com SA apresentou o menor teor total de carboidratos solúveis em lisianthus em ambas as temperaturas de armazenamento.

Palavras-chave: *Eustoma grandiflorum*; inibidores de etileno, açúcares, ácido salicílico.

1. INTRODUCTION

The post-harvest life of commercial flowers is affected by physical, environmental and biological factor; affecting plant water relation, disease, response to physical stress and carbohydrate status (KUMAR et al., 2016). Flowers after harvest, show high perishability due to intense catabolic physiological processes during post-harvest. Biochemical, physiological and structural changes lead to a process of disorganization and disintegration of tissues and organs, promoting senescence (FINGER et al., 2003).

The high energy required for flower growth and respiration requires substantial energy reserves in harvested cut flowers (REID and JIANG, 2012). The mobilization of storage carbohydrates and the import of sucrose go along with flower anthesis in most of cut-flowers specie (DOORN and MEETEREN, 2003). Fructose (with the highest concentration), glucose, and sucrose are the main soluble sugar in flowers (KARIMI and ASIL, 2017). The major soluble carbohydrates in petals of lisianthus are glucose and sucrose, while fructose is found in low concentrations (SHIMIZU and ICHIMURA, 2005).

Complex ranges of exogenous and endogenous signals (i.e., sugar signals) that initiate senescence are probably part of the lack or abundance of the metabolism of sugars (PALIYATH et al., 2009). Reserve carbohydrate (mostly starch) from leaves that function as a source, turns into soluble sugar, mainly sucrose and is transported to stem and reduced via hydrolysis by invertases action, releasing soluble sugars (glucose and fructose), consequently increasing its concentration (LARA et al., 2004).The contents of glucose, fructose and sucrose in the greenish petals of lotus (*Nelumbo nucifera*) floral cut at the commercial harvest stage and placed in water, decreased rapidly from day 0 of vase life and the lack of available sugars might be a cause for petal blackening in green petals (NETLAK and IMSABAI, 2016). The decrease of soluble carbohydrates concentration in petals of cut "Sonia" roses

DOI: http://dx.doi.org/10.14295/oh.v24i1.1108

[1] Received in 18/07/2017 and accepted in 26/02/2018

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was more responsible for termination of vase life than vascular occlusion (ICHIMURA et al., 2003).

The aim of this study was to analyze the endogenous levels of total soluble carbohydrates in leaves, flowers and buds of cut flowers of lisianthus (*Eustoma grandiflorum*) submitted to post-harvest treatments with refrigeration and inhibitors of ethylene action.

2. MATERIAL AND METHODS

Plant material was acquired from a commercial producer from Paranapanema (23°23'19"S and 48°43'22"W, altitude: 610 m, subtropical climate Cfa), São Paulo State, Brazil. Stems (35 cm) from plants grown in a greenhouse were harvested in the morning, had at least three fully opened flowers in the inflorescence. The base of the stems were put in the water and transported to the Biochemistry Laboratory of the Biosciences Institute, UNESP, Botucatu, São Paulo State, Brazil.

Lisianthus stems were exposed to eight different treatments: Control; 0.5 µl L^{-1} 1-MCP - 0.14% 1-MCP (EthylBloc™); SA - 1,000 mg L^{-1} salicylic acid; 1-MCP + SA - interaction between the two products at room temperature (24 ± 2 °C) and in pre-exposure to 9 ± 2 °C for 24 h. The treatment 1-MCP was performed in closed plastic boxes for 12 h at 24 ± 2 °C. SA pulsing solution was applied for 24 h. After treatment, the stems were placed in 1.5 L plastic containers with water. The water was changed every two days. Half of the stems were keeped at 9 ± 2 °C for a period of 24 h and the other half at room temperature (24 ± 2 °C) during the experiment. The plant material was collected every three days until the end of vase life and separated on flower buds, flowers and leaves, that were macerated in liquid nitrogen for the total soluble carbohydrates analyses.

The soluble carbohydrates were extracted from 25 mg (buds and flowers), and 50 mg (leaves) of fresh material macerated in liquid nitrogen, suspended in 10 mL of deionized water and kept for 40 min in 40 °C water-bath. The solution was centrifuged for 30 minutes at 5,000 rpm, and then an aliquot of 0.1 ml was used for total soluble carbohydrates determination. The sulfuric-phenol method (DUBOIS et al., 1956) was used to determine total soluble carbohydrates. Readings were made in a spectrophotometer at 490 nm wavelength. Total soluble carbohydrate concentrations were calculated as a function of the standard glucose curve. The concentration of total soluble carbohydrates was calculated as mg glucose 100 g^{-1} fresh matter of plant material.

The experimental design was a completely randomized 4×2 factorial, comprising four treatments and two different storage conditions. Each treatment consisted of three replicates with ten flower stems each. The results were submitted to variance analysis (F-test) and the means compared by Tukey test at $p \leq 0.05$ using the software SISVAR.

3. RESULTS AND DISCUSSION

During the storage, carbohydrate contents decreased in buds (Tables 1 and 2), flowers (Tables 3 and 4) and leaves of lisianthus (Tables 5 and 6), regardless of the post-harvest treatment and temperature.

Table 1. Total soluble carbohydrates concentrations (mg glucose 100 g^{-1} fresh mass) in lisianthus flowers pre-treted with ethylene inhibitors and storage at room temperature (24 ± 2 °C).

DAH (days)	Treatments			
	Control	1-MCP	SA	1-MCP + SA
0	19.6 aA	19.65 aA	19.65 aA	19.65 aA
2	13.7 bB	14.63 bA	12.09 bC	13.47 bB
5	12.2 cB	15.12 bA	11.36 cC	12.12 cB
8	12.5 cA	12.71 cA	11.01 cB	10.59 dB
11	10.7 dB	11.33 deA	-	-
14	10.7 dB	11.72 dA	-	-
17	9.03 eB	10.77 eA	-	-
20	-	-	-	-
C.V. (%)	1.74			
F Int	51.19**			

DAH – days after harvest

Averages followed by the same letter, upper and lower case in vertical do not differ by Tukey test ($p > 0.05$). ** Significant at 1% probability.

Table 2. Total soluble carbohydrates concentrations (mg glucose 100 g^{-1} fresh mass) in lisianthus flowers pre-treted with ethylene inhibitors and storage at cold temperature (9 ± 2 °C) for 24h.

DAH (days)	Treatments			
	Control	1-MCP	SA	1-MCP + SA
0	19.65 aA	19.65 aA	19.65 aA	19.65 aA
2	13.82 bB	15.55 bA	12.15 bC	12.36 bC
5	13.44 bB	14.09 cA	11.82 bC	11.33 cD
8	12.34 cB	12.96 dA	10.83 cC	10.00 eD
11	11.74 dB	12.24 eA	9.87 dD	10.38 dC
14	11.52 dB	12.10 eA	-	-
17	10.52 eB	11.36 fA	-	-
20	-	10.55 gA	-	-
C.V. (%)	1.51			
F Int	111.99**			

DAH – days after harvest

Averages followed by the same letter, upper and lower case in vertical do not differ by Tukey test ($p > 0.05$). ** Significant at 1% probability.

Pre-exposed flowers, buds and leaves in a cold chamber (9 ± 2° C) for 24 hours showed higher carbohydrate levels throughout the storage period when compared to those stored only at room temperature. This result is due to the effect of temperature on respiratory activity and consequently on carbohydrate consumption. Other studies show decrease in carbohydrate content during post-harvest. According Hew and Yong (2004), in orchids occur a decrease in carbohydrate levels due to increased respiratory activity. Carbohydrates, in general, act on respiration, water balance, metabolism and ethylene action, in addition to interacting with other plant hormones (SANTOS, 2008).

The highest reduction of soluble carbohydrates concentration was verified on SA treatment, with floral stems remaining viable until the eighth day after harvest, and the highest contents was observed on 1-MCP treatment. This was observed on leaves, buds and flowers for both storage conditions.

Figure 1. Yellowing and turgescense lost of lisianthus cut flowers in pre-tretament with salicilic acid.

The 1-MCP treatment delayed the reduction of carbohydrate contents in flowers until the fifth day of storage, regardless of the temperature used. On the other hand, this was not observed in buds stored at room temperature.

Generally, carbohydrates contents decrease during senescence due to oxidative processes that occur in plants after harvest. To prolong vase life, treatments that maintain the levels of carbohydrates in cut flowers are fundamental and according to Pun & Ichimura (2003), they are associated with alterations on the synthesis of ethylene. In our study, 1-MCP treatment may have reduced ethylene levels and consequently maintained carbohydrate contents, which may be significant to increase the vase life of lisianthus. Rose cut flowers treated with 1-MCP have a lower respiratory rate and small consumption of photo-assimilates (HUANG et al., 2017), which contributes to reduce carbohydrate consumption, thus a higher vase life of lisianthus.

The lisianthus pre-exposed in cold chamber (9 ± 2 °C) for 24 hours (Tables 2, 4 and 6) had higher carbohydrate levels during the storage period than those stored at room temperature (24 ± 2 °C) (Tables 1, 3 and 5).

Table 3. Total soluble carbohydrates concentrations (mg glucose 100 g^{-1} fresh mass) in lisianthus buds pre-treted with ethylene inhibitors and storage at room temperature (24 ± 2 °C).

DAH (days)	Treatments			
	Control	1-MCP	SA	1-MCP + SA
0	20.74 aA	20.74 aA	20,74 aA	20,74 aA
2	17.16 bB	19.01 bA	16,12 bC	16,36 bBC
5	14.12 cA	14.73 cA	12,27 cB	13,26 cB
8	12.92 cdAB	13.51 dA	11,15 dC	12,54 cB
11	11.78 dB	13.09 dA	-	-
14	10.48 eB	11.57 eA	-	-
17	9.85 eB	11.21 eA	-	-
20	-	-	-	-
C.V. (%)	2.76			
F Int	6.93**			

DAH – days after harvest

Averages followed by the same letter, upper and lower case in vertical do not differ by Tukey test ($p > 0.05$). ** Significant at 1% probability.

Table 4. Total soluble carbohydrates concentrations (mg glucose 100 g^{-1} fresh mass) in lisianthus buds pre-treated with ethylene inhibitors and storage at cold temperature (9 ± 2 °C) for 24h.

DAH (days)	Treatments			
	Control	1-MCP	SA	1-MCP + SA
0	20.74 aA	20.74 aA	20.74 aA	20.74 aA
2	14.86 bB	19.75 aA	15.68 bB	14.91 bB
5	13.69 bB	16.49 bA	10.00 dC	12.97 cB
8	11.57 cC	14.22 cA	12.96 cB	13.33 cAB
11	11.67 cB	12.79 dA	10.83 dB	11.44 dB
14	11.10 cB	12.42 dA	-	-
17	10.88 cB	12.15 dA	-	-
20	-	12.10 dA	-	-
C.V. (%)	3.40			
F Int	33.48**			

DAH – days after harvest

Averages followed by the same letter, upper and lower case in vertical do not differ by Tukey test (p > 0.05). ** Significant at 1% probability.

Low temperatures decrease respiration and transpiration, slow sugar reserves degradation, reduce ethylene production, prolong flowers durability (SONEGO and BRACKMANN, 1995), and consequently slows down degradation of carbohydrates (Srivastava et al., 2015). *Oncidium varicosum* 'Samurai' inflorescences kept at 5 and 10 ºC maintain the soluble carbohydrate contents constant, indicating that carbohydrates were little used during the senescence process and resulted in a longer vase life (MATTIUZ et al., 2010).

Total soluble carbohydrates contents were higher in buds (Tables 1 and 2) and flowers (Tables 3 and 4), than in leaves (Tables 5 and 6).

Table 5. Total soluble carbohydrates concentrations (mg glucose 100 g^{-1} fresh mass) in lisianthus leaves pre-treted with ethylene inhibitors and storage at room temperature (24 ± 2 °C).

Control	Treatments			
	Control	1-MCP	SA	1-MCP + SA
0	8.18 aA	8.18 aA	8.18 aA	8.18 aA
2	6.70 bAB	7.20 bA	6.46 bB	6.33 bB
5	5.16 bB	5.42 cA	4.24 cB	4.34 cB
8	4.91 cA	5.11 cA	3.51 dB	4.08 cB
11	4.74 cdA	4.99 cdA	-	-
14	4.79 cA	4.96 cdA	-	-
17	3.96 dB	4.29 dA	-	-
20	-	-	-	-
C.V. (%)	5.74			
F Int	7.13**			

DAH – days after harvest

Averages followed by the same letter, upper and lower case in vertical do not differ by Tukey test ($p > 0.05$). ** Significant at 1% probability.

Table 6. Total soluble carbohydrates concentrations (mg glucose 100 g^{-1} fresh mass) in lisianthus leaves pre-treated with ethylene inhibitors and storage at cold temperature (9 ± 2 °C) for 24h.

DAH (days)	Treatments			
	Control	1-MCP	SA	1-MCP + SA
0	8.18 aA	8.18 aA	8.18 aA	8.18 aA
2	6.50 bA	6.60 bA	5.38 bB	6.48 bA
5	6.07 bA	6.59 bA	5.03 bB	4.83 cB
8	5.03 cB	5.64 cA	4.43 cC	4.48 cdBC
11	5.00 cA	5.45 cA	4.21 cB	4.08 dB
14	4.58 cB	5.00 cA	-	-
17	4.50 cB	5.37 cA	-	-
20	-	5.27 cA	-	-
C.V. (%)	4.50			
F Int	16.31**			

DAH – days after harvest

Averages followed by the same letter, upper and lower case in vertical do not differ by Tukey test ($p > 0.05$). ** Significant at 1% probability.

Flowers usually act on plants as drains, due to high quantity of sugar needed to metabolism maintenance (MALAVOLTA et al., 2006). During cut flower post-harvest, leaves function as a source, mainly transforming starch into smaller molecules such as soluble sugars, which are translocated to flowers. Thus, in our study, the differentiated distribution of total soluble carbohydrates characterizes a competition among flowers, buds and leaves (source and drain) in lisianthus for assimilates, with high levels in of total soluble carbohydrates in flowers and low levels in leaves. The reduction on respiratory activity promoted by 1-MCP may be resulted from its effect on carbohydrate metabolism (HUANG et al., 2017). Treatment with 1-MCP was more efficient to maintain total soluble carbohydrates contents in lisianthus during the entirely post-harvest period on all storage conditions. This characteristic may be related to the capacity of 1-MCP to reduce considerably the respiratory activity and delay the climacteric phase (DONG et al., 2002), once 1-MCP inhibits ethylene action, which is responsible to accelerate fruit, foliage and flowers senescence. The association of 1-MCP with the pre-exposure of the material to cold chamber, besides keeping the carbohydrate contents higher in relation to the other treatments. Longevity depends on respiratory activity, once respiration is linked to growth and senescence and is a source of heat generation.

4. CONCLUSIONS

Total soluble carbohydrate contents decreased during the experimentation, characterizing the consumption of the reserves during lisianthus post-harvest. The 1-MCP treatment slowed the decrease of total soluble carbohydrate contents. SA was not an effective treatment for vase life

maintenance on lisianthus, either at room temperature or at low temperature. In this study, 1-MCP applied alone showed a beneficial effect to sustain carbohydrate levels, which will reflect in vase life, when combined with SA did not show satisfactory results to maintain carbohydrate levels. Our results showed that low temperature is the best treatment to maintain carbohydrate levels, thus conserving lisianthus vase life.

AUTHORS CONTRIBUTIONS

R.C.: conception and design of the research, obtaining data, analyze and data interpretation, statistical analysis, write and critically analyses of manuscript. **D.L.**: conception and design of the research, obtaining data, analyze and data interpretation, statistical analysis, write and critically analyses of manuscript. **A.R.T.**: analyze and interpretation of data, write and critically analyses of manuscript. **G.P.P.L.**: conception and design of the research, obtaining data, analyze and data interpretation, statistical analysis, write and critically analyses of manuscript and financial and obtaining funding.

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