




## SCIENTIFIC ARTICLE

# Hormonal endogenous changes in response to the exogenous 6-benzylaminopurine application in pre- and post-harvesting liliu flower stalks

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## Abstract

Phyto-hormones play a key role in regulating plant responses to stress. Cytokines are a type of phyto-hormones involved in the regulation of many important biological processes related to growth, development, and response to environmental variables. The exogenous application of cytokines increases the possibility of delaying senescence; however, this is a physiological process, and, under certain conditions, degradation processes may be triggered. The effect of 6-benzylaminopurine application and the endogenous hormonal changes involved in lilies floral stalks after their cutting were studied. In order to improve vase life and quality of *Lilium longiflorum* 'Brindisi' flower stalks, they were sprayed with 6-BAP, at a concentration of 300 ppm at pre-harvest, post-harvest, and pre- and post-harvest stages. After that, they were compared to non-sprayed control stalks. The application of 6-BAP caused endogenous hormonal changes in abscisic acid and cytokinin levels, and the most effective treatment was pre-harvest spraying. This treatment proved to be an appropriate method to improve the stalk tolerance to post-harvest stress as it delayed the appearance of senescence symptoms and reduced the speed of chlorophyll degradation with differences of up to 10% with respect to untreated stalks. In addition, the opening of flowers was delayed by up to 2 days, although there were no significant differences in total vase life.

**Keywords:** *Lilium longiflorum*; senescence; phytohormones; 6-benzylaminopurine; vase life.

## Resumo

### Alterações hormonais endógenas em resposta à aplicação exógena de 6-benzilaminopurina em pré- e pós-colheita de hastes florais de lírio

Os fitormônios desempenham um papel fundamental na regulação das respostas das plantas ao estresse. As citocinas são um tipo de fitormônios envolvidos na regulação de muitos processos biológicos importantes relacionados ao crescimento, desenvolvimento e resposta a variáveis ambientais. A aplicação exógena de citocinas aumenta a possibilidade de retardar a senescência; entretanto, este é um processo fisiológico e, sob certas condições, processos de degradação podem ser desencadeados. Foi estudado o efeito da aplicação de 6-benzilaminopurina e as alterações hormonais endógenas envolvidas, nas hastes florais de lírio japonês após seu corte. Para melhorar a vida de vaso e a qualidade das hastes florais de *Lilium longiflorum* 'Brindisi', estes foram pulverizados com 6-BAP na concentração de 300 ppm nas fases de pré-colheita, pós-colheita e pré e pós-colheita e foram comparados com hastes não pulverizadas. A aplicação de 6-BAP causou alterações hormonais endógenas nos níveis de ácido abscísico e citocinina, e o tratamento mais eficaz foi a pulverização pré-colheita. Este tratamento mostrou-se um método adequado para melhorar a tolerância da haste ao estresse pós-colheita, pois retardou o aparecimento dos sintomas de senescência e reduziu a velocidade de degradação da clorofila com diferenças de até 10% em relação às hastes não tratadas. Além disso, a abertura das flores foi atrasada em até 2 dias, embora não houvesse diferenças significativas na vida total de vaso.

**Palavras-chave:** *Lilium longiflorum*; senescência; fitohormônios; 6-benzilaminopurina; vida em vaso.

## Introduction

Lilies (*Lilium sp.*) have a worldwide demand due to their high commercial and ornamental value. However, the

quality and vase life of cut stalks are affected by pre- and post-harvest factors (Hajizadeh, 2016). This deterioration of cut flowers that affects the growth and development of plants is attributed to changes in the effects of endogenous

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enzymes, mechanical damage and/or environmental factors; all physiological responses are considered to be influenced by plant hormones (Lymperopoulos et al., 2018).

Cytokinins (CK) have profound effects on almost every biological event during plant development and defense, floral organ maturation and abscission, leaf/flower senescence, and in responses to various biotic and abiotic stresses (Smith et al., 2017; Bengoa Luoni et al., 2019). Previous experiments indicate the beneficial effects of these phytohormones in physiological and molecular processes, as well as in defense against biotrophic pathogens. Today cytokinins are known to regulate the metabolism and transport of amino acids and carbohydrates important for plant growth, as well as several macronutrients, including nitrogen, phosphorus, sulphur, and iron (Albrecht and Argueso, 2017).

During its life cycle, a plant can be exposed to various environmental conditions that can prematurely induce its senescence. Hormones can participate directly in its regulation or can work in an antagonistic way. This view is highly consistent with the findings that certain cultivars under stress conditions trigger premature senescence and penalize plant production (Lv et al., 2020).

Senescence is accompanied by numerous changes focused on initiating cellular structural degradation processes, at a cytological, physiological, and molecular level, in which phytohormones are heavily involved (Wojciechowska et al., 2018); leaf senescence implies gradual yellowing and wilting, mainly due to the plant's age and the transition to the reproductive stage, but they are also modulated by endogenous and exogenous signals that are integrated into the senescence program (Mayta et al., 2019). However, petal senescence is different because of the abundance of secondary metabolites. Hence, several phytohormones are involved in the regulation of petal senescence, and they are considered to act synergistically and antagonistically (Ma et al., 2018).

In cut flowers, the postharvest life is often limited, which reduces the commercial opportunities for these types of products. For this reason, the industry has developed a great variety of technologies whose objective is to replace the use of synthetic chemical preservatives, which are generally harmful to the environment. These technologies include temperature management, modified or controlled atmospheres, and hormonal treatments (Reid and Jiang, 2012).

Phyto-hormones are not only involved in regulating growth and developmental processes, but their exogenous applications may also help improve the plant yield (Li et al., 2016). Furthermore, as a well-established stress hormone, ABA plays a key role in plant responses to abiotic stresses (Shu et al., 2018); in addition, it has been shown that they can promote senescence, whereas others, such as CK, can suppress it (Khan et al., 2013; Zhang and Zhou, 2013). 6-benzylaminopurine (BAP) is well known as a master

growth modulator: it can build resilience by suppressing free radicals against abiotic or water stress (Kamran et al., 2021) or by inducing tolerance in perennial roots in *Lolium perenne* L. cv. Pinnacle (Ma et al., 2016). In general, the exogenous application of 6-BAP causes a delay in the foliar senescence. The exogenous application of cytokinin in potted and cut flowers was observed to delay leaf yellowing and decrease ethylene biosynthesis (Iqbal et al., 2017).

The visible signs of senescence are the late manifestation of events that begin even before the harvest. Therefore, early treatments (in pre-harvest or at harvest time) could be more effective in delaying the loss of quality in post-harvest and, thus, increasing the longevity of cut flowers (Haberer and Kieber, 2002). In a preliminary work, the effect of applications of 6-BAP in pre- and post-harvest on the vase life of cut roses was evaluated, and the best result was obtained with the application of 3 cm<sup>3</sup> L<sup>-1</sup> three days before harvest<sup>1</sup>.

Currently, one goal in postharvest technology of cut flowers is to develop environmentally-friendly techniques that allow optimizing both the quality of the final product and its vase life, based upon the exogenous application of phytohormones. Focusing on pre-harvest and post-harvest treatments, we tested whether CKs apply increases cytokinin biosynthesis and delays postharvest senescence of *L. longiflorum*.

Therefore, the objectives of the study were:

- to determine the content of cytokinins in lilies stems subjected to different treatments of exogenous application of BAP at the beginning and at the end of the post-harvest phase;
- to make correlations with the concentrations of total phytohormones;
- to correlate these values with vase life, and foliar and floral senescence.

## Material and methods

Plant material was obtained from a crop of cut flower *L. longiflorum* cv. *Brindisi* carried out in a 6.4 × 24 m metal greenhouse with a 150-micron thermal polyethylene cover, equipped with passive lateral and zenithal ventilation, and an automated irrigation system, in 0.4 m wide × 0.3 m deep × 20 m long flower benches, with perlite substrate.

Bulbs were obtained from a local representative, and these were 16–18 cm gauge. Planting was done on July 15<sup>th</sup> at a bed density of 20 bulbs m<sup>-2</sup>, and the stems were harvested on October 30<sup>th</sup>. The average air temperature during cultivation was 24.9°C with an average minimum of 20.4°C and an average maximum of 30.3°C, and a relative humidity of 82%.

Fertigation was applied daily, using drip belts with 1 L/h per emitter nominal flow rate, with 20 emitters per linear bed meter. The irrigation dose was established according to the evapotranspiration estimated by the Penman-Monteith

1 According to: Mascarini L, Divo De Sesar M, Caporale G, Longoni P, Vilella F. Longevidad en vaso de Rosa hybrida cv. Exótica para flor de corte suplementada con 6-bencilaminopurina en pre y poscosecha. In: 3er. Congreso Argentino de Floricultura. Libro de resúmenes. Buenos Aires: Instituto Nacional de Tecnología Agropecuaria; 2006. p. 191-194.

method modified by FAO (Allen et al., 1998), the applied lamina being between 2 to 3 L day<sup>-1</sup> m<sup>-2</sup> (bench square meter). Fertilization started after the emergence of the stems, approximately 20 days after planting. The formula applied was (mg L<sup>-1</sup>): 189.15 N, 91.24 P<sub>2</sub>O<sub>5</sub>, 189 K<sub>2</sub>O, 211 SO<sub>4</sub>, 186 Ca, 47 mg along vegetative stage; and (mg L<sup>-1</sup>): 189 N, 38 P<sub>2</sub>O<sub>5</sub>, 285 K<sub>2</sub>O, 345 SO<sub>4</sub>, 186 Ca, 47 mg along reproductive stage (modified from Álvarez-Sánchez et al., 2008).

When this crop reached commercial maturity stage (marked by the presence of the first colored bud), 6-BAP was applied at different times (Mascarini et al., 2006) (Table 1). A group of 24 homogeneous, both in their physiological development and number of buds stalks were selected, and 12 of them were sprayed with 6-BAP three days before harvest at a concentration of 3 cm<sup>3</sup> L<sup>-1</sup> in a uniform way, in the morning (8am) to avoid high temperatures that could evaporate the product. The spray was fine, covering all the vegetative parts of the plant, from the base to the extreme part of the inflorescence up to drip point. In the same way, the other 12 plants were sprayed with distilled water. During the operation, the plants were sprayed without the presence of strong air currents and surrounded by polyethylene sheets to minimize the risk of drift and precipitation to soil.

At the time of harvest, the 24 stalks were trimmed to 60 cm in length and transported with constant hydration to the post-harvest room. Stalks were weighed and then placed in distilled water in 250 cm<sup>3</sup> graduated test tubes previously disinfected, which were sealed with a plastic film to prevent direct evaporation; 6 stalks were left untreated, 6 were left only with the pre-harvest treatment, and the remaining 12, including the 6 previously treated in pre-harvest stalks, were again sprayed with 6-BAP at the same concentration.

Throughout the post-harvest period, the stalks were kept in distilled water (pH 5.2, TDS 5 ppm total dissolved solids, EC 0.5 µS/cm electrical conductivity), daily replenishing the water consumed by stems with a syringe. The post-harvest laboratory was kept at an average temperature of 18°C, 56% relative humidity, and with a 12-hour light photoperiod, under 36 W white fluorescent lamps, programmed with an electric timer.

Morphological changes were determined descriptively, using a scale of degrees of openness from degree zero (0) in ascending order to degree six (6). Thus, grade I (button closed showing color), grade II (initial opening of the button), grade III (anthesis), grade IV (turgid tepals), grade V (dehydrated tepals, color changes), and grade VI (total abscission of tepals) (adapted from Arrom and Munné-Bosch, 2012).

At the beginning and at the end of the post-harvest test, samples of leaves and tepals were taken to determine hormonal changes. CQ and ABA extraction and HPLC determination were performed following Hoyerová et al. (2006) and Dobrev and Kamínek (2002) methodologies.

The following variables were recorded: longevity of the stalks in the vase (vase life), opening speed and flower abscission or senescence, daily water consumption (recording the level of distilled water remaining in the test tube), daily variation of entire stalk fresh weight, and chlorophyll concentration in the youngest leaf measured by the SPAD index, taking 3 measures per leaf (SPAD -502 Konica Minolta Sensing, Inc).

The design was completely randomized, with 6 repetitions per treatment. Data were analyzed by ANOVA using InfoStat / Professional V1.1 software.

## Results

### Changes in endogenous phytohormone concentrations

The application of exogenous cytokinin provoked significant changes in the endogenous concentration of natural cytokinins, ZT (zeatin) and ZTR (zeatin riboside), and Q (quinetine); however, their intensity was different in leaves and tepals, and varied with the moment of application (Table 2). Changes in ABA (abscisic acid) and AIA (indolacetic acid) levels were also observed in leaves, but not in tepals.

In the leaves, ZT content was 32% lower, ZTR was 61% higher, and Q 147% higher in T2 than in the control; AIA also increased by 30% and ABA, by 573%.

In tepals, the application of 6-BAP at any of its variants caused a decrease of between 7 and 10 times the concentration of ABA, but untreated stalks showed up to 95% of decrease, without significant differences among treatments. On the other hand, Q has a strong decrease in the control treatment, therefore, the application of 6-BPA caused a significant difference in the Q concentration in tepals, between 1.2 up to 3.2 times compared with control treatment. whereas ZTR only showed a significant change in T3; and ZT, in T2 and T4, but in this case, the final concentration decreased with respect to the control treatment (Table 2).

### Water consumption

No differential response to water consumption was observed between treatments (Figure 1). However, a tendency to increase water consumption was registered between the sixth and twelfth day after cutting the stems under treatment 1. From the first day of the trial, consumption rate was higher at the beginning and gradually

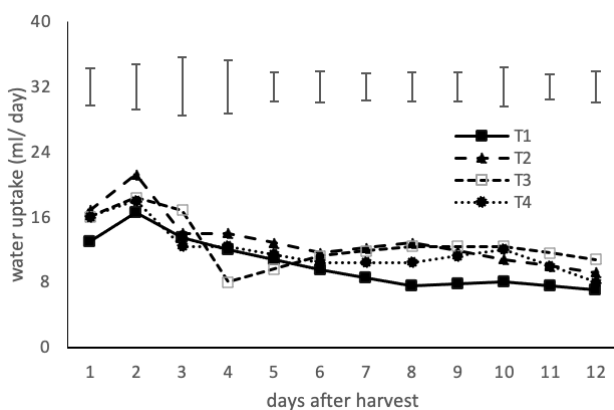
**Table 1.** Treatments applied to *Lilium longiflorum* cv. 'Brindisi' cut stalks.

Treatment	Description
T1	Control stalks sprayed with distilled water, 3 days before harvest
T2	Stalks sprayed with 6-BAP (300 ppm), 3 days before harvest
T3	Stalks sprayed with 6-BAP (300 ppm), at harvest
T4	Stalks sprayed with 6-BAP (300 ppm), 3 days before harvest and again at harvest

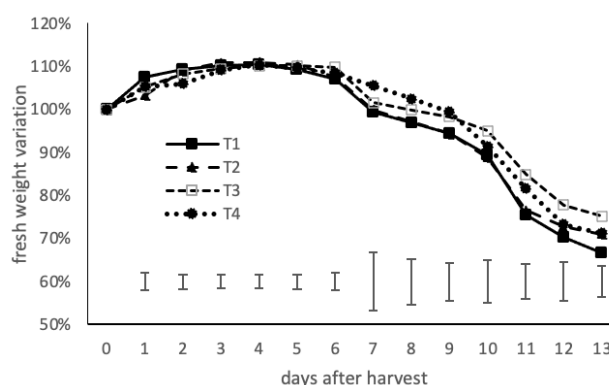
**Table 2.** Effects of BAP on the endogenous content of phytohormones (ng. g<sup>-1</sup>) at the beginning and at the end of the postharvest stage in tepals and leaves of cut stems of *Lilium longiflorum* cv. 'Brindisi', without hormonal treatment (T1) and after the application of 6-BAP in pre-harvest (T2), at the time of harvest (T3) or at both moments (T4).

Organ	Sampling time	Treatment	Phytohormones Concentration (ng g <sup>-1</sup> )									
			ABA		AIA		ZT		ZTR		Q	
Leaves	Initial	T1	0.3212	a	0.7845	a	0.0443	a	0.0145	a	nd	
		T2	0.4186	a	0.5351	a	0.0717	a	0.0357	a	nd	
		T3	0.1624	a	0.7993	a	0.0225	a	0.0121	a	nd	
		T4	0.266	a	0.7621	a	0.3503	b	0.0055	a	nd	
		Sign.	ns		ns		*		ns			
	Final	T1	7.35	a	321.2	ab	784.5	a	44.32	a	14.49	a
		T2	49.43	b	418.6	b	535.1	b	71.71	b	35.74	b
		T3	53.7	b	162.4	c	799.3	a	22.5	c	12.07	a
		T4	78.5	c	266	ac	762.1	a	350.3	d	5.482	c
		Sign.	*		*		*		**		**	
Tepals	Initial	T1	3.8	a	0.5923	a	0.0207	a	0.2033	a	0.442	a
		T2	0.2349	a	0.5489	a	0.0207	a	0.2245	a	0.0411	b
		T3	0.2576	a	0.5114	a	0.0143	a	0.2638	a	0.0167	c
		T4	0.254	a	0.5483	a	0.0346	b	0.1292	b	0.0304	b
		Sign.	ns		ns		*		*		*	
	Final	T1	0.0398	a	1.22	a	1.931	a	0.2232	a	0.0074	a
		T2	0.0226	a	0.8412	a	1.333	b	0.2108	a	0.0494	b
		T3	0.0237	a	1.074	a	1.697	a	0.3334	b	0.0537	b
		T4	0.0402	a	1.023	a	1.428	b	0.2298	a	0.0785	c
		Sign.	ns		ns		*		*		*	

ABA: abscisic acid; AIA: indolacetic acid; ZT: zeatin; ZTR: zeatin riboside; Q: quinetine; nd: not detectable; \*significant p≤0.05; \*\*significant p≤0.01; ns: not significant.



**Figure 1.** Daily water uptake in post-harvest of cut stalks of *Lilium longiflorum* cv. 'Brindisi', without hormonal treatment (T1) and after 6-BAP application at pre-harvest (T2), at the time of harvest (T3) or at both times (T4). Vertical bars represent DMS at p≤0.05.



**Figure 2.** Variation of relative fresh weight (day of harvest=100), in post-harvest of cut stalks of *Lilium longiflorum* cv. 'Brindisi', without hormonal treatment (T1) and after the application of 6-BAP in pre-harvest (T2), at the time of harvest (T3) or at both times (T4). Vertical bars represent the DMS at p≤0.05.

decreased towards the end, coinciding with the general deterioration of the stems and, specially, of the leaves.

### Fresh weight

Initial average weight was 112.7±7.17 g. There were no significant differences either in the initial weight or in those

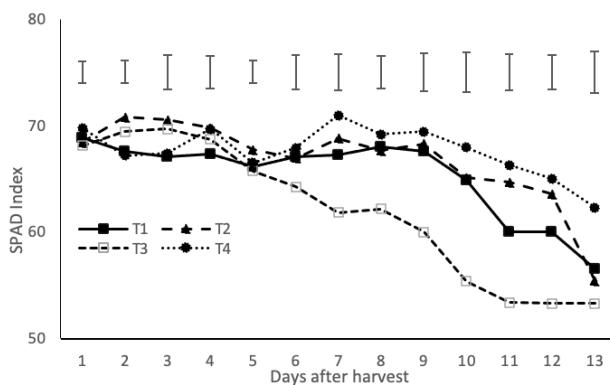
recorded on subsequent post-harvest days. Evolution of fresh weight measured as a percentage of initial weight did not show significant differences between treatments either (Figure 2). In contrast to water consumption, changes in fresh weight were slight until the sixth day, however, they increased towards the end.

### SPAD index

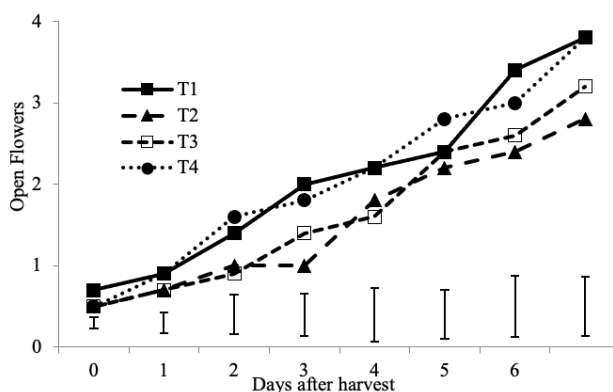
Chlorophyll content measured by green index (SPAD) showed significant differences for the first three days in all variants of 6-BAP application (Figure 3). The treatment that worked best to maintain greenness was T2, with up to 5% difference over the control. After the fourth day, T3 treatment showed a sustained drop in the SPAD index, whereas in the control, dropping started on day 9. The treatment that worked best to maintain greenness was T4, which at the end of the trial showed a 10% difference over the control.

### Vase life and first flower senescence

Although there were no significant differences regarding the total duration in the vase, in all the variants of 6-BAP application, a delay in the flower opening rate could be achieved (Figure 4). A similar evolution was observed in the control flowers, in those that received double hormone treatment (T4), as well as in those that only received one treatment (T2 and T3).

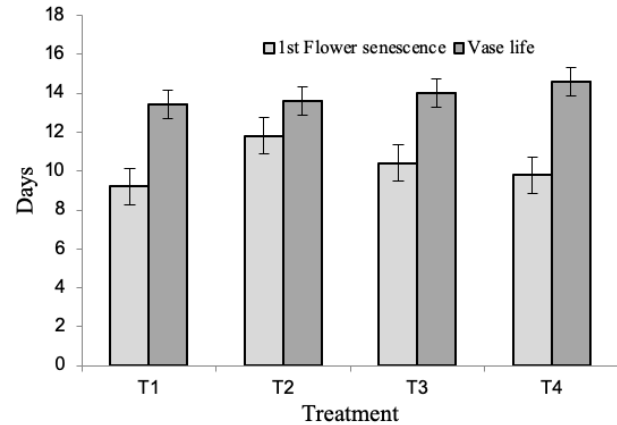


**Figure 3.** Evolution of SPAD chlorophyll index values, in post-harvest of cut stalks of *Lilium longiflorum* cv. 'Brindisi', without hormonal treatment (T1) and after the application of 6-BAP in pre-harvest (T2), at the time of harvest (T3) or at both times (T4). Vertical bars represent DMS at  $p \leq 0.05$ .



**Figure 4.** Individual flowers opening rate, at post-harvest of *Lilium longiflorum* cv. 'Brindisi' cut stalks without hormonal treatment (T1) and after the application of 6-BAP at pre-harvest (T2), at the time of harvest (T3) or at both times (T4). Vertical bars represent DMS at  $p \leq 0.05$ .

It was also observed that hormone treatment in all its variants delayed the senescence of the first flower, T2 being the most effective (Figure 5). However, there were no significant differences regarding total vase life.



**Figure 5.** Days to senescence of the first flower and total vase life, at post-harvest of cut stems of *Lilium longiflorum* cv. 'Brindisi', without hormonal treatment (T1) and after the application of 6-BAP at pre-harvest (T2), at the time of harvest (T3) or at both times (T4). The vertical bars represent the DMS at  $p \leq 0.05$ .

## Discussion

Usually, the first days after harvesting a floral stalk are crucial due to the impact of the initial conditions on the total vase life. An important part of these events is determined by the hormonal changes caused by post-cutting stress.

A distinctive feature of plant hormones is their cooperation with other hormones. One example is exogenous auxin and cytokinin; whereas stems with a larger diameter are less efficient in recovering water conductivity, stems applied with CK achieve a better vascular tissue formation and, consequently, photosynthesis tissue nutrition is improved, enhancing immunity to different stressful factors, such as water stress (Sosnowski et al., 2019).

CKs acting as ABA antagonists can increase the stomatal conductance and, therefore, modulate both gas exchange in the leaves and availability of  $\text{CO}_2$ , the essential substrate for photo-synthetically active tissue (Ahanger et al., 2018). Broccoli cultivation has shown that if the heads are completely immersed in 6-BAP solutions during the post-harvest, an inhibition of the senescence process occurs, resulting in a delay in proteins degradation (Hasperué et al., 2011).

Another important fact that determines the shortening in vase life is the yellowing of the leaves on the harvested stalks due to the degradation of the chlorophyll once it has been separated from the plant (Matak and Hashemabadi, 2016).

Application of 6-BAP had a great effect in the first 3 days after the harvest, T1=T4, and the SPAD index was maintained and even slightly increased. Over the last

days, the stems sprayed at pre-harvest or at pre- and post-harvest performed better in maintaining the greenness of the foliage, whereas those that were only sprayed at post-harvest behaved similarly to the control stems. Here, the timing of application may be important, since an exogenous phytohormone must be absorbed and assimilated in order to trigger a response, and this can normally take some time.

CKs are known to be specifically transported either by the phloem (Isopentenyl adenine-type CKs) or the xylem (Zeatin-type CKs) between different plant parts (Schäfer et al., 2014). When applied exogenously to plants, they can stimulate a series of physiological, metabolic, and biochemical processes (Iqbal et al., 2017). Since the mobility of synthetic CKs is low within plant tissue, liliium applications were only located in leaves and tepals. In liliium, it has been reported that changes in both endogenous ABA and gibberellins depend on application time of exogenous CK, due to a progressive loss in hormonal sensitivity during senescence progression (Cubría-Radío et al., 2017).

A third important phenomenon in the culture of cut flowers is the loss of ornamental quality due to tissue senescence. This can be delayed by the addition of the anti-senescing agent BAP, by altering the hormonal balance (Sobieszczuk-Nowicka et al., 2016).

Previous experiments have shown that CK delay flowering senescence in different ornamental species (Reid and Jiang, 2012). The analysis of endogenous hormone contents in different *Lilium* stalk tissues has revealed that cytokinin levels mostly increased in tepals before anthesis and subsequently decreased during senescence (Krstulović et al., 2018). CK exogenous application to plant tissues delays senescence, maintains chloroplast activity, decreases chlorophyll degradation, and enhances protein and nucleic acid production (Clarke et al., 1994; Xu et al., 2013).

Extent of vase life is not the only objective of postharvest management. From a commercial point of view, slowing the natural process in those cut flowers harvested at a mature physiological stage before the opening of the flowers is important. In *Nelumbo nucifera*, various combinations of 6-BAP, sucrose and time of pulsing were investigated to find the optimum combination to keep quality, based on weight loss, wilting, petal falling, and other quality parameters (Chathuri and Sarananda, 2011). However, 6-BAP has been used in numerous ornamental species to extend vase life. Rojas-Morales et al. (2017) reported that the double application of BAP slightly increased the number of days in the vase for *Lisianthus (Eustoma grandiflorum (Raf.) Shinnners)*. In *Anthurium andreanum*, vase life was extended up to 17 days when flower stalks were sprayed with 6-BAP (Favero et al., 2020). In our study, the application of 6-BAP succeeded in retarding the opening rate of individual flowers and the first opened flower senescence, resulting in a longer commercial life span although total vase life was not altered.

After flower opening, ZT decreased both in the external and internal tepals during senescence (stages III-VI), whereas AIA also decreased, but only in the leaves. Between grades IV and V, ZTR increased in the outer and inner tepals, whereas ABA increased only in the tepals, indicating the

role that CK plays in the regulation of senescence, as already observed in previous studies that show CK's determination in several model plants development, in which ZTs is high at seed stage, turns lower at young developing plants, and increases again when plants stop growing and begin to age (Schäfer et al., 2015).

## Conclusions

Although not all treatments responded positively to CK applications in the post-harvest period, BAP applications to the *lilium* stalks at pre-harvest or at pre- and post-harvest were conclusive. Stalks post-harvest life improved, both in the leaves and in the tepals, delaying the signs of senescence symptoms in the leaves and in the first flower. This can be crucial, considering this product is highly perishable and has an extremely limited commercial lifespan: once the flower is open, the product loses value or is not acceptable for sale. In general, the application of CKs was an excellent alternative to extend the shelf life of freshly cut lilies, stored for 15 days at 18 °C, but in few treatments. Future research should experiment with higher doses and at different times of pre-harvest application. The use of phytohormones, especially 6-BAP, are possible alternatives that deserve further investigation, and it could become a low-cost, easy-to-implement, and environmentally friendly postharvest handling technique.

## Author contribution

GM: Conceptualization, data curation, formal analysis, writing original draft. GAL: Conceptualization, formal analysis, writing original draft, writing review and editing. LM: Conceptualization, formal analysis, writing original draft, writing review and editing.

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