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ARTICLE

Effects of water stress on the post-harvest quality of cut *Lilium flowers*

Efeito do estresse hídrico na qualidade pós-colheita de flores de *Lilium longiflorum*

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Abstract: The limited availability of water due to agricultural, industrial, technological, and urban expansion requires a greater effort to make its use more efficient and achieve substantial savings in ornamental productions without affecting the commercial value quality, and final presentation of flowers and plants. Water stress is an environmental factor that causes physiological effects in plants and accelerates the senescence of leaves and flowers on cut stems. Considering crop evapotranspiration (ETc), an alternative way to reducing water consumption in intensive crops is the use of controlled, restricted irrigation, coupled with the application of phytohormones in pre-harvest to mitigate the effects of water stress, i.e. 6-Benzylaminopurine (6-BAP). The objective of this study was to determine the quality and vase life of Lilium stems under restricted irrigation combined with phytohormones application. An experiment with four treatments was designed, concerning normal (NI, 100% ETc) or restricted irrigation (RI, 35% ETc) and with or without application of 6-BAP (300 ppm). A completely randomized ANOVA design with 6 repetitions per treatment was used. The vase life of cut Lilium flowers was not significantly affected (13.9 vs. 12.5 days under NI and RI respectively). The application of 6-BAP had no influence on vase life, but delayed the foliage yellowing and therefore reduced the rate of deterioration of the floral stem. Restricted irrigation and the application of phytohormones can be useful tools to improve the water use efficiency in lily crops.
Keywords: 6-benzylaminopurine, *Lilium longiflorum*, phytohormones, senescence, vase life.

Resumo: A limitada disponibilidade de **água** devido ao desenvolvimento agrícola, industrial, tecnológico e urbano exige um maior esforço para tornar seu uso mais eficiente e obter economía substancial nas produções ornamentais, sem afetar o valor comercial, a qualidade e a apresentação final das flores e plantas. O estresse **hídrico é** um fator ambiental que causa efeitos fisiológicos nas plantas e acelera a senescência de folhas e flores em hastes de corte. Considerando a evapotranspiração das culturas (ETc), uma forma alternativa de reduzir o consumo de **água** nas culturas intensivas é o uso de irrigação controlada e restrita, somada à aplicação de fitohormônios na pré-colheita para mitigar os efeitos do estresse **hídrico, por** exemplo, 6-benzilaminopurina (6-BAP). O objetivo deste estudo foi determinar a qualidade e vida de vaso de hastes de Lilium sob irrigação restrita combinada com aplicação de fitohormônios. Foi delineado um experimento com quatro tratamentos, em irrigação normal (NI, 100% ETc) ou restrita (IR, 35% ETc) e com ou sem aplicação de 6-BAP (300 ppm). Foi utilizado um delineamento ANOVA inteiramente casualizado com 6 repetições por tratamento. A vida de vaso das flores de corte de Lilium não foi afetada significativamente (13,9 vs. 12,5 dias com NI e RI, respectivamente). A aplicação de 6-BAP não influenciou na vida de vaso, mas retardou o amarelecimento da folhagem e, portanto, reduziu a taxa de deterioração da haste floral. A irrigação restrita e a aplicação de fitohormônios podem ser ferramentas **úteis** para melhorar a eficiência do uso da **água** nas culturas de lírios.
Palavras-chave: 6-benzilaminopurina, fitohormônios, *Lilium longiflorum*, senescência, vida em vaso.

Introduction

Lilium (*Lilium longiflorum* Thunb.) is considered the fourth largest cut flower industry and one of the six most important flower bulb genera worldwide (CBI, 2016; Nasir and Sofi, 2018). Cut lilies (Lilium hybrids) are one of the most important ornamental plants worldwide that are used as cut flowers or pot plants and in gardening because of their excellent horticultural characteristics (Kang et al., 2021).

Around the world, the flower industry occupies about 1.3 million hectares, considering potted plants, nurseries, bulb production and cut flowers. It also contributes greatly to the economy of many countries. In a global context of increasing water scarcity situations, an important aspect of the environmental impact of flower production is the large water consumption of crops, estimated at 100 to 350 kg of water per kg of plant dry matter (Cassaniti et al., 2013). Ornamental crops concentrate a large demand for water per unit area, generally near large urban centers (Rafi et al., 2019). In Argentina, most of the centers producing flowers and ornamental plants are located on the periphery of large urban centers. The largest agglomeration of growers is located within a 150 km radius around the city of Buenos Aires (Morisigue et al., 2012).

During its life cycle, a plant is exposed to various environmental conditions, such as water stress. If it were a crop to produce cut flowers, stress situations can accelerate post-harvest leaf senescence and chlorosis and the opening of flowers, reducing the quality and vase life of the flower stalks (Krstulović et al., 2018). The water availability could determine the obtaining of high-quality products (Chantoiseau et al., 2018; López-Hernández et al., 2019), therefore, to apply controlled stress as a management technique, its effect on the productivity and quality of the final product must first be investigated.

Controlled deficit irrigation is a management technique that allows reducing plant water consumption without drastically reducing yield and/or quality. This technique has been successfully tested in numerous fruit and vegetable species, but few important precedents have been found in cut flowers. On the other hand, previous studies have reported on the role of phytohormones in mitigating water stress in lilium plants, finding the exogenous application of 6-BAP as a tool to obtain stronger plants, with a longer vase life. That is why, in recent years, research has been carried out with the aim of increasing the biosynthesis of endogenous cytokinin (Luo, et al., 2021), improving the understanding of the mechanism that regulates senescence (Kanojia and Dijkwel, 2018) and intervene to delay this process during the post-harvest of cut flowers and other horticultural products (Gill et al., 2023, Mantilla et al., 2021).

A greater understanding of the combined effect of restricted irrigation and the exogenous application of phytohormones on the post-harvest physiology of cut lily flowers could provide evidence to improve pre- and post-harvest treatments in a global context of water scarcity. The objectives of this work were: to study the physiological responses of the lily to the application of two irrigation regimes, and to pre-harvest applications of 6-BAP.

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Materials and Methods

The experiment with *L. longiflorum* cv. *Brindisi*, was carried out in a greenhouse between July and October 2021, located at the Faculty of Agronomy, University of Buenos Aires (34°35'S, 58°29'W), with natural radiation (DLI = 20 mol m $^{-2}$ day $^{-1}$, the average ambient temperature day/night was 25/17 ºC and the average relative humidity 82%. The bulbs gauge were 16-18 cm, they were planted in 0.4 m wide x 0.3 m deep x 20 m long benches with substrate 100% perlite, at a plant density of 20 bulbs m $^{-2}$.

The automated drip irrigation system was installed at random locations in each experimental unit, varying the irrigation level depending on the number of emitters. To assess the level of water restriction, the beds were subdivided and isolated during the experiment. The plants with normal irrigation (NI) were watered daily using drip belts with 1 L h^{-1} per emitter nominal flow rate, with 20 emitters per linear bench meter. The irrigation level was established according to the evapotranspiration estimated by the Penman-Monteith method modified by FAO (Allen et al., 1998), being between 2 to 3 L day^{-1} m^{-2}. The restricted irrigation (RI) was performed with 8 emitters per linear bench meter, it started from the stem emergence and was applied at the same frequency than NI throughout the crop cycle. Fertilization started from stem emergence, approximately 20 days after planting, with a complete nutrient solution applied with irrigation water for both NI and RI (Mantilla et al., 2021). Only stalks with 5 flower buds were considered for the postharvest test.

The plants were grown to commercial maturity (90 days), and the application of cytokinin 6-BAP (6-benzylaminopurine) was carried out (Tab. 1). To ensure the homogeneity of the plant material, only stalks with 5 flower buds were considered for the postharvest test. A group of 24 homogeneous stalks (12 from NI and 12 from RI) were selected. Six stalks of each group were sprayed with 6-BAP three days before harvest at a concentration of 3 cm^3 L^{-1} in a uniform way, covering all the vegetative parts of the plant, from the base to the extreme part of the inflorescence up to the drip point. The same procedure was carried out on the remaining stalks, but with distilled water. At harvest, the 24 stalks were trimmed to 60 cm in length and transported to the post-harvest room at an average temperature of 18 °C, a relative humidity of 56%, and a photoperiod of 12 hours of light. Stalks were weighed and then placed in distilled water (pH 5.2, total dissolved solids TDS 5 ppm, electrical conductivity CE 0.5µS cm^{-1}) in 250 cm^3 graduated test tubes previously disinfected, which were sealed with a plastic film to prevent direct evaporation; 12 stalks were left untreated, and the remaining 12 stalks previously treated in pre-harvest stalks, were again sprayed with 6-BAP at the same concentration.

Daily, postharvest life of each individual flower was determined daily by direct observation of tepal deterioration; water uptake was recorded daily by direct observation of the water level in the graduated test tube; fresh weight was measured for each flower stalk by means of a digital scale; and chlorophyll concentration was measured by the SPAD index (Spad -502 Konica Minolta Sensing, Inc.). Total vase life was recorded when all individual flowers on the cut flower stalk showed signs of senescence or wilting.

A completely randomized ANOVA design with 6 repetitions per treatment was used for the experiments. Analyses were carried out using InfoStat/Professional V1.1 software.

Tab. 1. Treatments applied to *L. longiflorum* cv. *Brindisi* cut stalks. Abbreviations: ETc: evapotranspiration of the crop. 6-BAP: 6-Benzylaminopurine.

Treatments	Description
T1	Adjusted irrigation to supply 100% ETc throughout the crop cycle. Control stalks sprayed with distilled water, 3 days before harvest and again at harvest.
T2	Adjusted irrigation to supply 100% ETc throughout the crop cycle. Stalks sprayed with 6-BAP (300 ppm), 3 days before harvest and again at harvest.
T3	Adjusted irrigation to supply 35% ETc throughout the crop cycle Control stalks sprayed with distilled water, 3 days before harvest and again at harvest.
T4	Adjusted irrigation to supply 35% ETc throughout the crop cycle Stalks sprayed with 6-BAP (300 ppm), 3 days before harvest and again at harvest.

Results and Discussion

Water uptake

A differential response between treatments to water consumption of up to 8 mL was observed by stem. In this work, the stalks of T2 were firmer, compared with the other treatments. The fresh weight of the lily floral stalks was significantly higher, in the order of 5 and 10% for treatments T2 and T3, compared to T1 and T4, respectively. In this sense, it has been reported that, in lily as well as in other plants, water consumption is closely related to development; as long as the water supply to the plant is adequate, growth increases or is not negatively affected (Rodríguez-Izquierdo et al., 2020). On the other hand, the three weeks prior to harvesting are critical due to the high demand for water. Indeed, the T4 values measured during the same hour decreased as the water supply was lower (Fig. 1), finding the lowest potential values at the end of the water restriction periods. The T3 treatments, with only a third of the irrigation, maintained water consumption, below the well-irrigated plants, reflecting the restricted irrigation condition, but above the plants with BAP restrictions (T4).

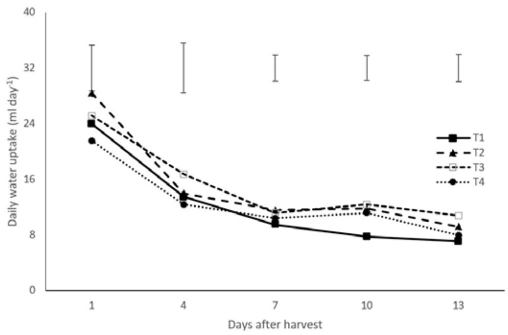

Fig. 1. Daily water uptake in post-harvest of cut flower stalks of *Lilium longiflorum* cv. 'Brindisi', cultivated under normal or restricted irrigation (NI or RI) and cut flower stalks sprayed 3 days before harvest and again at harvest with 6-Benzylaminopurine at 300 ppm (6-BAP) or distilled water (H_2O). T1: NI and H_2O, T2: NI and 6-BAP, T3: RI and H_2O, T4: RI and 6-BAP, Vertical bars represent DMS at $p \leq 0.05$.

Cumulative water uptake (CWU) was also enhanced under BAP treatments, the positive effect of cytokinins on delaying flower senescence has been demonstrated in many flowers (Trivellini et al., 2015), in both normal and restricted irrigation (Fig. 2). This result is consistent with the observation of Trevenzoli et al. (2020), suggested that cytokinins are known to regulate water uptake during postharvest while reducing plant respiration, which directly affects the water loss.

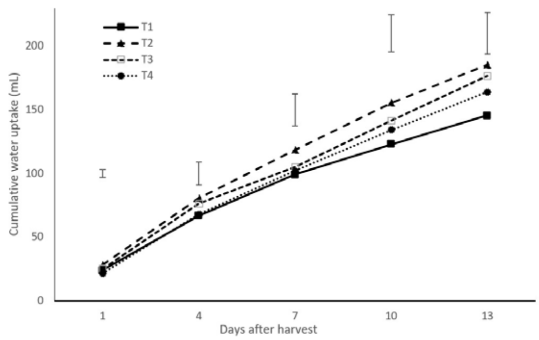

Fig. 2. Cumulative water uptake in post-harvest of cut flower stalks of *Lilium longiflorum* cv. 'Brindisi', cultivated under normal or restricted irrigation (NI or RI) and cut flower stalks sprayed 3 days before harvest and again at harvest with 6-Benzylaminopurine at 300 ppm (6-BAP) or distilled water (H_2O). T1: NI and H_2O, T2: NI and 6-BAP, T3: RI and H_2O, T4: RI and 6-BAP, Vertical bars represent DMS at $p \leq 0.05$.

Spad Index

The leaf senescence involves gradual yellowing and wilting, which starts at the base of the stems and proceeds acropetally (Van Doorn and

Han., 2011). However, petal senescence is distinct, due to the abundance of secondary metabolites. In addition, Ma et al. (2018), found that various phytohormones are involved in regulating petal senescence, and are thought to act both synergistically and antagonistically. In fact, BAP application, produced better results than the other hormonal treatments and a lower consumption of water was obtained compared to other treatments (Fig. 3) showing the physiological effect of the hormonal application.

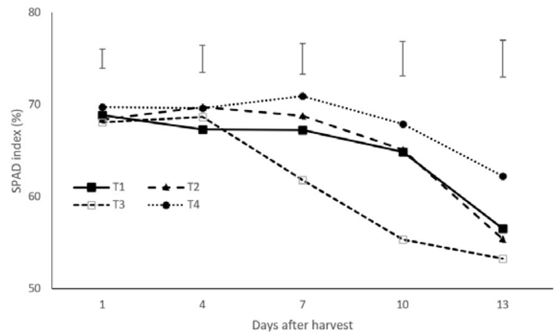

Fig. 3. SPAD chlorophyll index values, in post-harvest cut flower stalks of *Lilium longiflorum* cv. 'Brindisi', cultivated under normal or restricted irrigation (NI or RI) and cut flower stalks sprayed 3 days before harvest and again at harvest with 6-Benzylaminopurine at 300 ppm (6-BAP) or distilled water (H_2O). T1: NI and H_2O, T2: NI and 6-BAP, T3: RI and H_2O, T4: RI and 6-BAP, Vertical bars represent DMS at $p \leq 0.05$.

Chlorophyll content between the beginning and the end of storage, measured by green index (SPAD) showed there was a significant difference in those stems applied with T2 and T4, which suggests that BAP application regardless of irrigation, decreased senescence, decreasing ethylene levels. Furthermore, BAP-treated plants maintained higher leaf relative water content and membrane stability (Zhang et al., 2019), which led to higher chlorophyll content while those T3 treatments accelerated rates of chlorophyll degradation were found compared to T1, resulting in a yellowish coloration, high rates of chlorophyll degradation, increased senescence and leaf abscission. According to Domínguez and Cejudo (2021) chlorophyll breakdown is one of the most important events of chloroplast degradation in senescent leaves, so yellowing appears as a clear symptom of senescence. At the same time, the increase in chlorophyll percentages during senescence could be associated with changes in tissue turgor and leaf structure during aging (Villegas-Velázquez et al., 2022; Pizano et al., 2020). It has been determined that the onset of leaf yellowing can be delayed by treating leaves with plant hormones such as cytokinins, which modulate several important developmental processes, including the last phase of leaf development, known as senescence, which is associated with chlorophyll breakdown, disintegration of the photosynthetic apparatus and oxidative damage (Hönig et al., 2018).

Vase life and senescence

Exogenous application of phytohormones showed that the T2 treatment was able to prolong by 2.2 days the senescence in the first flower (Fig. 4) and the vase life together with the T3 treatment, compared to the other stalks with lower averages. The application of the phytohormone modified flower opening to some extend and delayed senescence symptoms despite the applied water restriction. However, possible undesirable effects such as accelerated opening in the first days, may have occurred in T4, which leading to precipitated senescence from the third day after harvest.

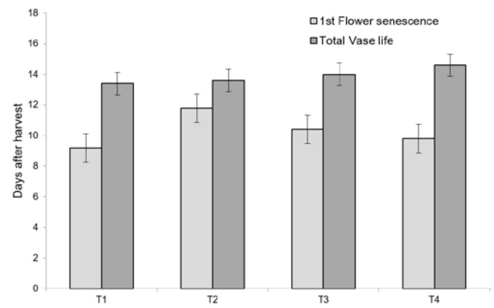

Fig. 4. Days to senescence of the first flower and total vase life, at post-harvest of cut flower stalks of *Lilium longiflorum* cv. 'Brindisi', cultivated under normal or restricted irrigation (NI or RI) and cut flower stalks sprayed 3 days before harvest and again at harvest with 6-Benzylaminopurine at 300 ppm (6-BAP) or distilled water (H_2O). T1: NI and H_2O, T2: NI and 6-BAP, T3: RI and H_2O, T4: RI and 6-BAP, Vertical bars represent DMS at $p \leq 0.05$.

According to Woltering and Van Doorn (1988), suggested that senescence is essentially the same in all senescing floral organs, but the sensitivity of some species to ethylene during the senescence phase accelerates this process. Vase life ends with the abscission of tepals that are still turgid or with the fall of the first buds. Therefore, changes in ethylene production during plant development are related to its phenological stages (Yang and Hoffman, 1984).

Flower opening depends on both internal and external factors. In this work, restricted irrigation (T3) and phytohormones application delayed individual flower opening, but this fact does not influence total vase life (Fig. 5). Pérez-Arias et al. (2019) pointed out that the BAP application in tuberose improves flower opening in tuberose due to a better water balance and a protective effect of cytokinins on cell turgor, probably associated with a decrease in ions outflow. On the other hand, restricted irrigation may affect rhythm of opening flowers by an alteration of hormonal balance (Kang and Zhang, 2004).

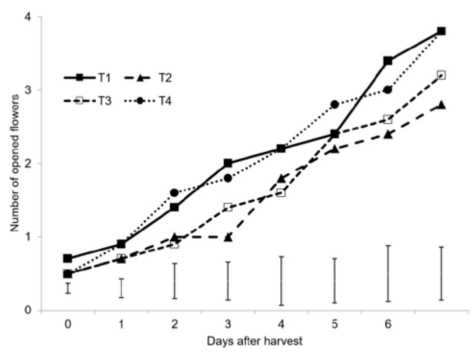

Fig. 5. Individual flowers opening rate, at postharvest of cut flower stalks of *Lilium longiflorum* cv. 'Brindisi', cultivated under normal or restricted irrigation (NI or RI) and cut flower stalks sprayed 3 days before harvest and again at harvest with 6-Benzylaminopurine at 300 ppm (6-BAP) or distilled water (H_2O). T1: NI and H_2O, T2: NI and 6-BAP, T3: RI and H_2O, T4: RI and 6-BAP, Vertical bars represent DMS at $p \leq 0.05$.

Fresh weight

No differences in fresh weight were detected at harvest time nor in postharvest period. (FW) increased 8% - 9% during postharvest period in both NI and RI conditions. The weight loss of the stalks under RI conditions was greater than under NI conditions (Fig. 6). In particular, the weight of the floral stems under water restriction conditions was better compared to the stems under normal irrigation conditions. These results confirm what was reported by De la Cruz Guzmán (2019) who points out that when they are placed in the vase, the stems increase their fresh weight and then decrease it significantly.

Similar results have been reported in cut roses by Arriaga (2016) where he shows the variability in stems with respect to the fluctuations of the water variables. And where FW and water uptake are related to metabolic processes in plants. The increase in weight of the treatments reflects that there was no influence of the length of the floral stem or number of buds, but of the initial hydration state.

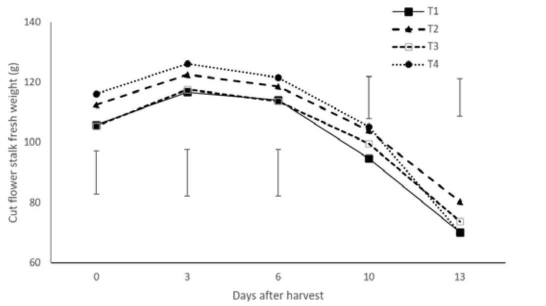

Fig. 6. Fresh weight (g) of cut stalks of cut flower stalks of *Lilium longiflorum* cv. 'Brindisi', cultivated under normal or restricted irrigation (NI or RI) and cut flower stalks sprayed 3 days before harvest and again at harvest with 6-Benzylaminopurine at 300 ppm (6-BAP) or distilled water (H_2O). T1: NI and H_2O, T2: NI and 6-BAP, T3: RI and H_2O, T4: RI and 6-BAP, Vertical bars represent DMS at $p \leq 0.05$.

The loss of fresh weight implies dehydration of the floral stem, and therefore represents the beginning of the senescence process by wilting. BAP treatment (T2 and T4) appears to slow the rate of dehydration. These results confirm what was reported by (Smékalová et al., 2014) who indicate that, in the face of stress, the exogenous application of phytohormones in small quantities, they play essential roles in plant growth and development with great capabilities to contribute to and improve abiotic stress tolerance.

Conclusions

L. longiflorum cv. Brindisi flower stalks, have a limited vase life and in this degenerative process there are changes in their quality, causing dehydration of individual flowers and yellowing of leaves. The use of the phytohormone in both NI and RI, mainly delayed yellowing. Meanwhile, vase life did not show significant differences, remaining at an average of 12 days.

The stalks with BAP application and RI presented the best result of SPAD index, and the stalks with BAP and NI presented lower dehydration, higher water uptake and higher vase life of the first individual flower.

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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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability Statement

Data will be made available on request.

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