Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Plant growth regulators' role in senescence and quality of lisianthus (Eustoma grandiflorum 'Mariachi Blue') cut flowers: Comparison Salicylic acid (SA), Sodium nitroprusside (SNP) and Spermidine (Sp)

Mohaddeseh Piri
Urmia University
zohreh jabbarzadeh (z.jabbarzadeh@urmia.ac.ir)
Urmia University https://orcid.org/0000-0003-2476-7284

Research Article

Keywords: Antioxidant enzymes, lisiantus, postharvest, vase life

Posted Date: May 15th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2878044/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Abstract

Post-harvest senescence is one of the crucial challenges limiting the commercialization of cut flowers. Long vase life is the most important factor determining the economic value of cut flowers. Major factors limiting the vase life of lisianthus cut flower included sensitivity to ethylene, inability to absorb water resulting in bending and suppression of bud opening and coloring, which must be considered at the post-harvest stage. Therefore, the present study was carried out to investigate three types of growth regulators, salicylic acid (SA), sodium nitroprusside (SNP) and spermidine (Sp), each with four concentrations and in four replicates, on the vase life and quality of Lisianthus cut flowers in post-harvest stage. The three regulators have a positive effect on the longevity of Lisianthus cut flowers and reduce ion leakage, malondialdehyde and hydrogen peroxide and increase the activity of the antioxidant enzymes, ascorbate peroxidase and guaiacol peroxidase, and preserving the degradation of lipid membrane and cell stability. The most appropriate concentrations in this study included 1- and 2-mM salicylic acid and/or spermidine as well as sodium nitroprusside concentrations of 40 and 60 µM.

Introduction

Lisianthus (*Eustoma grandiflorum*) is one of ten popular cut flowers in the world and originating from northern Mexico and the southern United States. This species belongs to the Gentianaceae family and is cultivated as cut flowers (Hohn et al. 2019). Lisianthus has been a very popular cut flower because of its floral colors and is a leading cut flower in Japan with sales of more than 129 million stalks in 2001, and was included in the list of top 10 cut flowers in Europe with a selling rate of at least 122 million stalks (Sitawati et al. 2018).

Putrescine, spermine, and spermidine are the important polyamines (PAs), found in all living organisms. PAs are formed by the decarboxylation of amino acids, and they facilitate cell growth and development via different cellular responses (Sagar et al. 2021). One of the important and proven effects of polyamines is to prevent the biosynthesis and effect of ethylene in plants. For this reason, this group of hormones is called anti-senescence hormones. High levels of polyamines in plant tissues act as a strong factor in preventing ethylene production. Polyamine and ethylene have completely opposite effects on ripening and senescence, and therefore the balance of these two hormonal groups in plants is extremely important for plant tissues, because the balance between the two opposite regulators delays or accelerates the senescence process (Asghari 2015). Recent investigations implicate extensive role of polyamines in regulation of flower senescence. In a previous study, polyamines used for improving postharvest quality of *Consolida ajacis* cut spikes, amplified the activity of various antioxidant enzymes viz, superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) to overcome the deleterious effects of reactive oxygen species (ROS). The electrolyte leakage of petals was profoundly reduced due to attenuated lipoxygenase (LOX) activity. These findings reveal conspicuous role of polyamines particularly Spm in modulation of flower senescence in cut spikes (Haq et al. 2021). In response of Gerbera (*Gerbera jamesonii*) cv. Great Smoky mountains to foliar application of spermidine at concentrations of 0 (distilled water as control), 50 and 100 $mg.l^{-1}$, it was observed that treatment with

100 mg.l^{-1} of spermidine gave the best results in terms of number of leaves, leaf area, relative chlorophyll content of leaves, flowering date, length and diameter of flower stalk, percentage of dry material in flowers and vase life (Mohammad Saeed et al. 2019).

Nitric oxide acts as an important phytohormone and messenger molecule in connection with many biochemical and physiological processes in plants, which may act as a gaseous internal growth regulator or a non-classical plant growth regulator. As a regulator, nitric oxide is an important physiological process depending on the concentration of the substance, including root organogenesis, hypocotyl growth, defense responses, stomatal conductivity, programmed cell death, hypersensitivity response, photosynthesis, growth and phytoalexin production under various conditions (Sami et al. 2017). The effect of the pulse treatment of sodium nitroprusside (SNP) was studied at four levels of 0, 20, 40, and 60 µM on the vase life of cut roses (*Rosa hybrida* L.), lisianthus (*Eustoma grandiflorum*), and sunflowers (*Helianthus annuus* L.), results showed that the longest vase life of cut roses (14.33 days) and sunflowers (14.5 days) were obtained from the application of 40 µM SNP whereas cut lisianthus flowers exhibited the extended vase life (14 days) when they were treated with 20 µM SNP. This treatment had the greatest effect in maintaining the dry matter of cut flowers. SNP significantly reduced ethylene synthesis in cut rose and Lisianthus vs control. However, the lowest ethylene synthesis in cut sunflower (0.03 nl L^{-1} h^{-1} g^{-1} FW) was obtained from 60 µM SNP treatment. SNP has significantly contributed to maintaining the protein content of the mentioned cut flowers. In general, it can be said that SNP improves postharvest longevity of roses, lisianthus, and sunflowers by suppressing ethylene synthesis and protecting proteins (Naziri Moghaddam et al. 2021).

The introduction of salicylic acid (SA), as a natural phenolic secondary metabolite, is well documented in various aspects of vital processes such as ethylene biosynthesis, stomatal conductance, respiration, senescence and activation of defense systems against various pathogens (Zeb et al. 2017). Salicylic acid (SA) has diverse regulatory roles in plant metabolism. It has many effects on cut foliage and flower species, among them, it can inhibit the upward gravitational bending of snapdragon, reduce the pH of the vase solution, reduce the rate of respiration, reduce moisture stress, improve the performance quality and vase life of cut flowers (Soffar and Taha 2019). A study was conducted to determine the vase life of *Vriesea incurvata* using distilled water (control) and a solution containing salicylic acid (50 µM) for 8 and 24 hours. Flower scapes kept in the solutions showed longer vase life by 16 days compared to the control (Pulido et al. 2021). In investigating the role of salicylic acid (SA) in regulating antioxidant enzymes and some biochemical characters in petals and stem bending of *Gerbera jamesonii* 'Sun Spot', cut flowers were treated with different concentrations (50, 100 and 200 µM) of SA for 24 hours. The findings showed that 50 µM SA significantly improved RWC, proline, anthocyanin, carotenoid, protein and soluble sugar contents and POX activity in petals, and significantly reduced water loss and MDA content in petals. (Hemati et al. 2019). Considering the importance of postharvest performance of cut flowers in the trade and also the importance of lisianthus among ornamental plants, as well as the effects of plant growth regulators in cut flowers, this research investigates the effect of salicylic acid, spermidine and sodium nitroprusside on the vase life and delaying the senescence of lisianthus cut flowers.

Materials and Methods

This research was conducted in the research and production greenhouses of Urmia University and the research laboratory of the Department of Horticultural Sciences, Faculty of Agriculture. The treatments of this study include vase solution containing spermidine at concentrations of 0, 0.5, 1 and 2 mM, salicylic acid at 0, 0.5, 1 and 2 mM, sodium nitroprusside at concentrations of 0, 20, 40 and 60 µM in post-harvest conditions.

Measurement of postharvest indicators

After the full opening of 3 flower buds, the lisianthus flowers were separated from the mother plant at a height of 30 cm and sent to the germinator with a temperature of 19 ± 2°C and a relative humidity of 65–70%, 14 hours of light and 10 hours of darkness and the light intensity was 15 µmol/m2/s provided by fluorescent light. In this research, 16 cut flower were used for each treatment and 4 replications were considered for each treatment. Some of the post-harvest characteristics considered in the research were: soluble sugar, protein, hydrogen peroxide, ion leakage, malondialdehyde and antioxidant enzymes including ascorbate peroxidase and guaiacol peroxidase in 4 different time periods of the first days, 4th, 8th and 12th. Vase life of cut flowers was also calculated.

Vase life

Vase life was calculated when 50% of the flowers have faded, the life of the lisianthus flower is over (Cho et al. 2001).

The amount of total soluble sugars

For measuring soluble sugars, to 0.1 ml of alcoholic extract, 3 ml of freshly prepared anthrone (150 mg anthrone + 100 ml 72% sulfuric acid) was added. The solution was placed in a hot water bath for 10 minutes to appear the color. After cooling the samples, their absorbance was read with a spectrophotometer at a wavelength of 625 nm (Irigoyen et al., 1992).

Measurement of total protein

Bradford (1997) method was used to determine soluble protein. First, 100 mg of Coomassie Blue was dissolved in ethanol (95% by volume, 50 ml). Then 100 ml of phosphoric acid (85%) was added to 1 liter and passed through filter paper. Bovine serum albumin was used to prepare the standard. 2.5 ml of the prepared Coomassie Blue solution was poured into disposable cells and then 100 µl of petal extract from each sample was added to the tube. Finally, the absorption rate of samples and standard solutions was read using a spectrophotometer at a wavelength of 595 nm. The amount of crude protein was obtained after drawing the standard curve and was expressed as mg per gram of fresh weight.

Ion leakage

Petal samples (0.2 g) were prepared from cut flowers. Then the samples were washed three times with distilled water. They were placed in vessels containing 20 ml of sterilized distilled water. The samples were placed in a water bath at 40°C for 30 minutes and then electrical conductivity (EC1) was measured. The samples then were placed in a water bath at 100°C for 10 minutes and were measured by EC meter. Ion leakage was calculated from following formula (Chakrabarty et al. 2009).

EL= (EC1/EC2) ×100

Peroxide Hydrogen

Hydrogen peroxide content was determined according to Velikova et al. (2000). 0.5 g of frozen petals were homogenized in an ice bath with 5 ml of 0.1% (w/v) trichloroacetic acid. The homogenate was centrifuged at 12,000 × g for 15 min, and 0.5 ml of the supernatant was added to 0.5 ml of 10 mM potassium phosphate buffer (pH = 7.0) and 1 ml of KI. The absorbance of the supernatant was measured at a wavelength of 390 nm. Hydrogen peroxide content was expressed as mM/g FW-1.

Malonedialdehyde

To measure malondialdehyde, 0.2 g of frozen petals were ground with 5 ml of 1% trichloroacetic acid (TCA), and then the plant extract was centrifuged at 8000 rpm for 10 minutes, then 1 ml of the supernatant was centrifuged with 4 cc of TCA solution 20% and TBA 0.5%. The samples were placed in a hot water bath at 95°C for 30 minutes and immediately cooled with ice water. They were centrifuged again at 5000 rpm for 5 minutes, the supernatant was removed, and the absorbance of the samples was read at 600 and 532 nm and malondialdehyde content was calculated with following formula (Horst and Cakmak 1991).

MDA (µmol/g FW) = $(A_{532} – A_{600} / 155)*100$

Measurement of enzyme activity

Extraction of plant extracts to measure enzymes

The methods of Kang and Saltveit (2002) were used to prepare plant extracts to determine antioxidant enzyme activity. 0.5 g of fresh petal was transferred to a cold mortar and treated with 3 ml Tris buffer solution with pH = 7.5 (Tris buffer containing 0.50 M hydrochloric acid, 3 mM magnesium chloride and 1 mM EDTA). It was ground with a fine mortar. The extraction buffer for measuring ascorbate peroxidase activity also contained 0.2 mM ascorbate. The resulting homogenate was centrifuged at 4000 rpm for 4

minutes at 4°C. The resulting supernatant was used as a crude extract to measure the activity of antioxidant enzymes

Measurement of Guaiacol peroxidase

The reaction mixture consisted of 1 ml of 1% guaiacol, 1 ml of 1% hydrogen peroxide, 2.5 ml of 50 mM phosphate buffer (pH = 5.7) and 0.1 ml of extract. Then, the activity of guaiacol peroxidase enzyme was calculated as the increase in absorbance for 1 minute at 420 nm using a spectrophotometer. To measure the activity of guaiacol peroxidase, the extinction coefficient of 26.6 cm-1 mM-1 was used. The activity of guaiacol peroxidase was calculated according to following formula: (Updhyaya et al. 1985).

$$Units\ \frac{mM}{min} = \frac{\frac{do\ D}{\min(sl\ ope)} \times vol.of\ assay\ (0.0003)}{Etinction\ coeffcient(26.6)}$$

Measurement of ascorbate peroxidase activity

Ascorbate peroxidase activity was measured using the methods of Nakano and Asada (1981). The reaction solution consisted of 2.5 ml of 50 mM phosphate buffer pH = 7 (Phosphate buffer: 0.1 mM EDTA, 1 mM sodium ascorbate, 0.2 ml (200 µl) of 1% H_2O_2) and 0.1 ml (100 µl) of the extract. Then, using a spectrophotometer with the following formula, the activity of ascorbate peroxidase enzyme was calculated as a decrease in absorbance during one minute at a wavelength of 290 nm. To measure the activity of this enzyme, the extinction coefficient of 2.8 cm-1 mM-1 was used.

$$Units\ \frac{mM}{min} = \frac{\frac{do\ D}{\min(sl\ ope)} \times vol.of\ assay\ (0.0003)}{Etinction\ coeffcient(2.8)}$$

Statistical analysis of data and software used

SAS software version 9.1 was used to perform variance analysis and mean comparision. Means were compared using Tukey's multiple range test method at the 1% probability level.

Discussion and results

Soluble sugar

The average comparison graph showed that the amount of soluble sugar decreased during vase life period and the highest soluble sugar was in the first time, which shows a significant difference compared to other times (Fig. 1A). Also, in relation to the effect of growth regulators, positive effect on sugar, except for salicylic acid treatment of 0.5 mM, other treatments were significantly different from the control. Among the treatments, the highest amount of soluble sugar was related to the concentration of 60 µM sodium nitroprusside, which had an almost two-fold increase in soluble sugar compared to the control (Fig. 1B).

Total protein

Comparison of average data shows that during vase life period, the amount of protein decreases as well as soluble sugars, so that in all treatments, the highest amount was at 1st day and the lowest at 12th day. The reduction at different times was different depending on the type and concentration of growth regulators. It should be noted that all the treatments were effective in preventing the reduction of protein, but among them, the effect of sodium nitroprusside was greater than spermidine and salicylic acid, especially its concentration of 40 µM could increase the amount of protein compared to other treatments (Fig. 2).

According to the comparison of the mean data, it can be seen that during vase life period of Lisianthus cut flowers, the amount of soluble sugar and protein decreases. Senescence is a genetically regulated process that can be observed at the level of a cell, tissue, organ, or whole plant. This process is mainly an oxidative one, characterized by cessation of photosynthesis, disintegration of organelle structures, loss of chlorophyll, proteins, dramatic increase in lipid oxidation, breakdown of cell wall components, and disruption of cell membranes leading to loss of cell/tissue structure. Senescence, the final stage of growth in the life cycle of a plant, can be accelerated by a set of abiotic and biotic factors (Setia and Setia 2018), so it is one of the reasons for the reduction of soluble sugar and protein. In Lisianthus, during vase life period, the plant enters the senescence stage. After water, carbohydrates are one of the most abundant compounds in plant tissues. Plants, like other higher organisms, use sugar and other oxidizable organic molecules as an energy source. Some of these carbohydrates produced in photosynthesis are converted into large starch molecules and stored in chloroplasts, and the rest of the carbohydrates are transferred to other parts of the plant (Ruan 2014). When the flower is separated from the mother plant, it actually experiences stress and by creating stress in the plants, many destructive enzymes start to function in the plant cells. One of these enzymes is chlorophyllase, which increases its amount in the cell and causes the breakdown of chlorophyll and ultimately reduces photosynthesis (Ershad Langroudi et al. 2020). Therefore, with the reduction of photosynthesis, the amount of carbohydrates also decreases, and probably one of the reasons for the decrease in soluble sugar during vase life period, is the decrease in photosynthesis due to separation from the mother plant and damage to chlorophyll.

As the results show, the use of different concentrations of salicylic acid, spermidine and sodium nitroprusside has a better effect in preventing the decrease of soluble sugar and protein during the vase life period of lisanthus. Chlorophyll reduction is considered as a symptom of oxidative stress conditions, which may be due to the production of reactive oxygen species (ROS), which causes damage to chlorophyll a and lack of light absorption by the plant. Therefore, in such a case, photosynthesis is reduced or stopped. It seems that the above anti-ethylene treatments (Spermidine as polyamine, SA and SNP as nitric oxide donor) preserve chlorophyll by inhibiting the activity of the chlorophyllase enzyme and creating chloroplasts and ultimately reducing the amount of soluble sugar in sensitive cut flowers such as alstroemeria, lilium, chrysanthemum and stock (Ershad Langroudi et al. 2020). Polyamines also act as anti-senescence agents in a number of plant species by inhibiting ethylene synthesis (Setia and Setia 2018). Salicylic acid also increases vase life, delays senescence, and increases cell metabolism by affecting ethylene synthesis (Vazirimehr and Rigi 2014). Nitric oxide also increases the storage life of a wide range of flowers, fruits and vegetables by down-regulating ethylene production (Sankhla et al. 2005). Therefore, one of the reasons that can be mentioned to prevent the reduction of Lisianthus sugar by spermidine, salicylic acid and sodium nitroprusside compared to the control treatment in the present study is the anti-ethylene and anti-senescence activity of these compounds that depending on the concentrations used, their effectiveness is different. In senescence, the amount of free radicals in plant cells increases, which can damage the protein structure and reduce the protein content in the cell (Mittler 2006). The decrease in protein level may be due to the decrease in the activity of nitrate reductase, glutamine synthase and glutamine aminotransferase. In addition, proteins oxidize free radicals due to their high tendency to react with them. Nitric oxide as a free radical in high concentrations has the ability to damage membranes, proteins and nucleic acids in plant cells, but in lower concentrations it is necessary for plant activity and as an antioxidant against radicals. Free radicals act in the plant's defense system (Asghari 2014). Probably, in the present study (where low concentrations of this substance were investigated), the amount of protein reduction was lower than the control due to the antioxidant activity of sodium-nitroprusside. There is also a possibility that salicylic acid, as an antioxidant, neutralizes the damage of free radicals caused by oxidation and destruction of the protein structure and thus increases the protein content (Zarei et al. 2013). In the case of spermidine, polyamines can improve the vase life of cut flowers and increase carbohydrate and protein levels by affecting free radicals and reactive oxygen species (ROS) or by changing their synthesis mechanism (Danaee and Abdossi 2018). One of the reasons for the short life of cut flowers is the lack of carbohydrate storage. The initiation of hydrolysis of proteins and carbohydrates is the initiation of an aging process in response to the lack of free sugars used in respiration (Ezhilmathi et al. 2007). Therefore, it may be argued that the growth regulators salicylic acid, sodium nitroprusside, and spermidine are effective in increasing cell lifespan by preventing the hydrolysis of carbohydrates and proteins. Salicylic acid, sodium nitroprusside and spermidine delay the senescence of flowers by inducing the activity of antioxidants and strengthening the antioxidant system of cells and control the reduction of sugar and soluble protein. In confirmation of the results of this study, in relation to the effect of spermidine on the amount of protein and soluble sugar, studies have shown that polyamines increase the amount of photosynthetic pigments and carbohydrates in the plant, and decrease the protein hydrolysis of cut flowers of lily, gerbera and carnation and delayed senescence

(Amorim et al. 2017; Vieira et al. 2017). Research has also shown that salicylic acid increases protein levels in plants by preventing the destruction of cell structures and increases carbohydrate (Apostolova et al. 2008). Other studies have reported that salicylic acid inhibits protein destruction in plants with antioxidant activity and reduces oxidative activity in plants (Ershad Langroudi et al. 2020). In relation to the studies conducted with sodium nitroprusside, according to the results of this study, nitric oxide is known as a signaling molecule that plays a role in the tolerance of biotic and abiotic stresses and increasingly to extend the life of cut flowers such as Rose, gladiolus and carnation have been used to prevent protein breakdown and cell wall destruction as well as preserve carbohydrates (Naing et al. 2017; Kazemzadeh-Beneh et al. 2018).

Hydrogen Peroxide (HO)

As can be seen in Fig. 3, the amount of hydrogen peroxide increased during vase life period, and the growth regulators used in this study were able to prevent the increase of hydrogen peroxide compared to the control. In this study, among the growth regulators, 1 mM spermidine and all concentrations of sodium nitroprusside showed a better effect than other treatments. Spermidine was more effective than salicylic acid in reducing hydrogen peroxide, but in general, all three growth regulators reduced the amount of hydrogen peroxide compared to the control and increased the vase life in Lisianthus.

Malonedialdehyde (MDA)

According to the results, malondialdehyde, as well as hydrogen peroxide, was increased during vase life period. All the treatments used in this study, significantly reduced the amount of malondialdehyde compared to the control. Also, among the regulators, spermidine at a concentration of 1 mM had the best effect, which reduced the amount of malondialdehyde more than the control. The highest amount of malondialdehyde was related to the control, especially on days 8th and 12th (Fig. 4).

Ion leakage

Ion leakage, such as malondialdehyde and hydrogen peroxide, increased during vase life period, and growth regulators prevented a large increase in ion leakage compared to the control, resulting in a longer vase life of Lisianthus flowers. However, all three regulators significantly reduced ion leakage compared to the control on days 4th, 8th, and 12th. The best effect of salicylic acid was at a concentration of 1 mM, which decreased the amount of ion leakage in all 3 time periods respectively, and spermidine with a concentration of 1 mM had a significant effect on reducing ion leakage. In relation to nitric oxide, such as salicylic acid and spermidine, ion leakage decreased in all 3 time periods, so that the lowest amount of it was related to the concentration of 20 µM sodium nitroprusside, as well as the highest amount of ion leakage related to the control. It should be noted that spermidine had the best effect among the regulators (Fig. 5).

As can be seen, salicylic acid, spermidine and sodium nitroprusside in all three concentrations had a positive effect on reducing the parameters of hydrogen peroxide, ion leakage and malondialdehyde. Senescence of cut flowers is associated with a set of physiological and biochemical processes such as protein degradation, lipid peroxidation and membrane leakage, macromolecule degradation, flower shriveling, color change, leaf yellowing and weight loss (Nasibi et al. 2014). Ion leakage occurs in the plant when the plant enters the senescence stage and the petals lose their freshness (Tatte et al. 2015). Hydrogen peroxide also reacts with the superoxide radicals and forms hydroxyl radicals, these hydroxyl radicals by reacting with methylene groups, unsaturated fatty acids (which are the main components of membrane lipids) cause membrane lipid peroxidation and increase the level of malondialdehyde (Gupta et al. 2019). Malondialdehyde is also a product of lipid peroxidation of cell membrane and its content in intracellular conditions can determine the degree of degradation in plants and cell membrane homeostasis. In plants, the production of reactive oxygen radicals increases with senescence. The increase in the production of reactive oxygen species (due to the disruption of the balance of reactive oxygen species radicals and antioxidant enzymes) causes secondary oxidative damage such as lipid peroxidation of the membrane and eventually loss of membrane permeability (Hernandez and Almansa 2002). Lipid peroxidation is a complex process that usually begins after the removal of hydrogen atoms from unsaturated fatty acids, which ultimately leads to the generation of lipid radicals. The increase in lipid peroxidation is determined by changing the concentration of malondialdehyde along with the increase in lipoxygenase activity. Peroxidation products cause membrane dysfunction (Zeng et al. 2011). Therefore, in the present study, sodium nitroprusside, salicylic acid, and spermidine increased the vase life of cut Lisianthus flowers by helping to delay senescence. Vase life has a positive correlation with enzyme antioxidant activity and lipid peroxidation (Kazemzadeh-Beneh et al. 2018). Also, salicylic acid may reduce lipid peroxidation by increasing the activity of antioxidant enzymes, which is one of the most effective anti-senescence mechanisms, reduce the amount of hydrogen peroxide, and subsequently prevent the increase of malondialdehyde and ion leakage, and inhibit the breakdown of the cell membrane. Salicylic acid can reduce ROS with its antioxidant properties, and on the other hand, by reducing the activity of the lipoxygenase enzyme, it reduces the degradation of membrane lipids (Ershad Langroudi et al. 2020). The positive effect of polyamines in reducing ion leakage of hydrogen peroxide and malondialdehyde with their ability to bind to membrane phospholipids and other membrane anion components can be explained by increasing the stability of the plant cell structure and inhibiting the activity of the lipoxygenase enzyme, preventing the lipid destruction of the membrane (Vieira et al. 2017). Polyamines also prevent the activity of reactive oxygen species and free radicals with their antioxidant activity and increase the activity of antioxidant enzymes, thus reducing the amount of hydrogen peroxide and malondialdehyde in the cell (Kamiab and Zamanibahramabadi, 2016). By increasing the activity of enzymes, especially ascorbate peroxidase, which is known as the most important antioxidant in plants, nitric oxide reduces the degradation process of membrane lipids and thus prevents the increase of hydrogen peroxide, ion leakage, and malondialdehyde (Kazemzadeh-Beneh et al. 2018). According to the results of this study, other studies on the effect of salicylic acid on cut flowers of gladiolus, rose, gerbera, lilium and carnation have shown that salicylic acid has an effect on hydrogen peroxide level, lipid membrane breakdown and reduces malondialdehyde by affecting the activity of antioxidant enzymes

and increases the vase life of cut flowers (Jalili Marandi et al. 2011; Ershad Langroudi et al. 2020). Also, the effect of spermidine in confirming the obtained results, it has been reported that polyamines increase the vase life of lilium, carnation, gerbera and rose cut flowers by reducing the amount of ion leakage and reducing the activity of lipoxygenase enzyme and preventing membrane lipids decline (Amorim et al. 2017; Danaee and Abdossi 2018). Regarding the effect of sodium nitroprusside, it has been reported that in gladiolus and other horticultural plants such as cut flowers and fruits, this substance improves the antioxidant system, photosynthetic efficiency, and reduces the accumulation of ROS, ion leakage, and the decomposition of lipid components of the cell membrane to a significant extent (Bilal et al. 2020; Kazemzadeh-Beneh et al. 2018).

Ascorbate peroxidase and Guaiacol peroxidase enzyme

By comparing the mean data, it can be seen that ascorbate peroxidase activity increased during vase life period in Lisianthus cut flowers. Also, all the treatments used in this study increased the activity of the enzyme compared to the control. Among the treatments, salicylic acid with a concentration of 2 mM showed a better effect than spermidine and sodium nitroprusside (Fig. 6).

Comparison of mean data shows that the activity of guaiacol peroxidase enzyme in cut flowers of Lisianthus increased during vase life period and salicylic acid, spermidine and sodium nitroprusside treatments also increased its activity compared to the control. All three growth regulators significantly increased guaiacol peroxidase activity, but salicylic acid at a concentration of 2 mM showed the best results during vase life period (Fig. 7).

According to the results, it can be seen that treatment with salicylic acid, spermidine and sodium nitroprusside reduces the ion leakage, malondialdehyde and hydrogen peroxide and preserves carbohydrates and proteins, because of their effect on the activity of antioxidant enzymes and ethylene synthesis. As seen in the present study, the treatments used increased the activity of the antioxidant enzymes guaiacol peroxidase and ascorbate peroxidase. In the post-harvest stage, one of the main causes of damage is the production of reactive oxygen species that change the structure of membrane compounds and antioxidants. Plants remove and decompose hydrogen peroxide and oxygen free radicals through two enzymatic (ascorbate peroxidase, peroxidase, superoxide dismutase and catalase) and non-enzymatic systems. Under normal conditions, reactive oxygen species are constantly produced in different parts of the plant, including chloroplasts and mitochondria, plasma membranes, etc., which increase to a degree that is harmful to the plant in conditions of degradation and senescence. Under normal growth conditions, reactive oxygen species are removed by the plant's antioxidant systems, but when the plant is in a state of degradation, the production of oxygen free radicals increases dramatically, because the activity of the antioxidant system is overcome, oxidative stress occurs, which eventually causes damage to proteins, RNA, DNA and cell membrane and lipid peroxidation occurs (Kumar et al. 2014). The activity of antioxidant enzymes is a very important factor in controlling the senescence process. In fact, adapting and overcoming oxidative stress and preventing the production of free radicals

and reactive oxygen species requires regulating the synthesis and regeneration of proteins and increasing the antioxidant capacity of cells. Also, increasing the activity of antioxidant enzymes increases plant protection against oxidative stress, aging and cell death. Therefore, antioxidants oxidize reactive oxygen species by releasing free electrons and reversing the senescence process. Although plants are equipped with antioxidant enzyme systems such as superoxide dismutase and catalase, these systems may be much less robust in cut flowers and lack the antioxidant capacity to reduce the negative effects of free radical oxidation. Anti-ethylene compounds such as polyamines, salicylic acid, and sodium nitroprusside, which are capable of modulating stress resistance and reducing the activity of free radicals by strengthening antioxidant systems, are used for this purpose (Danaee and Abdossi 2018). Also, during the senescence of flowers, with the accumulation of H_2O_2, the activity of antioxidant enzymes such as ascorbate peroxidase increases. Increasing the ascorbate peroxidase enzyme reduces the accumulation of H_2O_2, which significantly delays the senescence process. The increase in the accumulation of reactive oxygen species associated with senescence is compensated by the increase in antioxidants and ubiquitous enzymes (Naderi et al. 2018).

Another oxidizing enzyme of phenolic compounds, which plays an important role in increasing antioxidant defense, is the guaiacol peroxidase enzyme, which acts as a receptor and collector of peroxides and reduces the destructive effects of these molecules (Zhou et al. 2011). Antioxidant enzymes play a role in reduction of the peroxidation of lipid membranes and the activity of reactive oxygen species in plants (Ershad Langroudi et al. 2020). Salicylic acid acts as one of the antioxidants in the chloroplast and inhibits the activity of reactive oxygen species, known as free radicals, when the plant is under stress. Such effects may be due to the protection of antioxidant systems, which are often associated with increased resistance to oxidative stress or control of free radicals in plant tissues (Shahmoradi and Naderi 2018). In the present study, increasing the activity of ascorbate peroxidase and guaiacol peroxidase decreased lipid membrane degradation and delayed senescence in lisianthus. By eliminating free radicals and reactive oxygen species, polyamines reduce membrane degradation and delay senescence in cut flowers by increasing the activity of antioxidant enzymes such as ascorbate peroxidase and guaiacol peroxidase (Tatte et al. 2015). It has been reported that nitric oxide is produced in response to various stresses (Asghari 2015). Nitric oxide protects heme-containing enzymes, ascorbate peroxidase and peroxidase, from reactive oxygen species. Nitric oxide free radicals also eliminate the toxic effects of reactive oxygen species (Neill et al. 2003). Therefore, in this study, according to the effects of all three growth regulators, it can be seen that increasing the activity of antioxidant enzymes reduces the production of hydrogen peroxide, ion leakage, and malondialdehyde, and finally increases the vase life of Lisianthus cut flowers.

Vase life

According to the comparison of the average data, it can be seen that the longest vase life is related to the application of 1 mM spermidine, which caused the freshness of cut flowers of Lisanthus up to 19 days (Fig. 8).

By reducing the ion leakage, malondialdehyde and hydrogen peroxide and increasing the activity of antioxidant enzymes, as well as increasing the content of soluble sugar and total protein by the growth regulators of salicylic acid, spermidine and nitric oxide, which can be seen in the results, the vase life of lisianthus cut flowers was increases. As various studies have shown that antioxidant enzymes preserve and stabilize cells by overcoming oxidative stress and preventing the production of free radicals and reactive oxygen species, also increasing the activity of antioxidant enzymes increases plant protection against oxidative stress, senescence, and cell death. Anti-ethylene compounds such as polyamines, salicylic acid and sodium nitroprusside increase the activity of antioxidant enzymes and thus reduce the activity of free radicals (Danaee and Abdossi 2018). Also, during flower senescence with H_2O_2 accumulation, the activity of antioxidant enzymes such as ascorbate peroxidase is increased. The increase in the enzyme ascorbate peroxidase reduces the accumulation of H_2O_2, which significantly delays the senescence process. The increased accumulation of reactive oxygen species associated with senescence is counteracted by an increase in antioxidants and scavenging enzymes (Naderi et al. 2018). Therefore, in the present study, sodium nitroprusside, salicylic acid and spermidine significantly increased the vase life of lisianthus cut flowers by helping to delay senescence and desiccation of tissue or flower surface. Vase life is positively correlated with enzymatic antioxidant activity and lipid peroxidation (Kazemzadeh-Beneh et al. 2018). As mentioned earlier, salicylic acid can increase the activity of antioxidant enzymes through lipid peroxidation, which is one of the effective mechanisms against senescence, reduce the amount of hydrogen peroxide and subsequently prevent the increase in malondialdehyde and ion leakage content thus prevents the degradation of cell membranes. Salicylic acid can reduce ROS on the one hand through its antioxidant properties and on the other hand by reducing the activity of the enzyme lipoxygenase, reduces the degradation of membrane lipids and increases the vase life of cut flowers (Ershad Langroudi et al. 2020) and polyamines, by binding to membrane phospholipids and other membrane anionic components, reduce ion leakage, hydrogen peroxide and malondialdehyde and increase plant cell structure stability and inhibit lipoxygenase activity and they prevent the lipid structure of the membrane from breaking down, thus delaying the senescence process in plants (Vieira et al. 2017). Nitric oxide also protects heme-containing enzymes, ascorbate peroxidase and peroxidase, from reactive oxygen species and scavenges free radicals, prevents membrane damage and maintains cell stability. Nitric oxide also eliminates the toxic effects of reactive oxygen species (Neill et al. 2003). Therefore, considering the effects of the three growth regulators, it is observed that increasing the activity of antioxidant enzymes reduces the production of hydrogen peroxide, ion leak and malondialdehyde, and ultimately increases the longevity of Lisianthus cut flowers.

Conclusion

The present study was conducted to investigate the effect of different concentrations of growth regulators salicylic acid, spermidine and sodium nitroprusside on Lisianthus cut flowers at the post-harvest stage. The growth regulators increased the longevity of Lisianthus cut flowers and all three regulators improved the post-harvest characteristics of Lisianthus cut flowers. In this study, the concentrations of 1- and 2-mM salicylic acid and spermidine as well as the concentrations of 40 and 60

µM sodium nitroprusside were appropriate, although all three concentrations had a positive effect compared to the control.

Declarations

No funding was received for this work.

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Author's contributions:

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by all authors. The first draft of the manuscript was written by Mohaddeseh Piri and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

References

1. Amorim TL, Medeiros DC, Oliveira AS, Paes AR, Júnior WS, Moreira DA (2017) Gibberellin and polyamines in plant growth, development and postharvest senescence of ornamental plants – a review. Amazon J Plant 1: 1-13.
2. Apostolova P, Yard nova R, Popova L (2008) Response of antioxidative defense system to low temperature stress in two wheat cultivars. Gen Appl Plant Physiol 34(3-4): 281-294.
3. Asghari MR (2015) classic plant growth hormones and regulators. University of Urmia, 352 p.
4. Bilal AR, Iqbal RM, Asim M, Naser AA, Nafees AK (2020) Role of nitric oxide in improving seed germination and alleviation of copper-induced photosynthetic inhibition in Indian mustard. Anim Behav Cogn 8(30): 1-40. doi: 10.3390/plants9060776.
5. Bradford MM (1997) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72: 248-254. doi: 10.1006/abio.1976.9999.
6. Chakrabarty D, Verma AK, Datta SK (2009) Oxidative stress and antioxidant activity as the basis of senescence in Hemerocallis (day lily) flowers. J Hortic Forest 1(6): 113-119.
7. Cho MS, Celikel FG, Dodge L, Reid MS (2001) Sucrose enhances the postharvest quality of cut flower of *Eustoma grandiflorum* (Raf.) shinn. Acta Hortic 543: 304-315. doi: 10.17660/ActaHortic.2001.543.37.
8. Danaee E, Abdossi V (2018) Effect of different concentrations and application methods of polyamines (Putrescine, Spermine, Spermidine) on some morphological, physiological, and enzymatic characteristics and vase life of *Rosa hybrida* cv. 'Dolce Vita' cut flower. J Ornam Plant 8(3): 171-182.

9. Ershad Langroudi M, Hashemabadi D, Kalate Jari S, Asadpour L (2020) Effects of pre- and postharvest applications of acid salicylic on the vase life of cut Alstroemeria flowers (*Alstroemeria hybrida*). J Hortic Postharvest Res 3(1): 115-124. https://doi.org/10.22077/jhpr.2019.2409.1053.
10. Ezhilmathi K, Singh VP, Arora A, Sairam RK (2007) Effect of 5-sulfosalicylic acid on antioxidant activity in relation to vase life of Gladiolus cut flowers. Plant Growth Regul 51: 99-108. DOI: 10.1007/s10725-006-9142-2
11. Gupta DK, Palma JM, Corpas FJ (2019) Nitric oxide and hydrogen peroxide signaling in higher plants. Springer, 275p.
12. Haq A, Farooq S, Lone ML, Parveen Sh, Altaf1 F, Tahir I (2021) Polyamines accentuate vase life by augmenting antioxidant system in cut spikes of *Consolida ajacis* (L.) Schur. Ornam Hortic 27(4): 495-504. https://doi.org/10.1590/2447-536X.v27i4.2368.
13. Hemati E, Salehi Salmi MR, Daneshvar MH, Heidari M (2019) The roles of sodium nitroprusside, salicylic acid, and methyl jasmonate as hold solutions on vase life of *Gerbera jamesonii* 'Sun Spot'. Adv Hortic Sci 33(2): 187-195. https://doi.org/10.13128/ahs-24261.
14. Hernández JA, Almansa MS (2002) Short-term effects of salt stress on antioxidant systems and leaf water relations of pea leaves. Physiol Plant 115(2): 251-257. DOI: 10.1034/j.1399-3054.2002.1150211.x
15. Hohn D, Peil RMN, Marchi PM, Grolli PR, Perin L, Rosa DSB (2019) Growth and quality of lisianthus [*Eustoma grandiflorum* (Shinn.)] cultivated in rice husk substrates in troughs with leaching recirculation. Rev Colomb De Cienc Hortíc 13(3): 458-465. DOI: 10.17584/rcch.2019vl3i3.9891.
16. Horst JH, Cakmak I (1991) Effects of Aluminum on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (*Glycin max*). Physiol Plant 83: 463-468. https://doi.org/10.1111/j.1399-3054.1991.tb00121.x.
17. Irigoyen JJ, Emerich DW, Sanchez-Diaz M (1992) Water stress induced changes in concentrations of proline and total soluble sugars in nodulated alfalfa (*Medicago sativa*) plants. Physiol Plant 84: 55-60. https://doi.org/10.1111/j.1399-3054.1992.tb08764.x.
18. Jalili Marandi R, Hassani A, Abdollahi A, Hanafı S (2011) Improvement of the vase life of cut gladiolus flowers by essential oils, salicylic acid and silver thiosulfate. J Med Plant Res 5(20): 5039-5043. https://doi.org/10.5897/JMPR.9000728.
19. Kamiab F, Zamani Bahramabadi E (2016) The effect of different polyamines on some physiological traits as ACC oxidase and superoxide dismutase enzymes activity in *Chrysanthemum morifolium* cv. 'Bright Golden Ann'. J Ornam Plant 6(2): 83-92.
20. Kang HM, Saltveit ME (2002) Chilling tolerance of maize, cucumber and rice seedling leaves and roots and differentially affected by salicylic acid. Plant Physiol 115: 571-576. doi: 10.1034/j.1399-3054.2002.1150411.x.
21. Kazemzadeh-Beneh H, Samsampour D, Zarbakhsh S (2018) Biochemical, physiological changes and antioxidant responses of cut gladiolus flower 'White Prosperity' induced by nitric oxide. Adv Hortic Sci 32(3): 421-431. https://doi.org/10.13128/ahs-23361.

22. Kumar S, Yadav P, Jain V, Malhotra SP (2014) Isozymes of antioxidative enzymes during ripening and storage of ber (*Ziziphus mauritiana* Lamk.). J Food Sci Technol 51: 329- 334. doi: 10.1007/s13197-011-0489-7.
23. Mittler R (2006) Abiotic stress, the field, environment and stress combination. Trend Plant Sci 11(1): 15-19. https://doi.org/10.1016/j.tplants.2005.11.002.
24. Mohammad Saeed AAJ, Abdulhadi MD, Salih SM (2019) Response of Gerbera (*Gerbera jamesonii*) cv. 'Great Smoky Mountains' to foliar application of putrescine, spermidine and salicylic acid. Int Conf Agric Sci 388: 1-11.
25. Naderi R, Ataei D, Khandan Mirkohi AA (2018) Effect of pre-harvest salicylic acid treatment on growth characteristics and flowering life of cut flowers lisianthus (*Eustoma grandiflorum* cv. Miarichi Grand white). Sci Technol Greenhouse Crop 8(1): 123-133. (in Persian with English abstract)
26. Naing AH, Lee K, Kim KO, Ai TN, Kim CK (2017) Involvement of sodium nitroprusside (SNP) in the mechanism that delays stem bending of different gerbera cultivars. Front Plant Sci 8: 1-10. doi: 10.3389/fpls.2017.02045.
27. Nakano Y, Asada K (1981) Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. J Plant Cell Physiol 22: 867-880. https://doi.org/10.1093/oxfordjournals.pcp.a076232.
28. Nasibi F, Farahmand H, Kamyab A, Alipour S (2014) Effects of arginine, cysteine and 5-sulfosalicylic acid on of vase life of tuberose cut flowers. Annal Agric Sci 2: 35-41.
29. Naziri Moghaddam N, Kaviani B, Safari Motlagh MR, Khorrami Raad M (2021) Effect of Sodium Nitroprusside on the Vase Life of Cut Rose, Lisianthus, and Sunflower. J Ornam Plant 11(3): 185-195.
30. Neill SJ, Desikan R, Hancock JT (2003) Nitric oxide signaling in plants. New Phytol 159(1): 11-35. doi: 10.1016/S0083-6729(05)72010-0.
31. Pulido E, Rejane Negrelle RR, Cuquel FL (2021) Sucrose, salicylic acid and citric acid solutions to extent vase life of *Vriesea incurvata* Gaudich. (Bromeliaceae) floral scapes. Acta Agron 70: 27-34.
32. Ruan YL (2014) Sucrose metabolism: gateway to diverse carbon use and sugar signaling. Plant Biol 65: 33-67. https://doi.org/10.1146/annurev-arplant-050213-040251.
33. Sagar NA, Tarafdar S, Agarwal S, Tarafdar A, Sharma S (2021) Polyamines: functions, metabolism, and role in human disease management. Med Sci 9(44): 1-21. doi: 10.3390/medsci9020044.
34. Sami F, Faizan M, Faraz A, Siddiqui H, Yusuf M, Hayat S (2017) Nitric oxide-mediated integrative alterations in plant metabolism to confer abiotic stress tolerance, NO crosstalk with phytohormones and NO-mediated post translational modifications in modulating diverse plant stress. Nitric Oxide 73: 22-38. doi: 10.1016/j.niox.2017.12.005.
35. Sankhla N, Mackay WA, Davis TD (2005) Effect of nitric oxide generating compounds on flower senescence in cut racemes of pink flowered *lupinus havardii* wats. Proc-Plant Growth Regul Soc of Amer-Ann Meet 32: 126.
36. Setia N, Setia RC (2018) Polyamines: An overview and prospects in crop improvement. Crop Improv Strateg App 21: 376-393.

37. Shahmoradi H, Naderi D (2018) Improving effects of salicylic acid on morphological, physiological and biochemical responses of salt-imposed Winter Jasmine. Int J Hortic Sci Technol 5(2): 219-230. https://doi.org/10.22059/ijhst.2018.259507.246.
38. Sitawati S, Nurlaelih E, Daraini M (2018) Improved quality of lisianthus (*Eustoma grandiflorum* raf. Shinn.) with plant spacing and pinching frequency. J Innov Sci Inform ServiceNet 15(1): 280-286.
39. Soffar RA, Taha AM (2019) Effect of some anti-senescence compounds on the postharvest quality and longevity of cut *Cyperus alternifolius* Stems. Alex J Agric Sci 64 (5): 299-307. doi: 10.21608/alexja.2019.80486.
40. Tatte S, Singh A, Ahlawat TR (2015) Effect of polyamines on postharvest quality and vase life of rose var. *samurai*. Bioscan, 10 (2): 675-678.
41. Updhyaya A, Sankhla D, Davis TD, Sankhla N, Smith BN(1985) Effect of paclobutrazol on the activities of some enzymes of activated oxygen metabolism and lipid peroxidation in senescing soybean leaves. J Plant Physiol 121(5): 453-461. https://doi.org/10.1016/S0176-1617(85)80081-X.
42. Vazirimehr MR, Rigi Kh (2014) Effect of salicylic acid in agriculture. Int J Plant, Animal Env Sci 4(2): 291-295.
43. Velikova V, Yordanov I, Edreva A (2000) Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective role of exogenous polyamines. Plant Sci 151: 59-66. doi: 10.1016/S0168-9452(99)00197-1.
44. Vieira MRS, Moura FD, Simões NA, Souza AV, Santos CMG, Paes RA, Leal HY (2017) Application of polyamine and boron improves quality of potted gerbera cv. Kosak. J Appl Hortic 19(1): 1-5. doi: 10.37855/jah.2017.v19i01.15.
45. Zarei M, Sinaki JM, Rahbari A, Abaspour H (2013) Effects of planting date and salicylic acid on physiological traits of forage maize hybrids. Iran J Plant Physiol 3(2): 687-693. doi: 10.30495/ijpp.2013.540680.
46. Zeb A, Fazal Ullah E, Syeda Leeda Gul E, Maaz Khan E, Zainub B, Noman Khan M, Amin N (2017) Influence of salicylic acid on growth and flowering of Zinnia cultivars. Sci Int (Lahre), 29(6): 1329-1335.
47. Zeng CL, Liu L, Xu GQ (2011) The physiological responses of carnation cut flowers to exogenous nitric oxide. Sci Hortic 127(3): 424-430. https://doi.org/10.1016/j.scienta.2010.10.024.
48. Zhou R, Li Y, Yan L, Xie J (2011) Effect of edible coatings on enzymes, cell membrane integrity, and cell-wall constituents in relation to brittleness and firmness of Huanghua pears (*Pyrus pyrifolia* cv. Huanghua) during storage. Food Chem 124: 569-575. https://doi.org/10.1016/j.foodchem.2010.06.075.

Figures

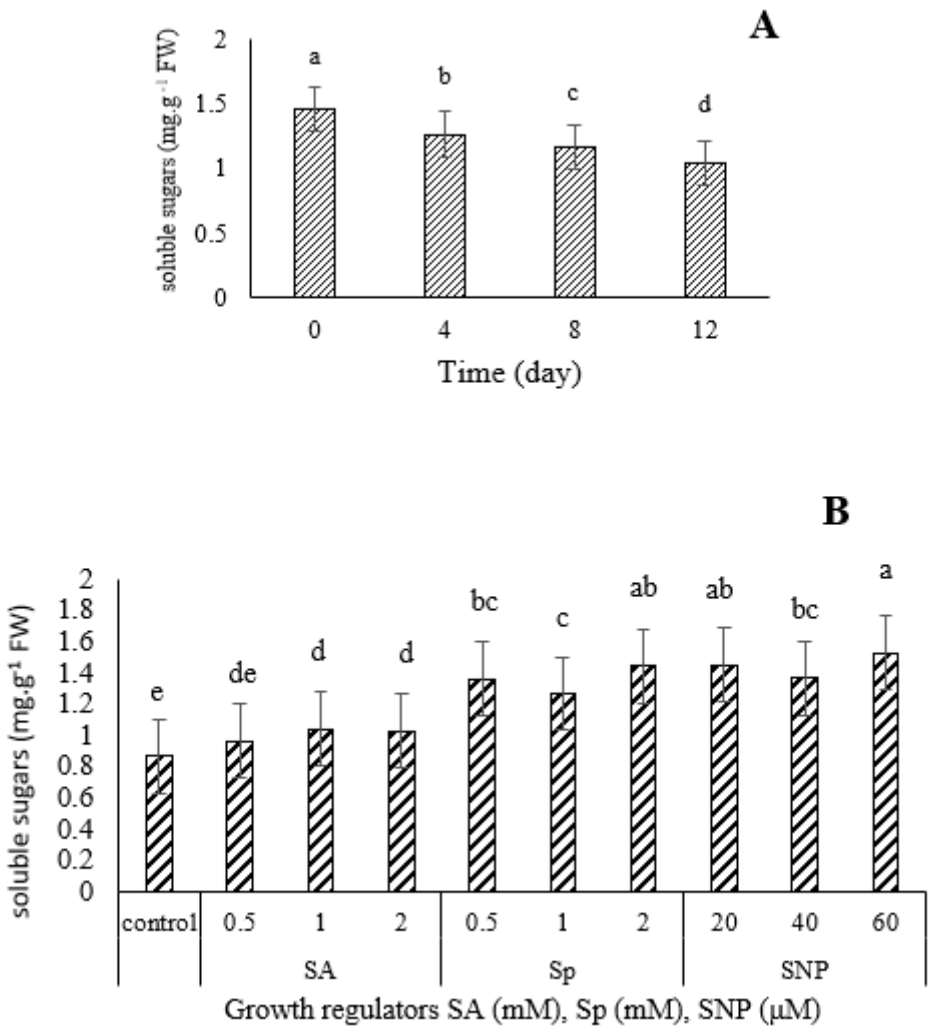


Figure 1

Effect of time (A) and growth regulators (salicylic acid (SA), spermidine (Sp) and sodium nitroprusside (SNP)) (B) on the amount of soluble sugar in Lisianthus cut flowers.

Different letters indicate statistically significant differences between treatments (Tukey = $P \leq 0.01$).

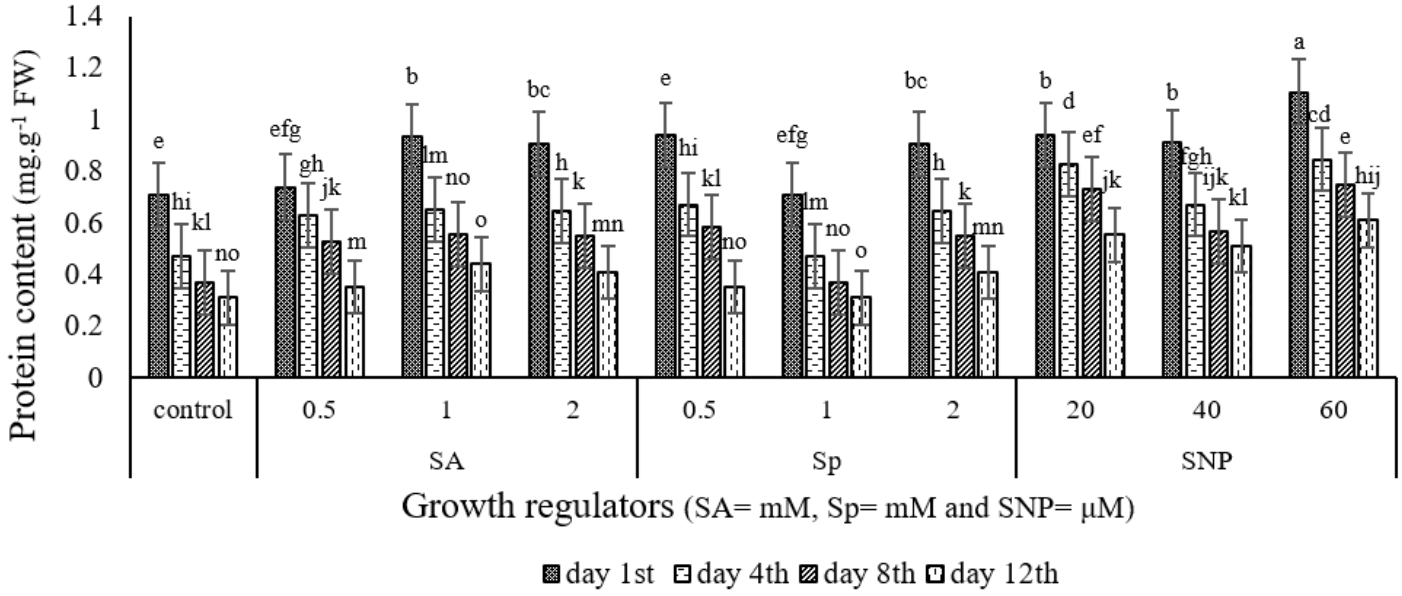


Figure 2

Effect of growth regulators (salicylic acid (SA), spermidine (Sp) and sodium nitroprusside (SNP)) on protein content in Lisianthus cut flowers during vase life period.

Different letters indicate statistically significant differences between treatments (Tukey = P≤0.01).

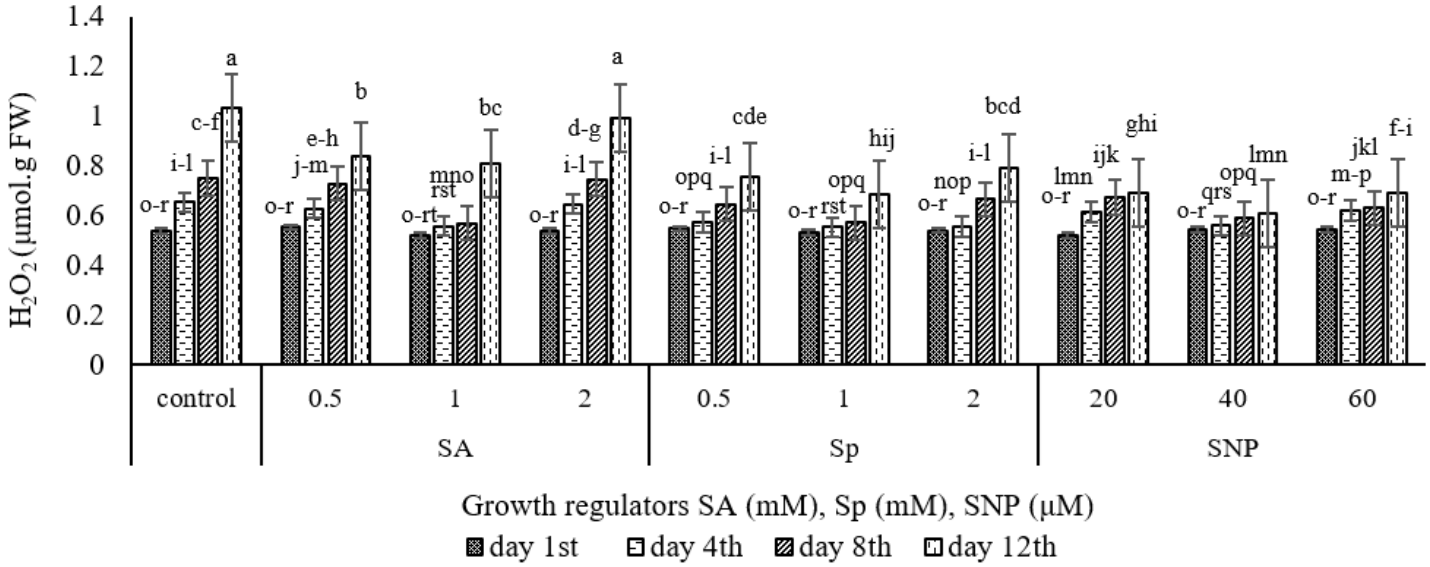


Figure 3

Effect of growth regulators (salicylic acid (SA), spermidine (Sp) and sodium nitroprusside (SNP)) on **hydrogen peroxide** content in Lisianthus cut flowers during vase life period.

Different letters indicate statistically significant differences between treatments (Tukey = P≤0.01).

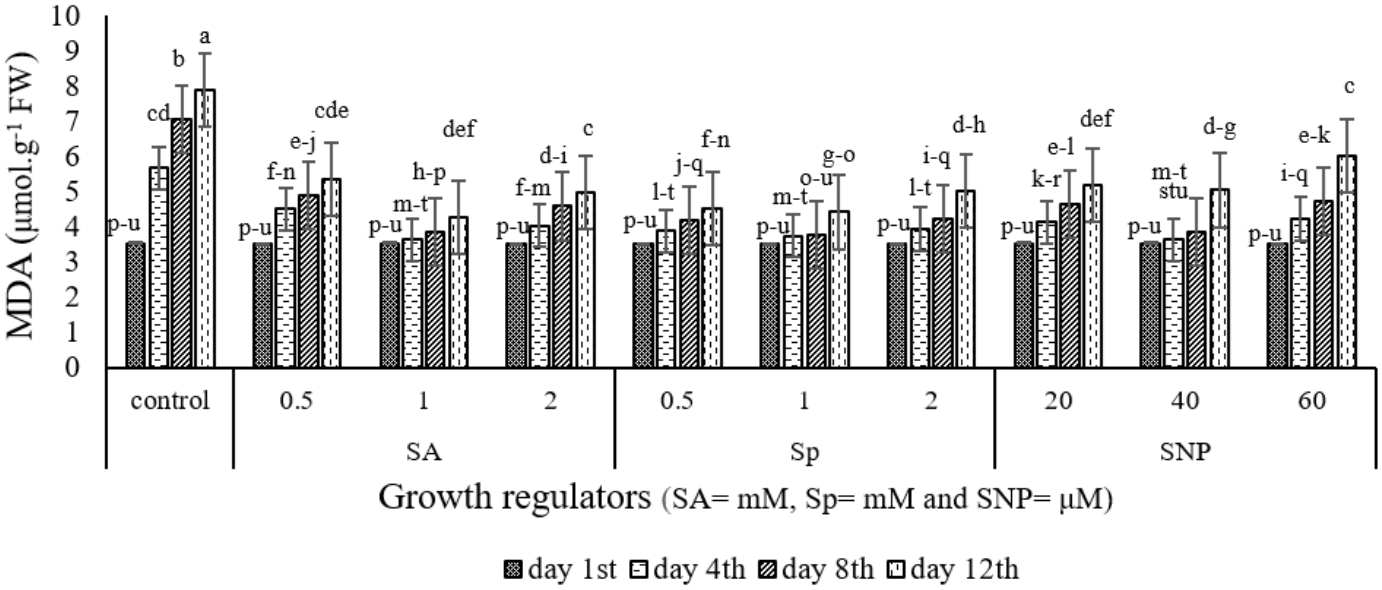


Figure 4

Effect of growth regulators (salicylic acid (SA), spermidine (Sp) and sodium nitroprusside (SNP)) on **malondialdehyde** content in Lisianthus cut flowers during vase life period.

Different letters indicate statistically significant differences between treatments (Tukey = P≤0.01).

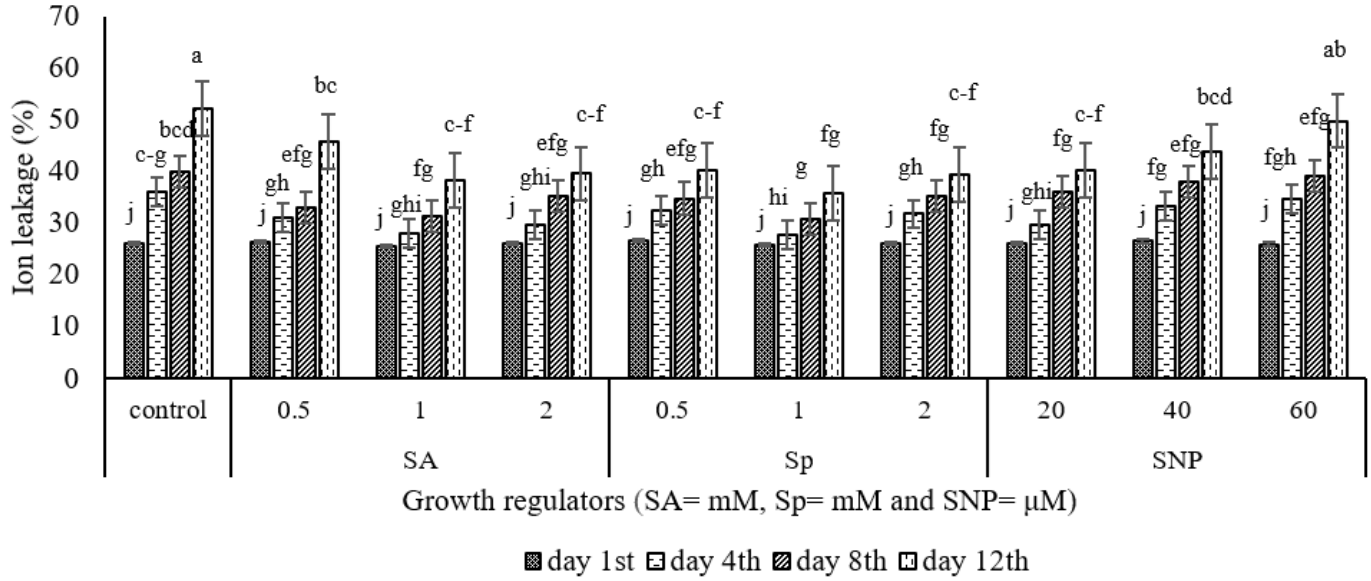


Figure 5

Effect of growth regulators (salicylic acid (SA), spermidine (Sp) and sodium nitroprusside (SNP)) on **ion leakage** in Lisianthus cut flowers during vase life period.

Different letters indicate statistically significant differences between treatments (Tukey = P≤0.05).

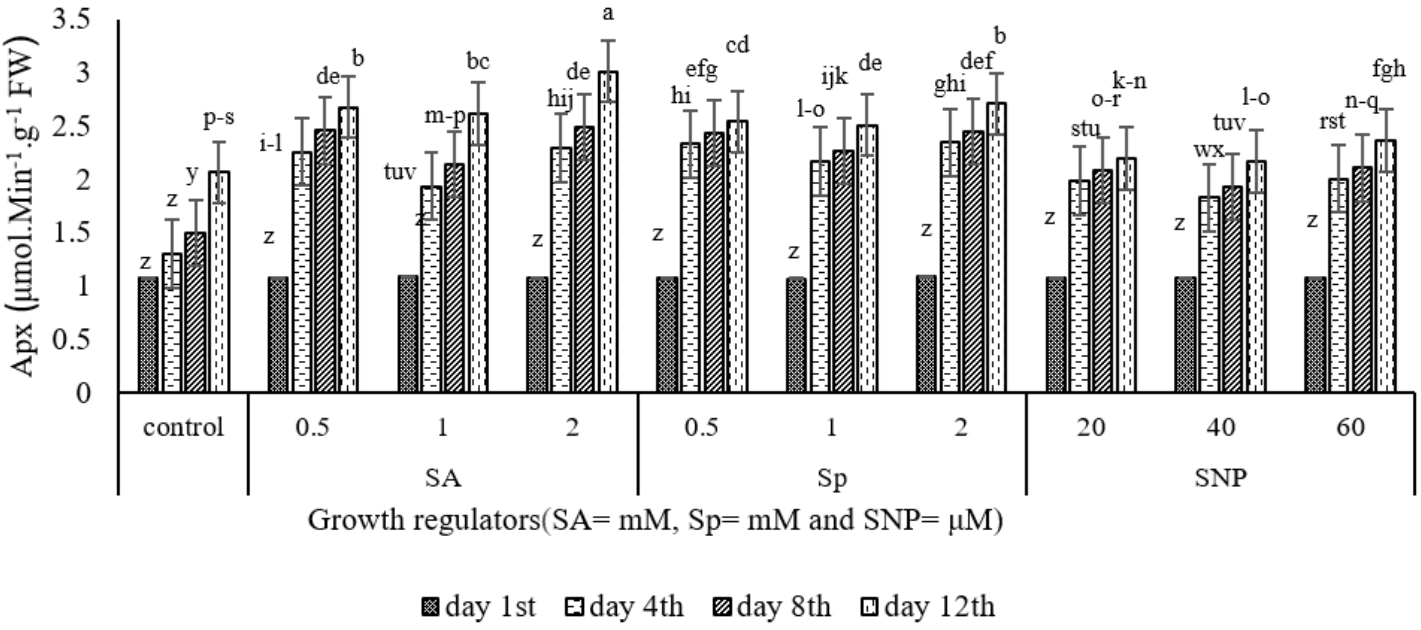


Figure 6

Effect of growth regulators (salicylic acid (SA), spermidine (Sp) and sodium nitroprusside (SNP)) on **the activity of ascorbate peroxidase** in Lisianthus cut flowers during vase life period.

Different letters indicate statistically significant differences between treatments (Tukey = P≤0.01).

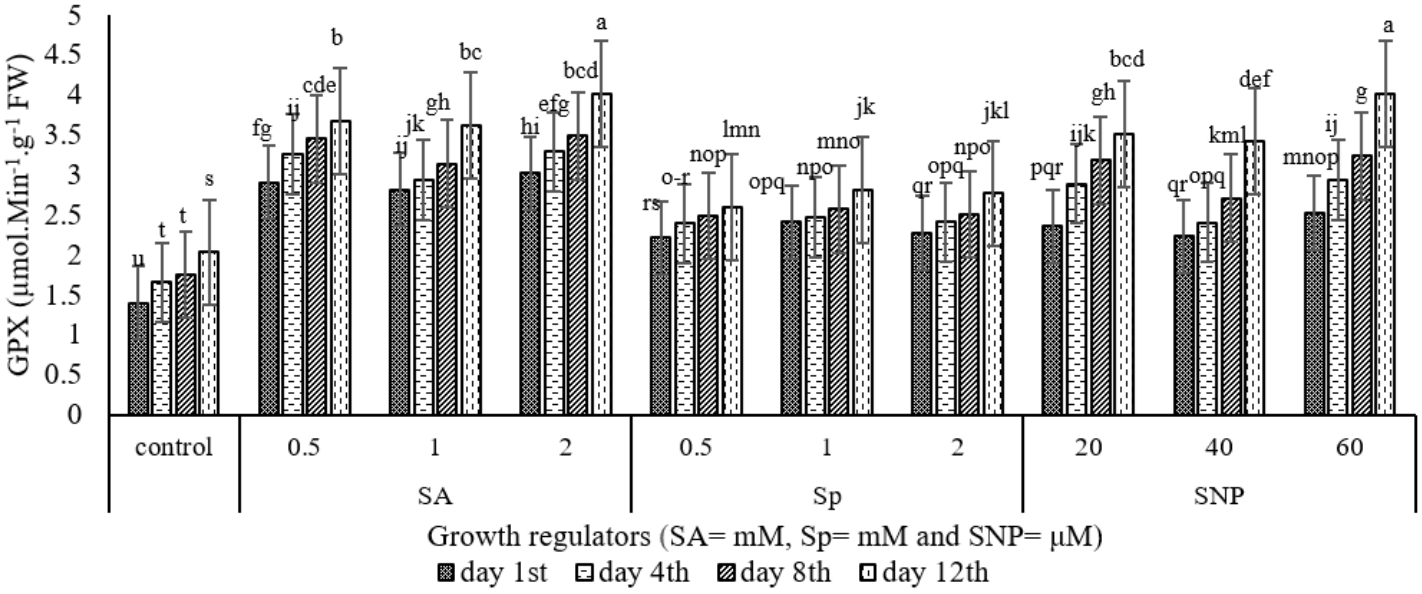


Figure 7

Effect of growth regulators (salicylic acid (SA), spermidine (Sp) and sodium nitroprusside (SNP)) on **the activity of guaiacol peroxidase** in Lisianthus cut flowers during vase life period.

Different letters indicate statistically significant differences between treatments (Tukey = P≤0.01).

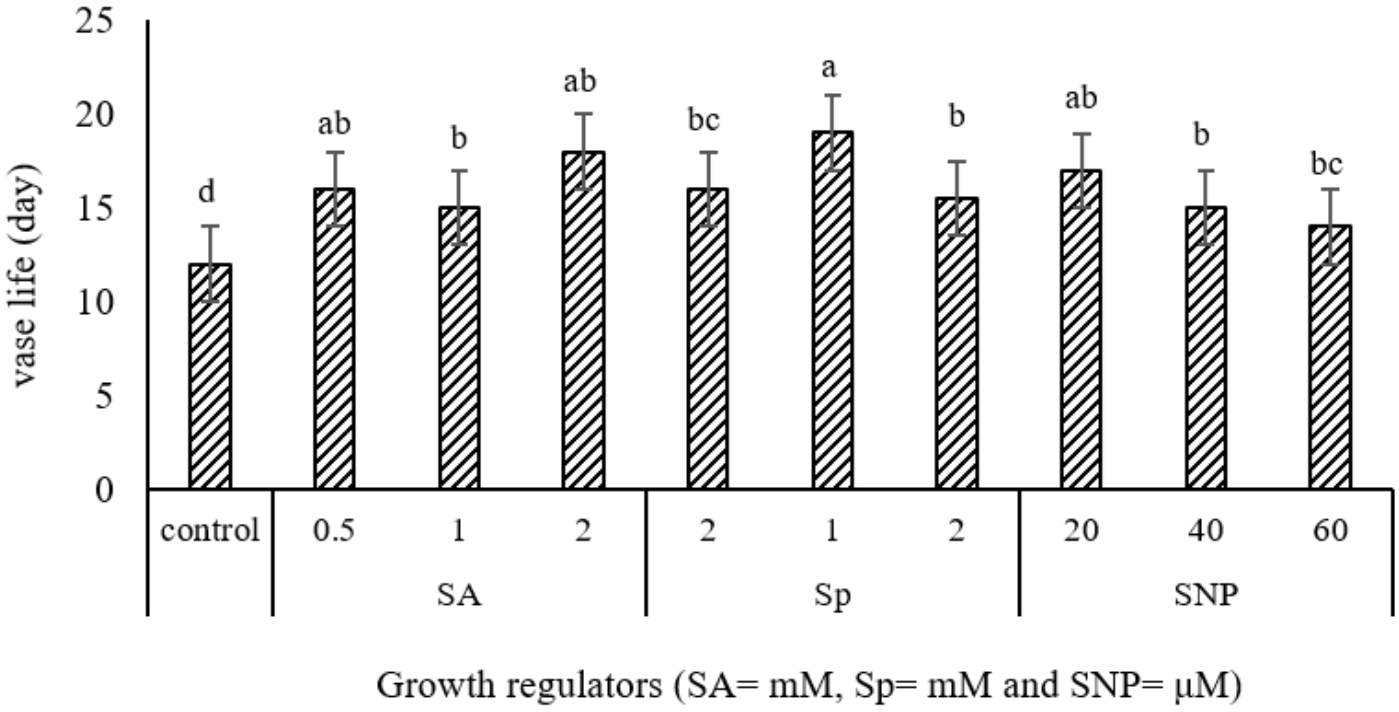


Figure 8

The effect of growth regulators (salicylic acid (SA), spermidine (Sp) and sodium nitroprusside (SNP) on the vase life of cut flowers of Lisianthus.

Different letters indicate statistically significant differences between treatments (Tukey = $P \leq 0.01$).