Ornamental Horticulture
ISSN 2447-536X | HTTPS://ORNAMENTALHORTICULTURE.EMNUVENS.COM.BR/RBHO
SBFPO Sociedade Brasileira de Floricultura e Plantas Ornamentais

SCIENTIFIC ARTICLE

Vase life and biochemical parameters of rose cv. Avalanche are affected by foliar application of sodium nitroprusside and putrescine

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Abstract

The effects of foliar spraying of sodium nitroprusside (SNP) and putrescine (Put) on rose cv. Avalanche were investigated. This experiment was conducted in a factorial trial with two factors, including SNP at four levels of 0, 50, 100 and 200 µM and putrescine at four concentrations of 0, 1, 2, and 4 mM with three replications in hydroponic conditions in the greenhouse. Some of the recorded traits included the number of leaves plant^{-1}, leaf area, fresh and dry leaf weight, as well as determination of soluble sugars, and total protein. Also, in the post-harvest stage, the recoded traits included malondialdehyde (MDA) content, relative fresh weight, and vase life. SNP at a concentration of 100 µM was found to improve morphological traits including fresh and dry weight of leaf (4.3 and 1.4 g, respectively) compared to the control. It also increased total protein (0.85 mg g^{-1} FW), decreased postharvest MDA content (0.32 µM g^{-1} FW), and increased vase life (23.66 days) at 100 µM level, and increased leaf area (7671 mm^2) and flower relative fresh weight (27.65%) at 200 µM level. Among different rates of putrescine, 4 mM putrescine was associated with higher leaf area (8056.7 mm^2), fresh and dry leaf weight (4.3 and 1.3 g, respectively), soluble sugars (4.63 mg g^{-1} FW), lower post-harvest MDA content (0.32 µM g^{-1} FW), and increased duration of vase life (26 days). In general, SNP and putrescine improved rose growth parameters and post-harvest traits.

Keywords: malondialdehyde, nitric oxide, polyamine, postharvest, *Rosa hybrida,* soluble sugars.

Resumo

Vida de vaso e parâmetros bioquímicos de rosa cv. Avalanche são afetados pela aplicação foliar de nitroprussiato de sódio e putrescina

Os efeitos da pulverização foliar de nitroprussiato de sódio (SNP) e putrescina (Put) em rosa cv. Avalanche foram investigados. Este experimento foi conduzido em ensaio fatorial com dois fatores, incluindo SNP em quatro níveis de 0, 50, 100 e 200 µM e putrescina em quatro concentrações de 0, 1, 2 e 4 mM com três repetições em condições hidropônicas em casa de vegetação. Algumas das características registradas incluíram o número de folhas planta^{-1}, área foliar, peso fresco e seco das folhas, bem como determinação de açúcares solúveis e proteína total. Além disso, na fase pós-colheita, as características recodificadas incluíram teor de malondialdeído (MDA), peso fresco relativo e vida de vaso. Verificou-se que o SNP na concentração de 100 µM melhorou as características morfológicas, incluindo o peso fresco e seco da folha (4,3 e 1,4 g, respectivamente) em comparação com o controle. Também aumentou a proteína total (0,85 mg g^{-1} FW), diminuiu o conteúdo de MDA pós-colheita (0,32 µM g^{-1} FW) e aumentou a vida de vaso (23,66 dias) no nível de 100 µM e aumentou a área foliar (7.671 mm^2) e flor peso fresco relativo (27,65%) no nível de 200 µM. Entre as diferentes taxas de putrescina, 4 mM de putrescina foi associada a maior área foliar (8.056,7 mm^2), peso fresco e seco de folhas (4,3 e 1,3 g, respectivamente), açúcares solúveis (4,63 mg g^{-1} FW), menor MDA pós-colheita conteúdo (0,32 µM g^{-1} FW) e aumento da vida útil do vaso (26 dias). Em geral, o SNP e a putrescina melhoraram os parâmetros de crescimento das rosas e as características pós-colheita.

Palavras-chave: açúcares solúveis, malondialdeído, óxido nítrico, poliamina, pós-colheita, *Rosa hybrida*.

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https://doi.org/10.1590/2447-536X.v29i3.2587
Received Jan 9, 2022 | Accepted Sept 5, 2023 | Available online Oct 5, 2023

Editor: Iftikhar Ahmad

Introduction

The genus Rosa of the Rosaceae family, with more than 150 species is one of the most important ornamental plants in the world. From a commercial point of view, cut roses play an essential role in the cut flower business (Raymond et al., 2018).

Nitric oxide (NO), a small, colorless and a diatomic gaseous molecule, is a lipophilic free radical that easily diffuses through the plasma membrane. These characteristics make NO an ideal autocrine (i.e., within a single cell) and paracrine (i.e., between adjacent cells) signalling molecule. As a chemical messenger, NO plays a crucial role in plant growth, development, and responses to biotic and abiotic stresses (Khan et al., 2023b). Further studies explored that NO is produced in living organisms via different enzymatic and nonenzymatic reactions and that NO can also be applied to plants in the form of different NO donors such as sodium nitroprusside (SNP) (Rahim et al., 2022). Sodium nitroprusside breaks down in plant tissue to release nitric oxide. NO consists of nitrogen and oxygen, therefore, can easily diffuse through cell membranes and can work as an excellent intracellular and intercellular chemical messenger in both plants and animals under normal and stressful conditions (Khan et al., 2023a). Under normal conditions, NO significantly contributes to the breaking of seed dormancy and induction of seed germination, root development, lateral root formation, primary root growth, adventitious root formation, root hair development, chlorophyll biosynthesis, vegetative growth, vascular differentiation, symbiosis nodule formation, stomatal moment, iron homeostasis, leaf senescence, flowering control, and fruit ripening (Simontacchi et al., 2015). NO also acts synergistically with auxin (AUX) to control a variety of plant responses, including root organogenesis, gravitropic responses, formation of root nodules, responses to iron deficiency, activation of cell division, formation of embryogenic cells, and stimulation of nitrate reductase (NR) activity (Pande et al., 2021). The role of NO as a chemical messenger has been studied extensively with respect to plant growth and development under normal and stressful conditions. Furthermore, NO has also been reported to significantly contribute to plant growth and development, symbiotic associations, defense responses against biotic and abiotic stressors, and vegetative and reproductive growth (Khan et al., 2023a; Simontacchi et al., 2015). Furthermore, NO significantly contributes to senescence-related processes in plant tissues. This contribution might have either a positive or negative effect depending on a specific organ. For example, NO delays senescence in leaves but promotes senescence in root nodules (Bruand and Meilhoc, 2019).

Polyamines (PAs) are small, low molecular weight, and ubiquitous polycations found in eukaryotic and prokaryotic cells (Alcazar et al., 2006; Groppa and Benavides, 2008). In higher plants, they could be found not only in the free form but also as conjugates bound to phenolic acids (hydroxycinnamic, coumaric, caffeic, or ferulic acid) or to biomacromolecules such as proteins and nucleic acids in order to regulate the free PAs intracellular levels or control enzyme activity, DNA replication, gene transcription, cell division, and membrane stability (Kaur-Sawhney et al., 2003; Chen et al., 2019). Among Polyamines, Put is the central product of the PA biosynthetic pathway and the most abundant PA in nature. PAs are considered a class of plant growth regulators (Xu et al., 2014). In general, enhanced plant growth and metabolism are associated with greater PA biosynthesis and higher PA content (Cai et al., 2006; Zhao et al., 2004). Among the main PAs, Put is the most abundant in leaves, and it is found to accumulate in the cytoplasm (Cai et al., 2006). It is also demonstrated that the chemical or genetic depletion of Put is lethal for many organisms, not only for plants, suggesting that Put may play an essential role in growth and development (Imai et al., 2004; Kusano et al., 2008). However, the molecular mechanisms behind these roles remain ambiguous. It was suggested that the enhancement of plant growth might be due to the fact that PAs act as hormonal second-messengers of cell proliferation and differentiation in many processes or regulate plant sensitivity to auxins/CKs ratio. In addition, the metabolism of PAs was related to the production of NO, which is considered an essential signaling component for plant growth (Pal et al., 2015). Put may influence the different stages of plant development, including flowering. Studies in Dendrobium nobile plants with higher levels of Put and Spd in the leaves showed that these plants had more flower buds, and they also presented not only more flowers but also with a larger average flower diameters (Li et al., 2014). Similarly, the application of 200 ppm of Put increased the number of flowers and their FW and DW in Dianthus caryophyllus and Gladiolus grandflorum (Nahed et al., 2009; Mahgoub et al., 2006). Antirrhinum majus plants treated with different concentrations of Put significantly increased the number of inflorescences per plant, yield of spike, and FW and DW of inflorescences per plant compared with untreated plants (Iman et al., 2018). Additionally, PAs were found to be involved in the reduction of electrolyte leakage and MDA levels, reflecting their role in protecting membrane integrity. These findings also suggest that PAs may replace Ca^{2+} and contribute to the maintenance of membrane integrity by binding to membrane phospholipids under the studied conditions (Gonzalez-Hernandez et al., 2022).

Rose has a high economic value in the agricultural industry, so in terms of production and consumption, it has the highest rank among cut flowers, and it is extremely important from an ornamental point of view. Regarding the effect of nitric oxide and putrescine on the improvement of plant growth and flowering, and the relationship between them, also considering that the effects of Put and NO are

different depending on the plant species and cultivars, in this research an attempt has been made to highlight the effects of Put and NO (various concentrations) as foliar application in different aspects of flower crop production and postharvest characteristics of rose plants cv. 'Avalanche'.

Materials and Methods

Before starting the experiment, a greenhouse water sample was analyzed; and the results of which are presented in Table 1 (in meq.L^{-1}).

Table 1. The results of greenhouse water analysis.

EC ($\times 10^6$)	pH	CO_3^{-2}	HCO_3^-	Cl^-	SO_4^{-2}	Total anions	Ca^{2+}	Mg^{2+}	Na^+	Total cations	Total hardiness ($CaCO_3$ based)
682	7.3	0	2.56	0.24	-	2.8	4.8	1.52	0.16	6.48	308.552

Growth medium and Nutritional Solutions

In this research, plants were grown in hydroponic conditions. The growing medium was peat moss: cocopeat: perlite (3:1:1 v v^{-1}). A commercial greenhouse nutrient solution with minor modifications (pH = 5.8-6.2) was used. The Tables 2 and 3 show the fertilizers used in the two stocks. Stock 1 consisted of potassium nitrate, calcium nitrate, ammonium nitrate, and iron, and stock 2 consisted of potassium nitrate, magnesium sulfate, magnesium nitrate, monopotassium phosphate, manganese sulfate, copper sulfate, borax, molybdenum, and zinc sulfate. The stock was added to 100 L of water until the EC of the solution reached 1.7 ds m-1.

Table 2. Fertilizers used in stock 1 (in g).

Potassium Nitrate	Ammonium Nitrate	Calcium Nitrate	Iron- EDDHA
62.5	6.625	490.625	20.5

Table 3. Fertilizers used in stock 2 (in g).

Sodium Molybdate	Sodium Borate	Zinc sulfate	Copper sulfate	Manganese sulfate	Mono Potassium phosphate	Magnesium Nitrate	Magnesium Sulfat	Potassium Nitrate
0.26	0.48	1.18	0.87	1.68	138.75	36.25	231.25	167.5

Plant materials and treatments

The effects of foliar spraying of SNP and putrescine on some morphological and physiological traits of 'Avalanche' rose were investigated in a factorial experiment in the form of a completely randomized design with two factors, including SNP in four concentrations, i.e. 0, 50, 100, and 200 µM and putrescine applied at four concentrations, i.e. 0, 1, 2, or 4 mM in three repetitions at two-week intervals for four months. Rose cv. Avalanche plants were grown in plastic pots (diameter 14 cm and height 11 cm). The day/night temperature of the greenhouse was set at 23-20/28-30 ˚C with 50%-70% relative humidity and 400-500 µmol m^{-2} s^{-1} illumination.

Growth characteristics

Measurement of morphological parameters started two weeks after the end of the foliar application. The leaf area was measured with the AM200 leaf area device, by which three mature leaves were randomly selected from the middle part of the plant and their area was estimated. The fresh leaf weight of the plants was measured with a PJ300 Mettler 0.0001-g digital balance. Then, the samples were dried in an oven at 72 °C for 48 hours to obtain the dry weight of the leaves using a digital scale. The number of leaves was counted for the whole plant.

Physiological measurements

Soluble sugars

To measure soluble sugars, the alcoholic extract was prepared. To prepare the extract, 0.5 g of fresh leaf tissue was crushed in a mortar containing 5 ml of 95% ethanol. Then the supernatant was transferred to a 25 mL tube, and 5 mL of 75% ethanol was added. Finally, 10 mL of the extract was centrifuged at 3500 rpm for 15 minutes. Then 0.1 mL of the extract was poured into the tube, and 3 mL of anthrone was added. The tubes were kept in a hot water bath for 10 minutes. After the samples were cooled, their absorbance was read with a spectrophotometer at 625 nm (Irigoyen et al., 1992).

Total protein

This content was estimated using the method of Bradford (1976). For this purpose, 100 mg of Coomassie Brilliant Blue was dissolved in 50 mL of 95% ethanol. Then, 100 mL of 85% sulfuric acid was added. Then it was filtered through filter paper. The standard was prepared from bovine serum albumin. Finally, the absorbance of samples and standard solutions was measured with a spectrophotometer at a wavelength of 595 nm, and the crude total protein content was determined after preparing a standard curve and expressed in mg.g^{-1} fresh weight (FW).

Postharvest assays

Measurement of flower vase life and relative fresh weight

Rose cv. Avalanche-cut flowers were harvested when they were almost fully opened. Then their stems were cut to 40 cm, and all the leaves except the top three were removed. Flowers were placed in vases containing 500 mL of a solution containing 200 mg L^{-1} HQC (Hydroxy Quinoline Citrate) and 20 mg L^{-1} sucrose. They were then stored in growth chambers (22 °C, 70% RH, 12.12 h day/night photoperiod, and 10 Mm m^{-2} s^{-1} illumination). The relative fresh weight of the flower was recorded every four days with a digital scale. The fresh weight of each cut stem was measured every four days with a digital scale. Relative fresh weight was measured using the Joyce and Jones method (1992), and was expressed in g per g of initial fresh weight per day by the following formula: '

$RFW = FW_i / FW_0$

FW_i= is the weight of stem (g) at the desired day

FW_0= the weight of the same stem (g) on the first day

The vase life of the flowers was determined based on petal wilting, petal discoloration, petal shedding, neck bending, and flower wilting (Lua et al., 2010).

Malondialdehyde (MDA) content

To measure MDA content in the postharvest period, flowers were sampled every four days at 0, 4, 8, and 12 days. The amount of MDA was measured on 0.1 g of freshly ground petal tissue in 5 ml of 1% trichloroacetic acid (TCA) and poured into the tube. The tube was centrifuged at 4000 rpm for 30 minutes. Then 1 mL of the centrifuged supernatant was poured into tubes containing 4 mL of 20% TCA and 0.5% thiobarbituric acid (TBA). The samples were kept in a water bath at 95 °C for 30 minutes, and then in ice water. Then they were centrifuged at 4000 rpm for 5 minutes and their absorbance was finally measured at 532 and 600 nm with a spectrophotometer. MDA content was calculated with the following equation (Horst and Cakmak, 1991):

$$\text{MDA (µmol / g FW)} = \frac{A_{532} - A_{600}}{155} \times 1000$$

Statistical analyses

The variance of the data was analyzed and their means were compared in the SAS software package (version 9.2). To compare the means, Tukey's test was used at $p < 1\%$ levels. Also, the graphs were drawn in MS-Excel (version 2010).

Results and Discussion

Growth characteristics

According to the results (Table 4), the largest significant leaf area (8056.7 mm^2) was observed in the plants treated with 4 mM putrescine as compared to the control. However, other SNP rates were not significantly different from each other or the control. It was found that combinations of SNP and putrescine had a significant effect on the number of leaves.

Table 4. Effect of sodium nitroprusside and putrescine at different concentrations on leaf area, number of leaves, fresh and dry weight of leaves, soluble sugars, total protein and vase life of rose cv.Avalanche (in each column similar letters indicate no significant difference at $p \leq 0.01$).

Sodium nitroprusside	putrescine	Leaf aera (mm^2)	Leaf number	Fresh Weigth of leaf (g)	Dry Weigth of leaf (g)	Soluble sugar (mg g^{-1} FW)	Total protein (mg g^{-1} FW)	Vase life (day)
Control	Control	5261.7^{f}	16^{cde}	3.1^{cd}	0.86^{c-f}	1.87^{d}	0.63^{bc}	16.33^{c}
	Put 1 mM	6844.7^{a-f}	18.62^{bc}	3.2^{bcd}	1.1^{bcd}	2.45^{cd}	0.69^{abc}	21^{abc}
	Put 2 mM	6260.3 $^{a-f}$	18.66^{bc}	3.6^{b}	1.2^{ab}	3.26^{a-d}	0.57^{c}	21.66^{abc}
	Put 4 mM	8056.7^{a}	17.33^{bcd}	3.1^{cd}	0.76^{ef}	4.63^{a}	0.71^{abc}	20.66^{abc}
SNP 50 µM	Control	6115.7^{def}	15^{cde}	2.7^{de}	0.94^{b-e}	2.43^{cd}	0.61^{bc}	22.33^{ab}
	Put 1 mM	5663.3^{ef}	17^{bcd}	2.4^{e}	0.74^{ef}	2.74^{bc}	0.6^{bc}	21^{abc}
	Put 2 mM	6868.7 $^{a-e}$	12^{e}	3.4^{bc}	1.1^{abc}	2.82^{bcd}	0.58^{c}	22^{abc}
	Put 4 mM	7658.3^{ab}	13de	3.2^{bc}	0.87^{cef}	4.19^{ab}	0.75^{abc}	26^{a}
SNP100 µM	Control	6432.3 $^{b-f}$	19.66^{bc}	2.4^{e}	0.74^{ef}	2.2^{cd}	0.70^{abc}	23. 66 ^{ab}
	Put 1 mM	5931.7^{def}	21.33^{ab}	2.5^{e}	0.72^{ef}	2.54^{cd}	0.72^{abc}	25.33^{ab}
	Put 2 mM	7670.7^{ab}	21.66^{ab}	3.3^{bc}	1.1^{abc}	2.44^{cd}	0.85^{a}	19.66^{bc}
	Put4 mM	7587.7^{abc}	21.66^{ab}	4.3^{a}	1.4^{a}	3.19^{a-d}	0.63^{bc}	20.33^{abc}
SNP 200 µM	Control	5647.3^{ef}	25^{a}	2.5^{e}	0.81^{def}	2.64^{bc}	0.8^{ab}	20^{bc}
	Put 1 mM	5868^{def}	19^{bc}	2.4^{e}	0.59^{f}	2.57^{cd}	0.86^{a}	23.33 ^{ab}
	Put 2 mM	7196 $^{a-d}$	18.66^{bc}	3.2^{bcd}	1b^{cde}	3.6^{abc}	0.69^{a-d}	22^{abc}
	Put 4 mM	7671^{ab}	17^{bcd}	3.4^{bc}	0.8^{def}	2.41^{cd}	0.76^{abc}	22^{abc}

The treatments of 100 µM SNP with 1, 2 or 4 mM putrescine and/or 200 µM SNP without putrescine were more effective in increasing the number of leaves. The highest number of leaves (25) was related to the treatment of 200 µM SNP. SNP and putrescine treatments had a positive effect on fresh leaf weight. In fact, the highest fresh leaf weight was 4.3 g corresponding to 100 µM SNP + 4 mM putrescine, which was significantly different from other levels and the control. Plants treated with 100 mM SNP + 4 mM putrescine showed the highest leaf dry weight of 1.4 g. Both SNP and putrescine treatments improved leaf dry weight when applied at higher rates, except SNP at 200 µM reduced this trait.

Polyamines (PAs) and nitric oxide (NO) are crucial signalling molecules that exhibit a promising role in a wide range of plant activities, including photosynthesis, growth, and development (Tripathi et al., 2023). In the present study, leaf number was affected by SNP. The highest number of leaves was obtained from plants exposed to 200 µM SNP. But this treatment did not have a significant effect on the leaf area or fresh and dry leaf weight, which can be explained by the fact that since the plants produce more leaves, they are not able to expand their area. On the other hand, the dry weight of leaves was slightly increased by SNP at lower concentrations, but the difference was not significant with the control. The lowest leaf dry weight was observed in plants treated with 200 µM SNP. The decrease in dry weight may be related to the decrease in leaf area and photosynthesis. It can also be caused by the increase in the number of leaves and as a result of creating shadows on each other, especially on the middle leaves. Then, some leaves do not intercept enough radiation even to meet their photosynthesis needs, and this greatly reduces photosynthesis and plant dry matter. A study on pepper showed that SNP did not affect the leaf area under any stress conditions (Badem and Söylemez, 2022). However, there is a report that SNP at 200 µM increases leaf area and number, indicating that SNP has diverse functions in different plant species (Salachna et al., 2016).

Polyamines are bioactive compounds involved in many physiological processes. Their cationic nature explains most of their biological activity. However, the numerous biological interactions where PAs are involved makes it difficult to determine their role in plant growth and development. Put is not only a signal molecule by itself, but also interacts with numerous molecules such as phytohormones such as auxin (Gonzalez-Hernández et al., 2022), Therefore, it can increase cell division and plant growth. In addition, polyamines directly affect cell enlargement and division, thus affecting growth. They also induce the production of growth hormones and thus help in cell enlargement and growth (Asghari, 2015). As a result, it can be said that as the surface area of the leaf expands, photosynthesis increases. Then, more dry matter accumulates in plants, and this ultimately improves their growth (Zhang et al., 2021).

As found here, putrescine increased leaf area and fresh and dry leaf weight. It also increased the number of leaves, but it was not significantly different from the control. One of the possible reasons for not increasing the number of leaves is the increase in leaf area because when the leaves expand, plants have less chance to increase their number of leaves and use their photosynthate to expand the leaf area. A study on roses reported that putrescine caused an increase in leaf area and fresh and dry weight compared to the control (Yousefi et al., 2021). Similarly, the investigation on cotton showed that putrescine increased the leaf area and thus increased the fresh and dry weight of the leaf (Kahrobaiyan et al., 2019).

Biochemical constituents

Soluble sugars

The comparison of means (Table 4) shows that putrescine increased soluble sugars, so the use of 4 mM putrescine without the use of SNP (4.63 mg g^{-1} FW) which increased this trait by 60% compared to the control. SNP application slightly reduced soluble sugars, but this reduction was mostly insignificant.

After water, carbohydrates are the second most abundant compound in plant tissues. Like all other higher organisms, plants use sugars and other organic molecules as a source of energy. In higher plants, the photosynthesis system captures solar energy in chloroplasts and converts atmospheric CO_2 into carbohydrates and similar compounds. Part of the carbohydrates synthesized during photosynthesis is converted into larger starch molecules that are stored in chloroplasts, and the remaining carbohydrates are mobilized to other parts of the plant (Hlahla et al., 2022). When the leaf area and number of leaves increase, photosynthesis and photosynthetic pigments increase and help to increase soluble sugars. On the other hand, it helps to protect macromolecules such as proteins and cell membranes (Vosnjak et al., 2021).

SNP increased leaf number but decreased leaf area. The increase in the number of leaves causes the lower leaves to be shaded, and this causes a decrease in sunlight reaching the plant, resulting in the loss of photosynthesis. Therefore, dry matter production is reduced. These are the possible reasons for the observed decrease in photosynthetic capacity and soluble sugars in NO-treated plants. These findings confirm research on pistachio, where SNP reduced soluble sugars under non-stressed conditions (Eslami et al., 2019).

We observed that higher putrescine levels were related to higher soluble sugars content in leaves, so that the highest levels of soluble sugars (4.63 mg g^{-1} FW) were observed in plants treated with 4 mM putrescine, which is consistent with the study of ornamental sunflower in which putrescine positively affects the photosynthetic system. As a result, it increased the soluble sugars (Faisal and Ibrahim, 2018). This increase in total carbohydrate content may be related to an increase in photosynthetic efficiency, which increases leaf net CO_2 as the primary carbohydrate product. Consistent with our findings, research on some plants such as cotton, wheat and Salvia, showed a positive effect of putrescine on soluble sugars content (Gonzalez-Hernández et al., 2022).

Total Protein

Comparing the means (Table 4) showed that the interaction between SNP and putrescine caused a relative increase in total protein. The highest amount of protein was obtained from the treatments of 100 µM SNP × 2 mM putrescine (0.85 mg g^{-1} FW) and 200 µM SNP × 1 mM putrescine (0.86 mg g^{-1} FW), which significantly increased the total protein content by 35% compared to the control. The lowest amount of total protein was obtained from 0 µM SNP × 2 mM putrescine (0.57 mg g^{-1} FW) and 50 µM SNP × 2 mM putrescine (0.58 mg g^{-1} FW), and they were not significantly different from the control.

As a free radical, SNP at high rates can damage the membrane, proteins, and nucleic acids in plant cells, but at lower rates, it is necessary for plant growth and development and acts as a defense system in plants (Asghari, 2015). The finding that the SNP increases the protein content may be related to the effect of the SNP on growth hormones, since these hormones in turn affect the protein content and prevent its degradation. Or it may be related to the role of the SNP in increasing the chlorophyll content. Our findings regarding the increasing effect of SNP on protein content are consistent with a research study on sunflowers, according to which the application of SNP under stress-free conditions increases the protein content of the plant. It also confirms the results of studies on tulips and roses about the effect of SNP on increasing total protein content with increasing chlorophyll (Salachna and Zawadzińska, 2018). Polyamines protect proteins in plants because polyamines covalently bind to biomacromolecules, such as proteins and nucleic acids, and inhibit the activity of RNAase and proteases, which are protein-degrading enzymes (Chen et al., 2019). From the results obtained, putrescine alone does not have a significant effect on the protein content, but when used in combination with SNP, the protein content is increased.

Postharvest parameters

Relative fresh weight of flowers

A look at the effect of different concentrations of SNP and putrescine on the relative fresh weight of flowers (Figure 1) shows that the highest value of this trait was obtained on the 4th day after harvest (26.33%).

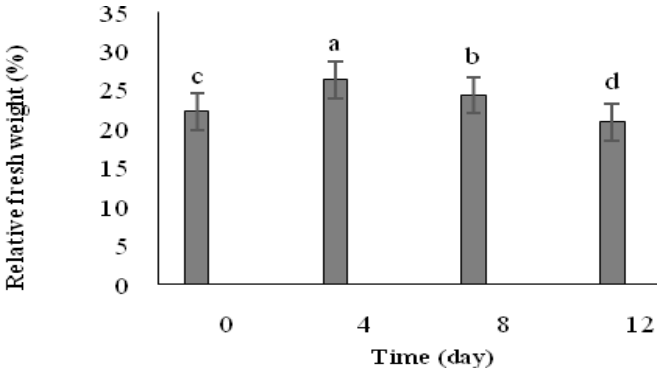

Figure 1. The effect of vase life period on flower relative fresh weight of roses cv. 'Avalanche' (Non-identical letters in each group of measured traits indicate a significant difference between the means with the Tukey test.).

Regarding the effect of SNP and putrescine at different rates on flower fresh weight (Figure 2), it was found that among different treatments, the treatment of 200 µM SNP with 4 mM putrescine (27.65%) showed the highest relative fresh weight of flowers, 60% more than which was obtained from the control.

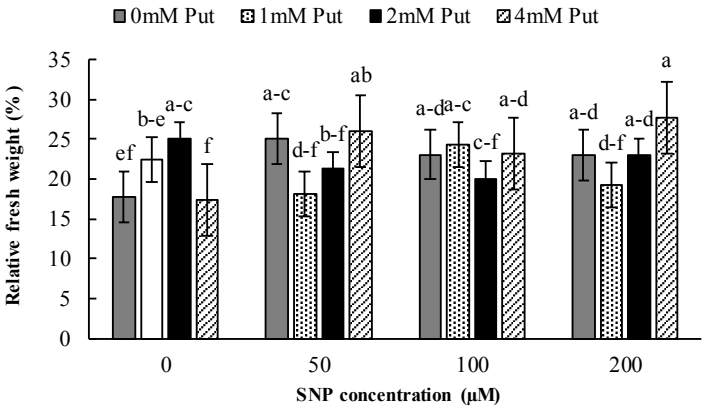

Figure 2. The effect of sodium nitroprusside and putrescine at different concentrations on flower relative fresh weight of roses cv. 'Avalanche' (Non-identical letters in each group of measured traits indicate asignificant difference between the means with the Tukey test.).

The fresh weight of the flower is a measure to evaluate the vase life, so the fresher the weight of the cut flowers, the longer the vase life. The main symptoms of petal senescence are loss of fresh weight, drying and wrinkling. In fact, as cut flowers approach senescence, their weight decreases due to reduced water absorption and transpiration. As a result, the water potential of flowers decreases and this reduces their fresh weight. SNP prevents vascular occlusion. Therefore, it plays a key role in improving water absorption and reducing water loss during the postharvest period (Naziri Moghaddam et al., 2021). Therefore, it can be concluded that SNP increases relative fresh weight, which is consistent with an experiment on gerbera that showed that NO increases relative fresh weight by increasing solute uptake by the plant, which inhibits vascular occlusion (Hemati et al., 2019).

In the present study, putrescine increased the relative fresh weight of flowers compared to the control. Putrescine binds to the cell membrane and thereby stabilising it and maintaining the wax of the cuticle layer. Therefore, it is important to reduce transpiration, water exchange with the surface (Qu et al., 2020).

Petal malondialdehyde

Based on the comparison of means (Table 5), the highest MDA content was related to day 12th of the plants treated with 200 µM SNP and no putrescine (2.53 µM g^{-1} FW), which significantly differed from the control.

Table 5. The effect of sodium nitroprusside and putrescine at different concentrations on petal's Malondialdehyde of roses cv. 'Avalanche' during vase life period. (Non-identical letters in each group of measured traits indicate a significant difference between the means with the Tukey test.). SNP: sodium nitroprusside (µM), Put: putrescine (mM), T: time (Day).

Malondialdehyde (µM/g.FW)																
	SNP 0				SNP 50				SNP 100				SNP 200			
	Put 0	Put 1	Put 2	Put 4	Put 0	Put 1	PPut 2	Put 4	Put 0	Put 1	Put 2	Put 4	Put 0	Put 1	Put 2	Put 4
T0	00.49 $^{r-y}$	00.77^{k-q}	00.32^{y}	00.45 $^{t-y}$	0.49 $^{r-y}$	0.32 ^{y}	0.41^{v-}	0.34^{x,y}	0.43 $^{u-y}$	0.45 $^{t-y}$	0.47^{s-y}	0.32 ^{y}	0.34^{x,y}	0.52^{q-y}	0.32^{y}	0.36^{w-y}
T4	00.95^{h-l}	00.87 $^{j-m}$	00.65^{x-v}	00.7 $^{l-t}$	0.46 $^{s-y}$	0.62^{m-w}	0.79^{k-q}	0.7 $^{l-t}$	0.55 $^{o-y}$	0.74 $^{l-q}$	0.83 $^{j-n}$	0.76 $^{k-r}$	0.71^{l-s}	0.59^{n-x}	0.67^{m-v}	0.7 $^{l-t}$
T8	10.07 $^{f-j}$	10.01 $^{f-k}$	10.26^{d-f}	00.76^{k-q}	0.52^{p-v}	0.59 $^{n-x}$	0.85 $^{j-n}$	0.72 $^{l-s}$	0.62 $^{m-w}$	0.79^{k-o}	0.72 $^{l-s}$	1.13 $^{e-l}$	1.23^{d-g}	0.95^{t-l}	0.72 $^{l-s}$	0.63^{l-u}
T12	10.47 ^{cd}	10.24^{d-g}	10.46 ^{cd}	00.75^{k-r}	0.88 $^{j-m}$	1.15 $^{e-i}$	1.36^{e-e}	1 $^{g-k}$	1.15 $^{e-i}$	1.72^{b}	1.55^{bc}	1.33 ^{de}	2.53^{a}	1.71^{b}	1.72^{b}	1.2 $^{e-h}$

MDA is produced from the peroxidation of cell membrane lipids, and its content in intercellular conditions can damage plants and detriment cell membrane homeostasis. In older plants, more active oxygen radicals are produced. Increased synthesis of reactive oxygen species (ROS) (due to the imbalance between reactive oxygen radicals and antioxidant enzymes) causes secondary oxidative damage such as membrane lipid peroxidation and ultimately leads to loss of membrane semi-permeability (Juan et al., 2021). Hydrogen peroxide reacts with superoxide radicals to hydroxyl radicals. Then these hydroxyl radicals react with the methylene groups of unsaturated fatty acids (which are the main components of membrane lipids) and cause lipid peroxidation of the membrane and thus increase the MDA content (Raymond et al., 2018).

SNP reduces cell membrane lipid peroxidation by several important mechanisms. SNP reacts with lipid alkyl and lipid peroxyl radicals and stops the lipid peroxidation chain from the direct pathway. As a result, the MDA content is reduced. Lipoxygenase is an oxidizing enzyme that causes membrane lipid peroxidation. According to the results, it can be said that the amount of MDA increased with the senescence of the flowers. Accordingly, SNP decreases MDA content compared to control, but increasing its rate to 200 µM increases MDA content. SNP reduces the degradation of membrane lipids by increasing the activity of enzymes, especially ascorbate peroxidase, which is known as the most important antioxidant in plants. Our results are consistent with studies conducted on gerbera, carnation, chrysanthemum, and *Echinacea angustifolia*, according to which SNPs reduce the breakdown of membrane lipids by affecting the activity of antioxidant enzymes (Shabanian et al., 2018).

Polyamines prevent membrane destruction by stabilizing lipid bilayers in the cell membrane through bonding with anionic compounds. Due to their antioxidant capacity, they are also effective in inhibiting free radicals and thus contribute to the cell wall and membrane stability and reducing MDA content. Polyamines can act as free radical scavengers and protect cell membranes from oxidation, thereby strengthening membranes. Polyamine treatments can increase internal polyamine content and contribute to membrane stability. Our results showed that higher putrescine content was associated with lower MDA content. We also found that during the vase life period, plant tissue degradation intensified and MDA content increased, although the degradation rate was slower in some treatments, and lipids were oxidized at a lower rate. This was observed in the treatment of 4 mM putrescine, so that the MDA content in plants exposed to this treatment did not change significantly with senescence. Similar findings were reported for berginia and rose according to which putrescine prevented the increase in MDA content by affecting lipoxygenase, an enzyme responsible for

membrane lipid peroxidation (Altaf et al., 2023; Yousefi et al., 2019).

Vase life

Comparison of means (Table 4) showed that both SNP and putrescine treatments had a positive effect on vase life, so the longest vase life was obtained from 50 µM SNP with 4 mM putrescine (26 days), which was 65% more than the control.

The vase life of a rose varies from 7 to 15 days. The vase life of cut flowers depends on genetic factors and varies greatly among cultivars. The difference in the vase life of different varieties of cut flowers is related to the diameter of the stem and their stiffness. Thicker stems are safe from bending and breaking and have enough storage for the flower to breathe, so flowers on thicker stems last longer. Light intensity is an important factor in determining the photosynthesis process and significantly affects the carbohydrate content. Flowers are sensitive to ethylene to varying degrees. As the flowers get senescence, they become more sensitive to ethylene (Dole and Wilkins, 2004).

SNP can inhibit ethylene synthesis and thus reduce the rate of degradation of cell wall polysaccharides. Phenolic compounds that are produced due to the NO-induced activation of the PAL enzyme have important antioxidant activity. The antioxidant activity of phenolic compounds is related to their oxidative and reductive properties. During the vase life period, a large amount of ROS and hydrogen peroxide are synthesized. Although ROS can act as secondary signals to activate cell defense systems against adverse conditions, their excessive accumulation can increase oxidation in biomolecules and cause cell death. It is believed that SNP is the factor that stimulates the postharvest synthesis of many antioxidants in products (Asghari, 2015). We observed that SNP increases the longevity of rose during postharvest storage, which may be due to its effect on the increase of plant stored carbohydrates, the activity of antioxidant enzymes, and their inhibitive effect on postharvest peroxidation of membrane lipids and hydrogen peroxides. This finding corroborates the results of studies on roses, oriental lilies, tulips and gerberas on the effect of SNPs on the extension of vase life through the preservation of membrane proteins (Salachna and Zawadzińska, 2018; Shabanian et al., 2018; Wang et al., 2018).

As plant cells and tissues aged, their polyamine content usually decreases. This accelerates ethylene synthesis and thus increases ACC-synthase activity or tissue sensitivity to ethylene activity. Polyamines reduce or inhibit ethylene synthesis by scavenging free radicals and inhibiting the expression of genes that encode these and other degrading enzymes, e.g. lipoxygenases, peroxidases and lipases. They also play a role in reducing the activity of decomposing enzymes by maintaining cell structures, reducing respiration and maintaining organic acids in cells (Asghari, 2015). We found that putrescine was partially effective in increasing vase life, which could be attributed to the anti-ethylene activity of polyamines and their effect on reducing electrolyte leakage and MDA content, which is consistent with research on gerbera and rose (Mohammadi et al., 2021; Hosseini Farahi et al., 2013).

Correlation analysis were carried out to find the factors affecting these results (Figure 3). The relationship between leaf number and leaf area, fresh leaf weight, soluble sugars and vase life is negative but other attributes are positive. In other words, when the number of leaves increases, the surface of the leaf decreases and, accordingly, the weight of the leaf decreases. On the other hand, due to the increase in the number of leaves, the shading of the leaves increases, the amount of photosynthesis decreases, and as a result, the carbohydrate storage decreases. The reduction of carbohydrate intake also decreases the vase life of flowers.

Pearson Correlation Coefficients, N = 16
Prob > |r| under H0: Rho=0

	larea	lnumber	lfw	lDw	solubles	totalpro	vaselife
larea	1.00000	-0.13886 0.6080	0.61177 0.0118	0.36715 0.1618	0.60675 0.0127	0.19424 0.4710	0.16780 0.5345
lnumber	-0.13886 0.6080	1.00000	-0.11169 0.6805	0.03127 0.9085	-0.20688 0.4420	0.44883 0.0812	-0.18332 0.4968
lfw	0.61177 0.0118	-0.11169 0.6805	1.00000	0.85258 <.0001	0.30493 0.2508	-0.29634 0.2651	-0.26997 0.3119
lDw	0.36715 0.1618	0.03127 0.9085	0.85258 <.0001	1.00000	0.10703 0.6932	-0.43207 0.0947	-0.29127 0.2737
solubles	0.60675 0.0127	-0.20688 0.4420	0.30493 0.2508	0.10703 0.6932	1.00000	-0.03342 0.9022	0.28512 0.2844
totalpro	0.19424 0.4710	0.44883 0.0812	-0.29634 0.2651	-0.43207 0.0947	-0.03342 0.9022	1.00000	0.17249 0.5230
vaselife	0.16780 0.5345	-0.18332 0.4968	-0.26997 0.3119	-0.29127 0.2737	0.28512 0.2844	0.17249 0.5230	1.00000

Figure 3. Correlation between leaf area, leaf number, fresh leaf weight, dry leaf weight, soluble sugars, total protein and vase life according to Pearson analysis.

Conclusions

According to the results, it can be concluded that SNP and putrescine improved some morphological, physiological, biochemical and postharvest parameters. Polyamines and nitric oxide are important signalling molecules that play promising roles in a wide range of plant activities, including photosynthesis, growth and development, so they can enhance plant growth. They also cause an increase in the content of soluble sugars and relative fresh weight of the flower and decrease the production of malondialdehyde, so increase the vase life of the flower. It was found that the highest number of leaves and relative fresh weight of flowers, the highest fresh and dry weight of flowers as well as, vase life and the lowest amount of MDA were obtained from 200 µM sodium nitroprusside with 4 mM putrescine.

Author Contribution

RA: performed the experiment, chemical analysis, statistical analysis and preparing the manuscript. ZJ: Assistance in designing the experiment, chemical and statistical analysis and writing the manuscript

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