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Impact of Exogenous Spermine Application on the Vase Life of Cut Rose Flowers 'Dolce Vita'

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Abstract

Senescence is an integral part of the normal developmental cycle of plants and can be traced in cell, tissue and organ level. This work was focused on the efficiency of post-harvest treatment with the free polyamine spermine (Spm) to extend the vase life of cut rose flowers as an anti-senescence compound. Spm treatment was applied at the rates of 0, 1, 2 and 4 mM. Some morphological, biochemical and physiological characteristics of tested cut roses (*Rosa hybrida* cv. 'Dolce Vita') such as flower diameter, water uptake, relative fresh weight, electrolyte leakage, malondialdehyde content, guiacole peroxidase activity, hydrogen peroxide content and vase life were evaluated. The effects of a pulsing application of the polyamine on the mentioned traits were significant, and treatment with Spm effectively delayed the senescence of roses, especially when applied at 4 mM concentration. Polyamine treatment resulted in a notable decrease in petal electrolyte leakage due to its cationic and anti-peroxidative characters. In addition to the anti-ethylene property, the cationic character of polyamines may play an important role in determining the vase life of cut flowers.

Keywords: Antiperoxidative, Climacteric flowers, Electrolyte leakage, Malondialdehyde, Polyamines, Water uptake

INTRODUCTION

Flowers are the most important and unique gift of nature. They are the adornments of the world with their valuable aesthetic, environmental, economic and medicinal properties. Critically important to the cut flower market are the form, shape, and colour of the flowers. Equally important and a key determinant of flower quality is longevity, not only to the end-user, but also for producer and marketer, who must subject the flowers to sometimes lengthy handling and transportation processes. A number of parameters are used to characterize the longevity and to define lifespan termination in cut flowers. Flowers become unacceptable because of sepal yellowing, petal wilting and petal abscission as well as the lack of bud opening or peduncle bending. The cut flowers deterioration is determined mostly by the environmental and storage conditions. The most adverse effects on several species of cut flowers are caused by ethylene in the storage atmosphere (Reid and Jiang, 2012). Based on post-harvest longevity, cut flowers are divided into two main groups: flowers having low ethylene-sensitivity with no flower bud or leaf abscission, and the flowers that are highly sensitive to ethylene. Chrysanthemums, tulips, irises and lilies do not have ethylene production peak or respiration quick raise during flower senescence; and even the exogenous ethylene has little or no effect on the senescence of their flowers (Seglie *et al.*, 2009). On the other hand, flowers from Campanulaceae, Caryophyllaceae, Geraniaceae, Ranunculaceae, and Rosaceae are highly sensitive to the ethylene exposure.

Many anti-ethylene compounds, such as silver thiosulfate (STS), 1-methylcyclopropene (1-MCP) and aminoethoxyvinylglycine (AVG) have been used to extend the vase-life of cut flowers. The broad anti-ethylene effects of polyamines are well-known, and they have been used in different assays. Polyamines (PAs) are low molecular weight cationic molecules implicated in various physiological and developmental processes in plants, animals, and microorganisms. In plants, di-amine putrescine (Put), tri-amine spermidine (Spd), and tetra-amine spermine (Spm) are frequently present in amounts varying from micromolar to more than millimolar (Pandey *et al.*, 2000). The various plant growth and developmental processes affected by PAs include stimulation of cell division, modulation of the response to environmental stresses, regulation of rhizogenesis, embryogenesis and fruit and flower development. The participation of PAs in senescence delay has been strongly suggested by certain studies.

At the ambient cellular pH levels, polyamines behave as cations and can interact with anionic macromolecules such as DNA, RNA, phospholipids and certain proteins (Pandey, 2000). They also regulate the rigidity and stability of cellular membranes. In addition to commonly occurring free polyamines, the importance of polyamines conjugated to low and high molecular weight compounds is now well recognized.

Post-harvest senescence is an integral part of the normal development cycle of plants and is genetically regulated at cellular level. Senescence is an oxidative process involving a general degradation of the cellular structure and the mobilization of the resultant products to the other parts of the plants (Pandey, 2000). The process is mainly characterised by the cessation of photosynthesis, intensive loss of chlorophyll and proteins, drastic increase in lipid peroxidation, membrane leakage, breakdown of cell wall components and disruption of cell membranes leading to cellular de-compartmentalisation and loss of tissue structures (Sood, 2008).

An increase in the endogenous levels of free PAs was noted during senescence of cut carnation flowers (Ebrahimzadeh *et al.*, 2013; Lee *et al.*, 1997), where, its post-harvest senescence was delayed by spermine application, which reduced ethylene production (Ebrahimzadeh *et al.*, 2013; Lee *et al.*, 1997). The ethylene biosynthesis could also be modulated by in vivo biosynthesis of PAs since both ethylene and PAs biosynthesis pathways share SAM (S-adenosyl methionine) for their synthesis and could compete for its availability during senescence (Pandey, 2000). The participation of PAs in senescence delay has been strongly suggested by several studies (Mahros *et al.*, 2011; Mahgoub *et al.*, 2006; Hosseini *et al.*, 2013).

'Dolce Vita' rose is among the most important cut rose cultivars and the goal of present

study was to evaluate the possible role of Spm in postharvest life of this ethylene-sensitive cut rose flower. Morever, the idea was to investigate the potential effect of Spm on some biochemical characteristics of the flowers.

MATERIALS AND METHODS

To investigate some morphological and biochemical changes during the time-course of rose petal senescence, 120 cut roses were examined. Flowers were re-cut to have 40 cm of stem length at day 0 after harvest (Fig. 1.).

Fig. 1. Handling of cut rose flowers from the green-house to the laboratory.

Flowers, then, received these treatments: a control group was kept in distilled water (dH2O) and the other three groups were held in 1, 2 or 4 mM Spm for 24 h. After the pulse treatments, stems were labelled, weighed (after removing the leaves, except for three leaves close to the flower), and immediately placed in dH2O (one flower per tube)(Fig. 2.), and the water was replaced every 24 h. During the experiment, flowers were kept in standard conditions at 20 ±1°C, 60% RH, and 13 µmol m^{-2} S^{-1} light using cool white light with a 12 h photoperiod. All groups of flowers were evaluated for morphological traits (such as vase life, fresh weight and water absorption) and membrane turgidity. Petals from 0, 2, 4, 6, 7, 8, 10 and 12-day-old flowers of each group were collected, frozen in liquid N2, and stored at -20 °C for biochemical assay.

Fig. 2. Effect of PAs on the vase life of cut rose flower cv. 'Dolce Vita'. After harvesting flowers at commercial maturity stage, pulsed for 24 h with different concentrations of PAs for vase life evaluation in standard conditions.

Vase life

During the vase-life period, the visual quality of cut stems was inspected on a daily basis. In our study, vase-life was defined as the period (days) from the time of cutting to when 50% of flower petals were wilted or abscised or flower necks were bent (Pompodakis *et al.*, 2005).

Relative fresh weight (RFW)

Fresh weight of the flowers was determined just before the immersion of the flowers into the solutions and repeated every day for all the treatments until the vase life of the flowers were terminated. Flowers were taken out of solutions for the shortest time as possible (20 to 30 s). The fresh weight of each flower was expressed as relative to the initial weight to represent the water status of the flower (Joyce and Jones, 1992).

$$\text{Relative fresh weight} = \text{Final weight} / \text{Initial weight} \times 100$$

Water uptake

Mean daily water uptake (g $stem^{-1}$ day^{-1}) was computed using the formula ($S_{t-1} - S_t$) where S_t is the weight of vase solution (g) at t = day 1, 2, 3, …, n (Hettiarachchi and Balas, 2005).

0.1 g petal samples were taken from each flower (3 replications for each treatment), rinsed with distilled water, and dried on filter paper. The petals were incubated in 30 mL mannitol (0.4 M) in 50 mL capped polypropylene test tubes at 24°C for 20 h on a rotary shaker (80 rpm). The conductance of the samples was measured using a conductivity meter. Samples, then, were autoclaved at 120°C for 3 min, cooled to room temperature and the volumes adjusted to 30 mL. The conductivity of the samples was measured again to determine the total electrolyte content of the tissue.

Malondialdehyde content

Measurements were carried out by the method of Stewart and Bewley (1980), with a slight modification. Petal tissue (0.2 g) from the explants was homogenized in 1.8 ml of TCA 0.1%, and centrifuged at 16,000 × g for 15 min. The supernatant was collected, and 0.5 mL of it was mixed with 2.5 mL of 0.5% TBA. The mixture was boiled for 30 min, then quickly cooled in an ice-bath and centrifuged at 10,000 × g for 30 min. The supernatant was collected and used to measure the absorbance at 532 and 600 nm.

H2O2 concentration

H2O2 concentration was determined by the trichloroacetic acid (TCA) reaction as described by Junglee *et al.* (2014).

Measurement of antioxidant enzyme activity (guaiacol peroxidases (GPX))

About 0.2 g petal tissue was homogenized by buffer phosphate. The homogenate was centrifuged at 4°C for 15 min at 15000 g and the supernatants were used for determination of enzyme activity. The activity of GPX was determined by the methods as described by Tatiana *et al.* (1999).

Experimental design and statistical analysis of the data

Experiments were performed with a Complete Randomized Design (CRD) with 4 treatments, 3 replications for biochemical and 5 replications for morphological traits. Data were subjected to analysis of variance (ANOVA) using the SAS program. In case of significant treatment effects, mean comparisons were afforded using LSD test (P= 0.05).

RESULTS AND DISCUSSION

Vase life

The least vase life of *Rosa hybrida* 'Dolce Vita' was 6 d in distilled water and it was 8, 9 and 11 d when exposed to 1, 2 and 4 mM Spm (Fig. 3).

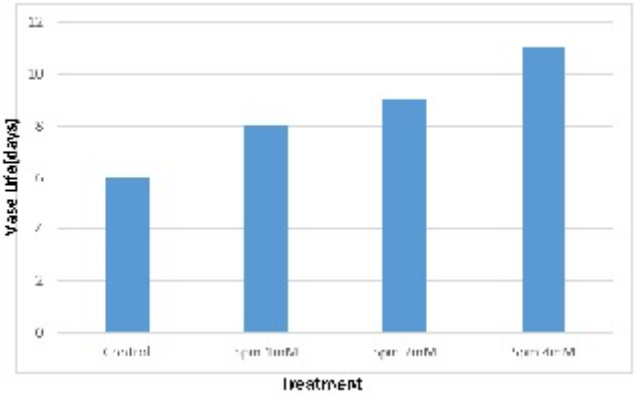


Fig. 3. Vase life of 'Dolce Vita' rose flowers submitted to different ranges of Spm (0, 1, 2, 4 mM). Different letters above the column indicate significant difference between the treatments at P< 0.01.

Spm prolonged flower longevity compared with untreated flowers. Macnish *et al.* (2010) previously demonstrated a difference in vase life of modern rose cultivars ranging from 5 to 19 days. Five of the 38 tested cultivars were insensitive to ethylene, while the others were sensitive. 'Dolce Vita' is one of the ethylene-sensitive cultivars of cut roses and holds longer vase life in 4 mM Spm (11 days) compared to the other treatments. Our results suggest that the flowers responses to the treatments was concentration dependent, which is in agreement with the findings of Hosseini *et al.* (2013) and Mahros *et al.* (2011).

Relative fresh weight

Spm treatment on cut roses had significant effect on the RFW (Fig. 4), reflecting the findings of Hosseini *et al.* (2012), Mahros *et al.* (2011) and Mahgoub *et al.* (2006).

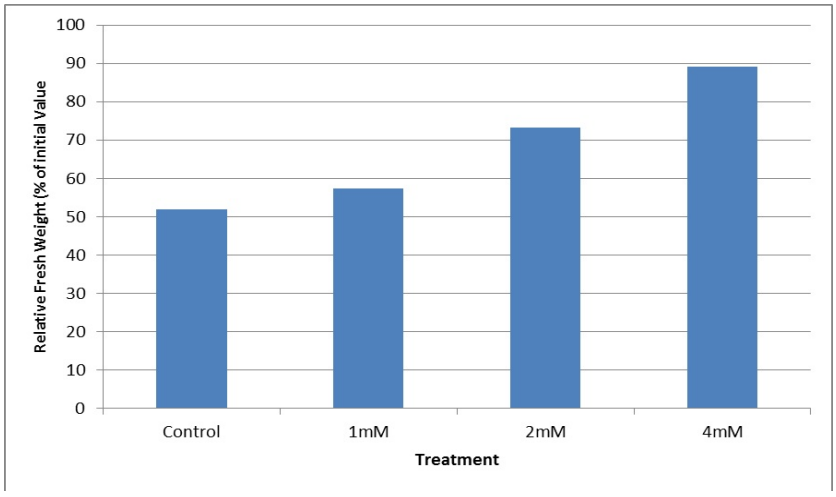


Fig. 4. Effect of Spm treatment on RFW of cut flower rose 'Dolce Vita'.

Using Spm at a 4 mM concentration was the most effective treatment when compared to all other treatments. The relationship between vase life and RFW was clear. Spm at 4 mM significantly improved RFW and vase life of *Rosa hybrida* 'Dolce Vita'. Petal expansion involved a rhythmic increase in FW. The FW of rose petal increased until the flower was fully open. These data are consistent with an opening mechanism involving cell expansion without an increase in cell number.

The effects of putrescine on fresh weight of cut roses is seemingly due to the impact of the compound on the photosynthesis rate and relate with the more assimilates agglomeration at the cell compartments and plant tissues.

Water uptake

Total water uptake by the stems indicated that Spm treatment with Spm had significant effect on the water uptake (Fig. 5).

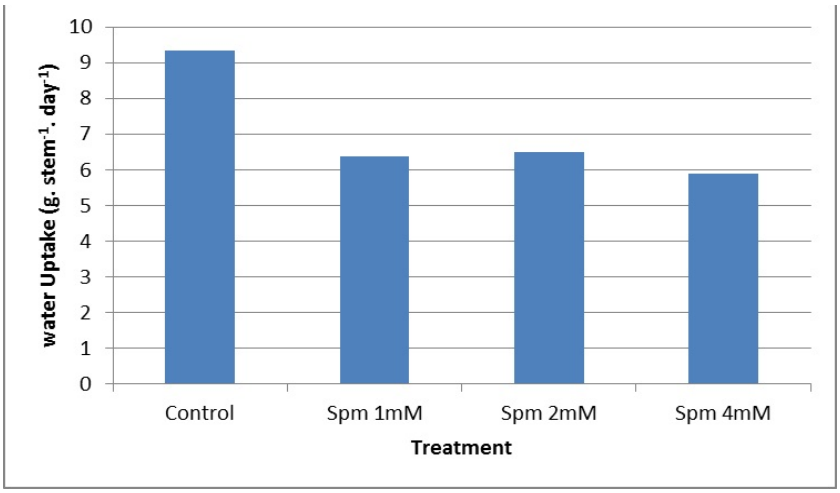


Fig. 5. Effect of Spm on water uptake of cut flower 'Dolce Vita'.

Flowers treated with Spm had less water uptake compared to control. From the above, it is still not clear, whether it is water alone which leads to the bud opening or some other physiological and biochemical processes are also responsible. Results from the present study suggest that, Spm may play an important role in increasing the tolerance of flowers to water loss which could be controlled by stomatal closure. We can hypothesize that exogenously applied Spm resulted in higher accumulation of endogenous polyamine pool and then activates cellular signaling pathway related to the stomatal movements. The higher polyamines may stimulate stomatal closure (Shi *et al.*, 2010).

It is well accepted that water balance in plant cells and tissues is a multifactor dependent phenomena and will be influenced by plant type, cell membrane integrity, cell wall components, cytoplasm composition and pH and hence, in this case will be so problematic to mention the real related criterion influencing water uptake and drainage unless, we have more detailed information at the sub-cellular level.

Membrane stability index

Spm had significant effect on MSI (Fig. 6). Linear increase was occurred in MSI with the increase in spm concentration. Significantly, higher MSI values were observed in Spm 4 mM as compared with other concentration ($P<0.05$).

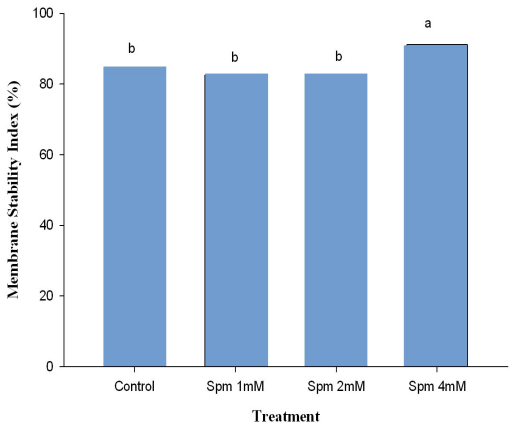


Fig. 6. Effect of polyamine Spm on MSI of rose (*Rosa hybrida* 'Dolce Vita').

Degradation of phospholipids and the resultant loss of membrane stability are of metabolic changes accompanying petal senescence (Ranchana and Kannan, 2015). However, during senescence of both ethylene sensitive and insensitive flowers, marked changes occur in the biochemical and biophysical properties of the cell membranes. In cut flowers, both the growth and senescence phases are accompanied by a decrease in membrane fluidity (Ranchana and Kannan, M. 2015).

Malondealdehyde (MDA)

As mentioned earlier, one of the most characteristic features of senescence is the initiation of membrane disassociation and a consequent loss of intracellular compartmentalization predominantly due to lipids degradation. Lipid peroxidation is commonly used as an indicator for the accumulation of free radicals in tissues. Lipid peroxidation not only threatens the integrity and function of membranes and membranous proteins but also produces a variety of toxic aldehydes and ketones. One of the main products of lipid peroxidation is MDA that can cause protein damage by the reactions with lysine amino groups, cysteine sulfhydryl groups and histidine imidazole substitutes (Refsgaard *et al.*, 2000). In this experiment, after 9 d Spm treatment at 0 and 1 mM had much less MDA content and, the MDA content with Spm at 2 and 4 mM were about at the level of harvest measurements (Table 1).

Table 1. Effects of time and Spm treatment on MDA content (nM g^{-1}) of *Rosa hybrida* 'Dolce Vita'.

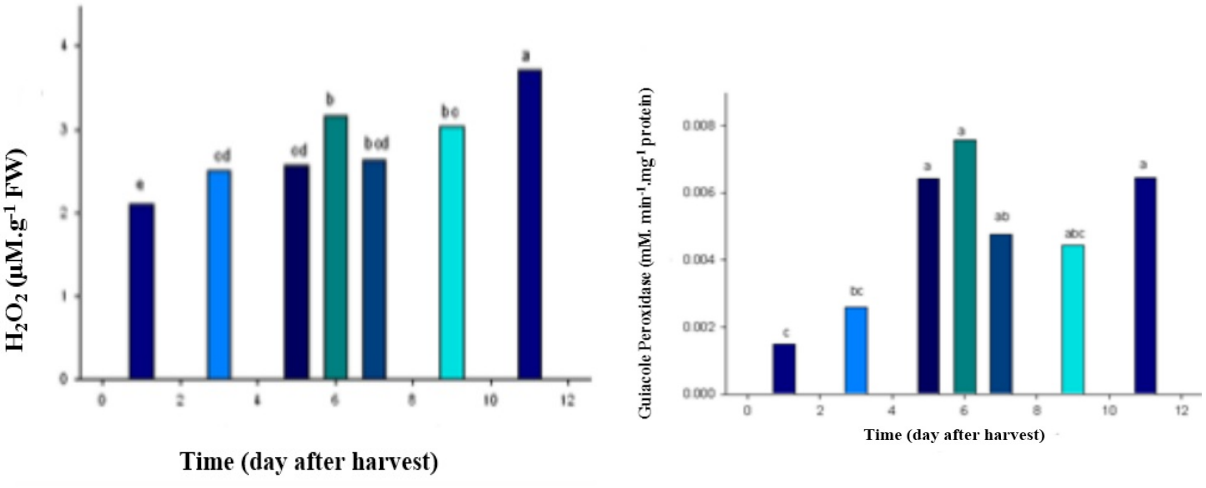
Treatment	Days (after harvest)					
	1	3	5	6	7	9
Control	189.62^{ba}	142.21^{ebdc}	111.79^{edc}	169.08^{bac}	-	-
Spm (1 mM)	179.03^{ba}	187.81^{ba}	136.01^{ebdc}	153.26^{bdac}	149.4^{ebdac}	-
Spm (2 mM)	204.08^{a}	140.75^{ebdc}	155.97^{ebdc}	179.93^{ba}	142.28^{ebdc}	192.65^{ba}
Spm (4 mM)	173.28^{ba}	139.82^{ebdc}	100.49^{ed}	194.27^{ba}	135.8^{ebdc}	169.01^{bac}

*Similar letter (s) in each column show insignificant differences at the 1 and 5% probability level according to the LSD test.

Numerous data demonstrate that PAs influence membrane structure and function. This could be explained by the interactions of PAs with the hydrophilic heads of either phospholipid bilayer or membrane bound proteins. This results rise from the specific charge–density distribution and specific configurationally states. PAs can lay parallel to membrane surface interacting with more than one negative charge. Thus, PAs could bridge membrane components through lipid-lipid and/or protein–lipid interactions forming 'packed' surface structures (inner organization of membranes seems to be not perturbed by PAs). As a consequence, complexation, rigidifying and stabilization of membranes occur and, this leads to the alterations of membranes permeability and variations in active transport properties. Antiperoxidative action of PAs (based on the formation of ternary complex between PAs, phospholipid heads and Fe^{3+}) also contributes to this end. Exogenously applied polyamines have also been shown to affect the membrane integrity and function in plants (Edreva, 1996).

Hydrogen peroxide and antioxidant enzyme GPX

H2O2 content increased with all the treatments during the postharvest days and significantly higher activity of GPX was observed in petals when, cut rose flowers were treated with spermine at 4 mM. Moreover, GPX activity was significantly higher during the postharvest days (Fig. 7 and 8).



Figs. 7 and 8. Effects of time on hydrogen peroxide and guiacole peroxidase

Plants produce reactive oxygen species (ROS) in nearly all common metabolic processes and when they suffer some kinds of stresses. Thus, ROS may be generated during the post-harvest phase of flowers, which according to Sharma *et al.* (2012) would cause oxidative damage in plants. The accumulation of these ROS molecules could promote damage to the lipids and proteins and encourages the production of H2O2.

In contrast, plants have antioxidative enzymes such as CAT and GPX that diminish the damages caused by excess peroxides.

It has been suggested that PAs may play a role in antioxidative system and protect membrane from peroxidation (Sharma *et al.*, 2012). It may be due to one or more of the following factors:(1) the activation of antioxidative defense system, (2) the suppression of the levels of H2O2, (3) the reductions of ROS levels through quenching of singlet oxygen, (4) the reduction of membrane leakage and lipid peroxidation and decreased MDA contents, (5) the stabilization of membrane damage due to maintenance of its polycationic nature, (6) the increase in GPX and CAT activity as well as SOD levels, (7) the increase in other organic solutes, such as polyamines that are involved in important biological processes, e.g., ionic balance and DNA, RNA and protein biosynthesis.

CONCLUSIONS

The present research demonstrated the efficacy of spermine in extending flower vase life in *Rosa hybrida* cv. 'Dolce Vita'. Spermine prolonged flower longevity as compared to untreated flowers and significantly affected the fresh weight of flowers. Meanwhile, cut flowers that were treated with spermine had less water uptake. Improvements occurred in membrane stability index with the increase in the concentration of spermine, and after 9 d, Spm treatment at 0 and 1 mM had much less MDA content and Spm at 2 and 4 mM had the same level of harvest measurements.

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