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Protective effects of polyamines on regulation of senescence in spray carnation cut flowers (*Dianthus caryophyllus*'Spotlight')

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ABSTRACT

This study was conducted to evaluate the effect of three polyamines (PAs) on antioxidants capacity, free radical scavenging and vase life improvement of spray carnation cut flowers. Hence, the cut flowers were dipped in different concentrations (0, 1, 2 and 3 mmol) of putrescine (Put), spermidine (Spd) and spermine (Spm) for 24 h. After treatment, the cut flowers were placed in distilled water and kept at 20 °C ± 2 °C, 70-80 % RH. All concentrations of Put treatment improved the vase life of cut spray carnation flowers as compared to control. The highest positive influence on vase life (with 13 days) was related to 2 mmol Put treatments. Result showed that applying Put and Spm treatments at 1 or 2 mmol concentration significantly minimized the percentage of mass loss compared to the control. A significant inhibition of anthocyanin degradation was observed with Put 1 or 2 mmol and Spm 1 mmol Significantly higher activities of catalase and DPPH radical scavenging activity were observed in petals when cut carnations were treated with 1 mmol Put. It can be concluded that application of polyamines such as putrescine can play a key role to prevent or delay deterioration in cut flowers.

Key words: anthocyanin; catalase activity; cut flowers; polyamines; vase life

IZVLEČEK

ZAŠČITNI UČINKI POLIAMINOV NA URAVNAVANJE SENESCENCE REZANIH MNOGOCVETNIH NAGELJČKOV (*Dianthus caryophyllus*'Spotlight')

V raziskavi je bil ovrednoten vpliv treh poliaminov (PAs) na antioksidacijsko sposobnost, odpravljanje prostih radikalov in izboljšanje trajanja rezanih večcvetnih nageljčkov. Odrezani poganjki s cvetovi so bili izpostavljeni različnim koncentracijam (0, 1, 2 in 3 mmol) putrescina (Put), spermidina (Spd) in spermina (Spm) za 24 h. Po obravnavanju so bile odrezane rastline premeščene v destilirano vodo pri 20 °C ± 2 °C, 70-80 % RH. Vse koncentracije putrescina so podaljšale trajanje rezanega cvetja v primerjavi s kontrolo. Največji pozitivni učinek na trajanje cvetja (13 dni) je imelo obravnavanje z 2 mmol putrescina. Rezultati so pokazali, da je uporaba putrescina in spermina v koncentracijah 1 ali 2 mmol značilno zmanjšala upad mase cvetja v primerjavi s kontrolo. Pri obravnavanjih z 1 ali 2 mmol putrescina in 1 mmol spermina je bilo opaženo značilno zmanjšanje razgradnje antocianina. V venčnih listih rezanih nageljčkov, tretiranih z 1 mmol putrescina sta bili značilno povečani aktivnosti katalaze in DPPH nevtralizacije radikalov. Zaključimo lahko, da ima lahko uporaba poliaminov kot je putrescin ključno vlogo pri preprečevanju in odlogu propadanja rezanega cvetja.

Ključne besede: antocianin; aktivnost katalaze; rezano cvetje; trajanje; poliamini

1 INTRODUCTION

Recently, spray type of cut carnation flowers have become more popular (Hanks et al., 2015). Hence its postharvest senescence is a major limitation to the marketing and considerable efforts have been devoted to developing postharvest treatments to extend the marketing period (Nichols, 1977; Rattanawisalanona et al., 2003, Karimi et al., 2012). On the other hand the main challenge of florists in the global flower trading is the quality of cut flowers (Seglie et al., 2012). Polyamines (PAs), putrescine, spermidine and spermine

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are low molecular weight compounds and present naturally in all living organisms (Kao, 1997). Many studies well documented the role of PAs as anti-senescent agents in reduction of respiration rate, ethylene production and retard color changes and increase flowers vase life (Niklas et al. 1998; Valero et al., 2002; Genk et al., 2009; Rani & Singh, 2014; Tiburcio e al., 2014). The polyamine and ethylene biosynthesis pathways are interrelated, due to the competition on S - adenosylmethionine (SAM) (Bouchereau et al., 1999). Also, ethylene has been shown to play an important role in flower senescence regulation. Treatment of carnation cut flowers with ethylene inhibitors significantly extended vase life and CAT, SOD and POD enzyme activity (Hunter et al., 2004; Karimi et al., 2012; Karimi et al., 2013). Meanwhile, more studies suggested that treatment with PAs significantly improved fresh mass, uptake of vase solution, flower opening, vase life and more parameters of ornamental plants (Pandey et al., 2000; Rubinowska & Miachałek, 2009; Kandil et al., 2011; Mahgoub et al., 2011; Hosseini Farahi et al., 2013; Ataii et al., 2015). The effects of different concentrations of putrescine on flower characters, total carbohydrates and photosynthetic pigments of *Chrysanthemum* were investigated by Kandil et al. (2011). They concluded that all flower characters were significantly increased by foliar application of putrescine at different concentrations (100, 200 and 300 ppm). In this regards, Mahgoub et al. (2011) reported that PAs delayed senescence and improved vase life of cut *Dahlia pinnata* Cav. by improving membrane stability of cells. In another related study Ataii et al. (2015) evaluated the effects of different levels of exogenous putrescine on vase life of cut *Lisianthus* flowers. They suggested that Put treatment enhanced the activities of antioxidant system (catalase and ascorbate peroxidase) and limited the accumulation of H_2O_2. Hence according to the importance of vase life of cut flowers, the present study was conducted to investigate the effects of exogenous application of different PAs on maintaining quality, extending vase life and antioxidant capacity of spray carnation.

2 MATERIALS AND METHODS

In order to evaluated the protective effects of polyamines on regulation of senescence in cut sprayed carnation (*Dianthus caryophyllus*'Spotlight') a potted experiment was conducted in Sari Agricultural Sciences and Natural Resources University in north of Iran during the spring of 2016. The uniform cut flowers of spray carnation (*Dianthus caryophyllus* 'Spotlight') were obtained from a commercial grower and packed in plastic bags, and transferred to the laboratory immediately. At purchasing time, the flowers were harvested commercially at the usual flowering time (the first flower of the six to eight buds on the stem was almost fully opened). The stems were shortened up to 40 cm, immersed with their cut ends at 0, 1, 2 and 3 mM diamine putrescine (Put), tetramine spermine (Spm) and triamine spermidine (Spd) solutions for 24 h. After treatment, the cut flowers were placed in a 500 ml flask with 400 ml of distilled water and kept at 20 °C ± 2 °C, 70-80 % relative humidity, and 12 h photoperiod with 15 µmol m^{-2} s^{-1} irradiance from warm fluorescent lamps throughout the experiment.

2.1 Evaluation of vase life and fresh mass loss

Vase life and fresh mass loss were determined as the time to wilting of more than one third of the flowers in each pot (Karimi et al., 2012). The fresh mass of each flower (stem + flower) was expressed relative to the initial fresh mass to represent the percentage of mass.

2.2 Measurement of anthocyanin concentration

Anthocyanin content of the petals was determined on day 5 (when the vase life of the control flower was terminated). Petal slices were extracted with 100 % methanol containing 1 % HCl at 4 °C overnight. The absorbance of the extract was measured at 530-700 nm with a spectrophotometer (3600 UV/ Vis, UNICO, USA) (Paliyathet al., 2008).

2.3 Evaluation of 1,1-dyphenyl-2-picrylhydrazyl (DPPH) scavenging activity

At first 4 mg DPPH was dissolved in 100 ml of methanol. The stock solution of the flower extract was prepared in 95 % methanol to achieve the desired concentration and to 0.2 ml of sample 2 ml of DPPH was added and the solution was incubated for 20 min. Absorbance was measured against reagent blank at 517 nm spectro-photometrically (3600 UV/ Vis, UNICO, USA) (Miliauskas et al., 2004). The IC 50 value of the sample, which is the concentration of sample required to inhibit 50 % of the DPPH free radical, was calculated using log dose inhibition curve (Sidduraju et al., 2002). The ability of the essential oil to scavenge DPPH radical was calculated as percent inhibition by the following equation: Percent inhibition = [(Acontrol – Asample) / Acontrol] x 100

2.4 Catalase enzyme assays (CAT; EC 1. 11.1.6)

Catalase (CAT) activities assay were quantified spectro-photometrically (Aebi, 1983). The reaction mixture contained 15 mmol H_2O_2, up to 100 µl of homogenate

(7 mg protein ml^{-1}) with 0.2 % (v/v) Triton X-100 in 50 mmol potassium phosphate buffer (pH 7.0)

2.5 Experimental design and statistical analysis

This experiment was conducted in a completely randomized design with four replications. Results were analyzed using SAS software. Mean comparisons to identify significant difference between treatments were performed using the least significant difference (LSD).

3 RESULTS

3.1 Vase life determination

The Put at all tested concentrations improved the vase life of cut spray carnation flowers as compared with control (Distilled water). The best results were achieved with the 2 mmol Put variant/ 13 days compared to control variant/ 9 days. However, there were no significant differences between 1 mmol spermine and 1 or 2 mmol Put treatment (Fig. 1).

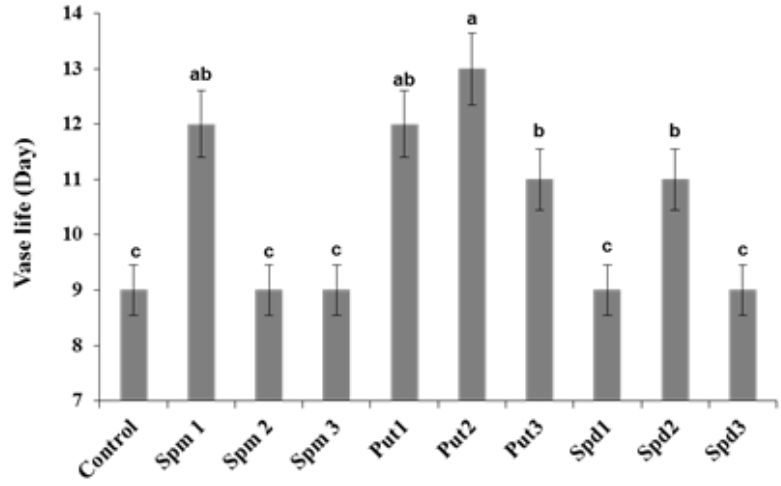

Figure 1: Vase life of cut carnation flowers in response to pretreatment with different concentrations of polyamines Put, Spm and Spd at 0, 1, 2 and 3 mmol. Diversified letters indicate significant differences ($p<0.05$).

3.2 Anthocyanin content

A significant inhibition of anthocyanin degradation was observed with Put 1 or 2 mmol and Spm 1 mmol. There were no significant difference between Spm 2, Spd 1 or 3 mmol, and control (Fig. 2).

3.3 Fresh mass loss

Result showed that applying Put and Spm treatments at 1 or 2 mmol dose significantly minimized the mass loss compared to control until the ninth day (Tab. 1). However, both 2 and 3 mmol treatments affected more positively the fresh mass than 1 mmol treatment. The lowest percent of mass loss on 9^{th} day (when the vase life of the control flowers was terminated) was obtained by 2mmol Put treatment (with 12.82 %). However, the results of the control flowers showed the maximum percentage of mass loss (48.32 %) on day 9 (Tab. 1).

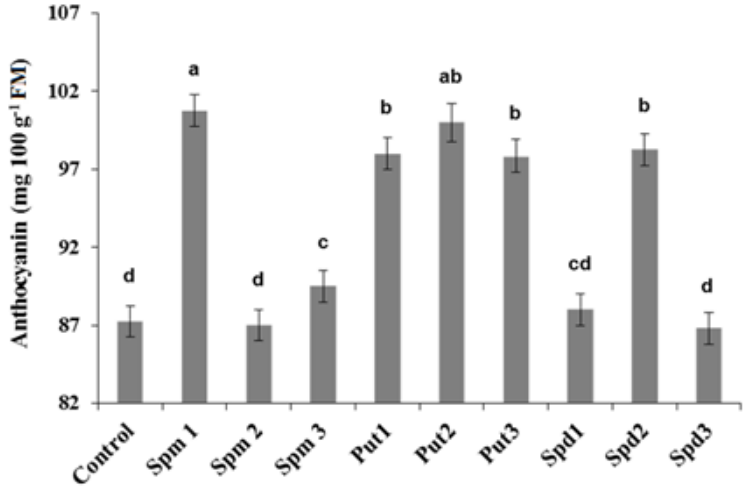

Figure 2: Anthocyanin contentment of cut carnation flowers in response to pretreatment with different concentrations of Put, Spm and Spd at 0, 1, 2 and 3 mmol. Diversified letters indicate significant differences ($p<0.05$)

3.4 Effect of polyamines on antioxidant metabolism

Significantly higher activities of CAT and DPPH radical scavenging activity were observed in petals when cut carnations were treated with 1 mmol Put. Although there were no significant differences between 1and 2 mmol Put and 1 mmol Spm (Tab. 1).

Table 1: Means ± standard errors of fresh mass loss, DPPH scavenging activity, catalase activity of cut carnation flowers in response to pretreatment with different concentrations of Put, Spm and Spd at 0, 1, 2 and 3 mmol

Treatments (mmol)		Fresh Mass Loss (%) 9th day	DPPH scavenging activity (mg/ml)	CAT activity (nmol $min^{-1}Pro^{-1}$)
CONTROL	0	48.32±2.07a	0.21±0.26d	0.50±0.39d
PUT	*1*	11.12±4.31c	0.49±0.17a	1.24±0.25a
	2	12.82±4.01c	0.47±0.17a	1.21±0.25a
	3	41.32±2.24A	0.21±0.26b	0.76±0.32c
SPM	*1*	42.12±2.21a	0.47±0.17a	1.20±0.26a
	2	47.30±2.09A	0.39±0.19bc	0.98± 0.28b
	3	46.32±2.11a	0.20±0.26d	0.59± 0.36d
SPD	*1*	44.18±2.16a	0.22±0.25d	0.58± 0.37d
	2	24.85±2.88b	0.34±0.19 c	0.99± 0.28b
	3	44.11±2.16A	0.39±0.20bc	1.04± 0.27b

*Means within each column followed by different letters are significantly different (p = 0.05)

4 DISCUSSION

The obtained results indicate that polyamines at 1 or 2 concentrations improved the vase life of cut spray carnation flowers respectively compared with the control. Meanwhile the highest vase life duration belonged to 1 mmol Put treatment. In plant cells, the Put, Spd and Spm constitute the major polyamines

(PAs). The increase in flowers vase life by using PAs may be due to inhibiting ethylene production, because, the polyamine and ethylene synthesis pathways are interrelated, which results from the competition for the common precursor, S – adenosylmethionine (SAM); hence, one synthesis pathway is stimulated and the other one inhibited (Bouchereau et al., 1999). The physiological effects of polyamines and ethylene on senescence are the opposite to each other (Fuhrer et al., 1982; Winer & Apelbaum, 1986). The time of the onset of ethylene production and the amount of ethylene produced in the flower vary with the carnation cultivar (Nukui et al., 2004). In our previous studies using ethylene inhibitors increased the vase life of carnation flowers (Karimi et al. 2012; Karimi et al., 2013; Hassanpour et al., 2013). Lee et al. (1997) reported that treatment of 1 mmol spermine extended the vase life of carnation flowers and reduced ethylene production. The highest vase life of *Rosa hybrida* 'Dolce vita' was obtained in solutions containing 0.5 mmol spermidine and also in solutions containing 1 mmol spermine (Hosseini Farahi et al., 2012). Color fading and discoloration are important factors in determining visual quality of flowers and in many cases they are the main reasons for determination of post-production quality (Basra, 2000). The major types of pigments contributing to the color of the flowers are carotenoids and anthocyanins (Basra, 2000). The improvement of petal color expression is at least partially due to the increase in anthocyanin contents. Ethylene has been known to cause petal color fading. Pretreatment with PAs could reduce ethylene production in cut flowers, which is an important factor involved in retaining bract discoloration. During senescence there is an over-production of free radicals such as superoxide anion (O^-_2), hydroxyl radicals (OH) and hydrogen peroxide (H_2O_2), which may cause damage, leading to cell death. The harmful free radicals are controlled and balanced by antioxidant systems (Khan, 2006). Several enzymes such as SOD, CAT and POD are involved in the scavenging of free radicals in the plant system (Celikel & Van Doorn, 1995). Larrigaudiere et al. (2004) suggested that ethylene was involved in ROS production. Our results showed that polyamines (1 or 2 mmol Put and Spm) treated cut flowers had significantly higher CAT and DPPH radical scavenging activity compared with the control (Tab. 1). This study on polyamines can be understood not only as experimental evidence confirming the hypothesis of a link between ethylene and free radicals generation in senescence, but also as a key to the development of adequate methods to prevent or delay deterioration in cut flowers.

Therefore, it could be concluded that polyamines treatments may be good candidates for extending vase life, maintaining the visual quality of flowers. The treatment with Put and Spm (1 and 2 mmol) retarded the decrease fresh mass loss and anthocyanin degradation and increased the CAT and DPPH radical scavenging activity measured in petals.

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