Ind J Plant Physiol. (April–June 2015) 20(2):186–190
DOI 10.1007/s40502-015-0154-7

SHORT COMMUNICATION

Modulation of flower senescence in *Nicotiana plumbaginifolia* L. by polyamines

Shaziya Nisar[1] · Inayatullah Tahir[1] · Syed Sabhi Ahmad[1]

Received: 31 December 2014 / Accepted: 15 April 2015 / Published online: 8 May 2015

Abstract An experiment was conducted to elucidate the effect of various polyamines on longevity of the isolated flowers of *Nicotiana plumbaginifolia*. Isolated flowers were harvested at brush stage (1 day before anthesis) at 08:00 h, cut to a uniform pedicel length of 1 cm and held in 10 mM concentration of polyamines, viz., putrescine, spermidine and spermine. A separate set of flowers held in distilled water designated the control. All the polyamines tested improved the flower longevity of isolated flowers of *N. plumbaginifolia* as compared to the control. The flowers held in spermine showed improvement in longevity by 3 days as compared to control besides; maintaining higher floral diameter, soluble protein content and α-amino acids. The flowers supplied with different polyamines maintained lower content of total phenols as compared to the control. Higher content of soluble sugars was recorded in the floral buds held in various polyamines. The flowers held in putrescine showed maximum content of sugar fractions. The present study revealed that polyamines are effective anti-senescence agents as they retard protein degradation and maintain increased content of sugar fraction in the isolated flowers, thereby increasing flower longevity of *N. plumbaginifolia*.

Keywords *Nicotiana plumbaginifolia* · Proteins · Putrescine · Spermidine · Spermine · Senescence · Sugars

✉ Inayatullah Tahir
inayatullahtahirku@gmail.com

[1] Plant Physiology and Biochemistry Research Laboratory, Department of Botany, University of Kashmir, Srinagar 190006, India

Senescence in plants is regulated by interplay between various growth regulators like ethylene, gibberellins, cytokinins, abscisic acid and auxins. Ethylene is the main candidate for senescence in ethylene sensitive flowers, while as it has little or no role to play in ethylene insensitive flowers (van Doorn and Woltering 2008). In ethylene dependent flower systems such as carnation, ethylene antagonists like amino oxyacetic acid (AOA), silver thiosulfate (STS) and amino vinyl glycine (AVG) have been shown to delay flower senescence (Satoh et al. 2005). Moreover, ethylene biosynthesis competitor, polyamines have been reported to show anti-senescence properties in ethylene dependent flower systems (Galston 1983; Sawhney et al. 2003). Polyamines delay senescence by retarding membrane deterioration, RNase and protease activity (Sawhney et al. 2003). The most abundant polyamines present in plants are putrescine, spermidine and spermine. The interplay between polyamines and ethylene appears to regulate flower development and senescence. Although, polyamines and ethylene share the common precursor, S-adenosyl methionine (SAM), but ethylene accelerates senescence, whereas polyamines tend to have delaying effect (Sawhney et al. 2003). The antagonistic roles of polyamines and ethylene can be ascribed to their competition for the common precursor, SAM (Galston 1983). The role of exogenously applied polyamines still remains elusive. The present study was persued to assess the effect of polyamines (putrescine, spermidine and spermine) on flower development and senescence in *N. plumbaginifolia* which follows a well defined senescence pattern, that can be physiologically monitored and documented.

Isolated buds of *N. plumbaginifolia* at brush stage, i.e., 1 day before anthesis (stage IV) were used for the present study (Figs. 1, 2). The pedicels (1 cm) were divided into three sets and held in 5 ml glass vials containing 3 ml of

Fig. 1 Flowers of *Nicotiana plumbaginifolia* L. in full bloom in the month of July in Kashmir University Botanic Garden

respective holding solutions. Each set was supplied with different polyamines, viz., putrescine, spermidine and spermine. The concentration of all the vase solutions was kept 10 mM after standardization of the optimal concentration in all the cases. A separate set of 35 buds held in distilled water served as control. The treatment effects were evaluated by keeping the experiment under natural conditions in the laboratory with temperature of 21 ± 2 °C under cool white fluorescent light with a mix of diffused natural light (10 W m^{-2}) for 12 h a day and relative humidity (RH) of 60 ± 10 %. The day of transfer of buds to distilled water (DW) was designated as day zero (D_0). Floral diameter was recorded on day 2 and day 4 of transfer to the vase solutions. Total soluble proteins, α-amino-acids, phenols and sugar fractions (reducing, non-reducing and total sugars) were estimated on day 3 of the transfer.

Proteins were estimated by the method of Lowry et al. (1951) using BSA as the standard. Reducing sugars were estimated by method of Nelson (1944) using D-glucose as the standard. Total sugars were estimated by enzymatic conversion of non-reducing sugars to reducing sugars. Non-reducing sugars were calculated as the difference between total and reducing sugars. α-amino acids were estimated by the method of Rosen (1957) using glycine as the standard. Total phenols were estimated by the method of Swain and Hillis (1959) using gallic acid as the standard. Experiment was conducted under complete randomized design. The data was analyzed statistically and LSD was computed at $P_{0.05}$ using SPSS 16 software.

Treatment of isolated buds of *N. plumbaginifolia* with different polyamines resulted in the improvement of its postharvest attributes. Maximum flower longevity was registered in the flower buds held in spermine, which showed an improvement in flower longevity by 3 days, followed by 2 days in putrescine and 1 day in spermidine (Fig. 3; Table 1). Earlier studies have shown that polyamines delay senescence in flower like *Dianthus* and *Narcissus*, but spermidine was found to be effective than putrescine and spermine (Lee et al. 1997; Sardoei et al. 2013). Delay in flower senescence by the application of polyamines can be ascribed to its suppression of ethylene biosynthesis and action (Sardoei et al. 2013).

The isolated flowers supplied with putrescine, spermidine and spermine in their test solutions maintained higher floral diameter than the control. Highest floral diameter was registered in the flowers held in spermine on day 2 of transfer to the test solution, followed by putrescine and spermidine (Table 1). The increase in floral diameter in the samples treated with polyamines can be attributed to greater water uptake and thereby turgidity of flowers, because of the maintenance of higher carbohydrates in the petal tissues (Dar et al. 2014). Increase in content of carbohydrates in petals may cause a more negative water potential in the flowers, which will ultimately result in increased water uptake, and thereby increased floral diameter (van Doorn 2004). The floral buds treated with putrescine, spermidine and spermine showed considerably higher values for soluble proteins and α-amino acids (Table 1). The floral buds held in spermine showed highest soluble protein and α-amino acid content as compared to other treatments including control. Similar results of increase in protein content by the application of polyamines

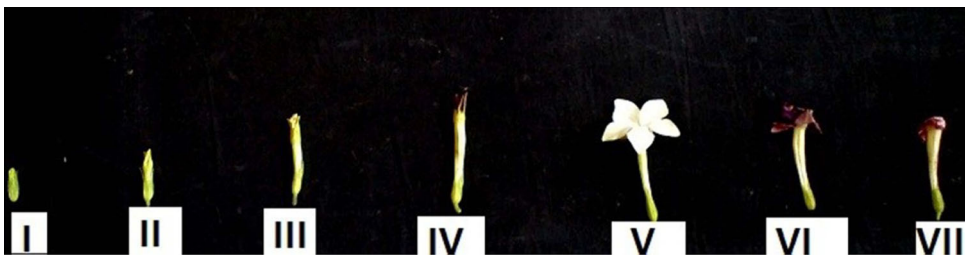

Fig. 2 Stages of flower development and senescence in *Nicotiana plumbaginifolia* L. The floral buds at stage IV (1 day before anthesis) were collected for experimentation

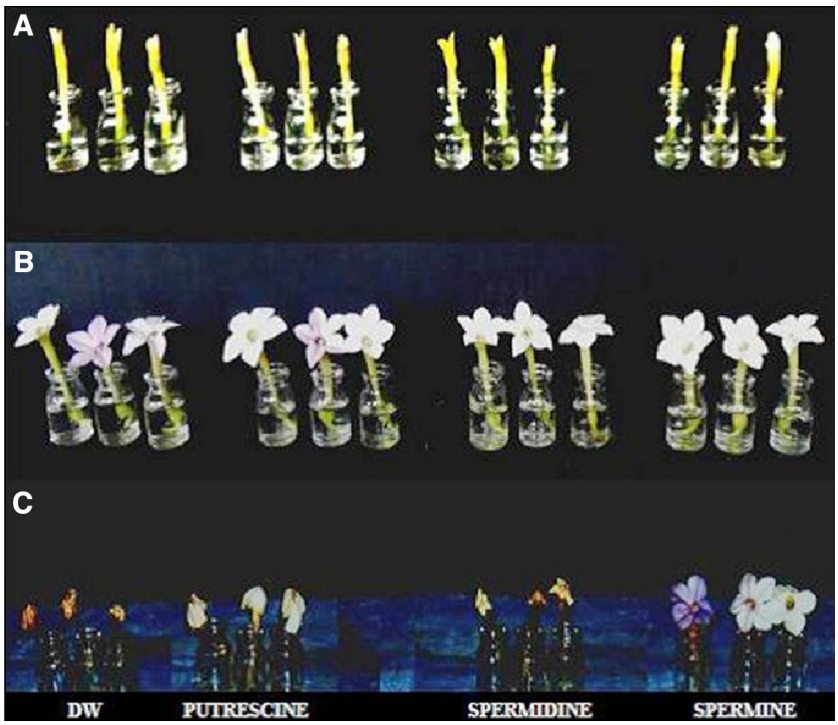

Fig. 3 Effect of putrescine spermidine and spermine on flower longevity in isolated flowers of *Nicotiana plumbaginifolia* L. on 0 (**A**), 2 (**B**) and 6 day (**C**) of transfer to the respective test solutions

have been reported in flowers like *Dianthus* and *Gladiolus* (Tassoni et al. 2006; Nahed et al. 2009). Polyamines are thought to delay flower senescence either by minimizing ethylene production or by stabilizing proteins. Polyamines bind to the carboxylic (COOH) group of the amino acids (proteins) and prevent them from the action by free radicals and other proteolytic enzymes (Galston 1983). Polyamines have also been found to delay protein degradation in various flowers such as carnation (Galston and Sawhney 1990). Protein degradation is considered to be the earliest step in the senescence of various flowers like *Petunia*, *Rosa*, *Iris*, *Dianthus*, *Hemerocallis*, *Sandersonia* and *Gladiolus* (Tripathi et al. 2009). The application of polyamines to the floral buds of *N. plumbaginifolia* resulted in decrease in total phenolic content (Table 1). The floral buds held in spermine showed lowest phenolic content. The increased phenolic content associated with senescent flowers corroborates the earlier findings on various flowers like *Iris* and *Dianthus* (Dar et al. 2014; Ahmad and Tahir 2015).

A marginal increase in sugar fractions (total, reducing and non-reducing) was registered in the floral buds treated with different polyamines. Maximum values for sugar fractions were recorded in the floral buds held in putrescine, followed by spermine and spermidine (Table 1). Our results are in agreement with the earlier work on *Gladiolus* (Nahed et al. 2009). The increase in the carbohydrate content due to the application of polyamines can be attributed to the regulated consumption of the storage carbohydrates through respiration by delaying the activity of ethylene (Galston and Sawhney 1990). Studies on various flower systems like *Iris* and *Dianthus* have suggested that increased carbohydrate levels are associated with increased floral longevity (Ahmad et al. 2013; Dar et al.2014). Moreover, exogenous application of sugars have been found to delay the rise in ethylene production mainly by shifting the polyamine-ethylene balance towards polyamines in various ethylene sensitive flower systems, thereby improving their postharvest performance (van Doorn 2004).

Our study reveals that the polyamines increase flower longevity by maintaining higher protein and carbohydrate content in the petal tissues. Among the polyamines tested, spermine was found to be the most effective in delaying flower senescence and maintaining the postharvest quality of *N. plumbaginifolia*.

Table 1 Effect of various polyamines (putrescine, spermidine and spermine) on flower longevity, floral diameter, soluble proteins, total amino acids, total phenols, total sugars, reducing sugars and non-reducing sugars on isolated flowers of *Nicotiana plumbaginifolia* L.

Days after transfer	Control (distilled water)	Putrescine (10 mM)	Spermidine (10 mM)	Spermine (10 mM)	LSD ($p \leq 0.05$)
Flower longevity (days)					
–	**3**	**4.8**	**4.2**	**6.05**	0.61
Floral diameter (cm)					
D2	2.3	3.5	3.3	4.2	0.09
D4	1.8	3.2	3.1	3.8	0.07
Soluble proteins (mg g^{-1} fr. wt.)					
D3	3.36	3.52	4.16	5.60	0.24
α-amino acids (mg g^{-1} fr. wt.)					
D3	3.38	3.85	3.87	4.83	0.17
Total phenols (mg g^{-1} fr. wt.)					
D3	6.24	3.89	4.42	3.13	0.11
Total sugars (mg g^{-1} fr. wt.)					
D3	1.96	8.79	3.91	6.85	0.34
Reducing sugars (mg g^{-1} fr. wt.)					
D3	0.98	4.06	2.94	3.92	0.09
Non-reducing sugars (mg g^{-1} fr. wt.)					
D3	0.98	4.73	0.97	2.93	0.07

Each value is a mean of ten independent replicates

Acknowledgments Syed Sabhi Ahmad thanks University Grants Commission (UGC), Govt. of India for providing JRF under (UGC-BSR) JRF scheme.

References

Ahmad, S. S., & Tahir, I. (2015). Storage protocol for improving the postharvest performance in cut scapes of *Iris versicolor*. *Acta Horticulturae, 1060*, 71–79.

Ahmad, S. S., Tahir, I., & Shahri, W. (2013). Effect of different storage treatments on physiology and postharvest performance in cut scapes of three *Iris* species. *JAST, 15*, 323–331.

Dar, R. A., Tahir, I., & Ahmad, S. S. (2014). Sugars and sugar alcohols have their say in the regulation of flower senescence in *Dianthus chinensis* L. *Scientia Horticulturae, 174*, 24–28.

Galston, A. W. (1983). Polyamines as modulators of plant development. *Bio Science, 33*, 382–388.

Galston, A. W., & Sawhney, K. R. (1990). Polyamines in plant physiology. *Plant Physiology*, *94*, 406–410.

Lee, M. M., Lee, S. H., & Park, K. Y. (1997). Effects of spermine on ethylene biosynthesis in cut carnation (*Dianthus caryophyllus* L.) flowers during senescence. *Journal of Plant Physiology, 151*, 68–73.

Lowry, O. H., Rosenbrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with folin phenol reagent. *Journal of Biological Chemistry, 193*, 265–275.

Nahed, G. A. A., Lobna, S. T., & Ibrahim, S. M. M. (2009). Some studies on the effect of putrescine, ascorbic acid and thiamine on growth, flowering and some chemical constituents of gladiolus plants at Nubaria. *Ozean Journal of Applied Sciences, 2*(2), 169–179.

Nelson, N. (1944). Photometric adaptation of Somogyi method for determination of glucose. *Journal of Biological Chemistry, 153*, 375.

Rosen, H. (1957). A modified ninhydrin colorimetric method for amino acids. *Archives of Biochemistry and Biophysics, 67*, 10–15.

Sardoei, A. S., Mohammadi, G. A., & Rahbarian, P. (2013). Interaction effect of salicylic acid and putrescine on vase life of cut *Narcissus* flowers. *International Journal of Advanced Biological and Biomedical Research, 1*(12), 1569–1576.

Satoh, S., Nukui, H., & Inokuma, T. (2005). A method for determining the vase life of cut spray carnation flowers. *Journal of Applied Horticulture, 7*, 8–10.

Sawhney, R. K., Tiburcio, A. F., Altabella, T., & Galston, A. W. (2003). Polyamines in plants: An overview. *Journal of Cell and Molecular Biology, 2*, 1–12.

Swain, T., & Hillis, W. E. (1959). The phenolic constituents of *Prunus domestica* L. The quantitative analysis of phenolic constituents. *Journal of the Science of Food and Agriculture, 10*, 63–68.

Tassoni, A., Accettulli, P., & Bagni, N. (2006). Exogenous spermidine delays senescence of *Dianthus caryophyllus* flowers. *Plant Biosystematics, 140*(1), 107–114.

Tripathi, S. K., Singh, A. P., Sane, A. P., & Nath, P. (2009). Transcriptional activation of a 37 kDa ethylene responsive cysteine protease gene, RbCP1, is associated with protein degradation during petal abscission in rose. *Journal of Experimental Botany, 60*(7), 2035–2044.

van Doorn, W. G. (2004). Is petal senescence due to sugar starvation? *Plant Physiology, 134*, 35–42.

van Doorn, W. G., & Woltering, E. J. (2008). Physiology and molecular biology of petal senescence. *Journal of Experimental Botany, 59*, 453–480.