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Effect of Nitric Oxide on Postharvest Quality and Vase Life of Cut Carnation Flower

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Abstract

Nitric oxide (NO) is a highly reactive signaling molecule and plays a variety of physiological roles in plants. The research on the application of NO to postharvest preservation of flowers and fruits shows great promise in recent years. However, the physiological mechanism of exogenous NO to affect cut flowers is not very clear, and NO donor treatment protected plants from damage by increasing the activity of antioxidative enzymes. Therefore, an experiment was conducted to study the effect of exogenous NO on the vase life and physiological basis of *Dianthus caryophyllus* L. 'Tempo'. The presence of the nitric oxide increased the activities of POD, while the production of MDA content and LOX activity were obviously decreased. The results showed that exogenous NO could significantly extend the vase life of cut carnation flowers (16.9 days). The results suggest that exogenous NO could delay petal wilting in carination cut flowers, maintain water metabolism, the antioxidative enzymes activity and mass-eliminate reactive oxygen species (ROS) and as well as cell membrane stability.

Keywords: Antioxidant enzymes, LOX, Senescence, *Dianthus caryophyllus*.

INTRODUCTION

As one of the fourth important cut flowers in the world, carnation (*Dianthus caryophyllus* L.) not only plays important role in the florist trade, but also performs well in the garden as a bedding plant (Ali *et al.,* 2008). Postharvest senescence is a major limitation to the marketing of many species of cut flowers and considerable effort has been devoted to developing postharvest treatments to extend the marketing period.

Silver ion, applied as silver thiosulphate (STS), is in widespread use to delay senescence in ethylene-sensitive cut flowers. Silver reduces ethylene- binding capacity and suppresses endogenous ethylene production (Van Doorn and Wothering, 1991) thereby delaying the appearance of characteristics such as premature wilting, petal inrolling and abscission of flowers and buds (Nichols, 1966; Wu *et al.,* 1991). However, concerns have been raised over the use of silver as it is a heavy metal salt and environmental toxin and many countries are actively working towards its elimination from commercial use (Nell, 1992; Serek *et al.,* 1995 a,b). 1-Methylcyclopropene (1-MCP) has been found to inhibit the action of ethylene and thereby extend the storage life of a range of cut flowers and potted flowering plants (Serek *et al.*, 1995 a,b; Porat *et al.,* 1995). Since 1-MCP is considered non-toxic to humans, studies have extended on fruit and vegetables where it has also been found to extend the postharvest life (Abdi *et al.,* 1998; Ku *et al.*, 2000; Wills *et al.,* 2002). 1-MCP has been approved for use with flowers in various countries and is seen as an environmentally acceptable alternative to STS.

Interesting in nitric oxide (NO) to extend the postharvest life of horticultural commodities is new. NO was first characterised in plants in 1996 (Leshem and Haramaty, 1996) and subsequent investigations have linked its occurrence to a range of physiological processes including modulation of endogenous ethylene and vegetative stress (Leshem and Pinchasov, 2000), water loss (Ku *et al.*, 2000), plant immunity (Hausladen and Stamler, 1998), anthocyanin biosynthesis and chlorophyll production (Giba *et al.*, 1998; Laxalt *et al.,* 1997), root growth and fruit and flower formation (Lamattina *et al.*, 2001). Postharvest application of NO has been shown to be effective in extending the postharvest life of a range of flowers, fruits and vegetables when applied as a short term fumigation treatment at low concentrations (Wills and Leshem, 1998; Leshem and Wills, 1998; Wills *et al*., 2000).

The objective of current study was to investigate the effect of different NO concentrations on the vase life, water uptake, peroxidase (POD), lipoxygenase (LOX), malondialdehyde (MDA) and chlorophyll in cut carnation 'Tempo' and finally determining the optimal concentration.

MATERIALS AND METHODS

cut carnation (*Dianthus caryophyllus* L. 'Tempo') were obtained from a commercial grower and transferred immediately to the laboratory and the experiments were established on the same day. The flower stems were recut under tap water to uniform length of 30 cm and placed in holding solutions, that containing of sodium nitroprusside (SNP, Na_2 [Fe $(CN)_5$ NO]· $2H_2O$) (Sigma-Aldrich), as NO donor (0, 25, 50, 75 and 100 µM) plus 7 % sucrose at a temperature of 20±1°C, under a 16:8 h light/dark cycle (irradiance 25 W m^{-2}) and 60 ± 5% RH for 24 hours. Control flowers were dipped in distilled water plus 7 % sucrose.

Vase life was determined at the wilting of more than one-third of the petals of flower. Water uptake was measured by periodically weighting the vase. For chlorophyll measurement, six circular disks, each 6.25 mm in diameter, were punched from the same general area of the leaf for which optical properties were measured. The disks were placed immediately into 8 mL of 100 % methanol, and pigments were allowed to extract in the dark at 30 ˚C for 24 h. Absorbance of the extracts was measured using spectrophotometer

at 652 and 665 nm.

Lipid peroxidation (MDA content) was determined by the method of Heath and Packer (1968). LOX activity was estimated according to the method of Bonnet and Crouzet (1977). The extraction buffer for POD contained 50 mM phosphate, 1 mM EDTA, 1 mM, 1 % (w/v) PVPP, at pH 7 and the assay mixture 50 mM phosphate, 45 mM guaiacol and 225 mM H_2O_2 (Chance and Maehly, 1955).

Statistical analysis

The experiment was carried out in completely randomized design with three replications. Three stems were used for each replication. Data were statistically analyzed using SAS software (version 9.2, SAS Institute Inc., Cary, NC, USA). Mean comparisons to identify significant differences between treatments were performed using Least Significant Difference (LSD) at the 0.01 level of probability.

RESULTS

Vase life and water uptake

The results indicated that 24 h pulse treatment with NO + sucrose at all concentrations significantly ($p \leq 0.01$). extended the vase life of carnation cut flowers compared to the control. except 25 µM The use of 100 µM NO resulted in a greater extension in vase life (16.9 days) than other treatments. Vase life of cut flowers held in distilled water was 13.6 days (Fig. 1).

The results show that adding NO to the vase solution improved the water uptake. The use of 100 µM NO resulted in a greater extension in water uptake (Fig. 2)

Chlorophyll content

The chemical analysis for the chlorophyll content of the leaves showed that NO treatments significantly inhibited the chlorophyll degradation in comparison with control. The use of 100 µM NO was more effective than other concentrations in this respect but there were no significant difference between 100 and 75 µM NO and there were no significant difference between 50, 25 µM NO with control (Fig. 3).

Lipid peroxidation (MDA) and LOX activity

During vase life, MDA concentration significantly increased in carnation petals in the NO treated and untreated flowers. A all concentrations of NO treated flowers had lower MDA content than the control over 13 days (Fig. 4).

LOX activity significantly increased in the control flowers during senescence. Over 13 days vase life, the treatment with NO caused reduction in LOX activity in comparison to control (Fig 5.).

Antioxidant enzymes activity

POD activity increased significantly in all concentrations of NO. By increasing NO concentration, POD activity increased, however, no difference among 0 and 25 µM NO. The present findings revealed that enzymatic antioxidant activities in carnation petals was substantially induced by NO application (Fig. 6).

DISCUSSION

NO has revealed as an exceptional molecule due to the versatility of its actions in the physiology and biochemistry of living organisms (Lamattina *et al.,* 2003). In recent years this molecule has caught considerable attention due to the evidence that NO plays an important role as signal molecule in plant growth and development (Shapiro, 2005;

Corpas *et al.,* 2006). For plant postharvest physiology, Leshem and Wills (1998) has found that exogenous NO could considerably prolong the shelf life of some leaf vegetables, flowers and fruits by inhibiting the emission of ethylene, implying that NO might take important roles in regulating aging process. However, whether NO could participate in the regulation of aging process in cut flowers and the possible physiological responses have not been examined so far. In organisms, the dual roles of NO were in dose-dependent manner (Beligni and Lamattina, 1999). In the present study, similar results was observed and 100 μM SNP could significantly extend the flower vase life of carnation cut flowers (Fig. 1).

The senescence of flower petals is associated with a series of highly regulated physiological and biochemical processes (Mayak and Halevy, 1980). Therefore, vase life mainly depends on development of adverse water relations, which results in a lack of flower opening, premature petal wilting and bending of the pedicel (Yamada *et al.*, 2007) suggesting that a good water uptake is one of the most important factor for a long vase life of cut flowers (Slootwet, 1995).

The visible symptom of leaf senescence is the loss of green color. This phenomenon is caused by chlorophyll degradation that is catalyzed by the chlorophyllase that converts the chlorophyll a and b to chlorophyllide and phytol (Matile *et al.*, 1997). Ethylene accelerates chlorophyll degradation of leaves in many cut flowers (Ferrante *et al.,* 2006). In the present study, NO treatment in all concentrations retarded chlorophyll degradation in comparison with the control (Fig. 3).

Increase in lipid peroxidation, usually determined from changes in MDA concentration, accompanies the increase in LOX activity while the products of peroxidation are considered to membrane degradation (Leverentz *et al.,* 2002). Our result showed that lipid peroxidation increased sharply from harvesting to senescence stage, while the treatment with SNP reduced the concentration of MDA and LOX activity.

Various studies have demonstrated that vase life of cut flowers is modulated by antioxidant enzymes (Baker *et al*., 1977). Our results showed that the activity of POD in the treatment with SNP was significant higher than those of the control (Fig. 6), suggesting that exogenous NO plays important roles in enhancing the ability of H_2O_2 detoxification in carnation cut flowers.

CONCLUSION

According to these results it is possible to conclude that, application of NO extend the vase life of cut carnation (*Dianthus caryophyllus* L. 'Tempo') flowers by acts as a ROS scavenger, thereby maintaining membrane integrity for extended period. However, the treatment of NO stimulated these antioxidant enzymes, and exhibited lipid peroxidation and LOX activity, increased the ROS scavenging activity of carination cut flowers. Therefore, the flower vase life of carnation cut flowers was markedly extended by NO.

Literature Cited

Abdi, N., McGlasson, W.B., Holford, P. and William, M. 1998. Responses of climacteric and suppressed-climacteric plums to treatment with propylene and 1-methylcyclopropene. Postharvest Biol. Technol. 14: 29_39.

Ali, A., Afrasiab, H., Naz, S., Rauf, M. and Iqbal, J. 2008. An efficient protocol for *In vitro* propagation of carnation (*Dianthus caryophyllus*). Pak. J. Bot. 1: 111–121.

Beligni, M.V. and Lamattina, L. 1999. Nitric oxide counteracts cyto-toxic processes mediated by reactive oxygen species in plant tissues. Planta. 208: 337–344.

Bonnet, J.L. and Crouzet, J. 1977. Lipoxygenase from tomato fruit partial purification and study of some properties. J. Food Sci. 42: 1999-2003.

Chance, B. and Maehly, A.C. 1995. Assay of catalases and peroxidases. Meth. Enzymol. 2: 764-817.
Corpas, F.J., Carreras, J.B.B.A., Barroso, J.B., Carreras, A., Valderrama, R., Palma, J.M., Leon, A.M., Sandalio, L.M. and del Rıo, L.A. 2006. Constitutive arginine-dependent nitric oxide synthase activity in different organs of pea seedlings during plant development. Planta. 224: 246–254.
Ferrante, A., A. Mensuali-Sodi, Serra, G. and Tognoni, F. 2006. Evaluation of postproduction performance of Salvia splendens potted plants for interiors use. Acta Hort. 723: 415-419.
Giba, Z., Grubisic, D., Todorovic, S., Sajc, L., Stojakovic, D. and Konjevic, R. 1998. Effect of nitric oxide-releasing compounds on phytochrome-controlled germination of Empress tree seeds. Plant Growth Regul. 26: 175-181.
Hausladen, A. and Stamler, J.S. 1998. Nitric oxide in plant immunity. Proc. Natl. Acad. Sci. USA. 95: 10345-10347.
Heath, R.L. and Packer, L. 1968. Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. Arch. Biochem. Biophys. 125: 189–198.
Ku, V.V.V., Wills, R.B.H. and Leshem, Y.Y. 2000. Use of nitric oxide to reduce postharvest water loss from horticultural produce. J. Hortic. Sci. Biotech. 75: 268-270.
Lamattina, L., Beligni, G.L., Garcia Mata, C. and Laxalt, A.M. 2001. US Patent. 6: 242-384 B1.
Lamattina, L., Garcia-Mata, C., Graziano, M. and Pagnussat, G. 2003. Nitric oxide: the versatility of an extensive signal molecule. Annu. Rev. Plant Biol. 54: 109–136.
Laxalt, A.M., Beligni, M.V. and Lamattina, L. 1997. Nitric oxide preserves the level of chlorophyll in potato leaves infected by *Phytophthora infestans*. Eur. J. Plant Pathol. 103: 643-/ 651.
Leshem, Y.Y. and Haramaty, E. 1996. The characterisation and contrasting effects of the nitric oxide free radical in vegetable stress and senescence of *Pisum sativum* Linn. Foliage. J. Plant Physiol. 148: 258-263.
Leshem, Y.Y., Wills, R.B.H., 1998. Harnessing senescence delaying gases nitric oxide and nitrous oxide: a novel approach to postharvest control of fresh horticultural produce. Biol. Plant. 41: 1–10.
Leshem, Y.Y. and Pinchasov, Y. 2000. Non-invasive photoacoustic spectroscopic determination of relative endogenous nitric oxide and ethylene content stoichiometry during the ripeneing of strawberries *Fragaria anannasa* (Dutch.) and avocados *Persea americana* (Mill.). J. Exp. Bot. 51: 1471-1473.
Leverentz, M.,Wagstaff, C., Rogers, H., Stead, A., Chanasut, U., Silkowski, H., Thomas, B., Weichert, H., Feussner, I. and Griffiths, G. 2002. Characterisation of a novel lipoxygenase-independent senescence mechanism in *Alstroemeria peruviana* floral tissue. J. Plant Physiol. 130: 273–283.
Matile, P., Schellenberg, M. and Vicentini, F. 1997. Localization of chlorophyllase in the chloroplast envelope. Planta. 201:96-99.
Mayak, S. and Halevy, A.H. 1980. Flower senescence. In: Rhimann, K.V. (Ed.), Senescence in Plants. CRC Press, Florida.
Nell, T.A. 1992. Taking silver safely out of the longevity picture. Growertalks. 6: 35-38. Nichols, R. 1966. Ethylene production during senescence of flowers. J. Hortic. Sci. 41: 279-290.
Serek, M., Sisler, E.C., Tamarini, G. and Borochov, A. 1995a. Inhibition of ethylene-induced cellular senescence symptoms by 1-methycyclopropene. Acta Hortic. 405: 264-268.
Serek, M., Sisler, E.C. and Reid, M.S. 1995b. 1-Methylcyclopropene, a novel gaseous inhibitor of ethylene action, improves the life of fruits, cut flowers and potted plants. Acta Hortic. 394: 337-345.

Shapiro, A.D. 2005. Nitric oxide signaling in plants. Vitam. Horm. 72: 339–398.
Slootwet, G. 1995. Effect of water temperature on water uptake and vase life of different cut flowers. Acta Hort. 405: 67–74.
Porat, R., Shlomo, E., Serek, M., Sisler, E.C. and Borochov, A. 1995. 1-Methylcyclopropene inhibits ethylene action in cut phlox flowers. Postharvest Biol. Technol. 6: 313-319.
Van Doorn, W.G. and Wothering, E.J. 1991. Developments in use of growth regulators for the maintenance of postharvest quality in cut flowers and potted plants. Acta Hortic. 298: 195-208.
Wills, R.B.H. and Leshem, Y.Y. 1998. Method for reducing the rate of deterioration of perishable horticultural produce. Australian Patent No. 738169.
Wills, R.B.H., Ku, V.V.V. and Leshem, Y.Y. 2000. Fumigation with nitric oxide to extend the postharvest life of strawberries. Postharvest Biol. Technol. 18: 75-79.
Wu, M.J., Zacarias, L. and Reid, M.S. 1991. Variations in the senescence of carnation (*Dianthus caryophyllus* L.) cultivars II. Comparisons of sensitivity to exogenous ethylene and of ethylene binding. Sci. Hortic. 48: 109-116.
Yamada, K., Ito, M., Oyama, T., Nakada, M.,Maesaka, M. and Yamaki, S. 2007. Analysis of sucrose metabolism during petal growth of cut roses. Postharvest Biol. Technol. 43: 174–177.

Figures

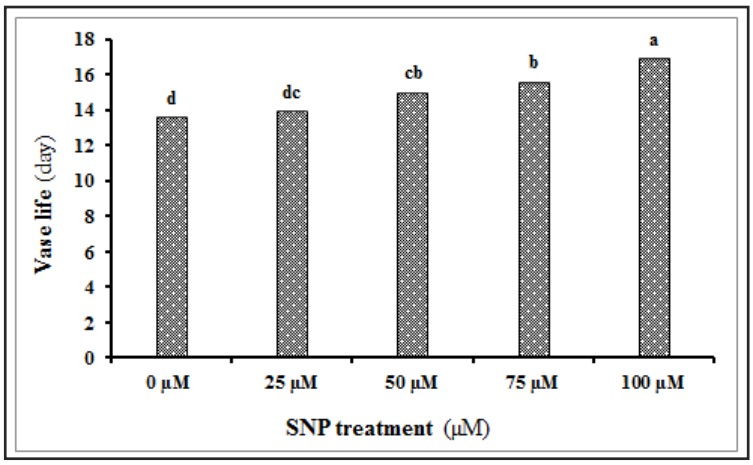

Fig. 1. Effects of exogenous SNP (as NO donor) on the vase life. Vertical bars with the same letters did not show significantly different at 1% probability level.

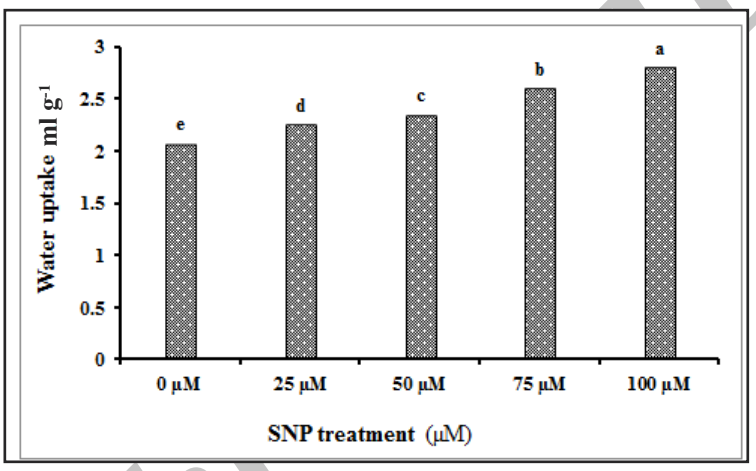

Fig. 2. Effects of exogenous SNP (as NO donor) on the water uptake. Vertical bars with the same letters did not show significantly different at 1% probability level.

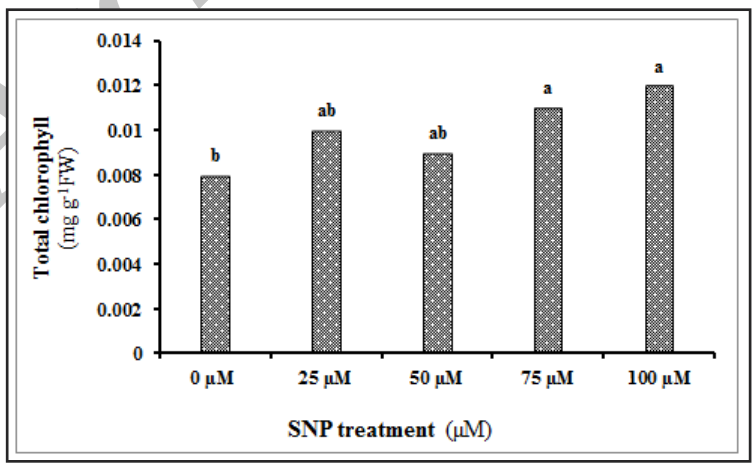

Fig. 3. Effects of exogenous SNP (as NO donor) on the changing of total chlorophyll in cut carnation flowers. Vertical bars with the same letters did not show significantly different at 1% probability level.

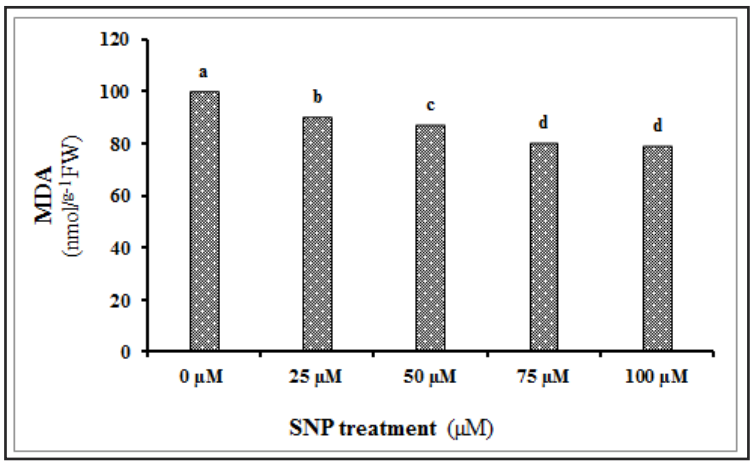

Fig. 4. Effects of exogenous SNP (as NO donor) on the content of MDA in cut carnation flower petals. Vertical bars with the same letters did not show significantly different at 1% probability level.

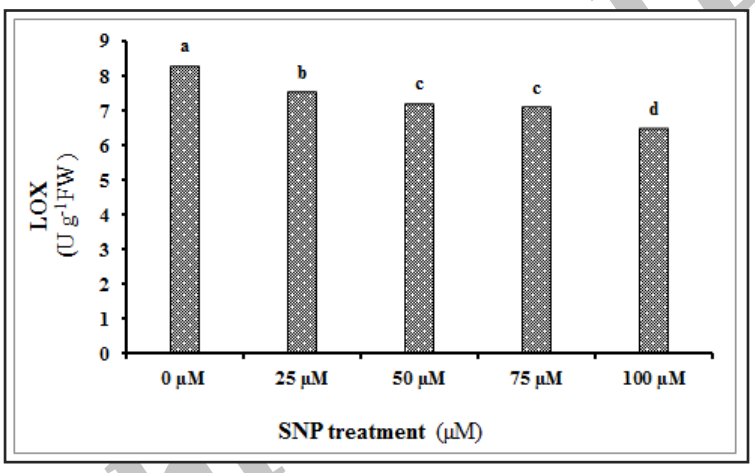

Fig. 5. Effects of exogenous SNP (as NO donor) on the LOX activity in cut carnation flower petals. Vertical bars with the same letters did not show significantly different at 1% probability level

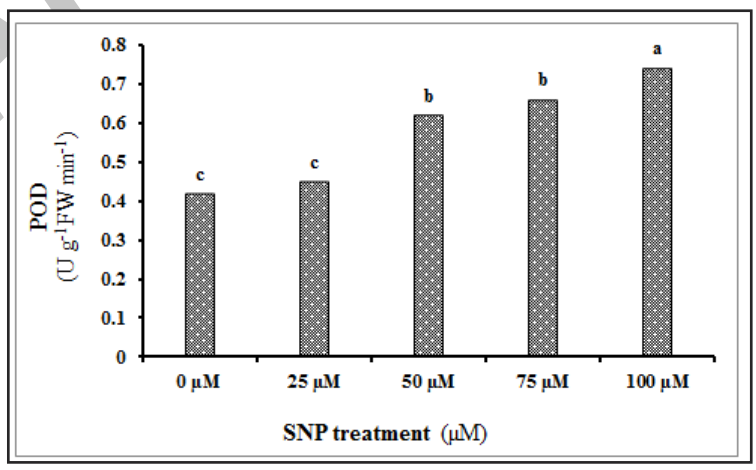

Fig. 6. Effects of exogenous SNP (as NO donor) on the POD activity in cut carnation flower petals. Vertical bars with the same letters did not show significantly different at 1% probability level.