

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

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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.

Abstract

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, Na₂ [Fe (CN)₅ NO] · 2H₂O) (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 H₂O₂ (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 μ M. The use of 100 μ M 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 μ M 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 μ M NO was more effective than other concentrations in this respect but there were no significant difference between 100 and 75 μ M NO and there were no significant difference between 50, 25 μ M 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 μ M 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 μ M 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 H₂O₂ 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.

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Figures

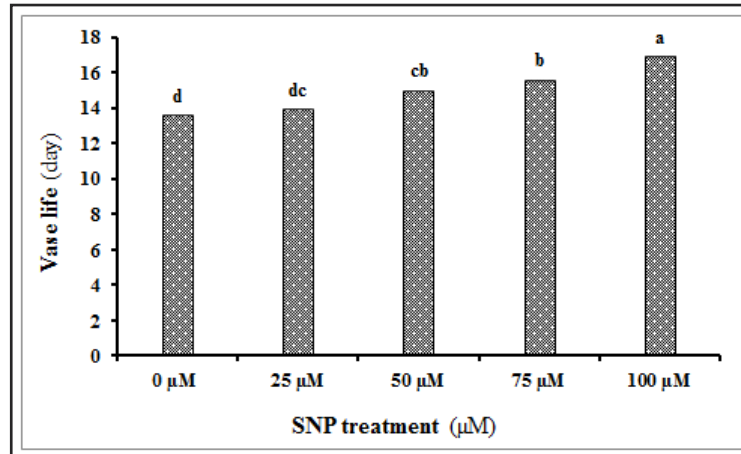


Fig. 1. Effects of exogenous SNP (as NO donor) on the vase life. Vertical bars with the same letters did not show significantly different at 1% probability level.

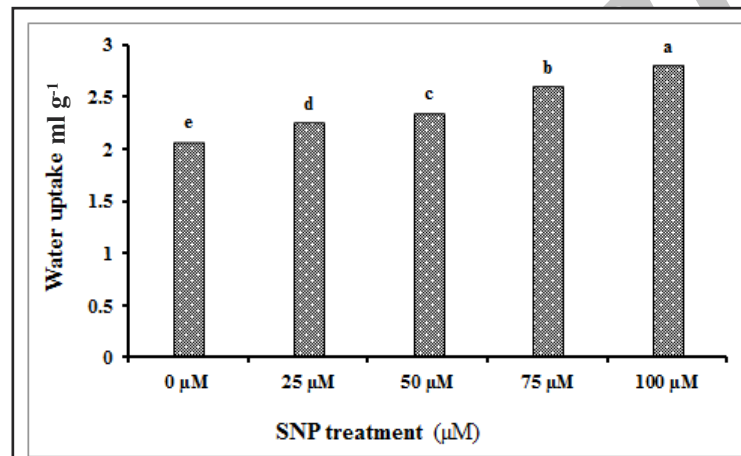


Fig. 2. Effects of exogenous SNP (as NO donor) on the water uptake. Vertical bars with the same letters did not show significantly different at 1% probability level.

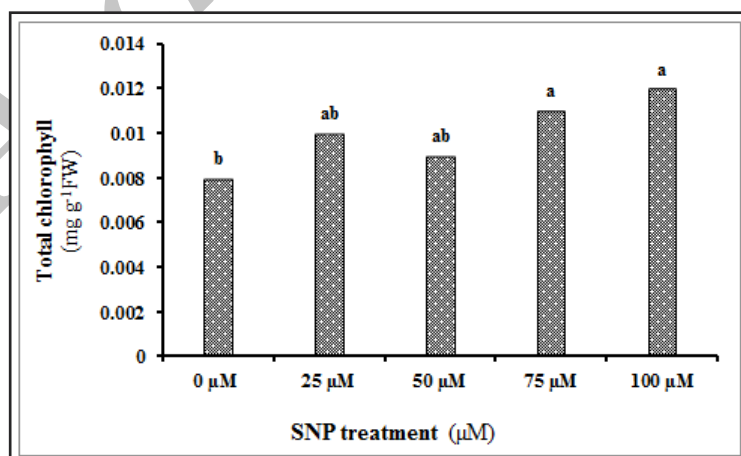


Fig. 3. Effects of exogenous SNP (as NO donor) on the changing of total chlorophyll in cut carnation flowers. Vertical bars with the same letters did not show significantly different at 1% probability level.

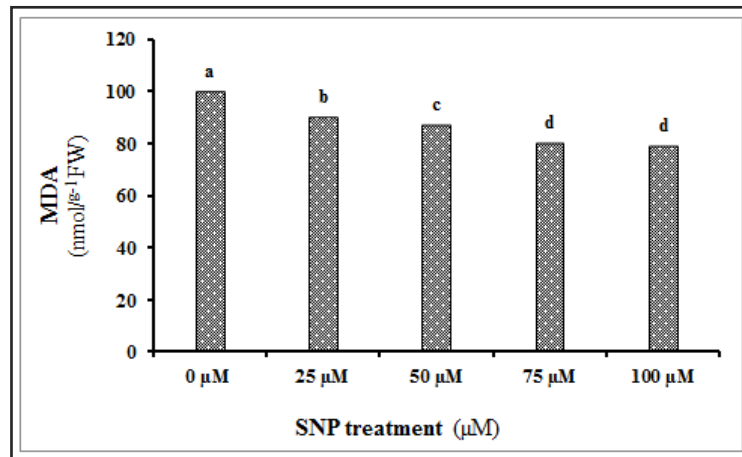


Fig. 4. Effects of exogenous SNP (as NO donor) on the content of MDA in cut carnation flower petals. Vertical bars with the same letters did not show significantly different at 1% probability level.

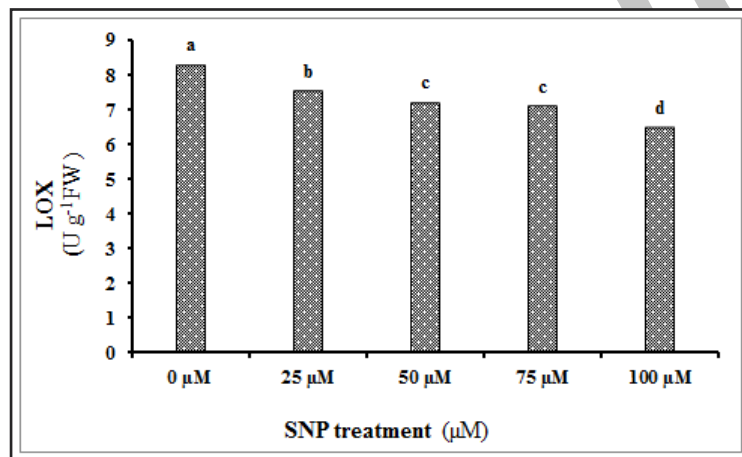


Fig. 5. Effects of exogenous SNP (as NO donor) on the LOX activity in cut carnation flower petals. Vertical bars with the same letters did not show significantly different at 1% probability level.

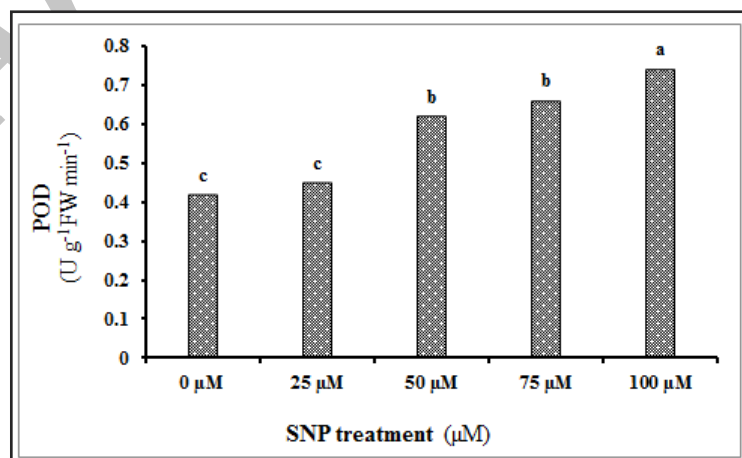


Fig. 6. Effects of exogenous SNP (as NO donor) on the POD activity in cut carnation flower petals. Vertical bars with the same letters did not show significantly different at 1% probability level.