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Role of Nitric Oxide Donor Compound to Extend the Vase Life of Lilium Cut Flower

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Abstract – **Nitric oxide is small diffusible and ubiquitous gaseous bioactive molecule and plays a variety of physiological and developmental 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. Therefore, an experiment was conducted to study the effect of exogenous NO on the vase life of LA –hybrid cv. Eyeliner. In the present study, Sodium nitroprussiate (SNP) was used as the NO donor and methylene blue (MB-1) was used as its scavenger. We evaluated the effects of sodium nitroprusside (SNP) on vase life and increase of fresh weight of cut lilium flowers. The results showed that exogenous NO could significantly extend the vase life of cut lilium flowers and markedly increase fresh mass. Moreover, the results indicated that MB-1 had the ability to reverse the active effects of NO on different physiological indexes. Therefore, the vase life of cut lilium flowers was markedly extended by SNP treatment.**

Keywords – **Nitric Oxide, MB-1, Fresh Weight, Lilium.**

I. INTRODUCTION

Flowers require sincere, patient, soft, affectionate as well as expert handling. All cut flowers are destined to die, and the challenge for postharvest researchers is to slow the processes controlling flower death to enable cut flowers to reach distant markets with a display life. All over the world, lily occupies a prominent place in horticulture as a cut flower, pot and garden plant. As a cut flower, lily is now ranked as the fourth most important crop in the Netherlands. However, postharvest senescence is a major limitation to the marketing of many species of cut flowers and considerable efforts have been devoted to developing postharvest treatments to extend the marketing period of them (Bowyer *et al.* 2003). It is well known that silver ion (applied as silver thiosulphate, STS) is widely used to delay senescence in ethylene-sensitive cut flowers because of its reducing ethylene-binding capacity and suppressing endogenous ethylene production (Van Doorn *et al.* 1991). However, as a heavy metal salt and environmental toxin, silver ion is prohibited by many countries in commercial use (Serek *et al.* 1995). Another ethylene inhibitor 1-methylcyclopropene (1-MCP), non-toxic to humans, has been demonstrated to extend the storage life of a range of cut flowers (Porat *et al.* 1995) and potted flowering plants, and it is seen as an environmentally acceptable alternative to STS. Although 1-MCP can prevent some of the effects of ethylene, it often does so only for a rather short period of time (Uthaichay *et al.* 2007). Therefore, a new, environment-friendly and more effective cut flowers preservative should be exploited.

Nitric oxide (NO) was first characterized in plants in 1996 (Leshem and Haramaty, 1996) and has since been linked to a range of physiological and developmental processes including signaling growth, development and adaptive responses to multiple stresses, delays senescence (Neill *et al.*, 2003) and modulation of endogenous ethylene. Postharvest application of NO by fumigation with NO gas has been shown effective in extending the postharvest life of a range of flowers, fruits and vegetables when applied as a short term fumigation treatment (Wills and Leshem, 1998). While NO and 1-MCP are alternative treatments to STS, the gaseous nature of both compounds increases commercial operational knowledge, requires infrastructure and is less suitable for developing countries. The importance of NO as a key regulator in mammalian physiology has led to substantial developments in NO donor technology, that is, solid compounds that store NO chemically but release it under appropriate physical conditions (Keefer, 1998). A major breakthrough in understanding the role of NO in plants relates to identification of multiple, enzymatic as well as nonenzymatic, NO generating systems, and widespread production, either constitutive or induced by biotic/abiotic stresses, of NO in plants (Crawford and Guo, 2005). Therefore, in last decade, the role of NO in plants and agricultural production received much attention. Leshem and Wills (1998) stated that NO was a natural senescence-delaying plant growth regulating agent acting primarily, but not solely, by down regulating ethylene emission. Badiyan *et al.* (2004) reported that the vase life of all eight flowers was extended by DETA/NO with an average extension of about 60% with the range being about 200% for gerbera to 10% for chrysanthemum compared with flowers kept in water, suggesting that DETA/NO appears to have widespread applicability to cut flowers and offers a simple technology to extend the vase life. This study explored this potential by examining the effects of exogenous NO on the vase life and increase of fresh weight of lilium cut flowers.

II. MATERIALS AND METHODS

The cut flowers of Lilium cv. Eyeliner utilized for the present study were grown at Sarsai farm of Indian Agricultural Research Institute, Regional Station, Katrain, Kullu (H.P.) and brought to the laboratory within half an hour of harvest. Flowers were immediately kept at room temperature and the stems immersed in simple tap water until treated. Cut stems of similar maturity and visual quality were selected and cut under water to a stem length of 50 cm. Leaves on the bottom 20 cm of stems that would otherwise be submerged in vase water were removed by hand. The stems were immersed into distilled water as low as 20 cm, and placed the cut flowers in test vases. The chemical reagent of SNP was used as donor of NO, and MB-1 as its scavenger. Both the reagents were dissolved in distilled water.

Optimal SNP concentration

Measuring cylinders of 500 ml capacity vases were surface sterilized with ethanol. Vases were filled with 250 ml of distilled water (control), 50, 100, 150, 200 and 500 ppm SNP solution. Three replicate stems of the lilium cut flowers were then placed individually into vases containing the different SNP concentrations. The pH of all the solutions was maintained at 3.5 since pH differences were shown to effect flower senescence (Halevy and Mayak, 1981). Stems were kept at an average room temperature 25.7^{0}C and 43% relative humidity, and a light intensity of 191.09 Lux. In the experiment, three replicates were designed (each replicates including three flowers), and only vase life was determined. The vase life of cut flowers was determined by counting the number of days to 50% petal colour fading.

Optimal MB-1 concentration counteracting SNP positive effect

Three stems of lilium cut flowers under study were distributed evenly to three sterile 500 ml measuring cylinders. Vases contained 250 ml of distilled water (control), 100 ppm SNP, 0.25ppm MB-1, 0.25ppm MB-1 + 100ppmSNP, 0.5ppm MB-1, 100ppmSNP +0.5ppm MB-1, 0.75ppm MB-1, 0.75ppm MB-1+ 100ppm SNP, 1.0 ppm MB-1, and 1.0 ppm MB-1+100ppmSNP, and the pH of all solutions were adjust to 3.5. Stems were held in vases under the same temperature, relative humidity and lighting regime described above. Three replicates were also designed (each replicate including three flowers), similarly, only vase life was determined in this experiment.

The physiological responses of cut flowers to SNP

The experiment was designed based on the results of above experiments. Five cut flowers were immersed into three 500 ml measuring cylinders. Cylinders contained 500 ml of different solutions, including (1) distilled water (control); (2) 100ppm SNP solution; (3) 100ppm SNP + 0.5 ppm MB-1 solution. The degree of flower wilting was determined according to the morphological stages as reported by Paulin *et al.* (1986). Longevity is determined by the duration from the starting time point to until flower wilting occurred (Yamamoto *et al.* 1992). Flower stems were briefly removed from vase solutions and weighed on day 0, and then every second day thereafter of vase life to determine the increase of fresh weight.

Experimental Design and statistical analysis

All experiments were arranged in a CRD in the laboratory. There were three repeats per treatment in the three experiments. For the experiments of optimal SNP concentration and MB-1 concentration counteracting SNP, only vase life was measured based on three repeated treatments (three flowers per repeat). However, data on the vase life and increase in fresh weight in the experiment 3 were five flowers per repeat. Data are presented as means ± standard errors, Statistical procedures were performed using the *COSTAT* software package. Where significant ($P \leq 0.05$) treatment effects were determined by ANOVA, data means were separated by the Duncan's multiple range test at $P = 0.05$.

III. RESULTS AND DISCUSSION

The optimal concentration of SNP and MB-1

Vase life of cut flower is an important integrative character resulted in internal and external cause. Under the same external conditions, the changes of internal physiological factors are likely to influence the vase life of cut flowers. Therefore, vase life was used to study the optimal concentration of SNP and MB-1 in the experiment. The effect of different concentrations of SNP on vase life of Lilium cut flowers were shown in Table 1. The results indicated that increasing the concentration of SNP in vase water from 50 ppm to 100 ppm extended flower display life as compared to control stems, while treatment with increasing concentration of SNP did not increase the vase life. The vase life of the inclusion of 100 ppm SNP was maximal. Therefore, the concentration of 100 ppm SNP was considered as the optimal one. Pulse treatments with solutions containing SNP slightly delayed the flower senescence of cut lilium flowers and slightly increased the vase life (Maryam and Syyed, 2013). Many studies suggested that NO was highly effective on flowers that having low sensitivity to ethylene (Woltering and Van Doorn, 1988). In another investigation DETA/NO has increased the vase life of eight flower species such as gerbera, iris, tulip, snapdragon, delphinium, oriental lily, rose and chrysanthemum (Badiyan *et al.* 2004). Also, DETA/NO (10, 100 and 1000 ppm) increased postharvest life of three native Australian cut flowers such as waratah, Kangraoo paw and ptilotus (Bowyer *et al.* 2003).

Based on the results of the optimal concentration of SNP, the experiment of optimal MB-1 concentration counteracting SNP positive effects was designed (Table 2). Inclusion of 0.5 and 0.75 ppm MB-1 did not extend the vase life of cut flowers whereas the addition of 1.0 ppm MB-1 decreased the flower display life. Similarly, placement of cut flowers into vase water containing 100 ppm SNP significantly extended the vase life by 3.33 days (36%) over control stems (Table 2). However, as a NO inhibitor or scavenger, different concentration of MB-1 had significant reverse effect on SNP. For example, exposure to the concentration of 0.5, 0.75 and 1.0 ppm MB-1 with 100 ppm SNP significantly decreased the vase life of cut flowers as compared to the treatment of 100 ppm SNP, due to the inhibition of positive effect of SNP. Actually, MB-1 is known to block cGMP (Iwamoto *et al.* 1992, Paciullo *et al.* 2010), thus inhibiting the action of NO resulting from the increased NO synthase (NOS) activity (Marczin *et al.* 1992). It has also the ability to inhibit NO production/action as well as synthesis (Mayer *et al.* 1993).

Vase life

The vase life of cut lilium flowers was significantly increased by SNP treatment. As indicated in Fig. 1, there was an increase of 2.0 days for the inclusion of 100 ppm SNP compared to control. However, when 0.5 ppm MB-1, a specific scavenger of NO, was applied, the vase life was similar to that of control, so SNP, as the donor of exogenous nitric oxide, while MB-1 had the ability to reverse the active effect of NO on vase life. In carnation, 5.0 µmolL^{-1} MB-1 inhibited the active effect of 0.1 mmolL^{-1} SNP (Chang-Li *et al.* 2011).

Increase of fresh weight

The senescence of flower petals is associated with a series of highly regulated physiological and biochemical processes. These include breakage of water balance, an increase in hydrolytic enzyme activity, degradation of macro-molecules, increased respiratory activity and loss of membrane integrity and cellular compartmentation. Each postharvest handling step has a potential to either maintain or reduce the quality of fresh cut flowers. 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). In the present study, increase of fresh weight in all treatments showed the trend toward increasing in the initial days and then decreasing (Fig.2). There was no difference in increase of fresh weight among the three treatments in the first 3 days, and then a distinct difference among them was observed. Rate of stem weight treated by SNP reached the maximum on the 4th day, and it was decreased from day 8 to day 9 (Fig.2). However, MB-1 held back the active effect of NO, resulting in the significant decrease in increase of fresh weight during the whole vase stage, suggesting that a good water uptake is one of the most important factor for a long vase life of cut flowers. SNP applications as NO donor compound resulted in keeping the relative fresh weight of flower stems by diminishing the rate of transpiration and slow down the water loss due to stoma closure (Steven *et al.* 2003) and also by good water uptake is one of the most important factor for a long vase life of cut flowers (Slootwet, 1995).

Table 1: Effect of different concentrations of SNP on vase life of Lilium cut flowers. Data are mean ± standard errors. Values with different letters in the same list are statistically significant by Duncan's multiple range test at 5% level.

Treatments	**Vase life (days)**
Control (DW)	10.33± 0.33^{bc}
SNP 50 ppm	10.33±0.88^{bc}
SNP 100 ppm	13.00±0.58^{a}
SNP 150 ppm	11.66±0.88^{ab}
SNP 200 ppm	8.66±0.88^{c}
SNP 500 ppm	5.33±0.33^{d}

Table 2: Effects of different concentrations of MB-1 with SNP on vase life of Lilium cut flowers. Data are mean± standard errors. Values with different letters in the same list are statistically significant by Duncan's multiple range test at 5% level.

Treatments	**Vase life (days)**
Control (DW)	9.33±1.20^{d}
SNP 100ppm	12.66±0.88^{a}
MB-1 0.25ppm	11.33±0.66^{abcd}
SNP 100ppm+ MB-1 0.25 ppm	12.33±0.33^{ab}
MB-1 0.5 ppm	12.00±0.57^{abc}
SNP 100 ppm + MB-1 0.5 ppm	10.00±0.57^{cd}
MB-1 0.75ppm	11.33±0.33^{abcd}
SNP 100ppm + MB-1 0.75ppm	10.33±0.33^{bcd}
MB-1 1.0 ppm	10.33±0.33^{bcd}
SNP 100ppm + MB-1 1.0ppm	9.33±0.33^{d}

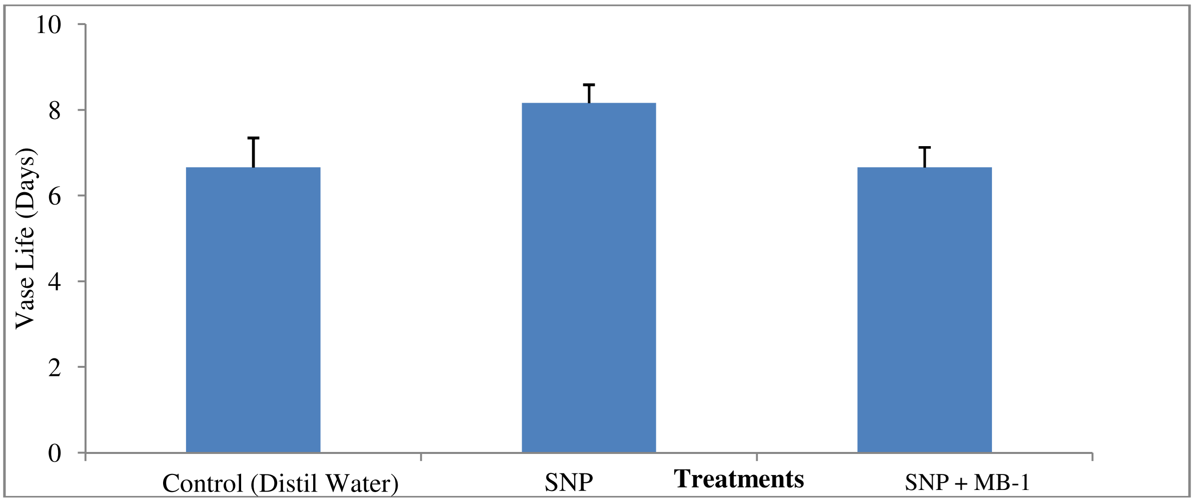

Fig.1. Effects of exogenous nitric oxide on the vase life. Vertical bar show standard errors of means. Values significantly different at P<0.05 according to Duncan's multiple range test.

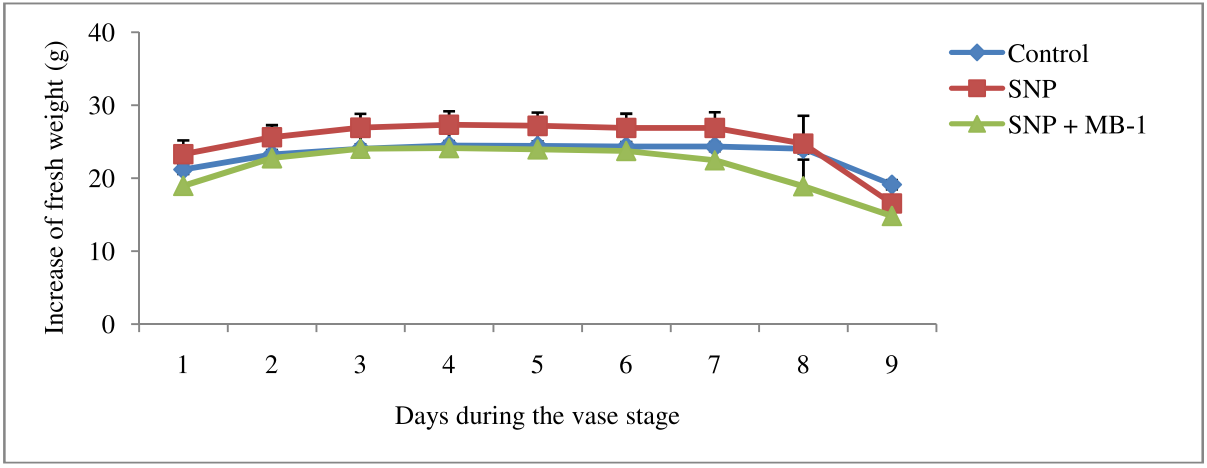

Fig 2. Effects of exogenous nitric oxide on the changing of fresh weight in cut flowers. Vases contained distilled water, 100ppm SNP, 100ppm SNP with 0.5 ppm MB-1 solution. Vertical bars show standard errors of means.

IV. CONCLUSION

The foregoing results and discussion indicated that as a solid that dissolves in water and with effectiveness from a single initial application at a relatively low concentration, also because of its low price, SNP could be introduced to apply in cut flower trading to extend vase life of flowers. Further work is required to assess the mechanism of SNP effects on postharvest life of other important lily cultivar groups.

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