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The physiological responses of carnation cut flowers to exogenous nitric oxide

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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. Therefore, an experiment was conducted to study the effect of exogenous NO on the vase life and physiological basis of *Dianthus caryophyllus* L. variety 'Monte'. In the present study, 0.1 mmol L^{-1} sodium nitroprussiate (SNP) was used as the NO donor, and 5 μmol L^{-1} methylene blue (MB-1) was used as its scavenger based on the preliminary experiment. We evaluated the physiological index including increase of stem weight, malondialdehyde (MDA) concentration and lipoxygenase (LOX) activity, and cell protection enzymes activities of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and ascorbate peroxidase (APX). The results showed that exogenous NO could significantly extend the vase life of cut carnation flowers and markedly increase fresh mass. The balance of water metabolism and the activities of SOD, POD, CAT and APX also showed improvement, while the production of MDA content and LOX activity were obviously decreased. 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. 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 carnation flowers was markedly extended by SNP treatment.

1. Introduction

As one of the fourth important cut flowers in the word, 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). However, 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 of them (Bowyer et al., 2003). It is well known that silver ion (applied as silver thiosulfate, STS) is widely used to delay senescence in ethylene-sensitive cut flowers because of it 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 it is seen as an environmentally acceptable alternative to STS. Although 1-MCP can prevent some of the effects of ethylene (Sisler and Serk, 1997), it often does so only for a rather short period of time (Uthaichay et al., 2007). For example, 1-MCP treatment did not seem a viable option for delaying the petal abscission in *Pelargonium peltatum* flowers (Cameron and Reid, 2001). Therefore, a new, environment-friendly and more effective cut flowers preservative should be exploited.

NO is a small diffusible and ubiquitous gaseous bioactive molecule, which participates in the broad spectrum of pathphysiological and developmental processes of living organisms (Lamattina et al., 2003). The cytotoxic or the cytoprotective roles of NO are thought to be due to its reactivity with ROS (Neill et al., 2003; Wendehanne et al., 2004). 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 the last decade, the role of NO in plants and agricultural production received much attention (Wendehenne et al., 2006). In fact, several investigations have demonstrated that fumigation with NO gas can delay senescence in selected fruits, vegetables and cut flowers. 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

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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 vase life. However, little has been reported on why exogenous nitric oxide can extend the vase life of cut flowers, and what on earth are physiological responses of cut flowers to exogenous NO, especially in variation of antioxidant enzymes system and lipid peroxidation. Therefore, the aims of the present study were: (1) to investigate the effects of exogenous NO on the vase life and increase of fresh weight, (2) to analyze the changes of cell protection enzyme systems and membrane permeability in carnation petals, and (3) to illuminate the relationship between the extension of vase life and physiological and biochemical indexes when exposure to NO solution.

2. Materials and methods

2.1. Plant material

Cut flowers of *D. caryophyllus* L. (cv Monte) were obtained from Wuhan Flower Centre, an organization of scientific research and exploitation, just 10 km away from our laboratory. Stems were graded for uniform quality, combined into bunches of multiple stems, and then re-cut to 50 to 60-cm length in accordance with commercial practice. Bunches were packed dry into commercial flower boxes and then were transported to the laboratory at Jianghan University for three times, just for the following experiments at different time.

2.2. General processing

Upon arrival in the laboratory, flower stems free of visible damage were trimmed to 45 to 50-cm length using cutters. The blades of cutters were surface sterilized by rinsing in 95% (v/v) ethanol prior to use. Leaves on the bottom 25 cm of stems that would otherwise be submerged in vase water were removed by hand. The stems were immerged into distilled water as low as 25 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. The both reagents were dissolved in sterile distilled water.

2.3. Experiment 1: optimal SNP concentration

Glass 250 ml capacity vases were surface sterilized with ethanol as described above for cutters. Vases were filled with 200 ml of sterile distilled water (control), 0.05, 0.1, 0.15, 0.2, and 0.5 mmol L^{-1} SNP solution. Five replicate stems of the carnation cut flowers were then placed individually into vases containing the different SNP concentrations. The pH of all solutions was maintained at 3.5 since pH differences were shown to effect flower senescence (Halevy and Mayak, 1981). Each vase opening was closed around the stem non-absorbent cotton to reduce vase solution evaporation and contamination from falling flowers and leaves. Stems were maintained in a controlled environment room at 22 °C and 60–70% relative humidity, and a light irradiance of 3 W m^{-2} for 12 h a day provided by cool white fluorescent tubes. In the experiment, three replicates were designed (each replicate including five flowers), and only vase life was determined.

2.4. Experiment 2: optimal MB-1 concentration counteracting SNP positive effect

Five stems of carnation cut flowers under study were distributed evenly to three sterile 250 ml glass vases. Vases contained 200 ml of sterile distilled water (control), 0.1 mmol L^{-1} SNP, 2.5, 5.0, 7.5, 10 μmol L^{-1} MB-1, 0.1 mmol L^{-1} SNP + 2.5 μmol L^{-1} MB-1, 0.1 mmol L^{-1} SNP + 5.0 μmol L^{-1} MB-1, 0.1 mmol L^{-1} SNP + 7.5 μmol L^{-1} MB-1, and 0.1 mmol L^{-1} SNP + 10 μmol L^{-1} MB-1, and the pH of all solutions were adjust to 3.5. Vase openings were closed non-absorbent cotton as described above. Stems were held in vases under the same temperature, relative humidity and lighting regime described above in experiment 1.Three replicates were also designed (each replicate including five flowers). Similarly, only vase life was determined in this experiment.

2.5. Experiment 3: the physiological responses of cut flowers to SNP

The experiment was designed based on the result of experiments 1 and 2. Ten cut flowers were immerged into three 500 ml glass vases. Vases contained 400 ml of different solutions, including (1) sterile distilled water (control); (2) 0.1 mmol L^{-1} SNP solution; (3) 0.1 mmol L^{-1} SNP + 5 μmol L^{-1} MB-1 solution. Three flowers in each vase were labeled and sampled to analyze non-destructive indexes of vase life and water loss. However, the remainder seven flowers in each vase were used to determine the destructive indexes such as cell protection enzymes, MDA and LOX activities. Other conditions were the same as described for experiment 1.

2.6. Biochemical analysis

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 as the following expressions:

$$\frac{\text{The fresh weight of flower during vase stage} - \text{the fresh weight of flower at day 0}}{\text{The fresh weight of flower at day 0}} \times 100\%$$

For the measurement of cell protective enzymes, MDA and LOX, the petals were sampled every 2 days. The petals were cut from the seven flowers per vase, and blended with each other (i.e. each vase as a repeat), and then, 1 g flower petals were weighted and homogenized in 1.5 ml of respective extraction buffer in a pre-chilled mortar and pestle by liquid nitrogen. The homogenate was filtered through four layers of cheesecloth and centrifuged at 15,000 rpm for 20 min at 4 °C. The supernatant recentrifuged again at 15,000 rpm for 20 min at 4 °C for determination of antioxidant enzyme activities. SOD (EC 1.15.1.1) activity was assayed by monitoring the inhibition of photochemical reduction of nitro-blue tetrazolium (NBT) according to the method of Bayer and Fridovich (1987). Flower petals were homogenized in 1 ml cold 100 mmol L^{-1} K-phosphate buffer (pH 7.8) containing 0.1 mmol L^{-1} ethylenediaine tetraacetic acid (EDTA), 1% (w/v) polyvinyl-pyrrolidone (PVP) and 0.5% (v/v) Triton X-100. One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the reduction of NBT as monitored at 560 nm. POD (EC 1.11.1.7) activity was measured by following the change of absorption at 436 nm due to guaiacol oxidation (extinction coefficient, 6.39 mM^{-1} cm^{-1}) following Pütter (1974). The activity was assayed for 5 min in a reaction solution composed of 50 mmol L^{-1} K-phosphate buffer (pH 7.0), 20.1 mmol L^{-1} guaiacol, 12.3 mM H_2O_2 and the required amount of enzyme extract from flower petals. For determination of CAT, flower petals were homogenized in 100 mmol L^{-1} sodium phosphate buffer (pH 7.0) containing 1 mmol L^{-1} EDTA under liquid nitrogen. Catalase (EC 1.11.1.6) activity was determined by following the consumption of H_2O_2 (extinction coefficient,