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Quality deterioration of cut carnation flowers involves in antioxidant systems and energy status

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ABSTRACT

Adenosine triphosphate (ATP) has played an important role in regulating senescence of horticultural crops. The physiological mechanism of exogenous ATP to affect the senescence of cut carnation during vase life was investigated. Fresh cut carnation flowers were treated with distilled water (control), 0.1 mM adenosine triphosphate (ATP) or 0.5 mM 2,4-dinitrophenol (DNP, an agent for uncoupling oxidative phosphorylation) and then held at 25 °C up to nine days. Exogenous ATP supply increased flower size, maintained fresh weight, extended vase life, reduced ethylene production rate and maintained membrane integrity. ATP treatment also reduced activities of phospholipase D (LD) and lipoxygenase (LOX), enhanced activities of superoxide dismutase (SOD) and catalase (CAT) and increased endogenous contents of ATP and adenosine diphosphate (ADP) and energy charge level at the later vase life. In comparison to control, exogenous application of DNP accelerated ethylene production and reduced vase life, increased membrane permeability, MDA content and activities of PLD and LOX and reduced activities of SOD and CAT. Furthermore, contents of ATP and ADP and energy charge level decreased in the DNP-treated flowers during vase life. Thus, exogenous ATP supply could maintain membrane integrity, increase antioxidant system and improve endogenous energy level, thereby suppressing ethylene production and retarding the senescence of cut carnation flower during vase life.

1. Introduction

Carnation is one of the most economically important cut flowers, comprising of 63% of the total cut flower production in the world and playing an important role in the florist trade (Önal, 2011). Currently, postharvest senescence is a major limitation to the marketing of cut carnation flowers, as shown by petal in-rolling and discoloration due to ethylene effect after harvest (Trippi and Paulin, 1984). Great effort has been made to develop postharvest handling to suppress ethylene production and extend vase life of cut carnation flowers. It is well known that inhibitors of ethylene effects, such as silver thiosulfate (STS) and 2,5-norbornadiene (2,5-NBD), can prevent effectively the senescence symptoms of carnation flowers by reducing ethylene-binding capacity or suppressing endogenous ethylene production (Peiser, 1989; Sylvestre and Paulin, 1987; Uda et al., 1996), but their toxicity to the environment limits the use (Serek et al., 1995). 1-Methylcyclopropene (1-MCP) as a non-toxic ethylene inhibitor is considered to be an environmentally acceptable alternative to STS and extends the storage life of carnation flowers (Asil et al., 2013; Seglie et al., 2011), but it shows a limited application as a gas when used in solution during vase holding time. Therefore, it is necessary to find an environment-friendly and efficient alternative to reduce senescence of cut carnation flowers.

Adenosine triphosphate (ATP), largely produced by mitochondrial oxidative phosphorylation, plays an important role in regulating senescence and ripening of postharvest horticultural crops (Jiang et al., 2007). For example, application of ATP exhibited potential to control browning and maintain quality of harvested litchi fruit (Song et al., 2006a). Exogenous ATP supply also alleviated membrane lipid peroxidation and enhanced activities of antioxidant enzymes and reduced pathogen infection in litchi fruit during storage (Yi et al., 2008, 2010). In the previous study, it was found that exogenous ATP supply preserved freshness and extended vase life of cut carnation flowers (Song et al., 2006b). However, the mode

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of action of ATP in reducing senescence of cut carnation flowers needs to be elucidated.

Membrane deterioration is an early and characteristic feature of petal senescence of cut flowers. Increased lipid peroxidation, mediated and sustained by phospholipid-degrading enzymes, such as phospholipase D (PLD) and lipoxygenase (LOX), results in a loss of membrane integrity, which has been noted in the senescing petal tissues (Brown et al., 1990; Fobel et al., 1987). On the other hand, plant cells possess both enzymatic and non-enzymatic antioxidant systems to alleviate membrane lipid peroxidation and protect cellular membranes from radical oxygen species (ROS) (Foyer et al., 1994). The irreversible senescence finally occurs due to oxidative membrane damage induced by the imbalance between ROS and ROS-scavenging systems. Recently, considerable studies have shown that senescence-related physiological attributes, such as increased membrane permeability and enhanced ROS production, may be related to limited availability of energy (Jiang et al., 2007; Veltman et al., 2003). For example, ATP production was involved in desaturation of acyl chains and membrane lipid synthesis (Crawford and Braendle, 1996). A direct relationship between energy metabolism and membrane lipid degradation in potato cell cultures was determined by Rawyler et al. (1999), who reported that membrane lipids were hydrolyzed when ATP synthesis rate fell into below a threshold. Kibinza et al. (2006) suggested that energy metabolism was a key factor to regulate ROS accumulation during sunflower seed deterioration. Furthermore, Yi et al. (2010) reported that increased ATP content and energy charge level of tissues could contribute to enhancement of antioxidant defense systems and reduction of pericarp browning incidence in postharvest litchi fruit during storage. Thus, it is suggested that the role of exogenous ATP in the senescence of cut carnation flowers may be related to high endogenous energy status involved in membrane integrity and antioxidant systems.

The aims of this present study were to investigate the effects of exogenous ATP on flower size, fresh weight, vase life and ethylene production, then analyze the changes in membrane permeability, lipid peroxidation, lipid-hydrolized enzymes and antioxidant enzymes, and finally determine energy status in cut carnation flowers when treated with exogenous ATP or mitochondrial uncoupler 2,4-dinitrophenol (DNP). The study can help to understand better quality deterioration of cut carnation flowers involved in antioxidant systems and energy status.

2. Plant materials and methods

2.1. Plant materials

Flowers of carnation (*Dianthus caryophyllus* L. cv. Master) were obtained from a local commercial flower center in Guangzhou and then transported by car to the laboratory within 6 h. The flowers were harvested naturally and were without any treatments such as STS. Flowers were collected at the pre-opening stage with a similar maturity, then re-cut in water to about 30 cm stem length, and finally inserted into 200-mL conical flasks containing distilled water (control), 0.1 mM ATP or 0.5 mM DNP. These concentrations were selected based on the preliminary study, which showed 0.1 mM ATP exhibited the most benefit in retarding the senescence of cut carnation flowers and 0.5 mM DNP had the most deleterious effect of the carnation petals. The volume of vase solution was maintained at a constant level (100 mL) by replenishing flasks daily up to nine days. There were three flowers per flask and five flasks per treatment. Stems were assigned to the various treatments in a randomized block design. Throughout the experimental period, the flowers were held at 25 °C and 70–80% relative humidity (RH) and a 12-h light period per day under irradiance of 12 W m^{-2} above flower using fluorescent tubes.

2.2. Measurements of flower size, fresh weight and vase life

Flower size was defined as the maximum width of each flower and measured by CS106 type of vernier calipers every 12 h. Fresh weight of the stems was recorded every two days throughout the vase life evaluation period. Vase life was evaluated every 12 h using a scale from one to five described by Song et al. (2006b), where 1, fresh without any deterioration; 2, slight discoloration, mold growth or wilting; 3, moderate discoloration, mold growth or wilting; 4, severe discoloration, mold growth or wilting; and 5, entirely discolored, substantial mold growth and/or wilting. All flowers were scored until they had deteriorated to score five. For each treatment, 15 flowers were selected for evaluation, and the means of flower size, fresh weight and vase life were determined.

2.3. Measurement of ethylene production rate

Ethylene production rate was measured according to the method of Serrano et al. (2001). Flower stems were trimmed to about 5 cm length and sealed in a 500-mL glass jar for 1.5 h. A 1-mL gas sample was then withdrawn from the jar using syringe. C_2H_4 concentration was determined by gas chromatography (Shimadzu GC 17A, Japan) and calculated on a fresh weight (FW) basis.

2.4. Measurements of membrane permeability and malondialdehyde (MDA) content

Membrane permeability, expressed by relative electrolyte leakage rate, was measured by the method of Jiang and Chen (1995) with a minor modification. Thirty outermost petal discs (10 mm in diameter) were immersed in 20 mL of 0.3 M mannitol solution at 25 °C, followed by shaking for 30 min. Electrolyte leakage was determined with a conductivity meter (Model DDS-11A, Shanghai Scientific Instruments). Total electrolyte leakage was determined after boiling the samples for 10 min and cooling to 25 °C. Relative electrolyte leakage rate was expressed as a percentage of total electrolyte leakage.

MDA content was measured according to the method of Health and Pakcer (1968) with a slight modification. Frozen outermost petal tissues (3 g) from 15 flowers were ground finely in liquid nitrogen, then homogenized in 15 mL of 10% trichloroacetic acid (TCA) and finally centrifuged at 5000 × *g* for 10 min. The supernatant phase was then collected. MDA content was determined by adding 5 mL of 0.5% thiobarbituric acid (dissolved in 10% TCA) to 0.5 mL supernatant. The solution was heated at 95 °C for 20 min, quickly cooled, and centrifuged at 10,000 × *g* for 10 min to clarify precipitation. In order to eliminate disturbance of red pigment, the same procedure with TCA was used as control. Absorbance at 532 nm was measured and subtracted from the non-specific absorbance at 600 nm. The concentration of MDA was calculated using an extinction coefficient of 155 m M^{-1} cm^{-1} as follows: MDA content (μM g^{-1} FW) = $(OD_{532} - OD_{600}) \times 15 \times 5.5/(0.5 \times 1 \times 155)$

2.5. Determinations of activities of PLD and LOX

PLD activity was measured by choline reinecke salt precipitation method described by Suttle and Kends (1980). Frozen outermost petal tissues (3 g from 15 flowers) were ground finely in liquid nitrogen and extracted with 15 mL of 0.1 M sodium acetate buffer (pH 5.6). The homogenate was centrifuged at 10,000 × *g* and 4 °C for 20 min and then the supernatant was used for PLD activity assay. To prepare the enzymatic substrate, 40 mg of 1,3-phosphatidyl choline was dissolved in 50 mL of ether and then the mixture