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The role of *N*-lauroylethanolamine in the regulation of senescence of cut carnations (*Dianthus caryophyllus*)

Yun Zhang[a,b], Wei-ming Guo[c,*], Su-mei Chen[c], Liang Han[d], Zheng-ming Li[d]

[a]*College of Life Science, Nanjing Agricultural University, Nanjing 210095, China*
[b]*Department of Biochemistry, Fujian Institute of Education, Fuzhou 350001, China*
[c]*College of Horticulture, Nanjing Agricultural University, Nanjing 210095, China*
[d]*National Key Laboratory of Elemento-organic Chemistry, Research Institute of Elemento-organic Chemistry, Nankai University, Tianjin 300071, China*

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Summary

N-acylethanolamines (NAEs) are a group of lipid mediators that play important roles in mammals, but not much is known about their precise function in plants. In this work, we analyzed the possible involvement of *N*-lauroylethanolamine [NAE(12:0)] in the regulation of cut-flower senescence. In cut carnation flowers of cv. Red Barbara, the pulse treatment with 5 μM NAE(12:0) slowed senescence by delaying the onset of initial wilting. Ion leakage, which is a reliable indicator of membrane integrity, was postponed in NAE(12:0)-treated flowers. The lipid peroxidation increased in carnation petals with time, in parallel to the development in activity of lipoxygenase and superoxide anion production rate, and these increases were both delayed by NAE(12:0) supplementation. The activities of four enzymes (superoxide dismutase, catalase, glutathione reductase and ascorbate peroxidase) that are implicated in antioxidant defense were also upregulated in the cut carnations that had been treated with NAE(12:0). These data indicate that NAE(12:0)-induced delays in cut-carnation senescence involve the protection of the integrity of membranes via suppressing oxidative damage and enhancing antioxidant defense. We propose that the stage from the end of blooming to the onset of wilting is a critical period for NAE(12:0) action.

Abbreviations: ABA, abscisic acid; ASC, ascorbate; APX, ascorbate peroxidase; CAT, catalase; EDTA, ethylene diaminetetraacetic acid; GR, glutathione reductase; GSH, reduced glutathione; GSSG, oxidized glutathione; LOX, lipoxygenase; MDA, malondialdehyde; NADPH, reduced nicotinamide adenine dinucleotide phosphate; NAEs, *N*-acylethanolamines; NAE(12:0), *N*-lauroylethanolamine; NBT, nitro blue tetrazolium; $O_2^{\bullet -}$, superoxide anion; ROS, reactive oxygen species; SOD, superoxide dismutase; PLD, phospholipase D.

*Corresponding author. Tel.: +86 25 84395543; fax: +86 25 84395266.
E-mail address: guowm@njau.edu.cn (W.-m. Guo).

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Introduction

N-acylethanolamines (NAEs) are fatty-acid amides, which are minor membrane lipid constituents of plant and animal cells. In animals, NAEs have been identified to be part of the endocannabinoid signaling system that regulates a variety of physiological functions (Chapman, 2004). Increasing evidence has indicated that some biological activities are attributed to NAEs in plants. It has been shown that NAEs are involved in plant defense signaling (Chapman et al., 1998; Tripathy et al., 1999), inhibit the activity of phospholipase Dα activity (Austin-Brown and Chapman, 2002), and disrupt normal seedling root development at elevated levels (Blancaflor et al., 2003). Recently, NAEs have been used to preserve the freshness of cut flowers and to delay the ripening of fruits (Chapman and Austin-Brown, 2001). However, the possible relationship between NAEs and post-harvest longevity has not yet been studied in flowers.

Loss of membrane integrity that results in an increase in membrane permeability is the final and irreversible phase of senescence (Marangoni et al., 1996), which is closely correlated with lipid modifications that are principally due to peroxidation (Paulin et al., 1986). The overall peroxidation process may be as follows: initially, phospholipases remove the polar heads of fatty acids, which are then more easily degraded. The peroxidation is then initiated and sustained by reactive oxygen species (ROS) and lipoxygenase (Paulin and Droillard, 1989; Marangoni et al., 1996). However, plant cells possess both enzymatic and non-enzymatic mechanisms that can overcome oxygen damage and delay the deleterious effects on membranes (Foyer et al., 1994).

Cut carnation flowers have been shown to be a convenient model system for the study of post-harvest physiological events during flower senescence (Paulin and Jamain, 1982). In the present investigation, we examined the possible relationship between NAEs and senescence and, moreover, attempted to follow the loss of membrane integrity via regulating oxidative damage and antioxidant defense levels during senescence that was delayed by the use of NAE(12:0).

Materials and methods

Plant material

Carnations, *Dianthus caryophyllus* L. cv. Red Barbara, were obtained from local commercial growers. Flowers were collected at the preopening stage (stage 1) as described below, and transported to the laboratory on the day of harvest. Flowers were trimmed to a peduncle length of $\sim$12 cm for subsequent treatments.

Chemicals

N-lauroylethanolamine [NAE(12:0)] was prepared by refluxing lauric acid with ethanolamine for 6 h and purified by recrystallizing in dichloromethane and acetone. Purity was greater than 99%, as determined by GC–MS.

Application of NAE(12:0)

Trimmed flowers were randomized and placed individually in test tubes (10 mL), then subjected to pulse treatment on the day of harvest (day 0 of the experiment). Pulse treatments were performed by holding the flowers in the vase solution (5 mL) containing 5 μM NAE(12:0) with a solvent of 0.1% (v/v) isopropanol and 0.001% (v/v) Tween-20 for 4 h. The concentration in preliminary experiments was found to be optimal for improving longevity in cut carnation flowers of cv. Red Barbara. Flowers were pulsed in solution with a solvent containing 0.1% isopropanol and 0.001% Tween-20, which served as controls, and no effect of isopropanol and Tween-20 on cut carnation flowers post-harvest was observed (data not shown). After pulse treatment, carnation flowers were transferred to deionized water for vase holding. Subsequently, the pulse treatment was performed again every 2 days until the experiment was complete. All flowers were held in an observation room at $21 \pm 2\,^{\circ}C$, $60 \pm 10\%$ relative humidity under white fluorescent light ($20\,\mu mol\,m^{-2}\,s^{-1}$) and 12-h photoperiod for 1 day.

Evaluation of development stages and longevity

To evaluate the senescence in response to NAE(12:0), four stages of development were arbitrarily distinguished according to Droillard et al. (1987). Stage 1, pre-opening: the petals form a right angle with the stem axis. Stage 2, opening: the petals form a 45° angle with the stem. Stage 3, beginning to withering: the petals have lost turgor and their edges are wrinkling. Stage 4, withering: the petals are wrinkled and discolored and there is a complete loss of turgor (Droillard et al, 1987). The post-harvest performance of flowers was evaluated twice each day, and the vase life was