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Effect of Age on the Auto-catalytic Ethylene Production and the Expression of Ethylene Biosynthetic Gene *Dc-ACS1* in Petals of Long-life Carnations

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Abstract

We studied the effect of aging after the full-open stage on auto-catalytic ethylene production and the expression of genes for ethylene biosynthesis and signaling pathway following exogenous ethylene treatment in long-life carnation flowers (Miracle Rouge [MR] and Miracle Symphony [MS]). As exogenous ethylene treatment time increased, auto-catalytic ethylene production increased in the petals of the control flower (White Sim), MR and MS, on Day 0 and 3, but it increased only slightly in the MR and MS flowers on Day 15. The levels of ethylene biosynthesis pathway gene *Dc-ACS1* in MR petals on Day 15 following 20 h exogenous ethylene treatment were around 1/10 of those on Day 3. In MS on Day 15, the levels were around 1/5 of those on Day 3. The levels of another ethylene biosynthesis pathway gene, *Dc-ACO1*, did not differ on both Day 3 and 15 in MR and MS. Levels of ethylene signaling pathway gene *Dc-EIL4* on Day 3 were higher than those on Day 15 at corresponding times. These results indicated that aging may affect the expression of *Dc-ACS1* and *Dc-EIL4*, but not the expression of *Dc-ACO1*, ethylene receptors, and *Dc-EIL1/2, 3*.

Discipline: Horticulture
Additional key words: *Dianthus caryophyllus*; ethylene signal transduction

Introduction

Ethylene, a gaseous plant hormone, regulates many important growth and developmental cues, such as flower senescence, fruit ripening, germination, and abscission, in numerous plant species[1,47]. The ethylene biosynthetic pathway can be summarized as follows: Methionine → *S*-adenosylmethionine (AdoMet) → 1-aminocyclopropane-1-carboxylate (ACC) → ethylene[19]. The conversion of AdoMet to ACC and the conversion of ACC to ethylene are catalyzed by ACC synthase (ACS) and ACC oxidase (ACO), respectively[19,51], and several genes encoding them have been cloned in many plant species. These steps are generally considered to be rate-limiting for ethylene biosynthesis. Components of the ethylene signal transduction pathway regulate a lot of ethylene-inducible genes[13,44]. Ethylene receptors that are studied for ethylene perception in plant tissue are considered to act as negative regulators[14,20]. Many ethylene signal components besides ethylene receptors have been studied in *Arabidopsis*[45]. *EIN3* and three *EIN3-LIKE* (*EIL1*, *EIL2*, and *EIL3*) genes were cloned, and these predicted proteins were nuclear-localized transcription factors[6]. Genes encoding EIN3/EIL have been cloned from other plant species, such as tobacco[33], tomatoes[40,52], mung beans[24] and carnations[16,42].

We have used the carnation as a model to study ethylene-induced petal wilting in flowers. It shows a climacteric-like rise in ethylene production during flower senescence, which is accompanied by the in-rolling of petals, a typical senescence symptom in carnations[1,3,11,21,32,43]. Petal in-rolling, which is one post-pollination development, is accelerated by ethylene[17]. The flower is a complex organ that produces ethylene at different times. Ethylene production from the gynoecium starts first in normal carnation flowers, such as White Sim (WS), during natural senescence. The careful removal of the gynoecium prolonged the life of the carnations[37], and the life of the detached petals was longer than that of the attached petals[39]. This data indicated that auto-catalytic ethylene production in the petals was induced

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from ethylene from gynoecium and/or surroundings.

Ethylene production increased with a rise in ACS and ACO mRNA levels during senescence and the genes for these mRNAs were up-regulated by exogenous ethylene[12,18,30,44,49]. Three ACC synthase genes (*Dc-ACS1*, *Dc-ACS2* and *Dc-ACS3*) and one ACC oxidase gene (*Dc-ACO1*) have been identified, and their levels during flower senescence have been characterized[18,30]. *Dc-ACS1* mRNA was the most abundant of all *Dc-ACS* genes in the petals[18,34]. *Dc-ACS2* and *Dc-ACS3* occur in the gynoecium but to a lesser extent[34]. Ethylene responsiveness, auto-catalytic ethylene production, and the senescence process in the flower organs implicate ethylene perception and ethylene signaling. It is possible that the levels of some senescence-related (SR) genes, including *Dc-ACS* and *Dc-ACO*, were directly or indirectly regulated by Dc-EILs[16]. EIN3/EIL, which are positive regulators, act as the last component in the primary ethylene-signaling pathway[16,41]. In general, EIN3/EIL are supposed to be regulated at posttranslational levels, because their genes did not change at transcript levels after ethylene treatment[10,31,50]. In carnations, *Dc-EIL*s appear to be regulated by ethylene at transcript levels in the flower and they will implicate in the up-regulation of SR genes[13,16]. Levels of *PhEIL1*, which is the EIN3-like gene in petunias, were induced by exogenous ethylene in normal plants and were not induced by ethylene in ethylene-insensitive mutants[35].

The flower life of a normal carnation, such as WS, was from 5 to 6 days under a constant air temperature of 23°C and RH of 70%. Miracle Rouge (MR) and Miracle Symphony (MS) were selected to improve the flower life of carnations by crossbreeding from 1992 to 2003 in a breeding research program of the National Institute of Floricultural Science, Japan[27]. The flower life of MR and MS is from 18 to 20 days under the same conditions, since their flowers produce slightly lower levels of ethylene during flower senescence[27]. The low levels of ethylene in MR and MS flowers have been implicated in the low levels of *Dc-ACS1*, *Dc-ACS2,* and *Dc-ACO1* in the petals and gynoecia[39], yet their ethylene sensitivity at the full open stage (Day 0) was the same as that observed in ethylene-sensitive Sim-type cultivars[29,38]. In general, the ethylene sensitivity of the flowers increases with age during senescence. However, ethylene sensitivity in long-life carnation cultivars decreased with age after anthesis[25,29]. A decline in ethylene sensitivity and auto-catalytic ethylene production with age may also contribute to long flower life in MR and MS. However, it is still unknown why ethylene sensitivity and auto-catalytic ethylene production decreases with age. To clarify whether the induction of ethylene biosynthesis and ethylene-signaling genes changes with age after exogenous ethylene treatment, we studied their levels in long-life carnation flowers. Furthermore, we discussed the influence of aging on the levels of genes responsible for auto-catalytic ethylene production.

Materials and methods

1. Plant material

Two long-life carnation (*Dianthus caryophyllus* L.) cultivars, Miracle Rouge (MR) and Miracle Symphony (MS), and a control cultivar, White Sim (WS), were grown under natural daylight conditions in a greenhouse[27,28]. WS has been used as a control flower in many studies of senescence in carnation petals. We defined the full open stage as anthesis. Flowers were harvested when the outer petals were horizontal.

2. Ethylene treatment

On Day 0, the stems of the harvested flowers were cut to 10 cm and held in distilled water under standard conditions at a constant air temperature of 23°C and a continuous photoperiod under cool fluorescent white lamps (10 μmol m^{-2} s^{-1}).

Flowers that were 0, 3, 6 or 15 days after harvest were placed in a 70-l chamber with 10 μl l^{-1} ethylene at 23°C. They were recorded every 1 h by a digital camera Caplio GX (Richo, Tokyo, Japan) to determine the onset of senescence symptoms.

3. Ethylene measurement

After the flowers were treated with ethylene (10 μl l^{-1}) for 0, 4, 8, and 20 h, they were exposed to fresh air for 1 h. They were placed in a 143- or 433-ml glass bottle, which was closed with a silicon cap and kept at 23°C for 1 or 2 h. To determine whether ethylene is produced mainly by the petals or the gynoecia, after ethylene treatment (10 μl l^{-1}), the gynoecia and petals were placed in a 15-ml glass vial or a glass bottle, which was closed with a silicon cap and kept at 23°C for 1 h. Gas samples (1 ml) were taken from the headspace and injected into a gas chromatograph, GC-13A (Shimadzu, Kyoto, Japan), equipped with an alumina column and a flame ionization detector.

To elucidate the dose-response relationships for the induction of auto-catalytic ethylene production, the flowers were treated with 0.01, 0.1, 1, and 10μl l^{-1} ethylene for 20 h. These flowers were kept in fresh air for 1 h and the ethylene production from the flowers was measured.

4. Real-time PCR analysis of *Dc-ACS1*, *Dc-ACO1*, *Dc-ETR1*, *Dc-ERS2*, *Dc-EIL1/2*, *Dc-EIL3*, and *Dc-EIL4* transcripts

Changes in the levels of ethylene-signaling pathway genes were investigated in order to understand how these genes are involved in the decrease in auto-catalytic ethylene production. We determined the levels of *Dc-ACS1*, *Dc-ACO1*, *Dc-ETR1*, *Dc-ERS2*, *Dc-EIL1/2*, *Dc-ERL3,* and *Dc-*

EIL4 mRNA in the petals after exogenous ethylene treatment using Real-time RT-PCR analysis. The total RNA was extracted from the petals of Day 3 and 15 flowers using the RNeasy Plant Mini Kit (Qiagen, Hilden, Germany), and 1 μg of it was used for the synthesis of the first strand cDNA by oligo (dT) primer and reverse transcripts using an Advantage RT-for-PCR kit (BD BioscienceClontech., Palo Alto, CA, USA). To confirm the amount of template RNA, a fragment of carnation actin (*Dc-ACT1-2*) was used as internal control[38].

Real-time PCR was achieved using a LIGHTCYCLER model 3.1 System (Roche Diagnostics, Mannheim, Germany). PCR was performed as described in a previous paper[38]. The identity of amplified fragments coded for partial *Dc-ACS1*, *Dc-ACO1*, *Dc-ETR1*, *Dc-ERS2*, *Dc-EIL1/2*, *Dc-ERL3,* and *Dc-EIL4* cDNA was confirmed by direct DNA sequencing.

The sequence of primers for *Dc-ACS1* forward, *Dc-ACS1* reverse, *Dc-ACO1* forward, and *Dc-ACO1* reverse were described in a previous paper[38]. Primers used for real-time PCR are shown in Table 1. The amplification of PCR products was monitored via the intercalation of SYBR-Green. The following program was applied: initial polymerase activation: 95°C, 10 min; 60 cycles at 94°C, 10 s; 57°C, 15 s; 72°C, 20 s for *Dc-ETR1*; 60 cycles at 94°C, 10 s; 60°C, 15 s; 72°C, 20 s for *Dc-ERS2*; 60 cycles at 94°C, 15 s; 60°C, 10 s; 72°C, 20 s for *Dc-EIL1/2*; 40 cycles at 94°C, 10 s; 60°C, 10 s; 72°C, 15 s for *Dc-EIL3*; 40 cycles at 94°C, 10 s; 60°C, 10 s; 72°C, 15 s for *Dc-EIL4*. Data is shown as the mean ± SE of three determinations for each sample.

Results

1. Reduction of auto-catalytic ethylene production induced by exogenous ethylene resulting from aging in long-life carnations

The flowers of MR and MS on Day 0 and 3 after anthesis showed petal in-rolling (inward rolling) following exogenous ethylene (10 μl l^{-1}) for 20 h, and flowers on Day 6 showed slight petal in-rolling (Fig. 1). However, the flowers of MR and MS on Day 15 did not show petal in-rolling following ethylene treatment. The petals of MR and MS became brown from the petal edge and eventually desiccated.

The auto-catalytic ethylene production of the flowers

Table 1. Primers used for real-time RT-PCR

Gene Name	Forward Primer Sequence	Reverse Primer Sequence
Dc-ETR1	CGTTCCGAGAGACCGATAATAG	CGGTCTCGTCAACCAGATAC
Dc-ERS2	TCAGAACACACTCAGAGCACG	TGGCCTCCTCTTATAGTGCG
Dc-EIL1/2	CAGCAGCTCGATCAATGC	GAAGAGATCGAGGAACAATCTCC
Dc-EIL3	GATCGGTGAGCTTATGTCAATG	CTGTGTAACAACGCAGCTCG
Dc-EIL4	AGCAGCTTGATCAATGCAGG	CGAACAGAAGGATTCGAGTCC

Fig. 1. Effect of exogenous ethylene treatment (10 μl l^{-1}) for 20 h on different aging MR and MS flowers
Flowers at Day 0, 3, 6 and 15 after the fully opened petals stage were used for this experiment. The flowers were held in distilled water at a constant air temperature of 23°C and a continuous photoperiod under cool fluorescent white lamps (10 μmol m^{-2} s^{-1}).

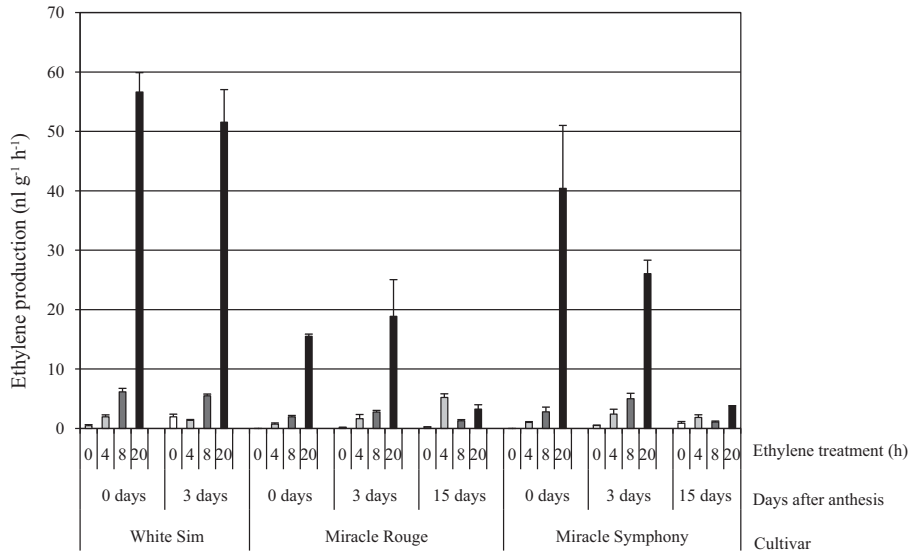

Fig. 2. Auto-catalytic ethylene production induced by exogenous ethylene (10 µl l^{-1}) in whole carnation flowers at Day 0, 3, and 15 after the fully opened petals stage

Flowers were held in distilled water after harvest and treated with ethylene for 0, 4, 8, and 20 h. After ethylene treatment, the flowers were kept in fresh air for 1h and their ethylene production was measured. The values are the mean ± SE of six replications.

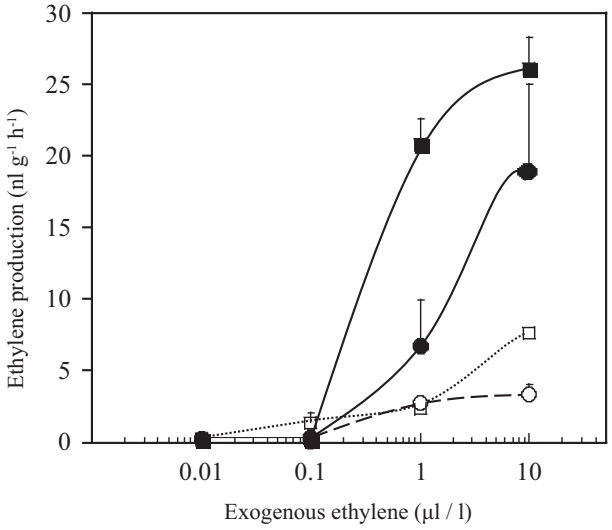

Fig. 3. Dose-ethylene production induced by the exogenous ethylene of flowers at Day 3 and 15 after anthesis

The values are the mean ± SE of three replications. ✧:Miracle Rouge 3d, ✳:Miracle Rouge 15d, ■: Miracle Symphony 3d, □:Miracle Symphony 15d.

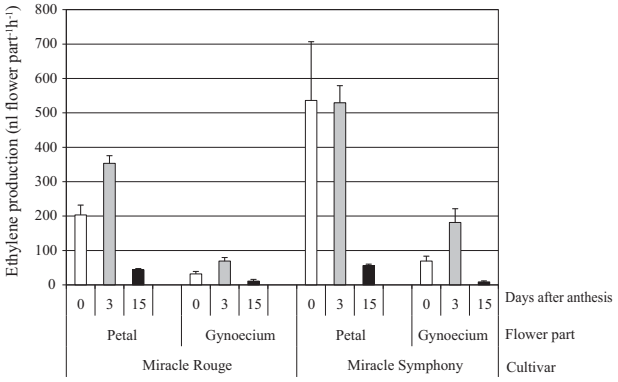

Fig. 4. Auto-catalytic ethylene production induced by exogenous ethylene in the petals and gynoecium at Day 3 and 15 after anthesis

The values are the mean ± SE of six replications.

of all three cultivars increased on Day 0 and 3 with the increasing time (0, 4, 8 and 20 h) of exposure to exogenous ethylene (10 µl l^{-1}) (Fig. 2). In WS flowers, levels of auto-catalytic ethylene production were no different between Day 0 and 3. However, the increase in the auto-catalytic ethylene of MR and MS flowers on Day 15 was only slight with the increasing time of ethylene treatment. MR flowers that were treated with ethylene for 20 h on Day 0 and 3 produced less ethylene than MS flowers.

Exogenous ethylene induced auto-catalytic ethylene production in whole MR and MS flowers on Day 3 in a dose-dependent manner (Fig. 3). In both cultivars, ethylene production on Day 3 was higher than that on Day 15 at 1 and 10µl l^{-1} of exogenous ethylene.

Levels of ethylene produced by the petals were higher than those of the gynoecia in MR and MS on all harvest days (Fig. 4). In MR, ethylene production from the petals was 203.3, 353.3, and 43.8 (nl flower part^{-1} h^{-1}) on Day 0, 3, and 15, respectively, and from the gynoecium, it was 32.0, 69.3, and 11.0 (nl flower part^{-1} h^{-1}) on Day 0, 3, and 15, respectively. In MS, ethylene production from the petals

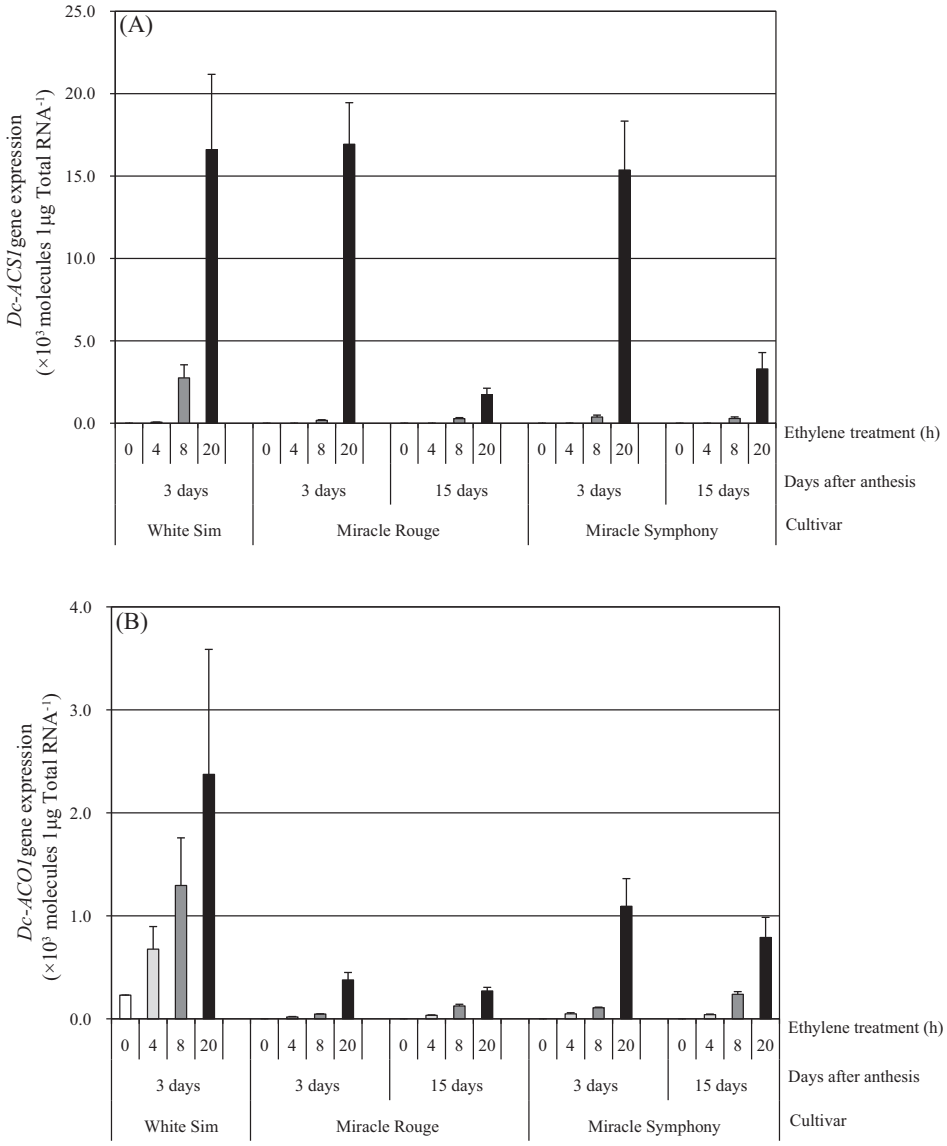

Fig. 5. Transcript levels of *Dc-ACS1* (A), and *Dc-ACO1* (B) in the petals of carnation cultivars. These are the results of three independent experiments (± SE)

was 536.0, 529.4, and 56.1 (nl flower part^{-1} h^{-1}) on Day 0, 3, and 15, respectively, and from the gynoecium, it was 69.1, 181.3, and 8.6 (nl flower part^{-1} h^{-1}) on Day 0, 3, and 15, respectively. Thus, the petals were the main producer of ethylene after exogenous ethylene treatment in these cultivars.

2. Levels of ethylene synthetic pathway genes in petals of long-life carnations

We analyzed changes in the ethylene synthetic pathway genes in the petals of WS, MR, and MS, because the petals produced much ethylene following exogenous ethylene treatment. Sequences of *Dc-ACS1*and *Dc-ACO1* were the same as those in the previous paper[31,44,49]. *Dc-ACS1* mRNA was induced in the petals of WS on Day 3 by ethylene treatment for 8 h, and reached the highest levels after 20 h of treatment (Fig. 5A). In MR and MS, levels of *Dc-ACS1* mRNA in the petals rose drastically on Day 3 after ethylene treatment, but increased only slightly on Day 15. In MR after ethylene treatment for 20 h, levels of *Dc-ACS1* on Day 15 (1.7×10^5 molecules 1μg Total RNA^{-1}) were much lower than on Day 3 (16.9×10^5 molecules 1μg Total RNA^{-1}). In MS, levels were lower (3.3×10^5 molecules 1μg Total RNA^{-1}) on Day 15 than on Day 3 (15.4×10^5 molecules 1μg Total RNA^{-1}). This indicated that the induction of *Dc-ACS1* in response to ethylene declines with the age of the flower.

Dc-ACO1 showed clear accumulation with increasing ethylene treatment time in the petals of MR and MS on Day

3 and 15 (Fig. 5B). Transcript levels of *Dc-ACO1* in WS petals on Day 3 were very high compared with those of other cultivars. *Dc-ACO1* in MR and MS was also increased by ethylene treatment on Day 3 and 15, suggesting that the induction of induced *Dc-ACO1* transcripts in response to ethylene was unchanged with the age of the flower.

3. Levels of ethylene signaling pathway genes in the petals of long-life carnations

Transcripts of ethylene receptor genes *Dc-ETR1* and *Dc-ERS2* showed a similar expression pattern (Fig. 6A,B). Their levels in WS on Day 3 increased after ethylene treatment for 4 h and decreased after 20 h of treatment. Their levels in MR and MS on Day 3 and 15 were relatively constant at 0, 4, 8 and 20 h after ethylene treatment.

We also studied the transcripts levels of three *EIN3-like* (*EIL*) genes, *Dc-EIL1/2*, *Dc-EIL3,* and *Dc-EIL4*. *Dc-EIL1* and *Dc-EIL1/2* were isolated from Nora and WS, respectively. The sequences of *Dc-EIL1* cDNA are very similar to those of *Dc-EIL1/2*; the deduced amino acid of Dc-EIL1/2 shares 98% identity with Dc-EIL1[16]. Levels of *Dc-EIL1/2* in WS and MR on Day 3 decreased with time following ethylene treatment (Fig. 7A). Their levels in MR and MS on Day 3 changed little. Levels in MS on Day 15 increased after ethylene treatment.

Levels of *Dc-EIL3* increased after ethylene treatment in MR and MS on Day 3 and 15 (Fig. 7B). *Dc-EIL3* levels in WS after 8 and 20 h of ethylene treatment on Day 3 were high compared to the other cultivars. *Dc-EIL3* in MR and MS was also increased by ethylene treatment on Day 3 and

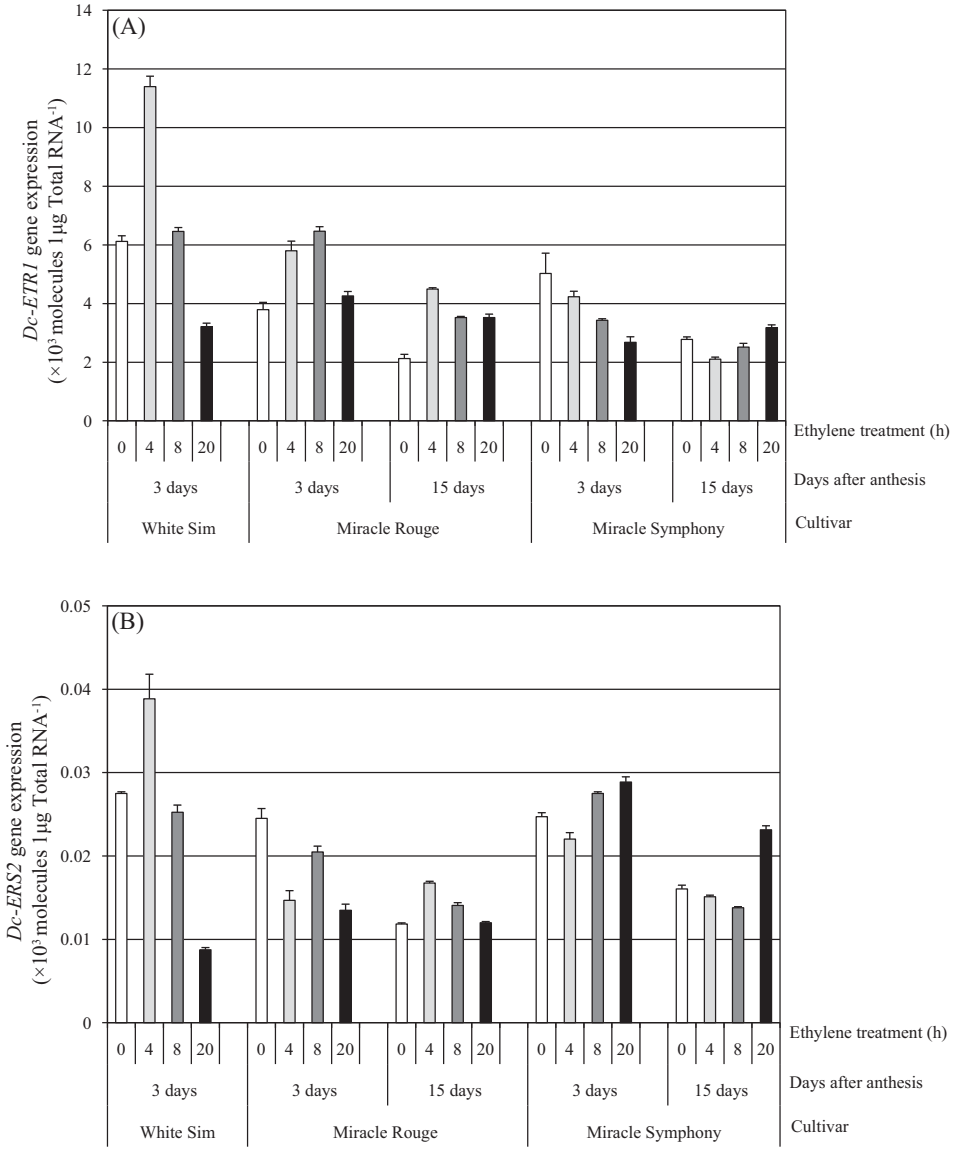

Fig. 6. Transcript levels of *Dc-ETR1* (A), and *Dc-ERS2* (B) in the petals of carnation cultivars. These are the results of three independent experiments (± SE)

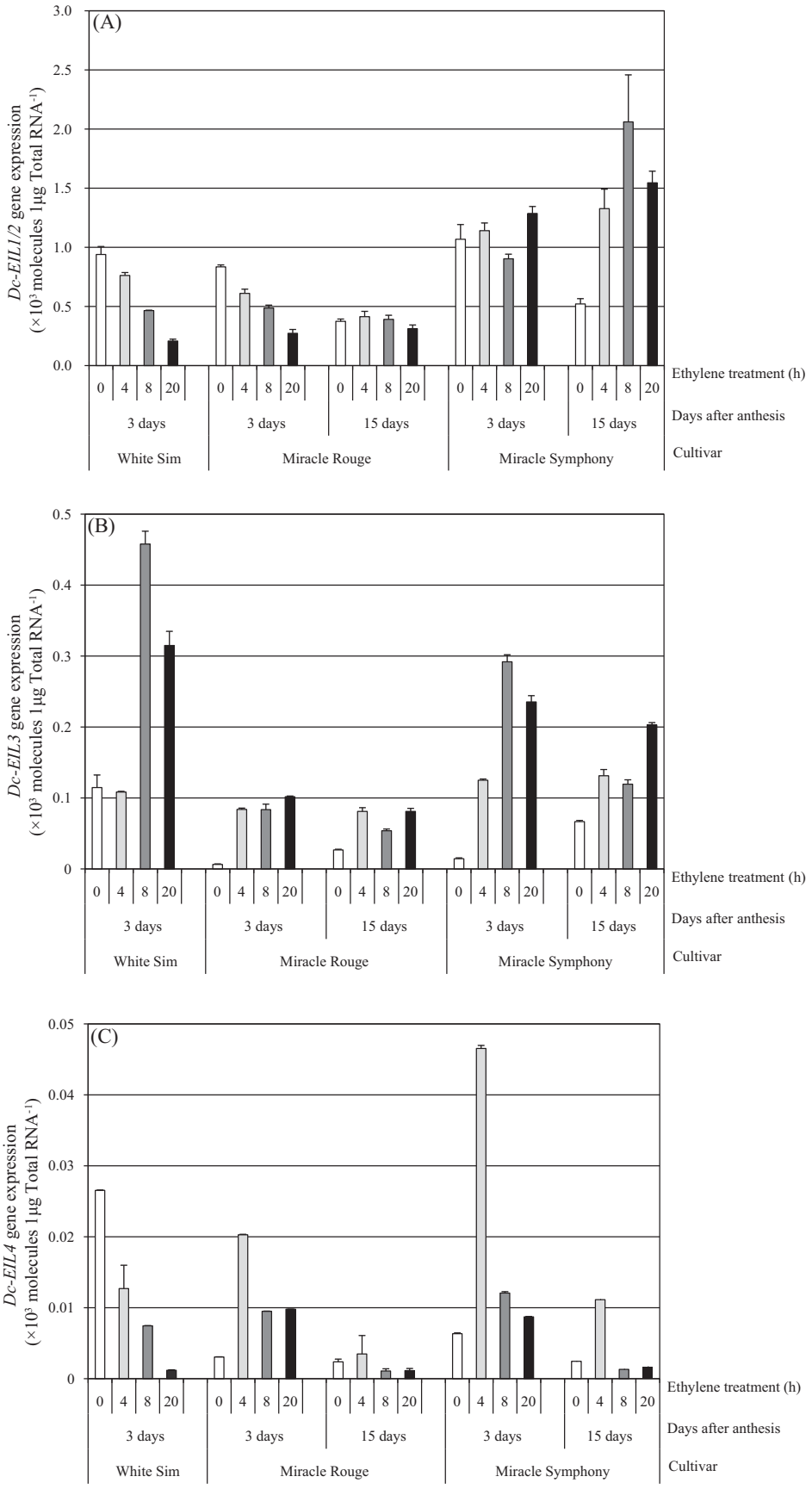

Fig. 7. Transcript levels of *Dc-EIL1/2* (A), and *Dc-EIL3* (B) and *Dc-EIL4* (C) in the petals of carnation cultivars. These are the results of three independent experiments (± SE)

15, and their levels on Day 3 were similar to those of Day 15.

Changes in transcripts of *Dc-EIL4* were different from the cultivars (Fig. 7C). *Dc-EIL4* levels declined in WS with increasing ethylene treatment time. In MR and MS, *Dc-EIL4* levels increased temporarily at 4 h and then decreased after 8 h. Levels of *Dc-EIL4* in MR and MS on Day 3 were higher than those on Day 15 at the corresponding times.

Discussion

To determine the effect of aging on auto-catalytic ethylene production in carnation petals induced by exogenous ethylene, we investigated the gene levels of *Dc-ACS1*, *Dc-ACO1*, *Dc-ETR1*, *Dc-ERS2*, *Dc-EIL1/2*, *Dc-EIL3,* and *Dc-EIL4*. Real-time PCR analysis showed that transcripts of *Dc-ACS1* and *Dc-ACO1* accumulated in WS, MR, and MS petals on Day 3 after ethylene treatment (Fig. 5A,B). Expression levels of these genes were related to the ethylene production of the petals[34]. In MR, *Dc-ACS1* in the petals on Day 15 following 20 h of ethylene treatment were lower than on Day 3 (10.1%). In MS, they were lower on Day 15 than on Day 3 (21.4%). In MR and MS, levels of *Dc-ACS1* transcripts in the petals following ethylene treatment differed widely between Day 3 and 15, but levels of *Dc-ACO1* were the same on Day 3 and 15 (Fig. 5A,B). These results strongly suggest that reduction in auto-catalytic ethylene production with age is the result of a decrease in *Dc-ACS1* induction.

In general, the ethylene sensitivity increases with age in many flowers, such as *Petunia hybrid*[46], *Pelargonium* × *domesticum*[7,8], *Eustoma grandiflorum*[15], and *Torenia fourni-eri*[9]. In carnation flowers, the ethylene sensitivity of the petals increased from bud to flower open[2,5,17,23,48]. Ethylene sensitivity, however, decreased with age from the flower full open stage to the senescence stage in long-life carnations[25,26,29]. Our study showed that both ethylene sensitivity and auto-catalytic ethylene production following exogenous ethylene treatment declined from the petal open stage to the flower senescence stage. As a consequence of the reduction in ethylene sensitivity, in-rolling was not observed in long-life carnations with age after ethylene treatment, and these flowers senesced gradually. Levels of ethylene receptor genes in the petals on Day 3 did not differ from those on Day 15 in MR and MS (Fig. 6A,B). A previous study reported that ethylene responsiveness cannot be attributed to ethylene receptor gene levels[36]. In addition, ethylene binding did not change during petal development[4]. Thus, ethylene receptors would not be related to the decrease in ethylene sensitivity and auto-catalytic ethylene production with aging, and aging does not affect the expression pattern of the ethylene receptors gene.

Three *EIN3-like* (*EIL*) genes, *Dc-EIL1/2*, *Dc-EIL3*, and *Dc-EIL4*, have been identified in carnations[13,16,42]. In contrast to findings in other plants[45], levels of the *Dc-EIL3* gene were regulated at the transcriptional level[16]. The pattern of *Dc-EIL3* was similar to that of some senescence-related (SR) genes, SR8 and SR12[22,41], and the up-regulation of EIL should relate to the up-regulation of ethylene biosynthesis genes[13]. Ethylene responsive elements were found in the promoter region of these SR genes, and might interact with EIL proteins[23]. Therefore, *Dc-EIL3* is a possible regulatory gene, which modulates levels of SR8, SR12 and ethylene biosynthesis genes[13,16]. In our study, levels of *Dc-EIL3* increased with exposure to ethylene in the petals of all cultivars, suggesting that its gene should be regulated by exogenous ethylene (Fig. 5B, 7B). Levels of *Dc-EIL3* were the same on Day 3 and 15 after ethylene treatment; therefore, its gene expression should not be affected by aging. On the other hand, levels of *Dc-EIL4* on Day 15 were lower than those on Day 3 in MR and MS (Fig. 7C). Thus, levels of *Dc-EIL4* should be affected by aging.

In this study, we have demonstrated that age after anthesis affects auto-catalytic ethylene production induced by exogenous ethylene. These results indicated that the decrease in auto-catalytic ethylene production with age is related to reduction in *Dc-ACS1* levels. We also showed that levels of *Dc-ACS1* and *Dc-EIL4* were affected by the aging of the petals. Additional research on the regulation of the *Dc-ACS1* gene should help us understand the effect of aging on auto-catalytic ethylene production in petals.

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References

1. Abeles, F.B., Morgan, P.W. & Saltveit, M.E.Jr. (1992) Ethylene in Plant Biology: Academic Press, Inc. New York.
2. Barden, L.E. & Hanan, J.J. (1972) Effect of ethylene on carnation keeping Life. *J. Am. Soc. Hort. Sci.*, **97**, 785–788.
3. Borochov, A. & Woodson, W.R. (1989) Physiology and biochemistry of flower petal senescence. *Hort. Rev.* **11**: 15–43.
4. Brown, J.H. et al. (1986) Ethylene binding to senescing carnation petals. *J. Exp. Bot.*, **37**, 526–534.
5. Camprubi, P. & Nichols, R. (1978) Effects of ethylene on carnation flowers (*Dianthus caryophyllus* L.) cut at different stages of development. *J. Hort. Sci.*, **53**, 17–22.
6. Chao, Q. et al. (1997) Activation of the ethylene gas response pathway in *Arabidopsis* by the nuclear protein ETHYLENE-INSENSITIVE3 and related proteins. *Cell*, **89**, 1133–1144.
7. Deneke, C.F., Evensen, K.B. & Craig, R. (1990) Regulation of petal abscission in *Pelargonium* × *Domesticum*. *Hortscience*, **25**, 937–940.

8. Evensen, K.B. (1991) Ethylene responsiveness changes in *pelargonium* × *domesticum* Florets. *Physiol. Plant.*, **82**, 409–412.
9. Goto, R. et al. (1999) Role of ethylene on flower senescence of *Torenia*. *J. Japan. Soc. Hort. Sci.*, **68**, 263–268.
10. Guo, H. & Ecker, J.R. (2003) Plant responses to ethylene gas are mediated by SCF(EBF1/EBF2)-dependent proteolysis of EIN3 transcription factor. *Cell*, **115**, 667–77.
11. Halevy, A.H. & Mayak, S. (1981) Senescence and postharvest physiology of cut flowers, Part 2. *Hort. Rev.*, **3**, 59–143.
12. Henskens, J.A. et al. (1994) Molecular cloning of two different ACC synthase PCR fragments in carnation flowers and organ-specific expression of the corresponding genes. *Plant Mol. Biol.*, **26**, 453–458.
13. Hoeberichts, F.A. et al. (2007) Sucrose prevents up-regulation of senescence-associated genes in carnation petals. *J Exp. Bot.*, **58**, 2873–2885.
14. Hua, J. & Meyerowitz, E.M. (1998) Ethylene responses are negatively regulated by a receptor gene family in *Arabidopsis thaliana*. *Cell*, **94**, 261–271.
15. Ichimura, K., Shimamura, M. & Hisamatsu, T. (1998) Role of ethylene in senescence of cut *Eustoma* flowers. *Post. Biol. Tech.*, **14**, 193–198.
16. Iordachescu, M. & Verlinden, S. (2005) Transcriptional regulation of three EIN3-like genes of carnation (*Dianthus caryophyllus* L. cv. Improved White Sim) during flower development and upon wounding, pollination, and ethylene exposure. *J. Exp. Bot.*, **56**, 2011–2018.
17. Jones, M.L. (2002) Ethylene responsiveness in carnation styles is associated with stigma receptivity. *Sex. Plant Rep.*, **15**, 107–112.
18. Jones, M.L. & Woodson, W.R. (1999) Differential expression of three members of the 1-aminocyclopropane-1-carboxylate synthase gene family in carnation. *Plant Physiol.*, **119**, 755–64.
19. Kende, H. (1993) Ethylene biosynthesis. *Ann. Rev. Plant Physiol. Plant Mol. Biol.*, **44**, 283–307.
20. Kieber, J.J. et al. (1993) *CTR1*, a negative regulator of the ethylene response pathway in *Arabidopsis*, encodes a member of the Raf family of protein kinases. *Cell*, **72**, 427–41.
21. Kosugi, Y. et al. (2000) Expression of genes responsible for ethylene production and wilting are differently regulated in carnation (*Dianthus caryophyllus* L.) petals. *Plant Sci.*, **158**, 139–145.
22. Lawton, K.A. et al. (1989) Molecular-cloning and characterization of senescence-related genes from carnation flower petals. *Plant Physiol.*, **90**, 690–696.
23. Lawton, K.A. et al. (1990) Regulation of senescence-related gene-expression in Carnation flower petals by ethylene. *Plant Physiol.*, **93**, 1370–1375.
24. Lee, J.H. & Kim, W.T. (2003) Molecular and biochemical characterization of VR-EILs encoding mung bean ETHYLENE INSENSITIVE3-LIKE proteins. *Plant Physiol.*, **132**, 1475–1488.
25. Mayak, S. & Tirosh, T. (1993) Unusual ethylene-related behavior in senescing flowers of the Carnation Sandrosa. *Physiol. Plant.*, **88**, 420–426.
26. Onozaki, T., Ikeda, H. & Shibata, M. (2004) Video evaluation of ethylene sensitivity after anthesis in carnation (*Dianthus caryophyllus* L.) flowers. *Sci. Hort.*, **99**, 187–97.
27. Onozaki, T. et al. (2006) Breeding and characteristics of carnation norin No.1 'Miracle Rouge' and No2.'Miracle Symphony' with long vase life. *Bull. Nat. Inst. Floric. Sci.*, **5**, 1–16.
28. Onozaki, T., Ikeda, H. & Yamaguchi, T. (2001) Genetic improvement of vase life of carnation flowers by crossing and selection. *Sci. Hort.*, **87**, 107–120.
29. Onozaki, T. et al. (2006) Breeding of carnations (*Dianthus caryophyllus* L.) for long vase life and rapid decrease in ethylene sensitivity of flowers after anthesis. *J. Japan. Soc. Hort. Sci.*, **75**, 256–263.
30. Park, K.Y., Drory, A. & Woodson, W.R. (1992) Molecular-cloning of an 1-aminocyclopropane-1-carboxylate synthase from senescing Carnation flower petals. *Plant Mol. Biol.*, **18**, 377–386.
31. Potuschak, T. et al. (2003) EIN3-dependent regulation of plant ethylene hormone signaling by two arabidopsis F box proteins: EBF1 and EBF2. *Cell*, **115**, 679–89.
32. Reid, M.S. & Wu, M.J. (1992) Ethylene and flower senescence. *Plant Growth Regul.*, **11**, 37–43.
33. Rieu, I., Mariani, C. & Weterings, K. (2003) Expression analysis of five tobacco EIN3 family members in relation to tissue-specific ethylene responses. *J. Exp. Bot.*, **54**, 2239–2244.
34. Satoh, S. & Waki, K. (2006) Repressed expression of DC-ACS1 gene in a transgenic carnation supports the role of its expression in the gynoecium for ethylene production in senescing flower. *J. Japan. Soc. Hort. Sci.*, **75**, 173–177.
35. Shibuya, K. et al. (2004) The central role of PhEIN2 in ethylene responses throughout plant development in petunia. *Plant Physiol.*, **136**, 2900–2912.
36. Shibuya, K. et al. (2002) Comparison of mRNA levels of three ethylene receptors in senescing flowers of carnation (*Dianthus caryophyllus* L.). *J. Exp. Bot.*, **53**, 399–406.
37. Shibuya, K. et al. (2000) Role of the gynoecium in natural senescence of carnation (*Dianthus caryophyllus* L.) flowers. *J. Exp. Bot.*, **51**, 2067–273.
38. Tanase, K. et al. (2008) Differential expression levels of ethylene biosynthetic pathway genes during senescence of long-lived carnation cultivars. *Post. Biol. Tech.*, **47**, 210–217.
39. ten Have, A. & Woltering, E.J. (1997) Ethylene biosynthetic genes are differentially expressed during carnation (*Dianthus caryophyllus* L.) flower senescence. *Plant. Mol. Biol.*, **34**, 89–97.
40. Tieman, D.M. et al. (2001) Members of the tomato LeEIL (EIN3-like) gene family are functionally redundant and regulate ethylene responses throughout plant development. *Plant J.*, **26**, 47–58.
41. Verlinden, S., Boatright, J. & Woodson, W.R. (2002) Changes in ethylene responsiveness of senescence-related genes during carnation flower development. *Physiol. Plant.*, **116**, 503–511.
42. Waki, K. et al. (2001) Cloning of a cDNA encoding EIN3-like protein (DC-EIL1) and decrease in its mRNA level during senescence in carnation flower tissues. *J. Exp. Bot.*, **52**, 377–379.
43. Wang, H. & Woodson, W.R. (1989) Reversible inhibition of ethylene action and interruption of petal senescence in Carnation flowers by norbornadiene. *Plant Physiol.*, **89**, 434–438.
44. Wang, H. & Woodson, W.R. (1991) A flower senescence-related messenger-RNA from Carnation shares sequence similarity with fruit ripening-related messenger-RNAs involved in ethylene biosynthesis. *Plant Physiol.*, **96**, 1000–1001

45. Wang. K.L., Li, H. & Ecker, J.R. (2002) Ethylene biosynthesis and signaling networks. *Plant Cell*, S131–51.
46. Whitehead, C.S. & Halevy, A.H. (1989) Ethylene sensitivity - the role of short-chain saturated fatty-acids in pollination-induced senescence of petunia-hybrida flowers. *Plant Growth Regul.*, **8**, 41–54.
47. Woltering, E.J. & van Doorn, W.G. (1988) Role of ethylene senescence of petals - Morphological and taxonomical relationship. *J. Exp. Bot.*, **39**, 1605–16.
48. Woodson, W.R. & Lawton, K.A. (1988) Ethylene-induced gene-expression in Carnation petals - Relationship to autocatalytic ethylene production and senescence. *Plant Physiol.*, **87**, 498–503.
49. Woodson, W.R. et al. (1992) Expression of ethylene biosynthetic-pathway transcripts in senescing carnation flowers. *Plant Physiol.*, **99**, 526–532.
50. Yanagisawa, S., Yoo, S.D. & Sheen, J. (2003) Differential regulation of EIN3 stability by glucose and ethylene signalling in plants. *Nature*, **425**, 521–425.
51. Yang, S.F. & Hoffman, N.E. (1984) Ethylene biosynthesis and its regulation in higher plants. *Annu. Rev. Plant Physiol.*, **35**, 155–189.
52. Yokotani, N. et al. (2003) Characterization of a novel tomato EIN3-like gene (*LeEIL4*). *J Exp. Bot.*, **54**, 2775–2776.