Mechanism of Senescence in Carnation Flowers

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Abstract

Senescence of carnation flowers is characterized by autocatalytic ethylene production from petals and subsequent wilting of the petals. Recent studies on the regulation of ethylene production and wilting in senescing carnation flowers revealed that (1) petal senescence is triggered by ethylene produced from the gynoecium during natural senescence, (2) there are two subsets of ethylene responses in the petals; one responsible for autocatalytic ethylene production and the other for wilting, and (3) in the petals ACC oxidase (*DC-ACO1*) and ACC synthase (*DC-ACS1*) genes are sequentially in this order. The expression of the *DC-ACO1* gene probably affects that of the *DC-ACS1* gene.

INTRODUCTION

Ethylene is the primary plant hormone involved in the senescence of cut carnation flowers. Natural senescence of carnation flowers culminates in autocatalytic ethylene production from petals and subsequent wilting of the petals. The whole mechanism of senescence of the flowers is far from being well understood. The gynoecium has been suggested to play a role in the induction of petal senescence, i.e., ethylene evolved from the gynoecium acts as a diffusible signal that is perceived by the petals and induces the onset of senescence in the petals (Nichols, 1977; Jones and Woodson, 1997; ten Have and Woltering, 1997). However, this proposal has remained a matter of dispute because petal senescence in the cut carnation flower was not delayed by the removal of gynoecium (Mor et al., 1980; Sacalis and Lee, 1987; Woodson and Brandt, 1991). Autocatalytic ethylene production and wilting occur simultaneously during petal senescence (Borochov and Woodson, 1989; Reid and Wu, 1992). However, there have been few studies on how these two phenomena are regulated in terms of gene expression involved in these events. Furthermore, the expression of ACC synthase (ACS) (*DC-ACS1*) and ACC oxidase (ACO) (*DC-ACO1*) genes is induced in carnation petals undergoing autocatalytic ethylene production. These two genes are usually expressed together, but little is known about the control mechanism for their simultaneous expression. We have studied the mechanism of these events in the natural senescence of carnation flowers.

ROLE OF THE GYNOECIUM IN NATURAL SENESCENCE OF CARNATION FLOWERS

Although the petals contribute substantially to the ethylene that is produced during natural senescence in carnation flowers, the gynoecium produces a significant amount of ethylene before the onset of production of the gas in the petals (Nichols, 1977; Woodson et al., 1992; ten Have and Woltering, 1997). During natural senescence, ACC content and ethylene production increase in the gynoecium before the increase in the petals (Nichols, 1977; Veen and Kwakkenbos, 1984; Hsieh and Sacalis, 1986). The expression of ACS and ACO genes (and ethylene production) first started in the ovary followed by the style and petals, in senescing carnation flowers (ten Have and Woltering, 1997). If the petals were detached from the flowers at the presenescent stage, they showed no ethylene production and had a prolonged life-span (ten Have and Woltering, 1997). These findings clearly suggest a role for the gynoecium in controlling petal senescence in carnation flowers.

However, removal of the gynoecium did not delay petal senescence of cut carnation flowers (Mor et al., 1980; Sacalis and Lee, 1987; Woodson and Brandt, 1991). These results are in apparent conflict with the proposed role of the gynoecium in controlling petal senescence. In contrast to previous investigations where the gynoecium was removed by forceps or scissors, Shibuya et al. (2000) carefully snapped them off by hand. This treatment prevented the increase in ethylene production and markedly prolonged flower life (Fig. 1). This experiment showed a decisive role of the gynoecium in controlling natural senescence of carnation flowers.

Treatment with exogenous ethylene induced autocatalytic ethylene production and petal wilting in the flowers with gynoecia removed. By contrast, abscisic acid (ABA) and indole-3-acetic acid, which induced ethylene production and accelerated petal senescence in carnation flowers, did not stimulate ethylene production in the flowers with gynoecia removed (Shibuya et al., 2000). These results strongly indicate that ethylene produced in the gynoecium triggers the onset of ethylene production in the petals, resulting in petal wilting in carnation flowers.

REGULATION OF ETHYLENE PRODUCTION AND WILTING IN CARNATION PETALS

So far, three ACS genes (*DC-ACS1*, *DC-ACS2*, *DC-ACS3*) and one ACO gene (*DC-ACO1*) have been identified from carnation (Wang and Woodson, 1991; Park et al., 1992; Henskens et al., 1994; Jones and Woodson, 1999). These genes are regulated in a tissue-specific manner during senescence. *DC-ACS1* is expressed both in the gynoecium and petals during senescence. *DC-ACS1* is expressed mainly in petals whereas *DC-ACS2* and *DC-ACS3* expression occurs mainly in the gynoecium (Henskens et al., 1994; ten Have and Woltering, 1997; Jones and Woodson, 1999).

Ethylene, emanating from the gynoecium, induces autocatalytic ethylene production and wilting in carnation petals. The autocatalytic ethylene production in the petals is due to expression of *DC-ACS1* and *DC-ACO1*. Petal wilting in flower senescence is caused by the decomposition of cell constituents by hydrolytic enzymes such as proteinase, lipase and nuclease. Cysteine proteinase (CPase) is probably one of the enzymes responsible for hydrolytic degradation of cell components leading to cell death during senescence of petals (Jones et al., 1995; Panavas et al., 1998, 1999). A CPase (*DC-CP1*) gene is up-regulated during natural and exogenous ethylene-induced senescence of carnation petals (Fig. 2). It is thought that the ethylene production and wilting are regulated in concert and cannot be separated, since the two events occur almost simultaneously.

Kosugi et al. (2000) examined the responses of petals of a transgenic carnation line to exogenous ethylene with regard to the induction of petal inward rolling, ethylene production and some genes involved in these events. They used the sACO-1 line, harboring an *sACO* transgene (*DC-ACO1* cDNA in sense orientation under the control of a strong constitutive promoter, CaMV35S, with additional enhancer sequences). Cut flowers of the sACO-1 line produced only a trace amount of ethylene during the senescence period, and had a vase life about 2-fold longer than flowers of the parent cv. Nora. Interestingly, the treatment with ethylene of petals detached from the sACO-1 flowers caused accumulation of transcript for *DC-CP1* and petal wilting, but it did not induce accumulation of transcripts for *DC-ACO1* and *DC-ACS1* and ethylene production (Fig. 3). These findings indicated that the exogenous ethylene-induced expression of *DC-ACS1* and *DC-ACO1* genes, leading to autocatalytic ethylene production, and that of *DC-CP1* gene, leading to petal wilting, were regulated separately in the petals of the sACO-1 flowers. Based on these findings, we propose the presence of two subsets of ethylene responses leading to the expression of respective genes in carnation petals as discussed later (Fig. 6).

EXPRESSION OF *DC-ACS1* AND *DC-ACO1* GENES IN CARANTION PETALS

The absence of accumulation of transcripts for both *DC-ACO1* and *DC-ACS1*

genes in the petals of sACO-1 flowers treated with exogenous ethylene indicated the simultaneous repression of expression of the two genes in the petals (Fig. 3). *DC-ACO1* transcript was not accumulated in the sACO-1 petals probably because of the action of the *sACO* transgene, since the introduced *sACO* transgene seemed to cause co-suppression of the endogenous *DC-ACO1* gene. However, this does not explain why the *DC-ACS1* transcript was not accumulated in the sACO-1 petals after treatment with exogenous ethylene, since the sensitivity of the petal to ethylene was unaltered, which was proved by the wilt- ing of petals, and since ethylene was administered exogenously at a sufficient concentration and treatment duration (10 µl L^{-1} ethylene for 18 h). What causes the simultaneous suppression of the expression of *DC-ACS1* and *DC-ACO1* genes in the exogenous ethylene treated petals of sACO-1 flowers? One explanation could be that the expression of *DC-ACS1* gene in the petals of carnation flowers requires simultaneous expression of *DC-ACO1* gene, and probably vice versa, by a still unknown mechanism. If this were the case, treatment with exogenous ethylene should cause no accumulation of *DC-ACO1* transcript in petals of transgenic carnation flowers in which the expression of endogenous *DC-ACS1* gene is suppressed.

We examined the expression of these genes in carnation petals in two transgenic carnation lines, the sACO-1 line, described above, and the 16-0-66 line harboring an *sACS* transgene (*DC-ACS1* cDNA in sense orientation), as compared with that in their parent cvs Nora and Ashley, respectively. Flowers of the 16-0-66 line produced ethylene in a trace amount during natural senescence, probably due to co-suppression by the *sACS* transgene of the expression of the corresponding endogenous *DC-ACS1* gene. Treatment with exogenous ethylene induced petal wilting and ethylene production from 'Nora' and 'Ashley' flowers at the pre-senescence stage (Fig. 4). The ethylene production was accompanied by an accumulation of *DC-ACO1* transcript followed by that of *DC-ACS1* one, confirming the previously shown sequential expression of the two genes in carnation petals (Fig. 5). On the other hand, in the sACO-1 and 16-0-66 transgenic flowers, treatment with exogenous ethylene caused petal in-rolling (Fig. 4), but did not induce ethylene production in a significant amount (Fig. 5). Exogenous ethylene caused little accumulation of *DC-ACO1* and *DC-ACS1* transcripts in the sACO-1 petals, whereas it caused accumulation of *DC-ACO1* transcript, but not of *DC-ACS1*, in the 16-0-66 petals (Fig. 5), suggesting that the expression of the *DC-ACO1* gene must precede that of the *DC-ACS1* gene in carnation petals. Based on these findings, we propose that in carnation petals not only are *DC-ACO1* and *DC-ACS1* genes expressed sequentially in this order, but also the expression of the *DC-ACO1* gene affects that of the *DC-ACS1* gene in a still unknown way (Fig. 6).

A similar regulation of gene expression has been reported in the expression of genes for phenylalanine ammonia-lyase (PAL) and cinnamic acid 4-hydroxylase (C4H). The two enzymes catalyze the conversion of L-phenylalanine into cinnamic acid and that of cinnamic acid into *p*-coumaric acid, respectively, at the entry point into the phenylpropanoid pathway. Blount et al. (2000) demonstrated that PAL activity was reduced in transgenic tobacco plant tissues in which C4H activity had been genetically down-regulated, whereas C4H activity was not reduced in plants in which PAL activity had been downregulated by gene-silencing. They suggested that the reduction in PAL enzyme activity in C4H down-regulated plants occurred, at least in part, by the down-regulation of PAL gene expression caused by cinnamic acid, which was shown to act as a negative feedback regulator of the expression and enzymatic activity of PAL. Regulation of *PAL/C4H* gene expression is one method of examining the expression of *DC-ACS1* and *DC-ACO1* genes are regulated in carnation petals. Recently, Jones (2003) reported that ACC induced the expression of ACS and ACO genes in carnation styles, indicating that ACC could act as a modulator of expression of these genes. If ACC could act as a negative feedback regulator of expression of *DC-ACS1* gene in the petals of carnation flowers, the down-regulation of *DC-ACO1* gene may result in the simultaneous down-regulation of *DC-ACS1* gene in the petals.

CONCLUSION

We showed some of our findings on the mechanism of senescence in carnation flowers. Removal of the gynoecium prevented the onset of ethylene production and prolonged the vase life of the flower. The results indicate that ethylene produced in the gynoecium triggered the onset of ethylene production in the petals, resulting in petal wilting.

Upon perception of ethylene, carnation petals begin autocatalytic ethylene production and the petals then also start to wilt. We showed that ethylene production and wilting are separately regulated in carnation petals. Based on this, we propose the presence of two subsets of ethylene responses leading to the expression of respective genes in carnation petals (Fig. 6). One subset leads to the expression of genes for *DC-ACO1* and *DC-ACS1*, in this order, resulting in autocatalytic ethylene production. The expression of *DC-ACO1* gene precedes and affects the expression of *DC-ACS1*. The mechanism of this regulation remains to be elucidated. The other subset leads to the expression of *DC-CP1* gene, and probably other genes for enzymes for hydrolytic degradation, such as aspartic proteinase (unpublished) and lipase (Hong et al., 2000), resulting in petal wilting. In carnation petals the *DC-CP1* gene is expressed substantially already at the full opening stage (day 0), but its expression is elevated by exogenous ethylene treatment (Fig. 2).

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Figures

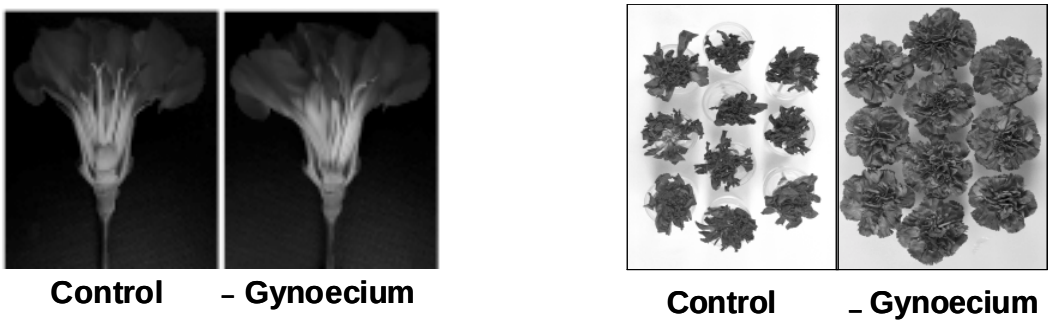

Fig. 1. Effects of the removal of the gynoecium on senescence of carnation flowers. Left, The gynoecium was removed or left intact at the stage of full opening. Right, Flowers 8 d after removal of gynoecia. The carnation cultivar used was ‘Reiko’.

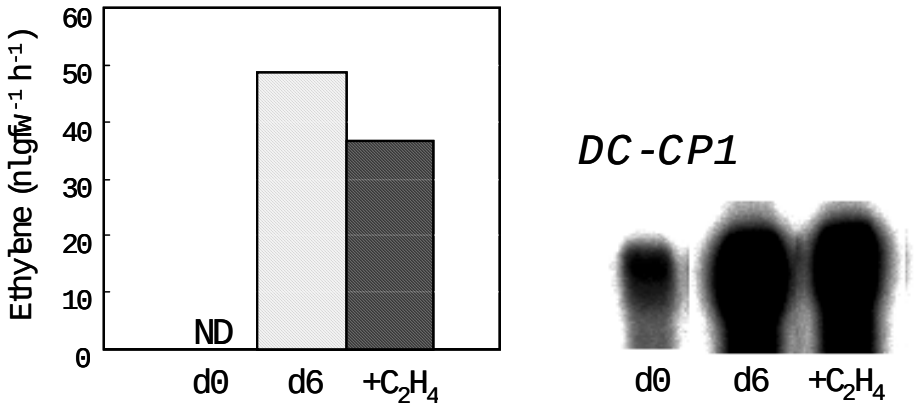

Fig. 2. Ethylene production and expression of a cysteine proteinase gene, *DC-CP1*, in carnation petals.

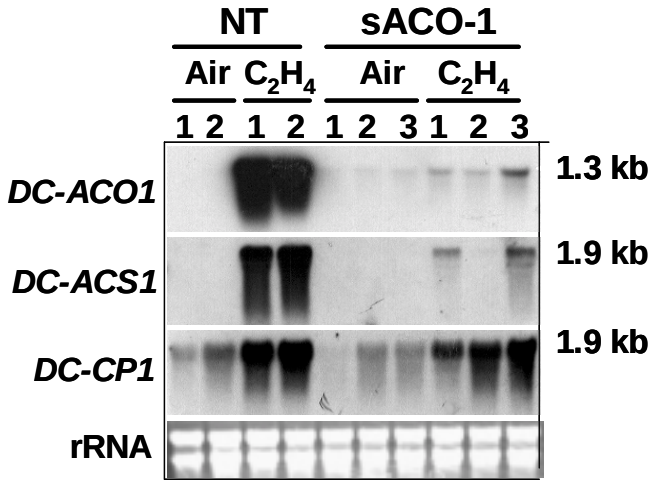

Fig. 3. Accumulation of transcripts for *DC-ACO1*, *DC-ACS1* and *DC-CP1* in petals of control (NT) and sACO-1 transgenic lines after treatment with ethylene at 10 µl L^{-1} for 18 h.

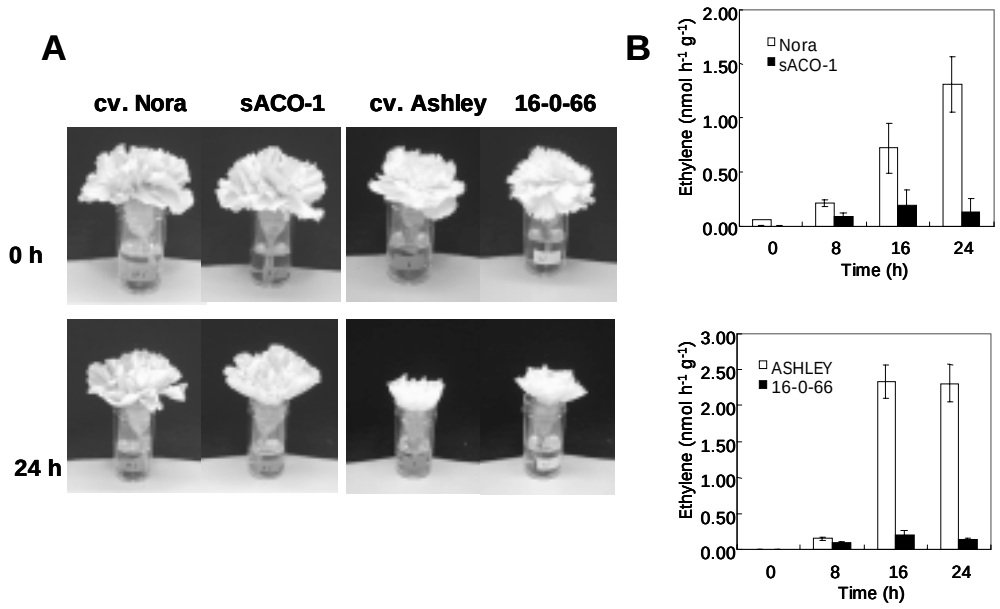

Fig. 4. Petal wilting (**A**) and ethylene production (**B**) in whole flowers of transgenic lines, sACO-1 and 16-0-66, and corresponding parent cvs Nora and Ashley, respectively. The sACO-1 line contains an *sACO* transgene, whereas the 16-0-66 line an *sACS* transgene. Flowers were photographed at 0 and 24 h after the start of ethylene treatment at 10 µl L^{-1}. Other sets of carnation flowers were subjected to measurement of ethylene production at given time of ethylene treatment.

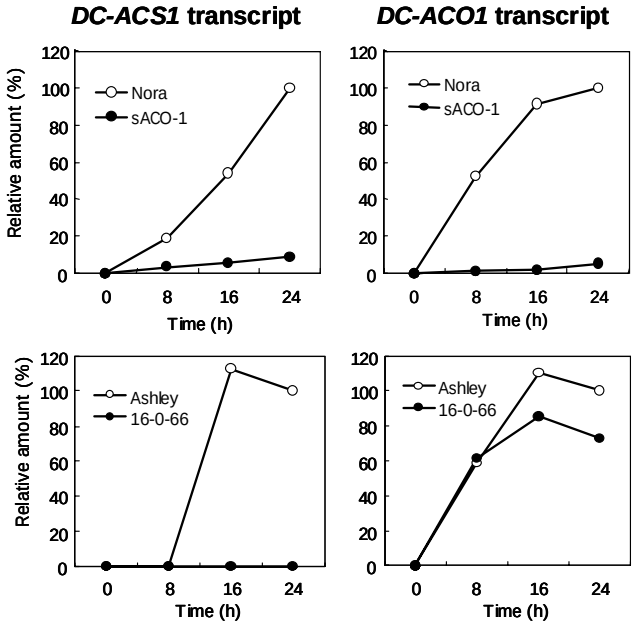

Fig. 5. Changes in the amount of *DC-ACS1* and *DC-ACO1* transcripts in the petals of flowers treated with ethylene at 10 µl L^{-1} of cv. Nora and the sACO-1 line as well as cv. Ashley and the 16-0-66 line. Data are shown by the relative amounts of transcripts when the amount of the respective non-transformed control at 24 h is 100%.

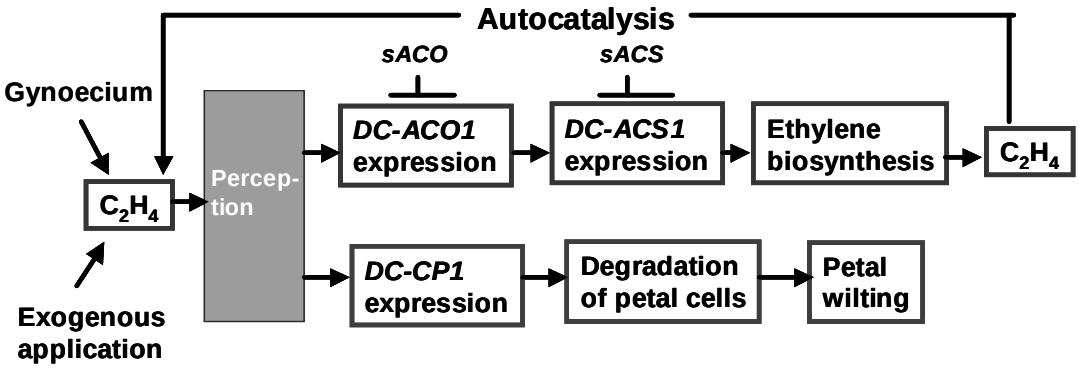

Fig. 6. Regulation of ethylene production and wilting in carnation petals. Three ACS genes (*DC-ACS1*, *DC-ACS2*, *DC-ACS3*) and one ACO gene (*DC- ACO1*) have been identified from carnation. These genes are expressed in a tissue-specific manner; only *DC-ACS1* gene, out of the three ACS genes, and *DC-ACO1* gene are expressed in the petals of carnation flowers during senescence. DC-CP1, a cysteine proteinase.