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Two Ethylene Signaling Pathways in Senescing Carnation Petals: Exogenous Ethylene-induced Expression of Genes for 1-Aminocyclopropane-1-Carboxylate (ACC) Synthase and ACC Oxidase is Different from That of the Gene for Cysteine Proteinase

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Abstract

Carnation petals exhibit autocatalytic ethylene production and wilting during senescence. The autocatalytic ethylene production is induced by the expression of 1-aminocyclopropane-1-carboxylate (ACC) synthase and ACC oxidase genes, whereas the wilting of petals is related to expression of the cysteine proteinase (CP) gene. Until recently, it has been believed that these two phenomena, autocatalytic ethylene production and wilting, are regulated in concert in senescing carnation petals, since the two phenomena occurred closely in parallel. Our studies with petals of a transgenic carnation harboring a sense ACC oxidase transgene and petals of carnation flowers treated with 1,1-dimethyl-4-(phenylsulfonyl)semicarbazide showed that the expression of ACC synthase and ACC oxidase genes and that of CP are regulated differently in carnation petals. Interestingly, in the petals of transgenic carnation, the transcript for CP was accumulated but the transcripts for ACC synthase and ACC oxidase were not accumulated in response to exogenous ethylene. Based on these results, we hypothesized that two ethylene signaling pathways, one leading to the expression of ACC synthase and ACC oxidase genes and the other leading to the expression of CP gene, are functioning in senescing carnation petals.

Introduction

Ethylene is the primary plant hormone involved in the senescence of cut carnation flowers (Abeles et al., 1992; Borochov and Woodson, 1989; Reid and Wu, 1992). A large amount of ethylene is synthesized several days after full opening of the flowers during natural senescence (Manning, 1985; Peiser et al., 1986; Woodson et al., 1992), or several hours after compatible pollination (Nichols, 1977; Nichols et al., 1983; Larsen et al., 1995) or after the treatment with exogenous ethylene (Borochov and Woodson, 1989; Wang and Woodson, 1989).

Ethylene is produced first in the pistil during natural (Shibuya et al., in submission) and pollination -induced (Jones and Woodson, 1999a) senescence. Then the produced ethylene, acting as a diffusible signal, is perceived by petals and induces autocatalytic ethylene production in the petals, resulting in in-rolling of petal margins and wilting of the whole petals. The ethylene produced by autocatalysis in the petals accounts for a large portion of ethylene produced by carnation flowers during senescence. On the other hand, exogenous ethylene applied to carnation flowers directly acts on petals, and induces autocatalytic ethylene production and wilting of the petals (Shibuya et al., in submission).

Ethylene is synthesized through the following pathway: L-methionine → S-adenosyl-L-methionene → 1-aminocyclopropane-1-carboxylate (ACC) → ethylene. ACC synthase and ACC oxidase catalyze the last two reactions (Yang and Hoffman, 1984; Kende, 1993). The increase in ethylene production in car-

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nation petals is accompanied by the expression of genes for both ACC synthase (*DC-ASC1*: Jones and Woodson, 1999b) and ACC oxidase (*DC-ACO1* corresponding to pSR120 cDNA: Park et al., 1992). Petal wilting in flower senescence is caused by the decomposition of cell constituents by hydrolytic enzymes such as protease and nuclease (Panavas et al, 1998, 1999). Cysteine proteinase (CP) may be one of the enzymes responsible for hydrolytic degradation of cell components leading to cell death during senescence of petals (Panavas et al., 1998; 1999). A CP gene is up-regulated during natural, pollination-induced and exogenous ethylene-induced senescence of carnation petals (Jones et al., 1995). So far, it has been believed that autocatalytic ethylene production and petal wilting are regulated in concert and can not be separated, since they occur closely in parallel. Recently, we found that the expression of ACC synthase and ACC oxidase genes in response to exogenous ethylene, could be regulated different from that of CP gene in petals of transgenic carnation harboring a sense ACC oxidase transgene and in the petals of carnation flowers treated with 1,1-dimethyl-4-(phenylsulfonyl)semicarbazide (DPSS). We also revealed the presence of transcripts for the components of ethylene signaling pathway in carnation petals, which are homologous to those in *Arabidopsis thaliana*. Based on these observations, we suggest the presence of two ethylene signaling pathways in carnation petals, one leading to the expression of ACC synthase and ACC oxidase and the other leading to the expression of the CP gene.

Difference of the expression of genes for ACC synthase and ACC oxidase from that of the gene for CP

Experiments with the petals of the transgenic carnation harboring a sense ACC oxidase transgene

We have recently generated more than 10 lines of carnation plants transformed with transgenes of carnation ACC synthase or ACC oxidase, which expressed respective genes in sense or antisense orientation (Kosugi et al., in submission). The line sACO1 is a transformant harboring at least 5 copies of a sense ACC oxidase transgene under the control of a strong constitutive promoter El2W (Mochizuki et al., 1999). The transgenic carnation plant grew and flowered normally like the non-transformed control plant. Cut flowers of the transgenic carnation produced only a trace amount of ethylene during the senescence period, and had a vase-life about 2-fold longer than that of the control flowers. The suppressed ethylene production was accompanied by the decrease in the activities of ACC oxidase and ACC synthase as well as ACC content in petals. In addition, there was no accumulation of transcripts for ACC oxidase and ACC synthase in the petals of the transgenic carnation, suggesting that the sense ACC oxidase transgene caused cosuppression of the expression of ACC oxidase gene, which was accompanied by the blockage of the expression of the ACC synthase gene resulting in the absence of autocatalytic induction of ethylene production.

Interestingly, the treatment with ethylene of petals detached from the transgenic carnation flowers caused the accumulation of the transcript for CP and petal in-rolling, but not the accumulation of the transcripts for ACC oxidase and ACC synthase and ethylene production (Figure 1). The findings indicated that the exogenous ethylene-induced expression of ACC synthase and ACC oxidase genes, leading to autocatalytic ethylene production, and that of CP gene, leading to petal wilting, were regulated differently in the petals of the transgenic carnation. Also, the findings indicated that the expression of ACC synthase gene in the petals of carnation flowers required simultaneous expression of ACC oxidase gene, and probably vice versa.

The mechanism for concerted expression of two genes remains to be elucidated.

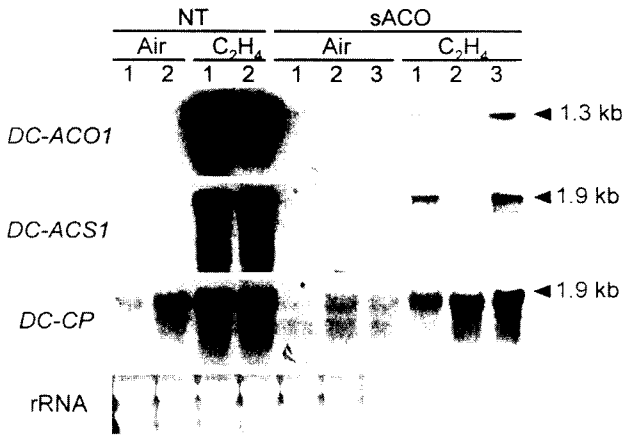

Figure 1. Changes in the levels of transcripts for ACC oxidase, ACC synthaase and CP in detached petals after the treatment with exogenous ethylene. Petals detached from flowers at full opening stage (day 0) were treated with or without 10 μL/L ethylene for 18 h. Two individual flowers (1 and 2) were used for the control line, and 3 individual flowers (1, 2 and 3) for the transgenic line. Ten μg of total RNAs isolated from petals was separated on an agarose gel and hybridized with DIG-labeled *DC-ACO1*, *DC-ACS1* or CP probes. NT, non-transformed control line (cv. Nora); sACO, the transgenic line sACO1.

Experiments with the petals of carnation flowers treated with DPSS, an antisenescent preservative

The investigation of the action mechanism of DPSS also indicated a difference of the regulation of genes for ACC synthase and ACC oxidase from that of the gene for CP in carnation petals. DPSS is an antisenescence preservative for cut carnation flowers (Midoh et al., 1996). DPSS prolongs the vase-life of carnation flowers by preventing ethylene production, but does not affect the ethylene-induced senescence in the flowers (Midoh et al., 1996). Application of DPSS inhibits the increase in activities of ACC synthase and ACC oxidase, which occurred in non-treated control flowers during natural senescence. DPSS does not inhibit *in vitro* activities of either ACC synthase or ACC oxidase obtained from senescing carnation petals (Satoh et al., 1997). Recently, we revealed that the inhibitory action of DPSS was specific to the ethylene production in carnation flowers undergoing natural senescence, and that DPSS did not inhibit the ethylene production induced by exogenous ethylene in carnation flowers, that induced by indole-3-acetic acid in mungbean hypocotyl segments and that induced by wounding in winter squash mesocarp tissue (Onoue et al., 2000).

On the other hand, in carnation flowers, abscisic acid (ABA) accumulated transiently before the onset of ethylene production during natural senescence, and exogenously applied ABA accelerated flower senescence by stimulating ethylene production (Onoue et al., 2000). These results suggest that ABA triggers the senescence of carnation flowers. Interestingly, we observed that DPSS prevented the accumulation of ABA in the pistil and petals 2 days before the onset of ethylene production in carnation flowers. Thus, DPSS seems to exert its inhibitory action on ethylene production in naturally-senescing carnation flowers through the action on the ABA-related process.

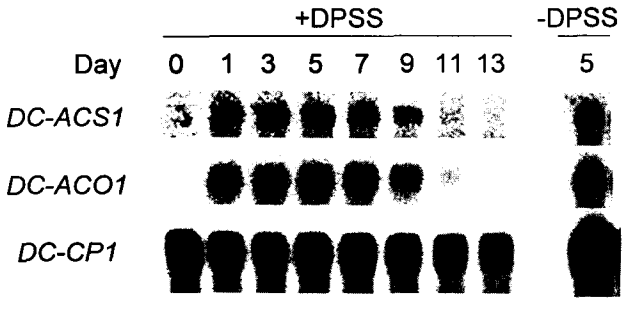

Figure 2. Changes in the levels of transcripts for ACC oxidase, ACC synthase and CP in petals of carnation flowers treated with DPSS. Carnation flowers at day 0 were treated with 0.1 mM DPSS for 24 h, then left for sampling of the petals at given time. Two *mU*g of mRNAs isolated from petals was separated on an agarose gel and hybridized with ^{32}P-labeled *DC-ACO1*, *DC-ACS1* or CP probes. For reference, the mRNAs isolated from petals of flowers, which underwent natural senescence for 5 days, were treated similarly.

Most recently, we found that the administation of DPSS to carnation flowers at the day of full opening induced the accumulation of transcripts for ACC synthase and ACC oxidase from the next day on, but not that for CP (Figure 2). The mechanism of the action of DPSS on the up-regulation of ACC synthase and ACC oxidase genes and no increase in the activities of the enzymes in carnation petals are now under investigation. Anyway, the findings clearly indicated that carnation petals have an ability to regulate the expression of genes for ACC synthase and ACC oxidase independent of that for CP.

The pathway of exogenous ethylene-induced expression of ACC synthase and ACC oxidase genes is different from that of the CP gene in carnation petals.

As mentioned above, senescence of carnation petals is accompanied by autocatalytic ethylene production and wilting of the petals; the former is caused by the expression of ACC synthase and ACC oxidase genes and the latter is related to the expression of CP gene. To our knowledge, there have been no reports that showed any difference between these two processes in senescing carnation petals. However, in the present studies with the petals of a transgenic line (sACO1) of carnation and petals treated with DPSS, we could show that the mechanism of the expression of genes for ACC synthase and ACC oxidase differs from that of the gene for CP in carnation petals. In other words, carnation petals regulate the expression of ACC synthase and ACC oxidase genes independent of the CP gene.

Although only the gene expression in response to exogenous ethylene was examined in this study, it is reasonable to speculated that similar regulation also takes place in the petals of carnation flowers undergoing natural senescence, in which the ethylene evolved from the pistil acts on the petals. Based on the present observations, we propose the presence of two pathways leading to the expression of respective genes in carnation petals as shown in Figure 3. One pathway leads to the expression of ACC synthase and ACC oxidase genes and autocatalytic ethylene production. The other leads to the expression of CP gene and probably other genes for enzymes for hydrolytic degradation, and petal wilting. We hypothesize that these two physiological ethylene-de-

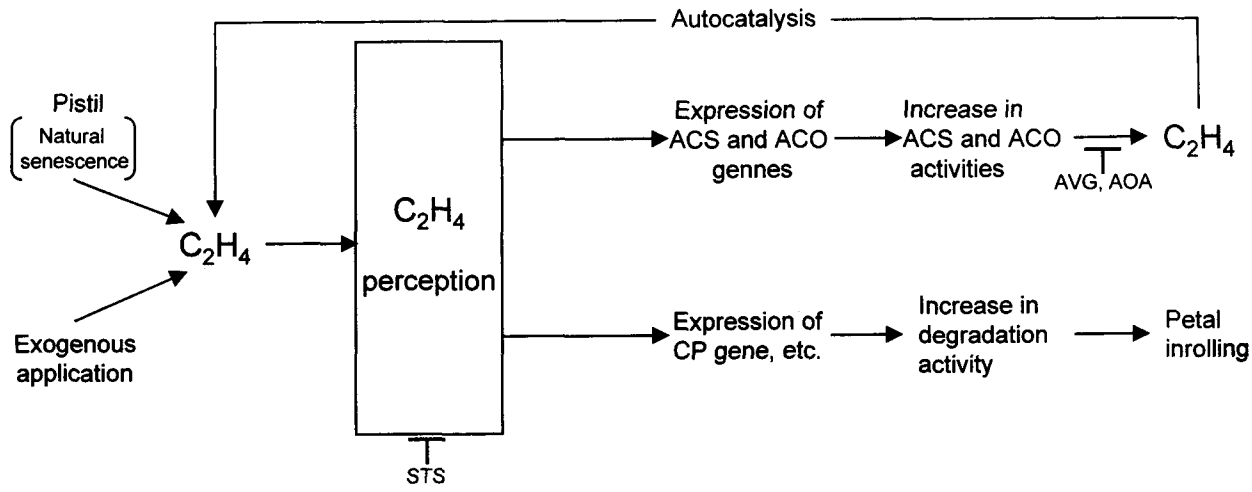

Figure 3. Two putative ethylene signaling pathways responsible for petal senescence in carnation flowers. Sites of action for known inhibitors [AVG (aminoethoxyvinyl glycine), AOA (aminooxyacetic acid) and STS] are shown in the figure.

pendent pathways represent two ethylene signaling pathways functioning in senescing carnation petals.

We have succeeded in cloning of cDNAs [i.e., *DC-CTR1*, *DC-CTR2* and *DC-EIL1*(*EIN3 Like 1*)]from carnation petals, which are counterparts of genes involved in ethylene signaling pathway in *A. thaliana* (Johnson and Ecker, 1998; Kieber, 1997; Woeste and Kieber, 1998). These results indicated that the ethylene signaling pathway in carnation petals is homologous to that in *A. thaliana* (Shibuya and Waki, unpublished). In addition, we have recently shown that the gene for ethylene receptors expressed in carnation petals during senescence is mainly DC-ERS2 (Shibuya et al., 1998), not DC-ERS1 (Charng et al., 1997), which were identified in carnation plants. It is of interest to investigate which one (or both) of the CTR homolog genes (*DC-CTR1* and *DC-CTR2*) is involved in each of the ethylene signaling pathways in carnation petals.

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