Towards Understanding the Onset of Petal Senescence: Analysis of Ethylene Production in the Long-Lasting Carnation cv. White Candle

Shigeru Satoh, Hideki Nukui, Sakiko Kudo and Takayuki Inokuma
Laboratory of Environmental Biotechnology, Graduate School of Agricultural Sciences
Tohoku University, Sendai 981-8555
Japan

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Abstract

In senescing carnation (*Dianthus caryophyllus* L.) flowers, ethylene is produced from the gynoecium, and acts as a diffusible signal received by petals to induce the expression of 1-aminocyclopropane-1-carboxylate (ACC) synthase (*DC-ACS1*) and ACC oxidase (*DC-ACO1*) genes in the petals. This results in autocatalytic ethylene production in the petals. We investigated ethylene production in cut flowers of cv. White Candle, which produce ethylene only in trace amounts and have a long vase life. The low ethylene production in these flowers was due to low ethylene production in the gynoecium, accompanied by low accumulation of ACC synthase transcripts. These findings further support the importance of ethylene production from the gynoecium in the senescence of carnation flowers. Cv. White Candle flowers had water relations that were different from those in cv. Light Pink Barbara, a cultivar that showed the normal climacteric rise of ethylene production during senescence. We discuss a factor that possibly induces ethylene production in the carnation gynoecium.

INTRODUCTION

Senescence in carnation flowers culminates in autocatalytic ethylene production and petal wilting. The ethylene production in the petals begins by the expression of ACC synthase (ACS) (*DC-ACS1*) and ACC oxidase (ACO) (*DC-ACO1*) genes upon perception of ethylene that comes from the gynoecium or is applied exogenously (Jones and Woodson, 1997; ten Have and Woltering, 1997; Shibuya et al., 2000). The ethylene production in the gynoecium is induced by compatible pollination or, in the case of nonpollinated flowers, it starts by itself several days after full opening of the flower.

Several papers deal with pollination-induced ethylene production in the carnation gynoecium (Nichols, 1977; Jones and Woodson, 1997). However, many carnation cultivars do not have anthers, and thus cannot be pollinated by their own pollen. They nonetheless show an increase in ethylene production in gynoecium, which must be induced by factors other than pollination. The mechanism of gynoecium ethylene production without pollination has thus far received little attention. It has been established, however, that the increase in ethylene production in unpollinated flowers starts in the ovary (part of the gynoecium), whereas in pollination-induced senescence it starts in the style of the gynoecium. We are interested in factors that induce ethylene production in the gynoecium of unpollinated carnation flowers.

In the present study, we investigated ethylene production in flowers of a cv. White Candle (WC), which has long lasting flowers, in comparison with that of the conventional cv. Light Pink Barbara (LPB). The work was undertaken to obtain further evidence of the role of ethylene production from the gynoecium in carnation flower senescence. Furthermore we explored a factor that induces ethylene production in the gynoecium of unpollinated senescing carnation flowers.

MATERIALS AND METHODS

We used carnation flowers (*Dianthus caryophyllus* L.) of the cvs. White Candle (WC) and Light Pink Barbara (LPB). Flowers were harvested at the full opening stage (day

0). Stem length was trimmed to 3 cm. The stem ends were placed in 20 ml distilled water in 50-ml glass vials (one flower per vial). The flowers were left under white fluorescent light (15 µmol $m^{-2} s^{-1}$) at 23 °C, and water was replaced daily. Flowers were observed daily to record senescence symptoms, and flower ethylene production was measured once each day.

For ethylene treatment, the vials with flowers on day 0 were placed in a glass chamber with ethylene at 2 µl L^{-1} for 0 to 18 h under the conditions described above. Flowers were taken at 6-h intervals from the glass chamber, and held in open air for 1 h to allow the accumulated ethylene to diffuse out of the flower tissue. Ethylene production from the whole flower was measured by enclosing the flowers in 350-ml glass containers (one flower per container) for 1 h at 23 °C. A 1-ml gas sample was taken from the container, and analyzed for ethylene with a gas chromatograph. Then the flowers were separated into gynoecium (ovary plus styles), petals, and the remaining parts (sepals plus stem). The gynoecium and petals were subjected to measurement of ethylene production by enclosing them for 1 h in glass containers of appropriate sizes. After the assay for ethylene production, the petals and gynoecium were weighed, immediately frozen in liquid N_2 and stored at – 80 °C until isolation of RNA.

Total RNA from petals and gynoecium was isolated using the SDS–phenol method (Palmiter, 1974). The presence or absence of transcripts of an ACO gene (*DC- ACO1*) and ACS genes (*DC-ACS1, DC-ACS2*, *DC-ACS3*) in total RNA fractions was determined by amplification by RT-PCR. RT-PCR was performed according to standard procedures with necessary optimization. The PCR amplicates were separated on a 2.0% agarose gel and visualized by ethidium bromide staining. To examine the identities of the operation of PCR and the amount of template RNA, we amplified the fragment of actin (*DC-ACT1,* Waki et al., 2001). The upstream and downstream primers for RT-PCR, the sizes of amplified cDNA fragments and their positions in the original cDNAs will be reported elsewhere.

Water uptake and transpiration of 'WC' and 'LPB' flowers were determined by daily measurement of flower fresh weight and the weight of the water that had been taken up from the incubation solutions.

RESULTS AND DISCUSSION

Ethylene Production in Whole Flowers, Gynoecium and Petals of Unpollinated 'WC' and 'LPB' Flowers

Fig. 1 compares ethylene production from whole flowers, petals and gynoecia in 'WC' and 'LPB' flowers. Ethylene production of whole 'WC' flowers was below the detection limit, throughout the 19 d study period. When petals and gynoecia were detached from the whole flowers, their ethylene production was also mostly below detection limit, throughout the 19 d period. By contrast, whole 'LPB' flowers produced ethylene in a significant amount. The production rate was maximal (2.70 nmol $g^{-1} h^{-1}$) on day 5. Isolated gynoecia and petals produced ethylene with a time course similar to that of whole flowers.

We determined whether the absence of ethylene production in the 'WC' flowers was a result of malfunction of ethylene biosynthesis in the gynoecia and/or the petals. We found that treatment of 'WC' flowers with exogenous ethylene caused both an increase in ethylene production (Fig. 2), and accumulation of transcripts for *DC-ACS1* and *DC-ACO1* genes in the gynoecia and petals (data not shown). This suggests that the machinery for induction of ethylene biosynthesis genes was fully intact, and that the resulting enzymes were fully functional.

Changes in the Levels of ACO and ACS Transcripts in the Gynoecium and Petals of 'WC' and 'LPB' Flowers during Natural Senescence

Since three genes for ACS (*DC-ACS1*, *DC-ACS2*, *DC-ACS3*) and one gene for ACO (*DC-ACO1*) have been identified in carnation (Wang and Woodson, 1991; Park et al., 1992; Henskens et al., 1994; Jones and Woodson, 1999), we determined the presence or absence of transcripts of these genes in the gynoecium and petals of unpollinated 'WC' and 'LPB' flowers (Fig. 3). In the gynoecium of the 'LPB' flowers, the *DC-ACS3* transcript was

detected on day 3 through day 9, the *DC-ACS1* transcript on days 5 and 6, and the *DC-ACS2* transcript, although in a small amount, on day 5. On the other hand, in the gynoecium of 'WC' flowers only the *DC-ACS3* transcript was detected in a small amount on day 9 through day 15.

The *DC-ACO1* transcript was present in a small amount already on day 0 and its amount increased, with a maximum on day 6 in the gynoecium of 'LPB' flowers. By contrast, the *DC-ACO1* transcript was absent on day 0, present abundantly on day 6 and decreased thereafter in the gynoecium of 'WC' flowers. The presence of *DC-ACO1* and *DC-ACS1* transcripts on day 6 through day 9 was obvious in the petals of 'LPB' flowers. The *DC-ACS1* transcript was absent in the petals of 'WC' flowers during the senescing period of 21 days, although the *DC-ACO1* transcript was present on day 9 through day 21 except for day 15.

Expression of the *DC-ACS1* and *DC-ACO1* genes in the petals of unpollinated carnation flowers is induced by ethylene that comes from the gynoecium (Shibuya et al., 2000). The presence or absence of the *DC-ACS1* transcript in the petals of 'LPB' or 'WC' flowers apparently resulted in presence or absence of the DC- ACS1 enzyme, respectively. This seems to explain the presence or absence of increased ethylene production in 'LPB' and 'WC' petals. The presence of the *DC-ACO1* transcript in the petals of senescing 'WC' flowers suggests that some ethylene was produced in their gynoecia, although its amount was too low to be measured. We hypothesize that the absence of the *DC-ACS1* transcript and the presence of the *DC-ACO1* transcript in the petals of 'WC' flowers was due to very low ethylene production in the gynoecium, insufficient to induce the expression of the *DC-ACS1* gene but sufficient to induce the *DC-ACO1* gene. By contrast, in unpollinated 'LPB' flowers, the amount of ethylene produced in the gynoecium was high enough to induce the expression of both *DC-ACO1* and *DC-ACS1* in the petals.

Accumulation of the *DC-ACO1* transcript in the gynoecium of both 'WC' and 'LPB' flowers indicated that the expression of *DC-ACO1* gene did not play a causal role in the difference in ethylene production from the gynoecium between 'WC' and 'LPB' flowers. Transcripts of *DC-ACS3* and *DC-ACS1* accumulated in a significant amount in the gynoecium of 'LPB' flowers, whereas only the *DC-ACS3* transcript was detected in a small amount on day 9 through day 12 in the gynoecium of 'WC' flowers.The repressed accumulation of ACS transcripts was therefore probably responsible for the low ethylene production in the gynoecium of 'WC' flowers.

Why is Ethylene Production Low in the Gynoecium of Unpollinated 'WC' Flowers?

The *DC-ACO1* transcript accumulated in the gynoecium of 'WC' flowers, although it produced ethylene in an undetectable amount (Fig. 3), and the *DC-ACS3* transcript also accumulated in the gynoecium of 'WC' flowers. This suggests that the expression of these genes occurred independently of ethylene action. We therefore hypothesized that the expression of *DC-ACO1* and *DC-ACS3* genes was induced by a factor(s) other than ethylene.

Regarding the possible factor(s) involved in the onset of ethylene production in unpollinated senescing carnation flowers, Onoue et al. (2000) reported that the ABA concentration in the gynoecium increased transiently, before the rise ethylene production. 1,1-Dimethyl-4-(phenylsulfonyl)semicarbazide (DPSS), a potent preservative for carnation flowers, inhibited the transient increase in the ABA concentration, apparently resulting in the suppression of petal ethylene production. Application of ABA and IAA to carnation flowers hastens petal senescence. They both act through induction of ethylene synthesis in the gynoecium (Shibuya et al., 2000). We now also included ABA or IAA in the vase water, which induced ethylene production in both 'WC' and 'LPB' flowers at their pre-senescent stage. Interestingly, there was a large difference in the concentrations of ABA and IAA required to induce ethylene production to a similar magnitude; 1 mM in 'WC' flowers, but 0.1 mM in 'LPB' flowers. This difference implied that there was a dif- ference in sensitivity to ABA or IAA of the gynoecium, between two carnation cultivars. We also found that the concentration of STS (silver thiosulfate anionic complex, an inhibitor of ethylene action)

required to counteract early flower senescence induced by exogenous ethylene in 'WC' flowers was 10 times higher than that required in 'LPB' flowers. Therefore, it seemed that the difference in the concentrations of ABA or IAA to induce ethylene production did not result from the difference in sensitivity to these hormones of the gynoecium. Rather, the difference might result from the difference in amounts of hormones taken up into flower tissues.

Preliminary studies on water relations in cut carnation flowers revealed that in the control 'LPB' flower the water balance, i.e. the net amount of water taken up into the cut flowers, decreased rapidly from the beginning of the experiment (day 0), whereas this decrease in water balance occurred much later in 'LPB' flowers treated with DPSS, as well as in untreated 'WC' flowers (Fig. 4). This rapid decrease in the water balance is indicative of a low water potential, i.e., drought stress. This may result in the accumulation of ABA. When taking these data together, we hypothesize that unpollinated cut 'LPB' flowers suffer from drought stress, which is accompanied by ABA accumulation. This in turn results in the induction of expression of ethylene biosynthetic genes in the gynoecium. Such apparently does not occur in cut 'WC' flowers.

CONCLUSION

We compared ethylene production and accumulation of transcripts for ethylene biosynthetic genes in the petals and gynoecium of unpollinated carnation flowers of the cultivar 'WC', which produces little ethylene and has a long vase life, and the cultivar LPB, which shows a climacteric rise of ethylene production and has a short vase life.

The findings showed that repressed ethylene production in unpollinated 'WC' flowers is primarily due to low ethylene production in the gynoecium, and that ethylene production from the gynoecium plays a role in the onset of petal senescence.

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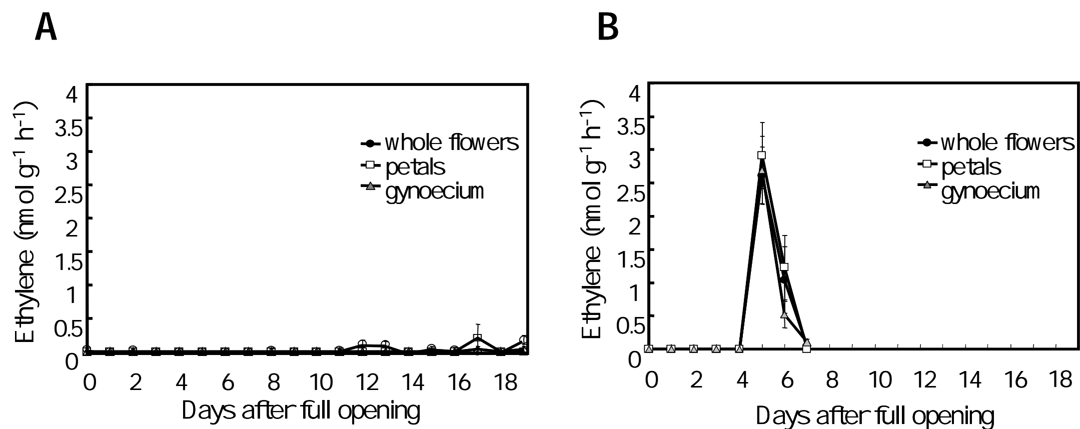
Figures



ig. 1. Ethylene production from whole flowers, gynoecium and petals of unpollinated 'White Candle' (**A**) and 'Light Pink Barbara' (**B**) flowers. After measurement of ethylene production in the whole flowers, the petals and gynoecium (ovary plus styles) were isolated and assayed for ethylene production. Each data point is the means (±SE) of 3 flowers.

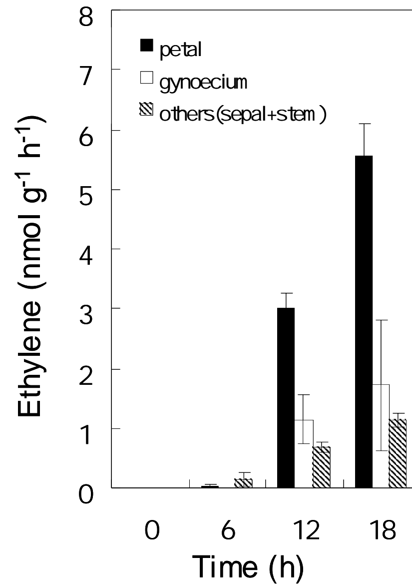


Fig. 2. Effects of ethylene treatment on ethylene production from 'WC' flowers. 'WC' carnation flowers on day 0 were treated with ethylene at 2 microliters per liter for 0 to 18 h. Samples of flowers were collected at 6-h intervals. The petals, gynoecium and the other parts were isolated from the flowers and assayed for ethylene production. Data are the means (±SE) of 5 flowers.

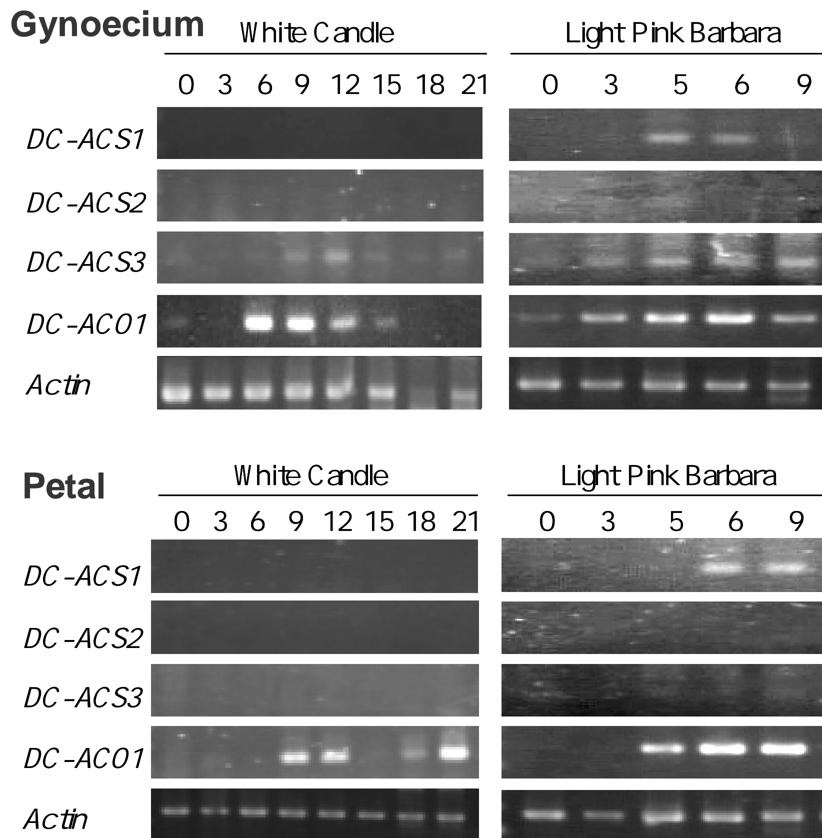


Fig. 3. Changes in transcript levels of ACS genes and an ACO gene in the gynoecium (Top) and the petals (Bottom) of unpollinated 'WC' and 'LPB' flowers. The petals and the gynoecium (ovary plus styles) were collected from 3 flowers, combined to make respective samples, and used for isolation of total RNA. The levels of ACS and ACO transcripts in total RNA fractions were determined by amplification by RT-PCR. An actin fragment (*DC-ACT1*) was amplified to check PCR operation and the amount of template RNA.

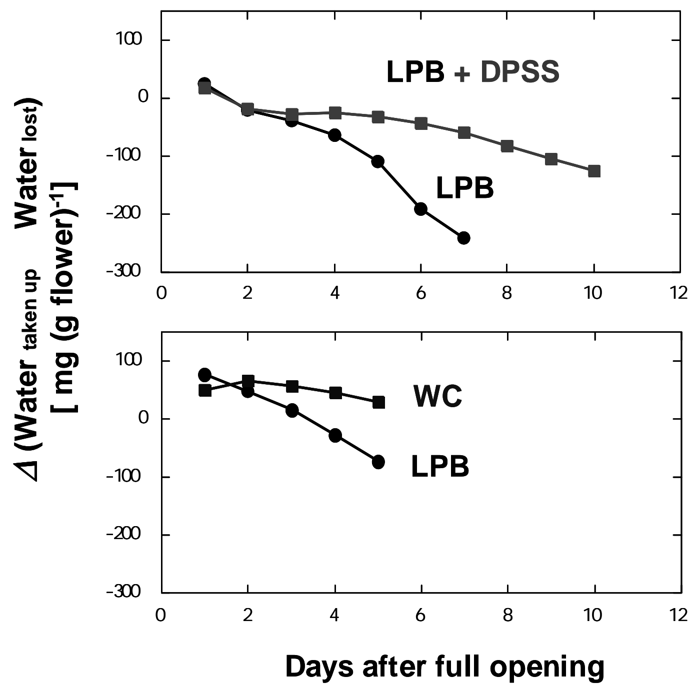


Fig. 4. Water relations of unpollinated 'LPB' flowers (treated with or without DPSS) and untreated unpollinated 'WC' flowers. The ordinate shows the difference between the amount of water taken up into the lowers and that lost from the flowers during the preceding day. In the DPSS treatment, the stem ends were placed in a 0.2 mM solution for 24 h (day 0 to day 1), and then in water for the remaining period. Each data point is the means of 10 flowers.