Senescence and Gene Expression of Transgenic Non-ethylene-producing Carnation Flowers

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Summary

Cut flowers of a transgenic carnation (Dianthus caryophyllus L.) line (sACO-1 line), which was transformed with a carnation 1-aminocyclopropane-1-carboxylate (ACC) oxidase cDNA in sense orientation (sACO transgene), had a longer vase life than had flowers of the non-transformed plant and produced only a negligible amount of ethylene during natural senescence. The suppressed ethylene production in sACO-1 line was not accompanied with the accumulation of mRNA for ACC oxidase (DC-ACO1) gene in the gynoecium or mRNAs for DC-ACO1 and ACC synthase (DC-ACS1) genes in the petals. Previously, it was shown that in naturally senescing carnation flowers, ethylene is first produced from the gynoecium; the evolved ethylene, acting as a diffusible signal which is perceived by petals, induces the expression of DC-ACO1 and DC-ACS1 genes that results in autocatalytic ethylene production in the petals (Shibuya et al., 2000). The results indicate that sACO transgene inhibits the expression of DC-ACO1, probably by cosuppression in the gynoecium, which then suppresses ethylene production in all flowers of sACO-1 line.

Key Words: ethylene production, flower senescence, gynoecium, petal, sACO transgene, transgenic carnation.

Introduction

Ethylene is a 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, mostly from the petals, several days after full opening of the flower during senescence (Manning, 1985; Peiser, 1986; Woodson et al. 1992). The increased ethylene production accelerates in-rolling of petals and wilting of the flower.

Inhibition of the synthesis or action of ethylene delays the onset of senescence and increases flower longevity. Hence, blockage of ethylene-induced senescence in flowers is of economic importance. Transgenic carnation plants with suppressed production or action of ethylene were generated in the past years; e.g., the line transformed with a carnation 1-aminocyclopropane-1-carboxylate (ACC) oxidase cDNA (corresponding to DC-ACO1) in antisense orientation (Savin et al., 1995), the line harboring an Arabidopsis thaliana ett1-1 allele, capable of ethylene insensitivity in carnation (Bovy et al., 1999), and the line (sACO-1 line) harboring an sACO transgene constructed from DC-ACO1 cDNA in sense orientation (Kosugi et al., 2000). Cut flowers of these transgenic lines have a prolonged vase life compared with those of the non-transgenic plants. The flowers of the transgenic line, transformed with the antisense ACC oxidase (ACO) cDNA, produce about 90% less ethylene than do the control during senescence. This decrease was accompanied by little or no accumulation of mRNAs of either ACO or ACC synthase (ACS) genes in petals (Savin et al., 1995). The ett1-1 transgenic carnation flowers had no detectable accumulation of ACO mRNA in petals during senescence, indicating that the expression of ett1-1 gene and action of the gene product prevented the autocatalytic induction of ethylene biosynthetic genes, such as DC-ACO1, in the petals (Bovy et al., 1999). That the petals of sACO-1 flowers exhibit no accumulation of mRNAs for DC-ACO1 and DC-ACS1 after treatment with exogenous ethylene (Kosugi et al., 2000) indicates that the ACO transgene caused cosuppression of the expression of endogenous DC-ACO1 gene in petals.

That the gynoecium plays a decisive role in control-
ling ethylene production during senescence of carnation flowers is well established (Overbeek and Woltering, 1990; Woodson et al. 1992; ten Have and Woltering, 1997; Shibuya et al., 2000). In carnation flowers, the gynoecium first produces a small amount of ethylene that acts as a diffusible signal which is perceived by the petals and induces the expression of ACS and ACO genes in the petals. This chain reaction results in autocatalytic ethylene production. Previous studies on the action of transgenes in carnation focused only on the petals and not on the gynoecium, in spite of its importance in the regulation of ethylene production. The results revealed that transgenes in carnation flowers were under the control of constitutive or floral tissue-specific promoters (Savin et al., 1995; Kosugi et al., 2000). Thus, it is reasonable to expect transgenes functioning in the gynoecium in addition to the petals.

In this paper, we characterized the sACO-1 transgenic line (Kosugi et al., 2000) and examined the probable function of sACO transgene in the gynoecium by Northern blot analysis of mRNA levels for ACO and ACS genes in the gynoecium and petals.

Materials and Methods

Plant materials

A generation of the transgenic line (sACO-1 line) of carnation (Dianthus caryophyllus L. cv. Nora) was described previously (Kosugi et al., 2000). The transgenic line, as well as the non-transformed (NT) control line, was cultured in a containment greenhouse.

Analysis of vase-life of cut flowers

Five flowers each of the NT control and sACO-1 lines were harvested at the full opening stage (day 0; their outermost petals were at right angles to the stem of flower). Stems were trimmed to 20 cm in length, and placed with their cut end in distilled water.

The flowers were left at 23 °C under a 16-hr photoperiod using white fluorescent light (15 μmol·sec⁻¹·m⁻²). The water was replaced daily. Senescing flowers were observed and photographed daily to record in-rolling and subsequent wilting of petals, the desiccation, and discoloration of the petal margins. Vase-life in days is expressed as the mean ± SE of 5 flowers.

Assay of ethylene production

Five flowering stalks from each of the NT control and sACO-1 lines, that were harvested at the full opening stage (day 0), were trimmed to 2-cm long, and the flowers placed in 30-ml glass vials with their basal end in distilled water. Ethylene production from carnation flowers was monitored daily by enclosing individual flowers in 450-ml glass containers (1 flower per container) for 1 hr at 23 °C. A 1-ml gas sample was drawn with a hypodermic syringe from inside the container through a rubber septum of a sampling port on the container and injected into a gas chromatograph (Model 263-30, Hitachi, Tokyo, Japan), equipped with an alumina column and a flame ionization detector to determine ethylene content.

RNA gel blot analysis

Total RNA was isolated by the SDS-phenol method (Palmiter, 1974) from the petals of cut flowers of the NT control and sACO-1 lines at 0, 3, 4, 5 and 7 days and from the gynoecium (ovary plus style) at 0 and 4 days after the full opening of flowers. Gel blot analysis for total RNAs was conducted with DIG-labeled probes for DC-ACS1 and DC-ACO1 mRNAs obtained from petals, but 32P-labeled probe was used for DC-ACO1 mRNA obtained from gynoecium as described previously (Kosugi et al., 2000).

Results and Discussion

Senescence profile of the sACO-1 transgenic flowers

Cut flowers of the sACO-1 line had a vase-life of 9.5 ± 0.3 days, whereas that of the NT control line was 5.8 ± 0.4 days (significant at P=0.05 by t test). Thus, the vase-life of flowers of the sACO-1 line was prolonged by 1.6-fold, compared with that of the NT control. The appearances of cut flowers during the senescence period of 10 days in the NT control and sACO-1 lines differed significantly (Fig. 1). Flowers of the NT control line remained turgid until day 5, showed in-rolling of petals on day 6, and completely wilted on day 9. By contrast, flowers of the sACO-1 line remained turgid without petal in-rolling until about day 10, but began to show desiccation and discoloration in the rim of petals on day 11 or later.

Petals in-rolling at the onset of wilting is a well-known characteristic of ethylene-dependent senescence of carnation flowers. By contrast, desiccation, discoloration, and browning of the rim of petals are characteristics of ethylene-independent senescence of the flowers. Thus, the treatment with silver thiosulfate anionic complex, an inhibitor of ethylene action, induces these symptoms in carnation flowers (Veen, 1979), whereas a transgenic carnation that harbors an antisense ACO transgene exhibits neither the climacteric rise of ethylene production nor petal in-rolling during senescence (Savin et al., 1995). These findings indicate little or no function of ethylene during the senescence of petals of the sACO-1 carnation.

Ethylene production of the sACO-1 transgenic flowers

Flowers of the NT control showed a climacteric rise in ethylene production, attaining a maximal rate on day 5, whereas flowers of the sACO-1 line produced a negligible amount of ethylene during the senescence period (Fig. 2). The lack of petal in-rolling and prolonged vase-life in flowers of this line indicate the suppression of ethylene production.
Fig. 1. Senescence profiles of carnation flowers of the non-transformed (NT) control (top) and the sACO-1 transgenic line (bottom). Five flowers each of both lines were harvested at full opening stage (day 0), their stems were trimmed to 20 cm in length, and then left in a glass vessel with their cut stem end in distilled water. Serial photographs of one flower, taken 0, 5, 6, 7, 8, 9 and 10 days after the start of the experiment, are typical of the respective lines.

Fig. 2. Ethylene production from cut carnation flowers during the senescence period in the NT control and the sACO-1 lines. Five flowers each of both lines were harvested at full opening stage (day 0) and their ethylene production monitored daily. Data are shown by the mean ± SE of 5 flowers. O, NT control; X, sACO-1.

mRNA levels for ACO and ACS in the petals and gynoeicum of the sACO-1 flowers

The presence of ACO and ACS mRNAs in the gynoeicum and petals of the NT control and their absence in the sACO-1 flowers on day 0 through day 7 are obvious (Fig. 3). So far, three genes encoding ACS (DC-ACS1, DC-ACS2, DC-ACS3) and one gene encoding ACO (DC-ACO1) have been identified in the carnation plant (Park et al., 1992; Henskens et al., 1994; Jones and Woodson, 1999; Wang and Woodson, 1991). These genes are regulated in a tissue-specific manner during flower senescence; DC-ACO1 is expressed in both the gynoeicum and petals of carnation flowers that are undergoing senescence, and DC-ACS1 is expressed mainly in petals whereas DC-ACS2 and DC-ACS3 occur in the gynoeicum (Henskens et al., 1994; ten Have and Woltering, 1997; Jones and Woodson, 1999). Since the probes for mRNAs of DC-ACS2 and DC-ACS3 were not available, the levels of DC-ACS1 mRNA were examined only in the petals in addition to those of DC-ACO1 mRNA in both the gynoeicum and petals.

In the gynoeicum of the NT control flowers, DC-ACO1 mRNA was already present in a significant amount on day 0 (at the time of full opening of flowers) and the amount increased on day 4 simultaneously with the onset of ethylene production. By contrast, DC-ACO1 mRNA was not detected on day 0 and day 4 in the gynoeicum of the sACO-1 flower. In petals of the NT control flowers, DC-ACO1 mRNA was detectable on day 4, its level attained a maximum on day 5 and remained almost constant until day 7. The levels of DC-ACS1 mRNA changed in a similar pattern to those of DC-ACO1 mRNA in petals of the NT control. On the contrary, no accumulation of mRNAs for DC-ACO1 and DC-ACS1 was detected throughout the incubation period in petals of sACO-1 line.

The results of Northern blot analysis revealed that the absence of DC-ACO1 and DC-ACS1 mRNAs in the petals corresponds to the decreased ACO and ACS activities in the petals and the suppressed ethylene production in the flowers. Kosugi et al. (2000) showed that the responsiveness to ethylene was not impaired in petals of the sACO-1 flowers. Therefore, sACO trans-
gene may act in the gynoecium in the sACO-1 flowers, inhibiting the expression of DC-ACO1 and suppressing ethylene production in the gynoecium and, subsequently, the expression of DC-ACO1 and DC-ACS1 in the petals. However, Kosugi et al. (2000) had revealed that no accumulation of DC-ACO1 mRNA occurred in the sACO-1 petals detached from flowers and treated with exogenous ethylene. These previous and present findings indicate that the sACO transgene acts in both petal and gynoecium of the sACO-1 flowers by suppressing the accumulation of DC-ACO1 mRNA. Hence, it is likely that its action in the petals, if any, is latent or plays a secondary role in the ethylene production during natural senescence of flowers.

**Fig. 3.** RNA gel blot analysis of mRNAs for DC-ACO1 and DC-ACS1 in petals and gynoecium of the NT control and the sACO-1 lines during natural senescence. Petals were isolated from cut flowers 0, 3, 4, 5, and 7 days, but gynoecium 0 and 4 days after full opening of flowers. Ten μg of total RNAs isolated from the petals were separated on an agarose gel and hybridized to DIG-labeled DC-ACO1 and DC-ACS1 probes. Thirty μg of total RNAs isolated from the gynoecium were separated on an agarose gel and hybridized to 32P-labeled DC-ACO1 probe. Equal loading of total RNAs was confirmed by ribosomal RNAs visualized by ethidium bromide staining of the agarose gel. NT, NT control line; sACO-1, sACO-1 transgenic line.

**Literature Cited**


エチレン非生産性形質転換カーネーションの花の老化と遺伝子発現

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摘　要

ACC酸化酵素（DC-ACO1）cDNAをセンス方向に発現するsACO遺伝子を導入したカーネーション（sACO-1系統）の、花の老化、エチレン生成、およびエチレン生成に関連遺伝子の発現を調べた。sACO-1系統の切り花は老化時にエチレンを生成せず、花の寿命が非形質転換体（NT、対照）の1.6倍に延長した。NTの切り花では、エチレン生成の始まる実験開始4日後から、離すと花弁でDC-ACO1 mRNAの蓄積がみられた。同時に、花弁でACC合成酵素（DC-ACS1）mRNAの蓄積も起こった。他方、sACO-1系統の切り花では、離すと、花弁の両方でDC-ACO1 mRNAの蓄積は起こらず、花弁でのDC-ACS1 mRNAの蓄積もみられなかった。

カーネーション切り花の自然老化時のエチレン生成は、離すいで生成したエチレンが拡散性のシグナルとして働いて花弁に受容され、花弁でのDC-ACO1とDC-ACS1の発現を誘導することによって自己触媒的に開始する（Shibuyaら、2000）。この知見と今回の結果から、sACO-1系統の切り花のエチレン非生産性は、sACO遺伝子が離すとにおいてDC-ACO1 mRNAの蓄積を阻害することが第一義的な原因と考えられた。

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