Recent Topics on Flower Opening and Senescence in Cut Carnation Flowers

4

Shigeru Satoh

Abstract

This chapter describes recent topics on flower opening and senescence of carnation flowers, highlighting new chemicals found to promote flower opening in carnation flowers, and the allelic nature of two variants (*DcACS1a* and *DcACS1b*) of a senescence-related 1-aminocyclopropane-1-carboxylate (ACC) synthase gene (*DcACS1*). Xyloglucan oligosaccharides (XGO) accelerated flower opening in some cultivars of spray-type carnation, suggesting the involvement of xyloglucan metabolism in petal cells undergoing cell expansion leading to petal growth. Isomers of pyridinedicarboxylic acid (PDCA), especially 2,3- and 2,4-pyridinedicarboxylic acids, accelerated flower opening in spray-type carnation, and eventually 3-pyridinecarboxylic acid, the most simple analog of PDCA, was highest in the acceleration of flower opening. ACC synthase gene *DcACS1,* the key gene acting in ethylene production in senescing carnation petals had two variants, *DcACS1a* and *DcACS1b*. Some carnation cultivars had only *DcACS1a*, and others *DcACS1a* and *DcACS1b*. A recent search revealed two carnation cultivars, 'Skyline' and 'Scarlett Plus', which had only *DcACS1b*, suggesting that *DcACS1a* and *DcACS1b* are present as alleles.

4.1 Introduction

This chapter describes our recent findings on chemicals which accelerate flower opening, eventually leading to the extension of vase life in some carnation flowers, and the allelic nature of two variants of a senescence-related carnation 1-aminocylopropane-1-carboxylate synthase gene. The vase life of cut carnation flowers is determined by the sum of the period for flower opening and that for flower senescence. Acceleration of flower opening followed by retardation of flower senescence would surely prolong the vase life of the flowers. There are many chemicals which have been used to retard the senescence of carnation flowers by inhibiting the synthesis and/or action of ethylene (Satoh et al. 2014). Whereas, to our best knowledge, no chemicals which accelerate the opening of carnation flower were not practically used previously. Therefore, we started to search for the chemicals which accelerate flower opening and help extend the vase life of the flowers. Our work to discover such the chemicals revealed xyloglucan oligosaccharide mixture (XGO) (Satoh et al. 2013) and isomers of pyridine-*di/mono*-carboxylic acids (Satoh et al. 2014, Satoh and Nomura 2019, Sugiyama and Satoh 2015). XGO is a mixture of enzymatic hydrolysis products of xyloglucan, a constituent of cell wall

S. Satoh (✉)
Faculty of Agriculture, Ryukoku University, Otsu 520-2194, Shiga, Japan
e-mail: jxymp688@yahoo.co.jp

T. Onozaki and M. Yagi (eds.), *The Carnation Genome*, Compendium of Plant Genomes,
https://doi.org/10.1007/978-981-15-8261-5_4

of dicot plants, and was shown to promote flower opening in some carnation cultivars. Its mechanism of action was suggested to participate in the process of cell wall loosening and promote petal cell expansion, and eventually leading to flower opening of carnation flowers. Vlad et al. (2010) were the first to report that 2,4-pyridinedi carboxylic acid (2,4-PDCA) inhibited ethylene production in detached flowers of 'White Sim' carnation and delayed senescence of the flowers. Then, Satoh et al. (2014) observed that 2,4-PDCA prolonged the vase life of cut flowers of spray-type carnation, by acceleration of flower opening in addition to delayed senescence of fully open flowers. The promotion of flower opening was caused by many isomers of pyridine-di/mono-carboxylic acids, although their mechanism of action has not been uncovered yet.

Senescence in carnation flowers is characterized by autocatalytic ethylene production from petals and subsequent wilting of the petals. A large amount of ethylene is synthesized several days after full opening of the flower during natural senescence (Woodson et al. 1992), or several hours after compatible pollination (Larsen et al. 1995; Nichols 1977; Nichols et al. 1983) or treatment with exogenous ethylene (ten Have and Woltering 1997; Wang and Woodson 1989). A substantial portion of ethylene comes from the petals of the flower. The increased ethylene production accelerates in-rolling of petals resulting in wilting of the flower.

Ethylene is synthesized in carnation petals, as well as in other plant tissues, via the following pathway: L-methionine → *S*-adenosyl-L-methionine (AdoMet) → 1-aminocyclopropane-1-carboxylate (ACC) → ethylene. The conversion of AdoMet to ACC is a rate-limiting step in ethylene biosynthesis and is catalyzed by ACC synthase (ACS) (Yang and Hoffman 1984). Three cDNAs coding for ACC synthase (*DcACS1*, *DcACS2*, and *DcACS3*) were cloned from carnation plants, suggesting that ACC synthase genes form a family in the plants, and tissue-specific or chemical-induced expression of mRNA has been studied (Jones 2003, Jones and Woodson 1999, ten Have and Woltering 1997).

DcACS1 is mainly responsible for autocatalytic ethylene production in carnation petals (Jones 2003, Jones and Woodson 1999, Satoh 2011, Shibuya and Ichimura 2010, ten Have and Woltering 1997); *DcACS1* showed ethylene-enhanced expression in petals (Jones 2003, Jones and Woodson 1999, ten Have and Woltering 1997), and carnation lines transformed with *DcACS1* transgenes showed suppressed ethylene production in flowers (Iwazaki et al. 2004).

About a decade ago genomic *DcACS1* gene was found to have two forms (variants), *DcACS1a* and *DcACS1b*, in which the sizes and nucleotide sequences of introns were different (Harada et al. 2011a). These two genes were suspected to make alleles of *DcACS1* gene, but this was not confirmed until recently.

4.2 Promotion by a Xyloglucan Oligosaccharide Mixture of Flower Opening in Carnation

Flower opening involves elongation, expansion, and outward bending of petals, which result from the enlargement of petal cells (Evans and Reid 1988; Kenis et al. 1985; Koning 1984). Cell-wall extensibility may be a growth-limiting factor for petal expansion (Yamada et al. 2009). An apoplastic enzyme, xyloglucan endotransglycosylase/hydrolase (XTH) is considered to be involved in cell-wall loosening in dicot plants (Carpita and McCann 2000). XTH catalyzes the transglycosylation of xyloglucan, which results in cleaving and reattachment among xyloglucan chains [xyloglucan endotransglycosylase (XET) activity], and realignment of the xyloglucan chain in different strata when newly synthesized xyloglucans are incorporated (Fry et al. 1992; Nishitani and Tominaga 1992; Vissenberg et al. 2000). By disturbing or inhibiting the cell-wall metabolism in the growing petals of opening flowers with an excess amount of xyloglucan oligosaccharides (XGO), the flower-opening process might be affected, resulting in a modified display time of the flowers.

A mixture of xyloglucan oligosaccharides (XGO) was prepared from xyloglucan (XG) of tamarind seed gum by digestion with *Aspergillus* XG-specific xyloglucanase and subsequent purification by ethanol fractionation. The XGO mixture contained XG7, XG8, and XG9 (Fig. 4.1) at the ratio of 1: 4: 5, which was almost identical to the literature value of constituent subunits ratio of 1.2: 3.8: 5.

The effect of XGO on flower opening was examined in several carnation cultivars. For example, cut buds, of cultivars 'Pure Red', 'Lillian' and 'Collin' were treated continuously with 1% XGO for 10 days. Figure 4.2 shows the time of flower opening over a period of 10 days and flowering profiles at 5 days ('Pure Red' and 'Collin') and 6 days ('Lillian') after the start of the experiment. XGO treatment promoted flower opening in 'Pure Red' and 'Lillian'. In 'Pure Red' carnation, the stimulation of flower opening was significant even one day after the start of treatment. Flower opening of 'Lillian' carnation was also promoted, but less than that of 'Pure Red' carnation. XGO treatment did not affect flower opening of 'Collin' carnation (Fig. 4.2) and 'Light Pink Barbara (LPB)' and 'Mule' carnations (data not shown). The reason why the XGO's effect varies among carnation cultivars has not been clarified yet.

The proposed mechanism of xyloglucan oligosaccharide on promotion of flower opening is as follows. Xyloglucan endotransglycosylase (XET) activity of XTH catalyzes the transglycosylation of xyloglucan, in which one chain of xyloglucan is cleaved and reattached to the non-reducing end of another xyloglucan chain (Fig. 4.3; Fry et al. 1992; Nishitani and Tominaga 1992; Vissenberg et al. 2000). Exogenously applied XGO might act as a substrate for XET, that is, an acceptor molecule of cleaved xyloglucan polymers (Fig. 4.3). Since XGO was not anchored to cellulose microfibrils, the formation of XGO-xyloglucan polymer complexes would cause more enhanced slippage of cellulose microfibrils, which was led by turgor in cells, resulting in the promoted expansion growth of petal cells.

Carnation plants have at least four genomic genes encoding xyloglucan endotransglycosylase/hydrolase (XTH) (*DcXTH1-DcXTH4*) (Harada et al. 2011b; Yagi et al. 2014). Harada et al. (2011b) suggested that two XTH genes (*DcXTH2* and *3*) are associated with petal growth and development during flower opening. It is interesting to investigate the in vivo and in vitro action of XGO on XTH enzymes.

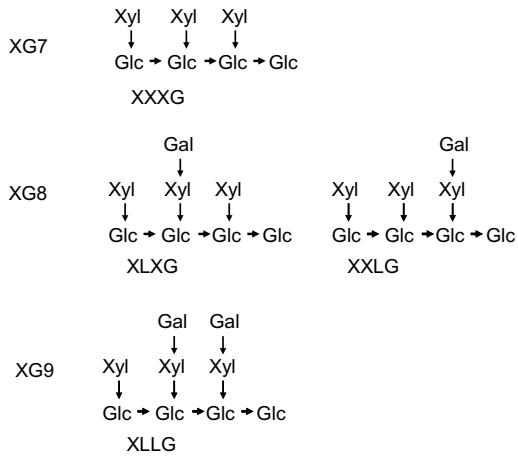


Fig. 4.1 Structures of XG7, XG8, and XG9 prepared from tamarind seed gum. Conventional abbreviations for respective XG oligosaccharides (XGO) are shown below the structure

4.3 Acceleration by Pyridinecarboxylic Acid Isomers of Flower Opening in Spray-Type Carnation

4.3.1 Acceleration of Flower Opening by Treatment with 2,4-Pyridinedicarboxylic Acid

Vlad et al. (2010) demonstrated that 2,4-pyridinedicarboxylic acid (2,4-PDCA) inhibited ethylene production in detached flowers of 'White Sim', a standard-type carnation which has one flower on a stem, and delayed senescence of the flowers. They suggested that 2,4-PDCA inhibited the activity of ACC oxidase by acting as a structural analog of ascorbate. Later, Satoh

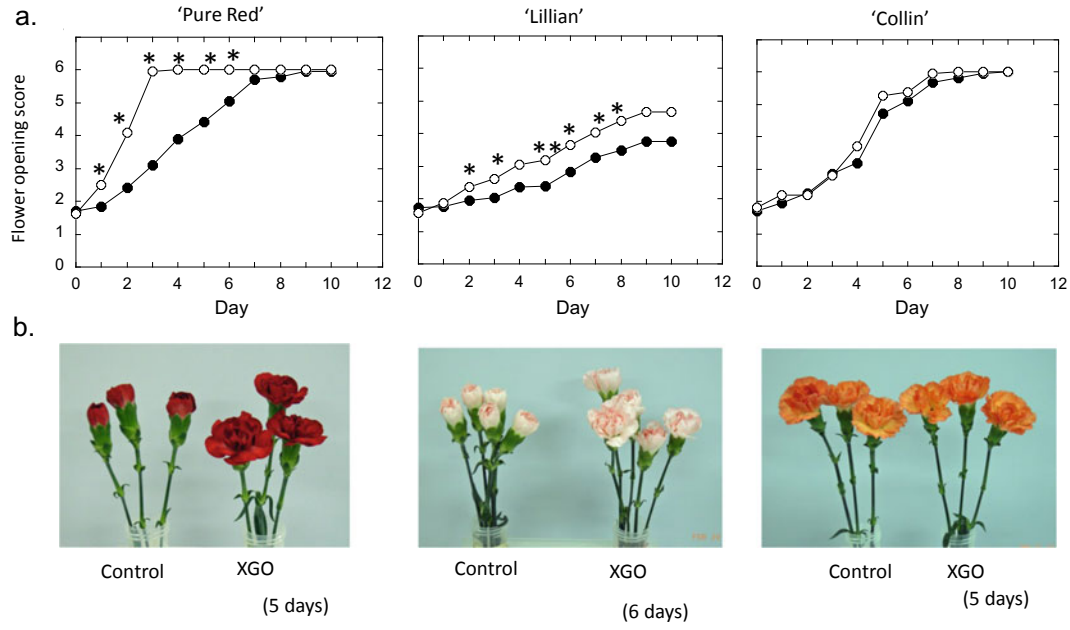


Fig. 4.2 Effects of 1% XGO on flower opening in 'Pure Red', 'Lillian', and 'Collin' carnation. **a** Time course of flower opening after treatment with 1% XGO (○) or with water (●). **b** Flower-opening profiles for respective cultivars at given days after the start of treatment. Data are the means of three replications, each with 6 or 7 flowers. * and ** show significant difference by Mann–Whitney's U-test at $P < 0.05$ and $P < 0.01$, respectively, on each day The flower-opening score was determined by separating flower opening profile into 6 stages according to Harada et al. (2011a): score 1, petals just emerged from buds; score 2, petals elongated vertically; score 3, petal clusters expanded; score 4, outer petals start to reflex (bend outwards); score 5, outer petals have reflexed; score 6, fully open flower with outer petals at right angles to the stem

et al. (2014, 2016) examined 2,4-PDCA action on ethylene production and senescence in 'LPB' carnation. Interestingly, they demonstrated the acceleration of flower opening by 2,4-PDCA as well as 2,3-pyridinedicarboxylic acid (2,3-PDCA) in the flowers, in addition to retardation of their senescence, resulting in prolonged display time. Eventually, 3-pyridinecarboxylic acid (3-PCA), the simplest analog of pyridinecarboxylic acid, was found to promote most markedly the flower opening of cut spray-type carnation. This section will describe the effects of the pyridinecarboxylic acid analogs on flower opening in cut spray-type carnation.

Usually, the time of flower opening and the vase life of carnation flower are determined by observing onset of flower opening and senescence, *i.e.*, in-rolling of petal margin leading to wilting of whole petals. This method has been used successfully for a standard type of carnation flower with only one flower per stalk (stem). However, in spray-type carnation flowers, which have a main stalk with several offshoots, each having one or two flowers at their tips, the time of flower opening and the vase life of the flowers are determined differently, since the onset of flower opening and senescence varies depending on each floret. Satoh et al. (2005, 2014) developed a method to determine the 'time to flower opening' and 'vase life', for characterizing flower opening profiles in cut spray-type carnation flowers. This newly developed method is demonstrated in the following with 'LPB' flowers treated with 2,4-PDCA.

Figure 4.4 shows the percentage of fully open and non-senescent (FONS) flowers of cut 'LPB' flowers treated with various concentrations of 2,4-PDCA (Sugiyama and Satoh 2015).

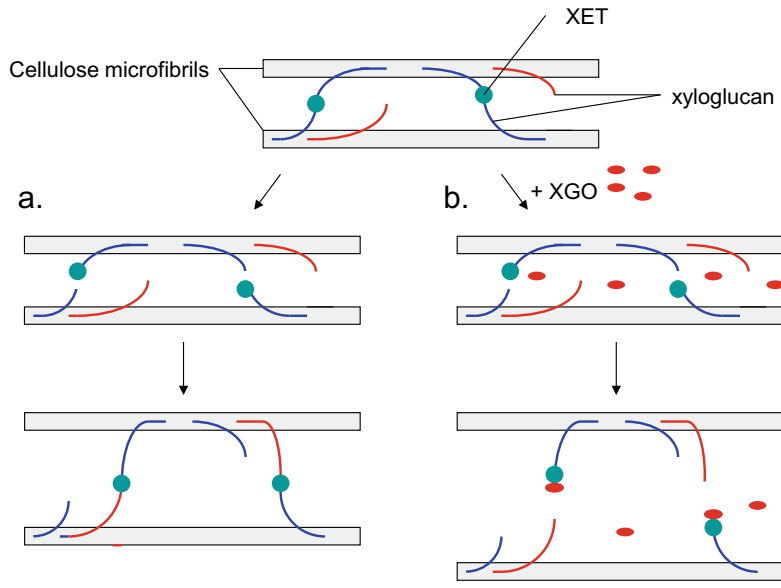


Fig. 4.3 Possible action mechanism of XGO on the promotion of flower opening in carnation. The drawing was made according to the figure shown in a textbook (Biochemistry and Molecular Biology of Plants, 2.38; Carpita and McCann 2000) with necessary modification. **a** During the ordinate process of petal cell growth, XET catalyzes cleavage and reattachment among xyloglucan chains, resulting in transient slippage of cellulosic microfibrils. **b** When XGO was present in excess, cleaved xyloglucan polymers were transferred to unanchored XGO, causing more enhanced slippage of cellulose microfibrils, eventually promoting flower opening

The percentage of FONS flowers was calculated from the proportion of those flowers to the total number of initial flower buds (25 buds per 5 flowers). The time to flower opening is the time in days from the start of experiment until the percentage of open flowers reached 40%. The vase life of spray-type carnation flowers was the period during which 40% or more were fully open and non-senescent (Satoh et al. 2005, 2014). The treatment with 0.3, 1 and 2 mM 2,4-PDCA tended to shorten the time to flower opening; that is, 4.3, 3.3 and 3.8 days, respectively, as compared to 4.4 days in the control, although the difference was significant only with treatment at 1 mM by Steel's multiple range test ($P < 0.05$). The results suggested that 2,4-PDCA accelerates flower bud opening. The vase life was significantly lengthened by treatment with 2,4-PDCA, attaining 53, 111, and 135% increases at 0.3, 1, and 2 mM 2,4-PDCA, respectively, as compared with the control (Satoh et al. 2014).

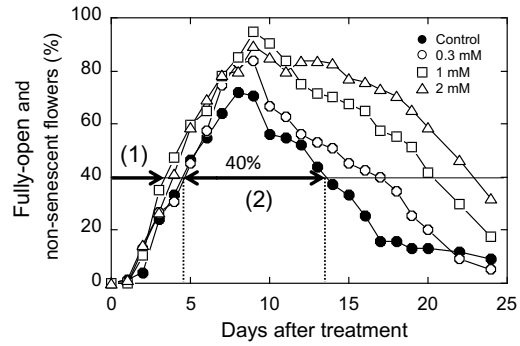


Fig. 4.4 Evaluation of flower opening by determining the time to flower opening and the duration of vase life in cut flowers of 'Light Pink Barbara' carnation treated continuously with different concentrations of 2,4-PDCA. This figure was adapted from Fig. 4.2 in Sugiyama and Satoh (2015). The percentage of fully open and non-senescent (FONS) flowers was calculated from the proportion of those flowers to the total number of initial flower buds (25 buds per 5 flowers). The time to flower opening (1) is the time in days from the start of experiment until the percentage of open flowers reached 40%. The display time of the flowers (2) is the duration when the percentage of FONS flowers was 40% or more

4.3.2 Acceleration by Various Pyridinedicarboxylic Acid Isomers of Flower Opening in Spray-Type Carnation

Pryridinedicarboxylic acids (PDCAs) have six isomers as shown in Fig. 4.5. Figure 4.6 shows the flower opening and senescing profiles of the flowers of 'LPB' carnation treated with 2,3-, 2,4-, 2,5-, and 3,4-PDCAs at 2 mM, which were chosen as typical specimens out of 3 replicates 0, 3, and 10 days after the start of the experiment. On day 3, there were more open pink flowers among the flowers treated with PDCAs, especially with 2,3- and 2,4-PDCAs, than in the control. These observations indicated that treatment with PDCAs accelerated flower opening compared with that in the untreated control flowers, and the acceleration effect was greatest with 2,3-PDCA among the PDCAs. On day 10, the control flowers remained as a mixture of buds, open flowers, and senesced flowers. The senesced flowers showed in-rolling and wilting of petals, typical symptoms of senescence in response to ethylene. On the other hand, almost all of the flowers treated with PDCAs were fully open with non-wilted and turgid petals. At the later stage, the PDCA-treated flowers withered with browning at the petal margins, as well as with jumbled, but turgid, fading petals, as reported previously (Satoh et al. 2014), or lost their vase lives by the breaking of flower stems.

Table 4.1 summarizes the effects of various PDCA isomers at 2 mM on the time to flower opening and the vase life of cut 'LPB' flowers. The control (H_2O) flowers reached the stage of flower opening (40%) on day 9.0. The treatment with all the PDCA isomers promoted flower opening by shortening the time to flower opening. The time to flower opening was the shortest (4.0 days) for 2,3-PDCA and 2,4-PDCA, followed by 2,5-PDCA and 3,5-PDCA (both, 4.7 days), then 2,6-PDCA and 3,4-PDCA. Four of the PDCA analogs (2,3-, 2,4-, 3,4-, and 3,5-PDCA) significantly lengthened the vase life compared with that of the control (8.3 days); the vase lives varied from 14.7 to 15.5 days.

A representative PDCA isomer 2,4-PDCA promoted flower opening of spray-type carnation cultivars, 'Barbara', 'Beam Cherry', 'Candle', 'Collin', 'Rascal Green', 'Scarlett Ostera', and 'Mule', as well as 'LPB'. Treatment of 'LPB' flowers for the initial 24 h with 2,4-PDCA at 5 and 10 mM was almost as effective as the continuous treatment with the chemical at 2 mM (Satoh et al. 2016).

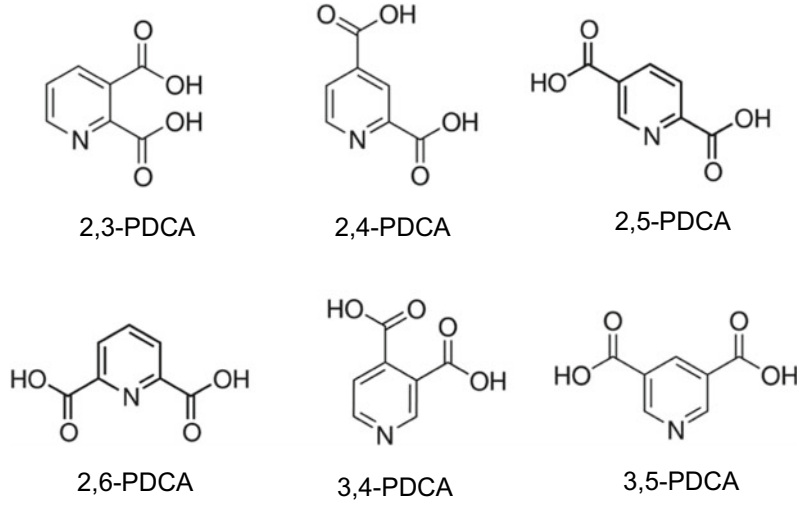


Fig. 4.5 Structures of pyridinedicarboxylic acid isomers (PDCAs). Drawings of the chemical structures were taken from HP of Sigma-Aldrich Co. LLC.

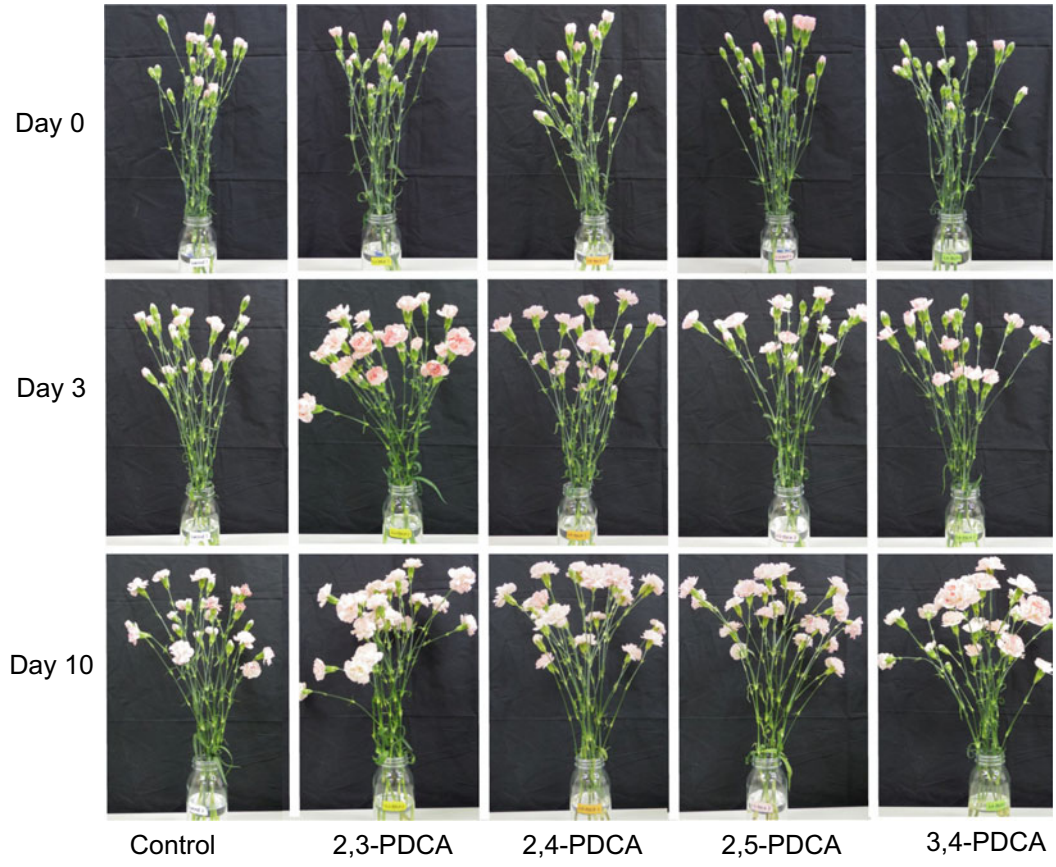


Fig. 4.6 Flower-opening and -senescing profiles of cut 'Light Pink Barbara' flowers treated with 2,3-, 2,4-, 2,5-, or 3,4-PDCA at 2 mM or water (control). Typical profiles for each treatment out of 3 replicates were chosen for 0, 3, and 10 days after the start of the experiment

Table 4.1 Effects of PDCA isomers at 2 mM on the time to flower opening and the vase life of cut spray-type 'Light Pink Barbara' carnation flowers

Chemicals	Time to flower opening (days)	Vase life (days)
Control (H_2O)	9.0±1.0	8.3±1.9
2,3-PDCA	4.0[a] ± 0.0	14.7[a] ± 0.3
2,4-PDCA	4.0[a]± 0.6	14.7[a] ± 1.7
2,5-PDCA	4.7[a]± 0.3	13.0±2.1
2,6-PDCA	5.3[a] ± 0.3	9.3±1.5
3,4-PDCA	5.7[a] ± 0.3	14.7[a] ± 2.4
3,5-PDCA	4.7[a] ± 0.7	15.5[a] ± 1.2

Data are the mean ± SE of 3 replicates, each with 5 flower stems with 5 florets (buds) on a stem

[a]Shows a significant difference from the control in each column by Dunnett's multiple range test ($P < 0.05$)

4.3.3 Acceleration by 3-pyridinecarboxylic Acid (3-PCA) of Flower Opening in Spray-Type Carnation

The treatment of pyridinecarboxylic acids (PCAs) revealed that 3-pyridinecarboxylic acid (3-PCA) and its derivative, 3-PCA amide, were most active in accelerating flower opening of cut 'LPB' flowers (Satoh and Nomura 2019). Figure 4.7 shows the changes in the percentage of FONS flowers of 'LPB' carnations that were treated on the first day with PCA and PDCA analogs at 5 mM, then left in water for another 29 days.

3-PCA and 3-PCA amide markedly promoted the opening of cut 'LPB' flowers and reduced the days to flower opening (Fig. 4.7a). It is possible that 3-PCA amide can be easily converted to 3-PCA, an expected active form, or vice versa. 2-PCA and 4-PCA also accelerated flower opening, but their effects were weaker than those of 3-PCA and 3-PCA amide. Moreover, the promotive effects of 3-PCA and 3-PCA amide were stronger than that of 2,3-PDCA, 2,4-PDCA, and

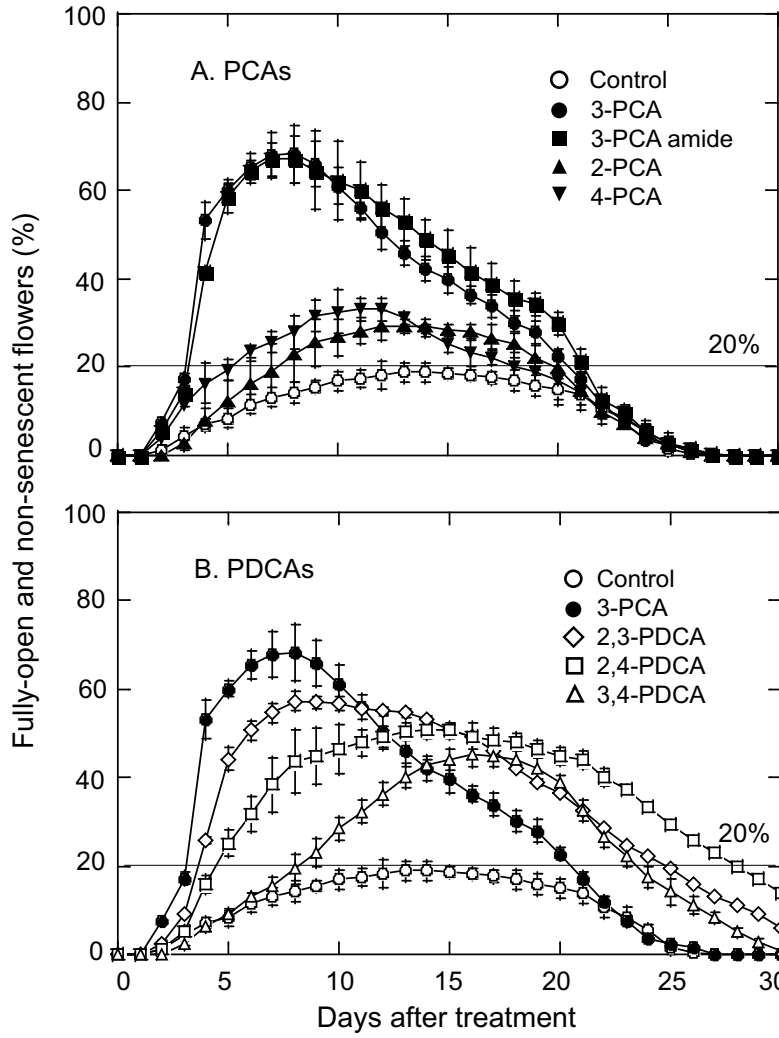


Fig. 4.7 Changes in the percentage of fully open and non-senescent 'Light Pink Barbara' flowers treated with or without pyridinecarboxylic acids (PCAs) (**a**) and pyridinedicarboxylic acids (PDCAs) (**b**) analogs at 5 mM for one day then kept in water until day 30. Data show the mean ± SE of three replicates, each with 25 flower buds. Data for the control and 3-PCA treatment are shown in both figures for comparison.

Table 4.2 Effects of PCA and PDCA isomers on the time to flower opening and the vase life of 'Light Pink Barbara' carnation flowers

Chemicals	Time to flower opening (days)	Vase life (days)
Control	(8.5[a])	(15.9[a])
2-PCA	6.8 ± 0.3	12.2 ± 0.5
3-PCA	3.1 ± 0.2	18.4 ± 1.1
3-PCA amide	3.1 ± 0.3	18.5 ± 1.7
4-PCA	4.0 ± 0.7	15.8 ± 3.4
2,3-PDCA	4.2 ± 0.6	16.6 ± 5.7
2,4-PDCA	(4.4[a])	(23.5[a])
3,4-PDCA	(8.4[b])	(14.5[a])

Data are the mean ± SE of 3 experiments, each with 3 replicates with 5 flower stems (25 buds in total) when the least display values for the flowers treated with respective chemicals surpassed 20%. However, if this was not the case, values from only one experiment ([a]) or the mean of two experiments ([b]) are shown in parentheses for reference. The threshold value of 20% was used because of low flowering vigor of a used flower sample as explained by Sugiyama and Satoh (2015)

3,4-PDCA (Sugiyama and Satoh 2015). Table 4.2 summarizes the days to flower opening and the vase life of the flowers as affected by the treatment with PCA and PDCA isomers, which were determined in three experiments including that shown in Fig. 4.7. Flower opening was earliest in the flowers treated with 3-PCA and 3-PCA amide (3.1 days), followed by 4-PCA (4.0 days), 2,3-PDCA (4.2 days), and 2-PCA (6.8 days) in this order. Among the PCA analogs, 3-PCA was more effective than 4-PCA and 2-PCA in promoting flower opening. These finding suggested that the carboxyl group at position 3 of the pyridine ring is needed for the promotion of flower opening.

4.4 Allelic Nature of Two Forms (Variants) of DcACS1 Gene, DcACS1a, and DcACS1b

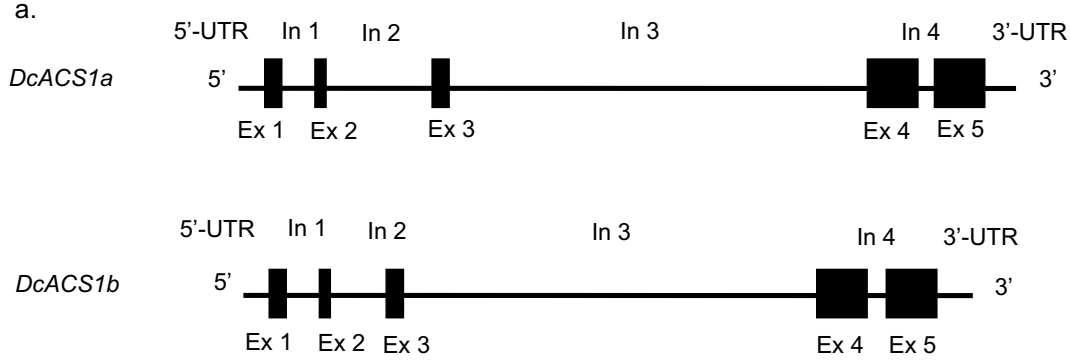
4.4.1 Genomic DNA Structure of a Carnation ACS Gene, DcACS1

DcACS1 was found to be mainly responsible for autocatalytic ethylene production in carnation petals (Jones 2003, Jones and Woodson 1999, Satoh 2011, Shibuya and Ichimura, 2010, ten Have and Woltering 1997), and the genomic DNA structure of *DcACS1* was determined by genomic PCR (Harada et al. 2011a). Interestingly, in the genome of 'LPB' carnation, two distinct nucleotide sequences, so-called variants, were found and were designated *DcACS1a* and *DcACS1b*. The former corresponded to a cDNA coding for the carnation *DcACS1* which was first cloned as *CARACC3* from 'White Sim' carnation (Park et al. 1992), and later confirmed by Henskens et al. (1994). The latter was a novel gene (Fig. 4.8a). Both *DcACS1a* and *DcACS1b* have five exons and four introns. These two genes (variants) had almost identical nucleotide sequences in exons, but not in some introns and 3′-UTR (Fig. 4.8b). Previous genomic PCR analysis of 32 carnation cultivars showed that 25 cultivars have only *DcACS1a* and seven cultivars have both *DcACS1a* and *DcACS1b*; that is, cultivars such as 'White Sim', 'Scania', 'Nora', and so on had only *DcACS1a*, whereas others such as 'LPB', 'Tanga', 'Kibo', and so on had both *DcACS1a* and *DcACS1b* (Table 4.3).

4.4.2 Allelic Nature of DcACS1a and DcACS1b Genes

There are two variants of *DcACS1* gene, *DcACS1a* and *DcACS1b*, which could be alleles of *DcACS1* gene. A search for carnation cultivars which are homozygotes of the *DcACS1b* gene was not successful (Harada et al. 2011a).

a.



b.

Region	5'-UTR	Exon 1	Intron 1	Exon 2	Intron 2	Exon 3	Intron 3	Exon 4	Intron 4	Exon 5	3'-UTR
DcACS1a	168*	174	217	132	769	161	2962	558	119	529	248
DcACS1b	166	174	296	132	445	161	2891	558	119	511	225
Identity (%)	87.5	100	69.6	100	56.4	100	65.6	99.5	100	94.7	55.8

* Sizes are in nucleotide number.

Fig. 4.8 Genomic structures (**a**) and sizes (**b**) in nucleotide number of five exons, four introns, 3′-UTR, and 5′-UTR of *DcACS1a* and *DcACS1b*

Table 4.3 Classification of 40 carnation cultivars by genomic PCR fragments of *DcACS1*

Cultivars harboring DcACS1a/DcACS1a			
Apollo	Candy	Chinera	Coral
Delphi	Eternal Green	Francesco	Killer
Kirk*	Lester	Light Cream Candle	Miracle Rouge
Miracle Symphony	Nora	Northland	Orange Range
Pallas	Peter	Pure Red	Scania
Tobias	Toy*	Uconn Sim	White Lucena
White Sim	William Sim	Yosooi	
Cultivars harboring DcACS1a/DcACS1b			
Barullo*	Beam Cherry	Carnet*	Cherry Tessino*
Fuji	Kibo	Light Pink Barbara	Scarlett Ostera*
Seto no Hatsushimo	Snow Crown Pink	Tanga	
Cultivars harboring DcACS1b/DcACS1b			
Scarlett Plus*	Skyline*		

In this table, eight cultivars (with an asterisk) were newly added to the previously shown 32 cultivars (Harada et al. 2011a)

However, very recently, genomic PCR analysis revealed two carnation cultivars 'Skyline' and 'Scarlett Plus', which had only the *DcACS1b* gene, suggesting these cultivars are homozygotes of the *DcACS1b* gene (Satoh et al. 2020). Furthermore, analysis of the gene structure of a

hybrid carnation between ordinal carnation and wild pink provided further evidence of the allelic nature of *DcACS1a* and *DcASC1b* genes (Satoh et al. 2020). Table 4.3 summarizes carnation cultivars thought to be homozygotes of *DcACS1a* or *DcACS1b*, and heterozygotes of both genes. In the table, eight cultivars were added to the 32 cultivars examined previously (Harada et al. 2011a). Analysis of transcript accumulation revealed that both *DcACS1a* and *DcACS1b* are expressed at a similar level in senescing petals of 'LPB' carnation, suggesting that *DcACS1a* and *DcACS1b* are codominant genes (Harada et al. 2011a).

Genomic ACC synthase genes, homologous to carnation *DcACS1* gene, were present as variants in other two *Dianthus* species; six variants in *D. petraeus* and seven variants in *D. superbus* var. *longicalycinus* (Satoh et al. 2011). All 13 sequenced homologous *ACS1* genes had five exons and four introns, similar to *DcACS1* genes in carnation. The exons had similar nucleotide sequences and consequently similar deduced amino-acid sequences. Satoh et al. (2011) suggested the possible origin of two variants (now alleles), *DcACS1a* and *DcACS1b*, by studying the intron structure in *DcACS1* homologous genes of *Dianthus* species. They suggested that (1) the variation in intron structure between two variants of carnation *DcACS1* is reminiscent of the variation that occurred universally in *Dianthus* species, (2) *DcACS1a* is probably a gene intrinsic to carnation, and (3) *DcACS1b* was acquired from another, as yet unknown, *Dianthus* species, in the course of breeding modern carnation cultivars. The present findings suggest that variants of *DcACS1* homologous genes in other *Dianthus* species are also alleles in the specific species; for example, *DsACS1a* and *DsACS1b* in *D. superbus* var. *longicalcinus* are also alleles in *Dianthus* species.

References

Carpita N, McCann M (2000) The cell wall. In: Buchanan BB, Gruissem W, Jones RL (eds) Biochemistry and molecular biology of plants. Am Soc Plant Physiol, Rockville, pp 52–108

Evans RY, Reid MS (1988) Changes in carbohydrates and osmotic potential during rhythmic expansion of rose petals. J Am Soc Hort Sci 113:884–888

Fry SC, Smith RC, Renwick KF et al (1992) Xyloglucan endotransglycosylase, a new wall-loosening enzyme activity from plants. Biochem J 15:821–828

Harada T, Murakoshi Y, Torii Y et al (2011a) Analysis of genomic DNA of *DcACS1*, a 1-aminocyclopropane-1-carboxylate synthase gene, expressed in senescing petals of carnation (*Dianthus caryophyllus*) and its orthologous genes in *D. superbus* var. *longicalycinus*. Plant Cell Rep 30:519–527

Harada T, Torii Y, Morita S et al (2011b) Cloning, characterization, and expression of xyloglucan endotransglucosylase/hydrolase and expansin genes associated with petal growth and development during carnation flower opening. J Exp Bot 62:815–823

Henskens JAM, Rouwendal GJA, ten Have 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

Iwazaki Y, Kosugi Y, Waki K et al (2004) Generation and ethylene production of transgenic carnations harboring ACC synthase cDNA in sense or antisense orientation. J Appl Hort 6:67–71

Jones ML (2003) Ethylene biosynthetic genes are differentially regulated by ethylene and ACC in carnation styles. Plant Growth Regul 40:129–138

Jones ML, Woodson WR (1999) Differential expression of three members of the 1-aminocyclopropane-1-carboxylate synthase gene family in carnation. Plant Physiol 119:755–764

Kenis JD, Silvente ST, Trippi VS (1985) Nitrogen metabolite and senescence-associated change during growth of carnation flowers. Physiol Plant 65:455–459

Koning RE (1984) The role of plant hormones in the growth of the corolla of *Gaillardia grandiflora* (Asteraceae) ray flowers. Am J Bot 71:1–8

Larsen PB, Ashworth EN, Jones ML et al (1995) Pollination-induced ethylene in carnation (Role of pollen tube growth and sexual compatibility). Plant Physiol 108:1405–1412

Nichols R (1977) Sites of ethylene production in the pollinated and unpollinated senescing carnation

(*Dianthus caryophyllus*) inflorescence. Planta 135:155–159

Nichols R, Bufler G, Mor Y et al (1983) Changes in ethylene production and 1-aminocyclopropane-1-carboxylic acid content of pollinated carnation flowers. J Plant Growth Regul 2:1–8

Nishitani K, Tominaga R (1992) Endo-xyloglucan transferase, a novel class of glycosyltransferase that catalyzes transfer as a segments of xyloglucan molecule to another xyloglucan molecule. J Biol Chem 267:21058–21064

Park KY, Drory A, Woodson WR (1992) Molecular cloning of an 1-aminocyclopropane-1-carboxylate synthase from senescing carnation flower petals. Plant Mol Biol 18:377–386

Satoh S (2011) Ethylene production and petal wilting during senescence of cut carnation (*Dianthus caryophyllus*) flowers and prolonging their vase life by genetic transformation. J Jpn Soc Hort Sci 80:127–135

Satoh S, Kosugi Y, Sugiyama S et al (2014) 2,4-Pyridinedicarboxylic acid prolongs the vase life of cut flowers of spray carnations. J Jpn Soc Hort Sci 83:72–80

Satoh S, Meng N, Harada T et al (2011) Possible origin of two variants of a carnation 1-aminocyclopropane-1-carboxylate synthase gene, DcACS1a and DcACS1b, as suggested by intron structure in homologous genes in Dianthus species. J Jpn Soc Hort Sci 80:443–451

Satoh S, Nomura Y (2019) Promotion of flower opening of spray-type carnation cut flowers by 3-pyridinecarboxylic acid, a simple analog of pyridinedicarboxylic acids (PDCAs) including 2,3- and 2,4-PDCAs. Hort J 88:293–298

Satoh S, Nomura Y, Kosugi Y et al (2020) Allelic nature of two carnation variants 1-aminocyclopropane-1-carboxylate (ACC) synthase genes, *DcACS1a* and *DcACS1b*. Hort J 89:311–318

Satoh S, Nomura Y, Morita S et al (2016) Further characterization of the action of pyridinecarboxylic acids: multifunctional flower care agents for cut flowers of spray-type carnation. J Appl Hort 18:3–6

Satoh S, Nukui H, Inokuma T (2005) A method for determining the vase life of cut spray carnation flowers. J Appl Hort 7:8–10

Satoh S, Tateishi A, Sugiyama S (2013) Preparation of a xyloglucan oligosaccharide mixture from tamarind seed gum and its promotive action on flower opening in carnation cultivars. J Jpn Soc Hort Sci 82:270-276

Shibuya K, Ichimura K (2010) Depression of autocatalytic ethylene production by high-temperature treatment in carnation flowers. J Jpn Soc Hort Sci 79:97–102

Sugiyama S, Satoh S (2015) Pyridinedicarboxylic acids prolong the vase life of cut flowers of spray-type 'Light Pink Barbara' carnation by accelerating flower opening in addition to an already-known action of retarding senescence. Hort J 84:172–177

ten Have A, Woltering EJ (1997) Ethylene biosynthetic genes are differentially expressed during carnation (*Dianthus caryophyllus* L.) flower senescence. Plant Mol Biol 34:89–97

Vissenberg K, Martinez-Vilchez IM et al (2000) In vivo colocalization of xyloglucan endotransglycosylase activity and its donor substrate in the elongation zone of *Arabidopsis* roots. Plant Cell 12:1229–1237

Vlad F, Tiainen P, Owen C et al (2010) Characterization of two carnation petal prolyl 4 hydroxylases. Physiol Plant 140:199–207

Wang H, Woodson WR (1989) Reversible inhibition of ethylene action and interruption of petal senescence in carnation flowers by norbornadiene. Plant Physiol 89:434–438

Woodson WR, Park KY, Drory A et al (1992) Expression of ethylene biosynthetic pathway transcripts in senescing carnation flowers. Plant Physiol 99:526–532

Yamada K, Takahashi R, Fujitani C et al (2009) Cell wall extensibility and effect of cell-wall-loosening proteins during rose flower opening. J Jpn Soc Hort Sci 78:242–251

Yagi M, Kosugi S, Hirakawa H et al (2014) Sequence analysis of the genome of carnation (*Dianathus caryophyllus* L.). DNA Res 21:231–241

Yang SF, Hoffman N (1984) Ethylene biosynthesis and its regulation in higher plants. Ann Rev Plant Physiol 35:155–189