



Further characterization of the action of pyridinedicarboxylic acids: multifunctional flower care agents for cut flowers of spray-type carnation

Shigeru Satoh^{1*}, Yoshihiro Nomura¹, Shigeto Morita^{2,3} and So Sugiyama²

¹Faculty of Agriculture, Ryukoku University, Otsu 520-2194, Japan. ²Graduate School of Life and Environmental Sciences, Kyoto Prefectural University, Kyoto 606-8522, Japan. ³Kyoto Prefectural Institute of Agricultural Biotechnology, Seika Town, Kyoto 619-0224, Japan. *E-mail: ssatoh@agr.ryukoku.ac.jp

Abstract

Pyridinedicarboxylic acid (PDCA) analogs accelerate flower opening and retard senescence, which markedly extend the vase life of spray-type ‘Light Pink Barbara (LPB)’ carnation. In the present study, we characterized the activity of these chemicals to develop a novel flower care agent for a practical use. A representative PDCA analog 2,4-PDCA is effective in a wide range of spray-type carnation cultivars, ‘Barbara’, ‘Beam Cherry’, ‘Candle’, ‘Collin’, ‘Rascal Green’ and ‘Scarlet Ostara’, as well as ‘LPB’ and ‘Mule’. 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.

Key words: pyridinedicarboxylic acids, spray-type carnation, carnation cultivars, flower bud, time to flower opening, vase life, gross flower opening

Abbreviations: ACC, 1-aminocyclopropane-1-carboxylate; FONS, fully-open and non-senescent; LPB, Light Pink Barbara; PDCA, pyridinedicarboxylic acid.

Introduction

Carnations are popular cut flowers and of highest economic importance in the floriculture industry in many countries. They are classified into standard and spray-type. The standard-type carnations have only one flower per stalk (stem), whereas the spray-type carnations have a main stalk with several offshoots, each having one or two flowers at the tip (inflorescence), making a plant with multiple flowers on a stalk, as a whole.

During the senescence of carnation flowers, a climacteric increase in ethylene production occurs, and the evolved ethylene induces in-rolling of petals, resulting in wilting of whole flowers (Abeles *et al.*, 1992; Satoh, 2011). The effect of ethylene on flower senescence can be diminished by treatment with inhibitors of ethylene biosynthesis or action, as discussed by Satoh *et al.* (2014). Treatment with these inhibitors prolongs the vase life of cut carnation flowers as well as other ethylene-sensitive ornamental flowers.

Vlad *et al.* (2010) reported that 2,4-pyridinedicarboxylic acid (2,4-PDCA) inhibited ethylene production in detached flowers of ‘White Sim’, which is a standard-type carnation, and delayed senescence of the flowers. They hypothesized that 2,4-PDCA inhibited 1-aminocyclopropane-1-carboxylate (ACC) oxidase by competing with ascorbate, a co-substrate of the enzyme action. Then, Fragkostefanakis *et al.* (2013) showed that 2,4-PDCA inhibited the *in vitro* activity of ACC oxidase prepared from tomato pericarp tissues. This observation supported the hypothesis that 2,4-PDCA inhibits ACC oxidase action by competing with ascorbate in carnation flowers.

Satoh *et al.* (2014) confirmed that 2,4-PDCA inhibited ACC

oxidase action using a recombinant enzyme produced in *Escherichia coli* cells from the carnation ACC oxidase gene (*DcACO1* cDNA). They also showed that 2,4-PDCA treatment significantly prolonged the vase life of cut ‘Light Pink Barbara (LPB)’ and ‘Mule’ carnation flowers, both of which belong to the spray type, from the percentage of open flowers to the total number of initial flower buds (Satoh *et al.*, 2005). Then, Sugiyama and Satoh (2015) evaluated the activity of 2,4-PDCA and its five analogs (2,3-, 2,5-, 2,6-, 3,4- and 3,5-PDCAs) to accelerate flower opening by determining the number of days to flower opening, in addition to their activity to extend the vase life of cut flowers of ‘LPB’ carnation. All six chemicals accelerated flower opening and extended vase life, although the effects varied with the chemical. They concluded that 2,3-PDCA and 2,4-PDCA were useful. Recently, Sugiyama *et al.* (2015) showed that PDCA analogs increased the number of open flowers according to their criterion ‘gross flower opening’. Satoh *et al.* (2014) suggested that gibberellin (GA) was involved in the promotive effect of 2,4-PDCA on flower opening in cut flowers of spray-type carnation, because 2,4-PDCA is a structural analog of 2-oxoglutarate (2-OxoGA) (Vlad *et al.*, 2010), and the latter acts as a co-substrate of enzymes responsible for GA biosynthesis and metabolism.

The foregoing studies (Satoh *et al.*, 2014; Sugiyama and Satoh, 2015; Sugiyama *et al.*, 2015) demonstrated that PDCA analogs increased the number of open flowers, accelerated flower opening, and lengthened the vase life by retarding senescence, thereby markedly extending of the vase life of cut flowers of ‘LPB’ carnation. To the best of our knowledge, this is the first report of a chemical with multiple effects on cut carnation flowers. This novel agent, PDCA, may be applicable as a flower care agent in the near future. In the present study, we explored the applicability

of 2,4-PDCA to cut flowers of spray-type carnation cultivars, including 'LPB' and 'Mule', and compared pulse treatment with continuous treatment.

Materials and methods

Carnation flowers: Flowers of seven cultivars of the spray-type carnation (*Dianthus caryophyllus* L.) were used, *i.e.*, 'Barbara', 'Beam Cherry', 'Candle', 'Collin', 'Light Pink Barbara (LPB)', 'Rascal Green' and 'Scarlet Ostara'. Flowers at the usual commercial stage of flowering, when the first flower out of six to eight flower buds on a stalk was partially open, were harvested with 65-cm-long stalks at the nursery of a commercial grower in Miyagi prefecture, Japan. The carnation flowers were harvested in the afternoon (in May and June 2015), immediately placed with their cut stalk ends in tap water, and sent in the next morning to the Faculty of Agriculture of Ryukoku University, Otsu city, Shiga prefecture, without supply of water during transportation. The flowers were not treated with any flower preservatives, including silverthiosulfate (STS), after harvest. Upon arrival the next day, the flowers were placed in plastic buckets with their cut stalk ends in tap water under continuous light from white fluorescent lamps ($14 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPF) at 23°C and 50-70% relative humidity, until experiments which were started several hours after they arrived.

Determination of the days to flower opening, the vase life and the gross flower opening of carnation flowers treated with PDCA: Three samples (bunches) of 5 flower stalks (trimmed to 60-cm long), each having 5 flower buds (25 buds in total per sample), were put in 0.9 L glass jars with their stalk end in 300 mL of test solutions (one sample per glass jar). The flowers were kept under continuous light from white fluorescent lamps ($14 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPF) at 23°C and 50-70% relative humidity for 24 days or 30 days ('Candle'). During this period the distilled water (control and the samples after pulse treatment) was replaced once a week, and test solutions were replenished as necessary in the continuous treatment. For pulse treatment (Fig. 2), flower samples were treated with 2,4-PDCA solutions at given concentrations for 24 h, and kept in distilled water thereafter. Fully-open and non-senescing (not wilted and turgid) flowers (FONS flowers), at flower opening stages Os 6 to Ss 2 (Harada *et al.*, 2010; Morita *et al.*, 2011), were counted daily and the percentage of these flowers to the total number (25) of initial flower buds per sample was calculated. Data are presented as changes of the percentages of FONS flowers during 24 days. Flower samples having 40% or more FONS flowers were regarded having display value.

Table 1. Effects of 2,4-PDCA on the time to flower opening, the vase life and the gross flower opening in cut flowers of various spray-type carnation cultivars

Cultivars	Time to flower opening (days)		Vase life (days)		Gross flower opening (scores)	
	Control	PDCA	Control	PDCA	Control	PDCA
Barbara	3.7 ± 0.3	2.4 ± 0.3	11.2 ± 0.9	$17.2^{**} \pm 0.5$	126.7 ± 17.0	$550.7^{**} \pm 26.6$
Candle	2.4 ± 0.1	2.0 ± 0.2	16.5 ± 0.2	$23.6^{**} \pm 0.2$	429.3 ± 51.7	$1138.7^{**} \pm 44.6$
Collin	2.8 ± 0.2	2.1 ± 0.2	10.0 ± 1.5	$18.2^{**} \pm 0.8$	208.0 ± 31.7	$713.3^{**} \pm 19.6$
Beam Cherry	6.3 ± 0.6	5.1 ± 0.5	10.8 ± 1.3	13.5 ± 0.9	250.7 ± 40.7	$548.0^* \pm 58.9$
Scarlet Ostara	3.0 ± 0.7	1.9 ± 0.2	13.1 ± 1.5	16.3 ± 0.9	205.3 ± 19.2	$534.7^{**} \pm 31.0$
Rascal Green	9.5 ± 0.0	$7.0^{**} \pm 0.5$	9.8 ± 0.5	$12.9^{**} \pm 0.1$	218.7 ± 33.7	$480.0^{**} \pm 31.2$

Data are shown by the mean \pm SE of 3 replicated samples. * and ** show significant differences from the control by Student's t-test at $P < 0.05$ and $P < 0.01$, respectively.

The vase life of flowers is expressed by the number of days during which the percentage of FONS flowers was 40% or more (Satoh *et al.*, 2014). The time to flower opening was determined as the number of days from the start of the experiment to the time when the percentage of FONS flowers reached 40% (Sugiyama and Satoh, 2015). The gross flower opening was shown by the cumulative daily percentage at 40% or more of FONS flowers during incubation, which was shown by 'scores' as the unit (Sugiyama *et al.*, 2015). The test solutions consisted of 2,4-PDCA at 0 (control), 2, 5, 10 and 30 mM dissolved in distilled water. The pH of these test solutions was not adjusted and 8-hydroxyquinoline sulfate at 100 mg L⁻¹ was added to the solutions as a germicide. The stock solution of 100 mM 2,4-PDCA was made by dissolving 2,4-PDCA as Na-salt and adjusted at pH 7 with 1 M NaOH, and diluted with distilled water before use. Data are shown by the mean \pm SE. 2,4-PDCA was purchased from Wako Pure Chemical Industries, Ltd., Osaka, Japan.

Statistical analyses: Statistical analyses were carried out by Student's t-test or Tukey's multiple range test using an online statistical analysis program, MEPHAS (<http://www.gen-info.osaka-u.ac.jp/testdocs/tomocom/>, July 17, 2015). Values with $P < 0.05$ were considered significant.

Results and discussion

Effects of 2,4-PDCA on flower opening characteristics of different carnation cultivars: Figure 1 shows percentage of FONS flowers in cut 'Barbara' flowers treated continuously with 0 (control) or 2 mM 2,4-PDCA. Data are shown by those of three independent replicated samples. Each sample consisted of 5 flower stalks with 25 initial flower buds in total. Using this figure, we determined (1) the time to flower opening, (2) the vase life and (3) the gross flower opening which are shown by arrows with 1, 2 and 3. The treatment with 2 mM 2,4-PDCA tended to shorten the time to flower opening, lengthen the vase and increase the gross flower opening compared with the untreated control flowers. The same experiment as in Figure 1 was conducted using other cultivars, and the values for these three criteria for the flower opening profile are summarized in Table 1. The time to flower opening tended to be shortened by 2 mM 2,4-PDCA in all the cultivars tested, although it was significantly shortened in 'Rascal Green' cultivar.

The vase life was significantly extended by the treatment with 2 mM 2,4-PDCA, in 'Barbara', 'Candle', 'Collin', and 'Rascal Green'. The extension of the vase life was the greatest in 'Collin' cultivar (1.82 fold), followed by 'Barbara' (1.54 fold), 'Candle'

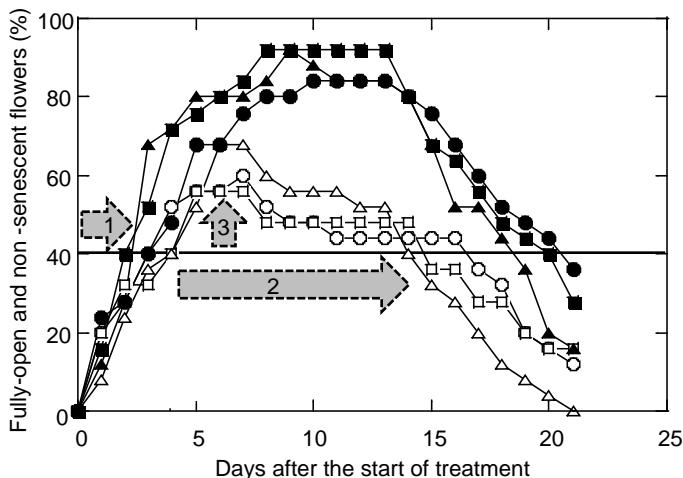


Fig. 1. Change in the percentage of fully-open and non-senescent flowers for cut flowers of 'Barbara' carnation treated with 2,4-PDCA. Bunches of cut flowers, each with 5 main flower stalks with 5 flowers (buds) on each stalk (25 flower buds in total), were treated continuously with 0 (control) or 2 mM 2,4-PDCA. Three replicated data for the control (\circ , \square , Δ) and 2,4-PDCA treatment (\bullet , \blacksquare , \blacktriangle) are shown. The time to flower opening (1), the vase life (2) and the gross flower opening (3) are shown by arrows with numbers.

(1.43 fold) and 'Rascal Green' (1.32 fold) cultivars. In 'Beam Cherry' and 'Scarlet Ostara', the vase life was extended by 2,4-PDCA treatment, 1.25 and 1.24 fold, respectively, though not significantly different with the control. The gross flower opening was increased by treatment with 2 mM 2,4-PDCA in all the carnation cultivars. The magnitude of increase in the gross flower opening was the largest with 'Barbara' (4.35 fold) followed by 'Collin' (3.43 fold) and 2.19 to 2.65 fold in other cultivars.

Previous studies demonstrated the promotive effect of 2,4-PDCA treatment on flower opening in cut flowers of 'LPB' and 'Mule' (Satoh *et al.*, 2014). The present findings further indicate that PDCA analogs, including 2,4-PDCA, promote flower opening and delay senescence in a wide range of spray-type carnation cultivars.

Comparison of the effect of 2,4-PDCA between the continuous and pulse treatments: In previous studies (Satoh *et al.*, 2014; Sugiyama and Satoh, 2015), PDCA was applied to cut carnation flowers continuously during experiments (the continuous treatment). This procedure sometimes caused detrimental side effects, resulting in browning of leaves or broken stalks (Sugiyama and Satoh, 2015), probably because of excess absorption of the chemicals. Also this procedure seems to be practically inadequate from application perspective of the chemicals, since it would need much labor work. Therefore, we tried to apply PDCA by a pulse treatment, in which the flowers were treated once after harvest for short period then kept in water (pulse treatment). For comparison, the cut flowers were treated continuously with 2 mM 2,4-PDCA (continuous treatment). The mean of 4 replicated samples each for the control and the 2,4-PDCA-treated samples, each with 5 flower stalks having 25 initial flower buds in total, is shown.

The time to flower opening was 6.0 ± 0.5 days in the control. The pulse treatment with 5 and 10 mM 2,4-PDCA shortened it to 5.0 ± 0.7 days and 3.1 ± 0.4 days, respectively, the latter value

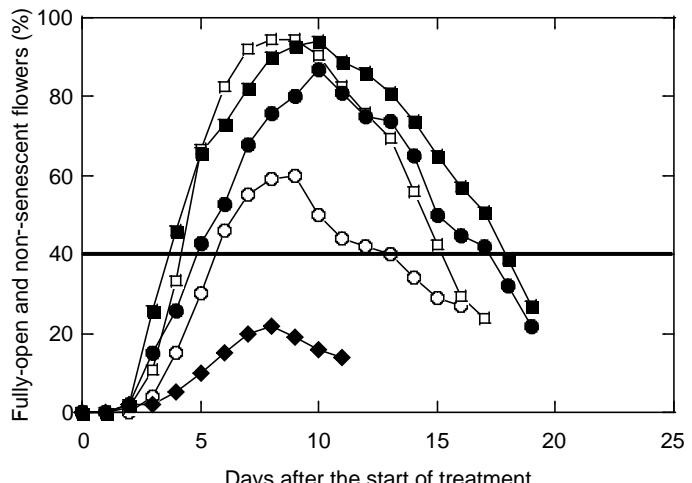


Fig. 2. Effects of pulse and continuous treatments with 2,4-PDCA on flower opening characteristics of 'Light Pink Barbara' carnation. Bunches of cut flowers, similar to those described in the legend to Fig. 1, were treated with 2,4-PDCA at 0 (\circ), 5 (\bullet), 10 (\blacksquare) and 30 mM (\blacklozenge) for 24 h, then kept in water (pulse treatment). The continuous treatment was conducted by leaving flower bunches continuously in 2 mM 2,4-PDCA (\square). Change in the percentage of fully-open and non-senescent flowers is shown by the mean of 4 replicated samples.

being significantly different from the control ($P < 0.05$ by Tukey's multiple range test). The continuous treatment with 2 mM 2,4-PDCA shortened the time to flower opening to 4.3 ± 0.3 days, though not significantly different with the control.

The vase life in the control was 5.7 ± 1.7 days. It was lengthened by the pulse treatment with 2,4-PDCA at 5 mM (11.9 ± 0.7 days) and 10 mM (14.2 ± 0.3 days), and by the continuous treatment with 2 mM 2,4-PDCA (11.2 ± 0.5 days), with a significant difference from the control value. The gross flower opening score in the control was 94.0 ± 35.7 . The pulse treatment with 5 and 10 mM 2,4-PDCA increased the score to 335.0 ± 28.8 and 495.0 ± 8.5 , respectively, and the continuous treatment with 2 mM 2,4-PDCA increased the score to 413.3 ± 74.2 .

The pulse treatment with 30 mM 2,4-PDCA severely inhibited flower opening, resulting in browning and death of stems and leaves. The pulse treatment with 5 and 10 mM 2,4-PDCA did not have this adverse effect. The present results demonstrated that the effect of pulse treatment with 5 or 10 mM 2,4-PDCA on the flower opening characteristics was similar to or greater than that of the continuous treatment with 2 mM 2,4-PDCA in 'LPB' carnation.

The present study revealed that 2,4-PDCA, probably other PDCA analogs as well, are effective on carnation cultivars, with spray-type flowers. These finding will help promote the practical use of PDCA analogs. Further studies are needed on the effect of PDCAs on cut flowers of other species with spray-type flowers, irrespective of the involvement of ethylene in the process of flower senescence, such as *Eustoma*, *Gypsophila*, and *Alstroemeria* flowers and spray-type *Chrysanthemum*.

References

- Abeles, F.B., P.W. Morgan and M.E. Saltveit, Jr., 1992. *Ethylene in Plant Biology*. 2nd ed. Academic Press, San Diego, CA.
- Fragkostefanakis, S., P. Kalaitzis, A.S. Siomos and D. Gerasopoulos, 2013. Pyridine 2,4-dicarboxylate down regulated ethylene production in response to mechanical wounding in excised mature green tomato pericarp discs. *J. Plant Growth Regul.*, 32: 140-147.

- Harada, T., Y. Torii, S. Morita, T. Masumura and S. Satoh, 2010. Differential expression of genes identified by suppression subtractive hybridization in petals of opening carnation flowers. *J. Exp. Bot.*, 61: 2345-2354.
- Morita, S., Y. Torii, T. Harada, M. Kawarada, R. Onodera and S. Satoh, 2011. Cloning and characterization of a cDNA encoding sucrose synthase associated with flower opening through early senescence in carnation (*Dianthus caryophyllus* L.). *J. Japan. Soc. Hort. Sci.*, 80: 358-364.
- 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. Japan. Soc. Hort. Sci.*, 80: 127-135.
- Satoh, S., Y. Kosugi, S. Sugiyama and I. Ohira, 2014. 2,4-Pyridinedicarboxylic acid prolongs the vase life of cut flowers of spray carnations. *J. Japan. Soc. Hort. Sci.*, 83: 72-80.
- Satoh, S., H. Nukui and T. Inokuma, 2005 A method for determining the vase life of cut spray carnation flowers. *J. Appl. Hort.*, 7: 8-10.
- Sugiyama, S. and S. Satoh, 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.
- Sugiyama, S., S. Morita and S. Satoh, 2015. Three criteria for characterizing flower opening profiles and display values in cut spray-type carnation flowers. *J. Appl. Hort.*, 17: 92-95.
- Vlad, F., P. Tiainen, C. Owen, T. Spano, F.B. Daher, F. Oualid, N.O. Senol, D. Vlad, J. Myllyharju and P. Kalaitzis, 2010. Characterization of two carnation petal prolyl 4 hydroxylases. *Physiol. Plant.*, 140: 199-207.

Received: October, 2015; Revised: November, 2015;

Accepted: December, 2015