Journal of Applied Horticulture, 17(2): 92-95, 2015

Three criteria for characterizing flower opening profiles and display values in cut spray-type carnation flowers

So Sugiyama, Shigeto Morita[1] and Shigeru Satoh[1]*

Graduate School of Life and Environmental Sciences, Kyoto Prefectural University, Kyoto 606-8522, Japan.
[1]Kyoto Prefectural Institute of Agricultural Biotechnology, Seika Town, Kyoto 619-0224, Japan.
**E-mail: ssatoh@agr.ryukoku.ac.jp*

Abstract

Previously we have developed a method, which uses two criteria, 'time to flower opening' and 'vase life', for characterizing flower opening profiles in cut spray-type flowers of carnation. These two criteria were used to evaluate the activities of flower preservatives, which accelerate flower bud opening, resulting in shortening the time to flower opening, and delay senescence, resulting in extension of vase life. In the present study, we developed the third criterion 'gross flower opening' which characterizes the ability of flower buds to open. Using this criterion the activity of analogs of pyridinedicarboxylic acids was successfully evaluated in addition to the previously-reported evaluation of their activity of acceleration of flower bud opening and extension of vase life.

Key words: Flower bud opening, display value, pyridinedicarboxylic acid, senescence, spray-type carnation, vase life.

Introduction

Carnation is one of the most popular cut flowers and of highest economic importance in the floriculture industry in many countries. Cut flowers of carnation are used in two forms or categories, *i.e.*, the standard type in which carnations have one flower on a stem and the spray type in which carnations have multiple flowers on a stem. In recent years, spray-type carnation flowers have become popular because they can be grown with less labor and meet modern consumer's demand. Carnation cultivars differ in the length of the vase life of cut flowers (Nukui *et al.*, 2004), which is one of the characteristics determining the commercial value of ornamental flowers. Usually the vase life of carnation flower has been determined by observing senescence profiles, *i.e.*, in-rolling of petal margin and wilting of whole petals as well as ethylene production. This method has been used successfully for cut carnation flowers of the standard type. However, in spray-type carnation flowers, the vase life of the flowers is determined by the sum of the flowering period of each flower, which required development of another method different from that for the standard-type flowers.

Previously, Satoh *et al.*(2005) established a method to determine the vase life of spray-type carnation flowers by observing the change in the percentage of open flowers to the total number of initial flower buds. In more detail, the vase life (in days) was determined by the number of days during which the percentage of fully open and non-senescent flowers was 40% or more. The vase life determined by this method was successfully used to evaluate the action of preservatives, such as sucrose and 1,1-dimethyl-4-(phenylsulfonyl)semicarbazide, on cut spray-type flowers of carnation.

Recently, Vlad *et al.* (2010) reported that 2,4-pyridinedicarboxylic acid (2,4-PDCA) could suppress ethylene production from carnation flowers, by inhibiting the action of 1-aminocyclopropane-1-carboxylate (ACC) oxidase, and prolong the vase life of cut standard-type flowers of 'White Sim' carnation. Satoh *et al.* (2014) confirmed that, using the above-described method for determining the vase life of cut spray-type carnation flowers, 2,4-PDCA lengthened the vase life of cut spray-type flowers of 'Light Pink Barbara (LPB)' carnation, as well as it inhibited ACC oxidase action using a recombinant enzyme produced in *E. coli* cells from a carnation ACC oxidase gene (*DcACO1*). Sugiyama and Satoh (2015) revealed that PDCA analogs in addition to 2,4-PDCA could accelerate flower opening of 'LPB' carnation, which was demonstrated by observing the shortened time to flower opening, in addition to the previously-shown extension of vase life. For characterizing the acceleration of flower opening by PDCAs, they developed another criterion 'time to flower opening', which was determined by the number of days from the start of the experiment until the time when the percentage of fully open and non-senescent flowers reached 40%. This second criterion was successfully used to describe the activity of PDCA analogs to accelerate flower (bud) opening (Sugiyama and Satoh, 2015).

Interestingly, moreover, Sugiyama and Satoh have noticed at the same time that PDCA analogs markedly increased the gross number of open flowers (unpublished results). The increase was probably caused by the elevation of ability of flowers buds to open by PDCA analogs as well as by the acceleration of flower (bud) opening and the delay of onset of senescence. Therefore, in the present study, we aimed to establish a method to determine the ability of flower buds to open, and to evaluate PDCA analogs in elevating the ability of flower buds in cut spray-type flowers of carnation.

Materials and methods

Original experiments and data: Data used for analysis of *gross flower opening*, which was defined in the present study and described later, came from those obtained previously and shown in two separate papers, Satoh *et al.* (2014) and Sugiyama and

Satoh (2015). Briefly, a carnation cultivar, *Dianthus caryophyllus* L. 'Light Pink Barbara (LPB)', which blooms spray-type flowers, was used. Treatment of carnation flowers with analogs of pyridinedicarboxylic acid (PDCA) was conducted as follows: three bunches of 5 flower stems (trimmed to 60-cm long), each having 5 flower buds (25 buds in total per bunch), were put in 0.9 L glass jars with their stem end in 300 mL of test solutions (one bunch per glass jar). The flowers were left under continuous light from white fluorescent lamps (14 μmol m^{-2} s^{-1} PPFD) at 23°C and 40–70% relative humidity for 24 days, and during this period the distilled water (control) was replaced every week and PDCA-containing test solutions were replenished as necessary. Fully open and non-senescent (not wilted and turgid) flowers, which were ranging from Os 6 to Ss 2 of flower opening stages (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 bunch was calculated. Data were presented as changes of the percentages of fully open and non-senescent flowers during 24 days. Flower bunches having 40% or more fully open and non-senescent flowers were regarded as those having a display value.

Determination of gross flower opening: In addition to the previously defined two criteria for describing flower opening profiles in cut spray-type carnation flowers, vase life (Satoh *et al.*, 2005) and time to flower opening (Sugiyama and Satoh, 2015), the third criterion, gross flower opening, was defined to describe the ability of flower buds to open. The gross flower opening was calculated by the sum of percentages at 40% or more of fully open and non-senescent flowers during incubation. The term 'scores' was employed as the unit for gross flower opening.

Statistical analyses: Statistical analyses were carried out by Steel's, Williams' or Dunnett's multiple range tests using an online statistical analysis program, MEPHAS (http://www.gen-info.osaka-u.ac.jp/testdocs/tomocom/; January 30, 2015).

Results and discussion

Previously, two criteria, vase life (Satoh *et al.*, 2005) and time to flower opening (Sugiyama and Satoh, 2015), were developed for characterizing flower opening profiles in cut spray-type carnation flowers, as described in Introduction. In the present study, we defined third criterion 'gross flower opening', for describing another flower opening profile, the ability of flower buds to open, and tried to use it for evaluating the action of PDCA analogs in cut spray-type flowers of 'LPB' carnation.

Fig. 1 shows changes in the percentage of fully open and non-senescent flowers for cut 'LPB' flowers treated with 2,4-PDCA at different concentrations; this figure came from Fig. 2 of Sugiyama and Satoh (2015) and Fig. 4 of Satoh *et al.* (2014). The time to flower opening was 4.4 days for the control, and it was shortened by treatment with 2,4-PDCA to 4.3 days at 0.3 mM, 3.3 days at 1 mM, and 3.8 days at 2 mM, although only the treatment with 1 mM 2,4-PDCA was significantly different from the control (Table 1; Sugiyama and Satoh, 2015). The vase life was significantly lengthened by treatment with 2,4-PDCA; from 8.3 days of the control to 12.7, 17.5 and 19.5 days at 0.3, 1.0 and 2.0 mM 2,4-PDCA, in this order (Table 1; Satoh *et al.*, 2014).

Data in Fig. 1 also suggested that 2,4-PDCA treatment increased the number of open flowers compared with that in the control. Therefore, we defined 'gross flower opening' as a measure of total number of open flowers during experiments, and calculated its values by the sum of percentages at 40% or more during experiments, as described in Materials and methods. The gross flower opening (the unit is 'scores') of the flowers treated with 2,4-PDCA at different concentrations was 187 at 0 mM (control), 258 at 0.3 mM, 504 at 1 mM, and 604 at 2 mM (Table 1). The latter two were significantly different from the control. These observations suggested that 2,4-PDCA treatment elevated the ability of flower buds to open. We considered that this procedure for determining 'gross flower opening' is useful to evaluate the ability of flower buds as affected (elevated) by treatment with chemicals such as 2,4-PDCA.

Recently, Sugiyama and Satoh (2015) showed that not only 2,4-PDCA but also other PDCA analogs, including 2,3-, 2,5-, 2,6-, 3,4- and 3,5-PDCA, had activities to accelerate flower opening as well as those to lengthen the vase life in cut flowers of 'LPB' carnation. In the present study, we determined the gross flower opening when treated with respective compounds using the original data (Fig. 4 and Table 1 in Sugiyama and Satoh, 2015). The results are shown in Table 2, with the data for time to flower opening and vase life, which were adopted from Table 1 of Sugiyama and Satoh (2015). In the experiment with PDCA analogs at 1 mM, the control (0 mM) flowers had the gross flower opening of 45 scores. All the PDCA analogs at 1 mM increased the gross flower opening, which ranged from 101 to 348 scores depending on each PDCA analog, although there was no statistical significance. In the experiment with PDCA analogs at 2 mM, the

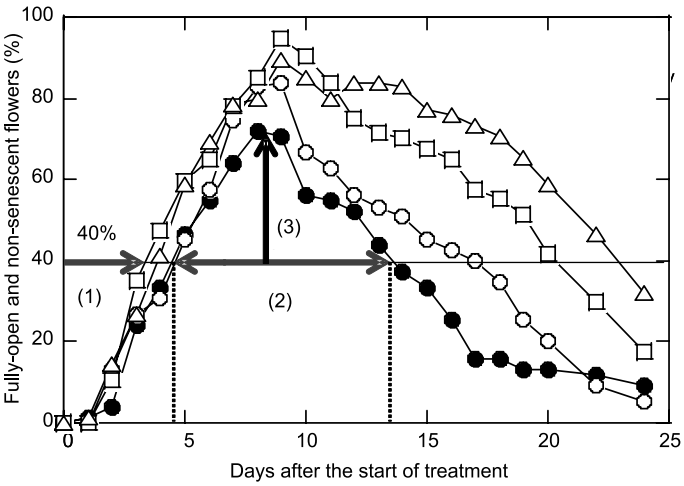

Fig. 1. Three criteria for characterizing the flower opening profiles in cut spray-type carnation flowers.

In addition to previously defined two criteria, time for flower opening (1) and vase life (2) (Sugiyama and Satoh, 2015), the third criterion, gross flower opening (3), was defined for evaluating 2,4-PDCA which improves displaying values of cut spray-type carnation flowers. The vase life of the cut flowers in days was defined as the duration when the percentage of fully open and non-wilted flowers was 40% or more. The time to flower opening was defined as the time in days from the start of the experiment until the percentage of open flowers reached 40%. Whereas, the gross flower opening (the unit is 'scores') was determined by the sum of percentages at 40% or more from the day when the percentage reached or surpassed 40% through the day when they declined to or fell below 40%. This figure came from the original and modified drawings that appeared as Fig. 4 in Satoh *et al.* (2014) and Fig. 2 in Sugiyama and Satoh (2015), respectively. Each point is the mean of 3 replicates, each with 5 flower stems with 5 flower buds (25 flower bud for each replicate). ●, Control (0 mM 2,4-PDCA); ○, 0.3 mM 2,4-PDCA; □, 1 mM 2,4-PDCA; Δ, 2 mM 2,4-PDCA.

Table 1. Evaluation by three criteria of 2,4-PDCA which improves display value of cut flowers of 'Light Pink Barbara' carnation

PDCA (mM)	Time to flower opening (days)	Decrease (%)	Vase life (days)	Increase (%)	Gross flower opening (scores)	Increase (%)
0 (control)	4.4	–	8.3	–	187	–
0.3	4.3	2	12.7*	53	258	38
1.0	3.3*	25	17.5*	111	504*	170
2.0	3.8	14	19.5*	135	604*	223

The open-flower stage good for display was defined as the duration when 40 % or more buds are in the fully-open and non-senescent stage [Os 6–Ss 2 (Harada *et al*., 2010; Morita *et al*., 2011)]. Data for the time to flower opening came from Fig. 2 in Sugiyama and Satoh (2015), and those for the vase life from Table 1 in Satoh *et al*. (2014). Data are shown as the mean of triplicate bunches, each with 5 flower stems with 5 buds (25 buds in total per bunch). * shows a significant difference from the control (0 mM) by Steel's multiple range test (MRT) ($P < 0.05$) for time to flower opening and by Williams' MRT($P< 0.05$) for vase life and gross flower opening.

Table 2. Evaluation by three criteria of PDCA analogs which improve display value of cut flowers of 'Light Pink Barbara' carnation

Chemicals	Experiment 1 (1 mM PDCA)			Experiment 2 (2 mM PDCA)		
	Time to flower opening (days)	Vase life (days)	Gross flower opening (scores)	Time to flower opening (days)	Vase life (days)	Gross flower opening (scores)
Control	5.5 (100)	4.0(100)	45 (100)	9.0 (100)	8.3 (100)	40 (100)
2,3-PDCA	3.3* (60)	14.0*(350)	244 (542)	4.0*(44)	14.7*(177)	531*(1328)
2,4-PDCA	4.3*(78)	14.0*(350)	189 (189)	4.0*(44)	14.7*(177)	423*(1058)
2,5-PDCA	5.0*(91)	17.0*(425)	348 (773)	4.7*(52)	13.0*(157)	412*(1030)
2,6-PDCA	4.3*(78)	12.7*(318)	201 (447)	5.3*(59)	9.3*(112)	283* (708)
3,4-PDCA	8.0(145)	8.0*(200)	101 (224)	5.7*(63)	14.7*(177)	441*(1103)
3,5-PDCA	5.3* (96)	14.3*(358)	206 (458)	4.7*(52)	15.5*(187)	506*(1265)

The original data for the time to flower opening and vase life were adopted from Fig. 4 and Table 1 in Sugiyama and Satoh (2015).Data are shown as the mean of triplicate bunches, each with 5 flower stems with 5 buds (25 buds in total per bunch), but data for time to flower opening and vase life in the control for Experiment 1 are the mean of 2 replicates since the remaining third replicate did not attain 40% open flowers. Figures in the parentheses show the percentages to the control.*shows asignificant difference from the control in each column by Dunnett's multiple range test ($P< 0.05$).

gross flower opening was significantly increased from the control value of 40 scores to much higher values from 283 to 531 scores depending on each PDCA analog. 2,3-PDCA gave the highest elevation of gross flower opening in this experiment. Judging from the combined results at 1 mM and 2 mM PDCA analogs, we suggest that PDCA analogs had activity to increase the gross flower opening, resulting in the elevation of the ability of flower buds to open. The order among PDCA analogs in terms of their action to elevate the gross flower opening was not clear, although it was evident that 2,3-PDCA gave the highest elevation in both concentrations. Previously, it was suggested that 2,3-PDCA and 2,4-PDCA were most effective among PDCA analogs for the acceleration of flower opening and the extension of vase life (Sugiyama and Satoh, 2015) in cut flowers of 'LPB' carnation. The present results on the gross flower opening partly agreed to the previous ones. We did not study further the reason for the difference of chemical structures of PDCA analogs among three actions, *i.e.*, acceleration of flower opening, extension of vase life and increase of gross flower opening, and its elucidation remains as a subject of future investigation.

In the original data (Fig. 3 in Sugiyama and Satoh, 2015), the maximum values of the percentage of fully open flowers of the untreated control, which slightly surpassed 40%, were much lower than those obtained previously with 'LPB' flowers, for example, 66–88% (Satoh *et al.*, 2014) and 100% (Satoh *et al.*, 2005). The low maximum percentage of open flowers might have resulted from differences in the ability to open among flower samples, which were cultivated and harvested in different years and seasons. It is likely that the low ability might be caused by shortage of sugars after harvest, which resulted from their consumption for respiration and reduced supply by depressed photosynthesis under low lightening condition. In fact, it is known that sugar accumulation in petal cells reduces the petal water potential and promotes water influx into the petal cells, resulting in cell enlargement and culminating in flower opening (Evans and Reid, 1988; Ho and Nichols, 1977; Ichimura *et al.*, 2003). Exogenously-applied sugars, such as glucose, fructose and sucrose (after being hydrolyzed by invertase forming glucose and fructose), result in the promotion of flower opening. Promotion of flower bud opening by exogenously-applied sugars was also found in our previous studies, in which 0.1 M sucrose slightly increased the number of fully open and non-senescent flowers in cut spray-type 'LPB' flowers (Satoh *et al.*, 2005) and sucrose and palatinose, both at 1%, accelerated flower (bud) opening in 'LPB' flowers (Satoh *et al.*, 2013). Some PDCA analogs at 2 mM accelerated flower (bud) opening and increased the gross flower opening; the percentage of open flowers surpassed 90% resulting in opening of almost all flower buds (Sugiyama and Satoh, 2015). These results suggested that the low ability of buds to open in cut spray-type 'LPB' flowers is not always caused by shortage of sugars in the flower tissues. Sugars may act as a signal to induce the opening of flower buds in addition to act as an energy source for flower opening. The action mechanism of sugars as well as PDCAs remains to be elucidated in the future.

The present study established a method to determine the gross flower opening, which evaluates the ability of flower buds to open for characterizing the flower opening profiles in cut spray-type carnation flowers. We think the three criteria, the present 'gross flower opening' and other two previously-defined criteria 'time to flower opening' and 'vase life', will be useful to characterize flower opening profiles in spray-type flowers of other ornamentals as well as carnation. On the other hand, from the point of view of flower preservatives, the three criteria would be useful to evaluate end-points of the chemicals, *i.e.*, to determine physiological steps of flower opening in which they will affect, and to elucidate the mechanisms for elevation of ability to open of flower buds,

acceleration of flower bud opening and extension of vase life of opened flowers. Keeping this notion in mind, we are currently testing the effects of PDCA analogs on flower opening profiles of some ornamentals with spray-type flowers other than carnation.

Acknowledgements

We thank financial support by a Grant-in-Aid (24580050 to S. Satoh) for Scientific Research from the Japan Society for the Promotion of Science.

References

Evans, R. Y. and M. S. Reid, 1988. Changes in carbohydrates and osmotic potential during rhythmic expansion of rose petals. *J. Amer. Soc. Hort. Sci*., 113: 884-888.

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.

Ho, L. C. and R. Nichols, 1977. Translocation of ^{14}C-sucrose in relation to changes in carbohydrate content in rose corollas cut at different stages of development. *Ann. Bot.*, 41: 227-242.

Ichimura, K., Y. Kawabata, M. Kishimoto, R. Goto and K. Yamada, 2003. Shortage of soluble carbohydrates is largely responsible for short vase life of cut 'Sonia' rose flowers. *J. Japan. Soc. Hort. Sci.*, 72: 292-298.

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.

Nukui, H., S. Kudo, A. Yamashita and S. Satoh, 2004. Repressed ethylene production in the gynoecium of long-lasting flowers of the carnation 'White Candle': role of gynoecium in carnation flower senescence. *J. Exp. Bot.*, 55: 641-650.

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., M. Miyai, S. Sugiyama and N. Toyohara, 2013. Palatinose-hydrolyzing activity and its relation to modulation of flower opening in response to the sugar in *Dianthus* species. *J. Japan. Soc. Hort. Sci*., 82: 337-343.

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.

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: March, 2015; Revised: April, 2015; Accepted: April, 2015