Fresh flowers (unopened fully matured flowers) from *J. sambac* were collected from randomly selected plants in the early morning hours around 5 am to 6 am during the entire period of study. The harvesting stages were classified based on the visual appearance of the flower bud. The different stages of harvesting are given in Table 1. Observations of E-nose generated Aroma Index (AI), ethylene emission rate and respiration rate was recorded for each stage of harvesting.

Table 1. Different harvesting stages of *J. sambac* flowers

Stage of harvest	Age of flower bud (days)
Stage I	5
Stage II	8
Stage III	10
Stage IV	12
Stage V	15

The matured unopened buds were harvested from the randomly selected plants. The flower buds were continuously observed using E-nose till it opened fully (*i.e.,* from morning to late evening). Postharvest physiological parameters such as E-nose generated Aroma Index (AI), CO_2 rate and ethylene emission rate were recorded at 10.00 am, 4.00 pm, 6.00 pm, 7.00 pm and 8.00 pm. The E-nose generated Aroma Index (AI) values for the above parameters were analysed statistically and least significant difference was applied to compare the differences among different time intervals at 5% as critical level of probability (α). The flower opening index was categorized based on the values in flower opening index chart (Table 2).

Identification of different fragrances was performed by using electronic nose, equipped with a metal oxide semiconductor sensor (MOS). E-nose has a great potential to discriminate fragrances and would be a useful tool for the fragrance of ornamentals.

Electronic Nose system for gradation of jasmine based on aroma characteristics comprised of two main components (i) The Sniffing Unit and (ii) Data Processing Unit. The sniffing unit consists of the sensory and sensing unit. The sniffing unit is the odor capture and delivery system to the sensor array and the data processing unit is responsible for data acquisition from the sensor array through a proper signal conditioning circuit and the acquired data is processed to generate and display the Fragrance Index.

The experimental sniffing cycle consists of automated sequence of internal operations: (i) headspace generation, (ii) sampling, (iii) purging before the start of the next sniffing cycle. Initially these MOS sensors require heating for at least one hour to be stable. Heating is done by supplying 5 Volts to the heater coils of the sensors. This heating phase of sensors is referred to as Pre-heating. The MOS sensors react to volatile compounds on contact; the adsorption of volatile compounds on the sensor surface causes a physical change of the sensor.

Table 2. Flower Opening Index chart of jasmine flowers (*J. sambac*) at different flower opening stages

Flower opening index	Flower opening pattern
0	Un-open bud
0.5	Nearly slight open
1.0	Slightly open
1.5	Nearly half open
2.0	Half open
2.5	Nearly full open
3.0	Fully open

Principle of operation: The samples to be tested were placed in a sample holder (be specific) in the E-Nose set-up. Data was recorded separately for flowers and buds. It has been observed that the system was able to identify the bud and also the blossoming state of Jasmine.

Air flow was blown into the sample container as pressure applied in time scale of second in order to ensure adequate concentration of volatiles in the air within the container. In the same time scale, output voltage was recorded with sensor when exposed to volatile substance influences. The time specified in seconds, for which the sensor array is exposed to fresh air in order to reestablish baseline values of the sensors. Due to the strong scent of jasmine, it is recommended that a purging time of at-least 15 minutes be used.

The sensor array was exposed to a constant flow of volatiles emanated from Jasmine flower at time duration of 50 seconds. Data from all the sensors were stored all through this sampling operation, but the steady-state value for each sensor is considered for computation purposes. During the purging operation of 100 secs, sensor heads were cleared through the blow of air so that the sensors can go back to their baseline values.

Data acquisition module: Sensor outputs were fed to this module. After signal conditioning, the channels were multiplexed and were fed to an data acquisition card. The DAQ output was fed to the processor for analysis and storage.

Flower opening index (FOI): The fragrance emission was determined during fresh flowers opening period which is under influence of their development stage varying from Stage I to stage V. There were significant differences in opening of flower from tight bud stage to fully opened condition as in V stage. The various flower opening indices recorded at different time intervals were categorized (Table 3).

Soxhlet extraction: The compounds responsible for fragrance emission in fully opened jasmine flowers were detected through the concrete extracted using Soxhlet extractor. The fresh samples of about 20 g were taken in the extraction chamber placed in a tube above the extraction solvent. The solvent used was food grade hexane (analytical reagent) to wash the sample using a reflux apparatus. When heated, the solvent evaporates into a gas, and then cools into a liquid in a condenser. It then refluxes back into the sample tube. This continuous cyclic process takes around 45 to 60 minutes per cycle until the concrete is separated from the sample. The solvent was evaporated off, by keeping it in the water bath and the amount of concrete was determined.

Gas chromatography: Later the extracted concrete samples at different interval of time were subjected to Gas Chromatography –Mass Spectrometry analysis. About one micro litre of sample concentrate was injected into a Thermo GC - Trace Ultra Ver: 5.0,Thermo MS DSQ II gas chromatograph equipped with a flame ionization detector. The column used was DB 35 - MS Capillary Standard Non - Polar Column. The specifications of Gas Chromatography used for analysis; Column: 50m × 0.25mm internal diameter (i.d.) coated with PEG 20 M, film thickness: 0.15 pm, Carrier gas: N, with a flow rate at 1.2 mL/min, oven temperature: 60°C (4 min) + 220°C, injection and detector temperature: 200°C Split ratio: 10: 1

Results and discussion

Fragrance parameters estimation at various development stages of flowers were recorded and given in the Table 3 and Table 4. Time taken for flower opening is an important character, which signifies the earliness or late flowering habit of the genotype. Both the habits are helpful in determining the availability of flowers for longer period (Khader and Kumar, 1995).

It was observed that there was no ethylene evolution observed till stage III while it was triggered up to 1 ppm during the stage IV. Then after, ethylene was released up to 3.2 ppm after Stage V of harvest. Under ambient conditions, ethylene evolution rate with the range of 2.0 ppm and 22.8 ppm after harvest of fresh flowers at different time intervals were observed in fully matured opened flower (Table 4). The results indicated that the rate of ethylene evolution increased rapidly after harvest upto 16 hours, after which the senescence of flower starts (Fig. 2). Similar results were observed by Mayak and Halevy (1974), Suttle and Kende (1978, 1980) and Borochov *et al*. (1997). Also it was observed that as the flowers started showing symptoms of wilting, there was a rise in level of ethylene emission rate.

The E-nose generated Aroma Index (AI) of jasmine flowers during different flower developmental stages were observed and it varied from 0.41 in I stage, 0.83 in II stage, 1.58 in III stage, 2.53 in IV stage to 4.26 in V stage. It clearly indicates that the Metal oxide Sensor (MoS) used in the E-nose instrument, sniffed the compounds responsible for fragrance and generated the Aroma Index (AI) as the stage of harvesting progressed. Fully matured harvested jasmine flowers were continuously observed from its closed unopened stage to fully opened condition. Based on our observation the E-nose generated Aroma Index (AI), gradually increased from 5.41 in 10.00 am (unopend closed bud stage) to 16.83 in 4.00 pm (nearly slight open stage), 24.58 in 6.00 pm (slightly opened stage), 32.53 in 6.00 pm (half opened) and 41.26 in 8.00 pm (fully opened). As the flower opens, the fragrance emission is higher and the senescence of flower takes place.

Table 3. E-nose generated aroma index (AI) of *J. sambac* at different flower developmental stages

Harvesting stages	E-nose Value (Aroma Index)	CO_2 Rate (ppm)	Ethylene Emission Rate (ppm)
Stage I	0.41	0	0
Stage II	0.83	0	0
Stage III	1.58	1.2	0
Stage IV	2.53	2.3	1.0
Stage V	4.26	8.4	3.2
SEm ±	0.02	0.1	0.1
LSD (*P*=0.05)	0.07	0.1	0.1

Table 4. Ethylene, CO_2 and Aroma Index at different Flower Opening Index (FOI) of *J. sambac*

Time	*Flower opening index (FOP)	E-nose value (Aroma Index)	CO_2 Rate (ppm)	Ethylene emission rate (ppm)
10.00 am	0	5.41	8.4	2.0
04.00 pm	0.5	16.83	27.8	9.5
06.00 pm	1	24.58	36.3	14.9
07.00 pm	2	32.53	42.6	18.6
08.00 pm	3	41.26	56.8	22.8
SEm ±		0.01	0.1	0.1
LSD (*P*=0.05)		0.21	0.3	0.4

Ethylene hormone has been known to play a crucial role in senescence of flowers, the sensitivity of which varies depending on the flower species (Redman *et al*., 2002). Ethylene reduces the longevity of some flowers causing rapid wilting of petals (*e.g.,* carnations), shedding or shattering of petals, or other changes to petal tissues, such as loss or change of colour.

Earlier reports (Naidu and Reid, 1989) have indicated that the flowers are ethylene sensitive based on the fact that though the flowers produce moderate ethylene during opening and senescence, they do not respond to exogenously applied ethylene (Veen, 1983) indicating that this hormone is not involved in their senescence. No report is available on *Jasminum* spp. with respect to ethylene evolution, however some records on wilting of flowers other than jasmine caused by ethylene have been discussed. Involvement of ethylene in wilting of flowers (Borochov *et al.*, 1997) has been observed in carnation (Ten Have and Woltering, 1997) and in *Gypsophila paniculata* (Vandoorn and Reid, 1992). It has also been noticed that the flower parts including petals, sepals, the ovary and labellum were the major site of ethylene production (Chao Chia *et al.*, 1991) and that ethylene promoted the accumulation of sugars and inorganic materials in the ovary, with a simultaneous loss of fresh and dry weight of the petals. These are some evidences of ethylene sensitive species where in, ethylene is the major cause of wilting of flowers.

Respiration rate: The minimum respiration rate of 8.4 ppm was observed at 10 am. It steadily increases and reached maximum of 56.8 ppm at 8.0 pm in the evening. The respiration of flowers started from III stage upto 1.2 ppm, followed by 2.3 ppm in IV stage and gradually increased in V stage upto 8.4 ppm (Table 3). All the flowers showed a climacteric rise in respiration rate after harvest. The lowest respiration rates were recorded immediately after harvest. This may be due to short supply of readily respirable substrates in the flowers due to onset of senescence. Similar results were reported by Coorts (1973) in cut flowers and Maxie *et al*. (1973) in carnation. Increased respiration leads to formation of free radicals with high oxidation potential. Free radicals promote senescence in tissues which in turn increases sensitivity to ethylene (Fig. 2). Respiration is the central process in living cells that mediates the release of energy through the oxidative breakdown of carbon compounds (starch, sugar and organic acids) and the formation of carbon skeletons necessary for maintenance and synthetic reactions after harvest (Wills *et al*., 1998). In the present study, a respiratory climacteric rise from the initial level and a decline thereafter was noticed with all the treatments.

With regard to *J. sambac* under ambient conditions a similar trend was noticed, recording minimum respiration rate and sufficient amount of carbohydrate levels. These significant levels of carbohydrates might have served as the substrate for respiration for a longer duration. Evidences supporting this fact have been reported in case of flowers supplied with exogenous sugar, wherein pool of dry matter and respirable substrates were maintained at favourable levels thus promoting respiration (Coorts, 1973) and in turn extending the longevity (Rogers, 1973). The

observation of Maxie *et al.* (1973) that the respiratory activity in flowers and the production of carbon-di-oxide by flowers was similar to the pattern in climacteric fruits, characterized by a rise in level of respiration with senescence also supports the present study.

Kaltaler and Steponkus (1976) have associated the decline in respiratory activity of aging rose petals with their inability to metabolise substrates consequent to decline in activity of mitochondria in the aging petals. Moreover, increased respiratory activity leads to the formation of free radicals with high oxidation potential and these free radicals have been found to promote senescence in the tissues, associated with an increased sensitivity to ethylene (Baker *et al*., 1977; Mishra *et al*., 1976). The typical climacteric respiratory rise reported in carnation *cv.* White Sim (Burger *et al*., 1986) and day-lily (Lukaszewski and Reid, 1989) is consistent with the present result. In contrary, Trippi and Paulin (1984) had reported a decrease in respiratory activity in carnation *cv.* White Sim.

GC-MS analysis: The concrete obtained from soxhlet extractor was injected into Gas Chromatography–Mass Spectrometry instrument and constituents were identified by comparison of both mass spectra and retention indices, strictly measured on the same instrument with those of authentic jasmine samples. The identified constituents listed in Table 5 and Table 6 with their respective chromatogram obtained shown in Fig. 1 and 2 exhibited significant compounds identified at specific time intervals (10.00 am and 8.00 pm). The GC-MS chromatogram at 10 am shows, the preliminary indication about the composition of some major volatile components. However, the quantitative composition of these compounds differs considerably from the other samples. The jasmine flower possesses maximum composition and recorded peak during this time. They are Eicosanoic acid, phenylmethyl ester (Benzyl icosanoate) (26.47%), 9-Octadecenoic acid (Z), phenylmethyl ester (Benzyl oleate) (24.04%), Nonadecane (17.41%) and 2,6-Octadien-1-ol, 3,7 dimethyl-(Z)- (cis-Geraniol) (14.24%).

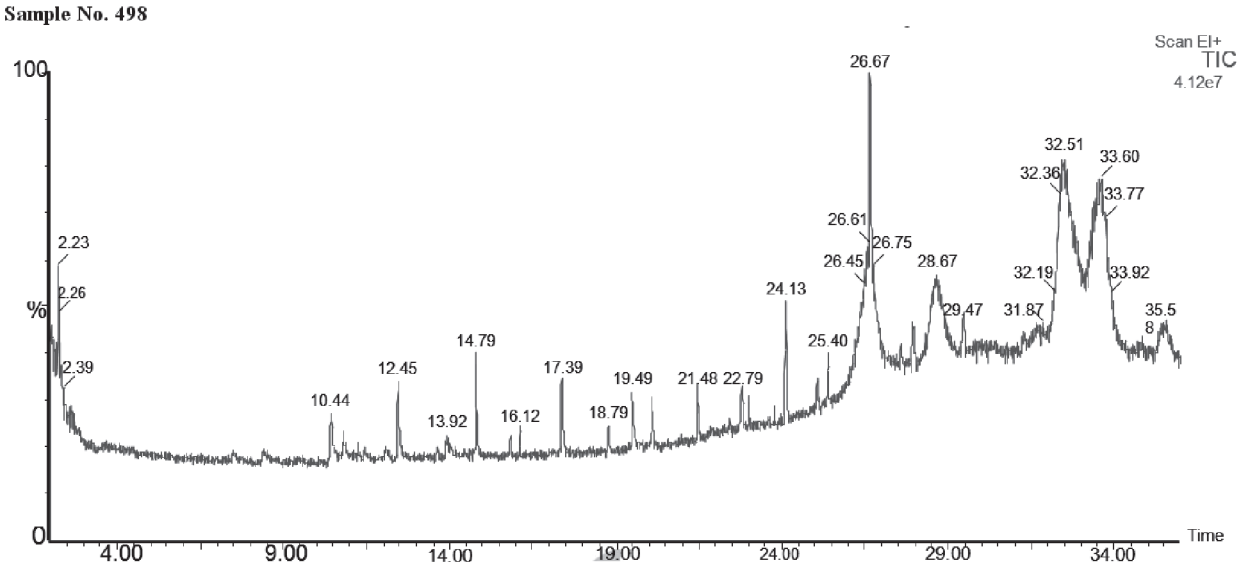

Fig. 1. GC-MS Chromatogram of *J. sambac* extract (soxhlet extraction) at 10.00 am

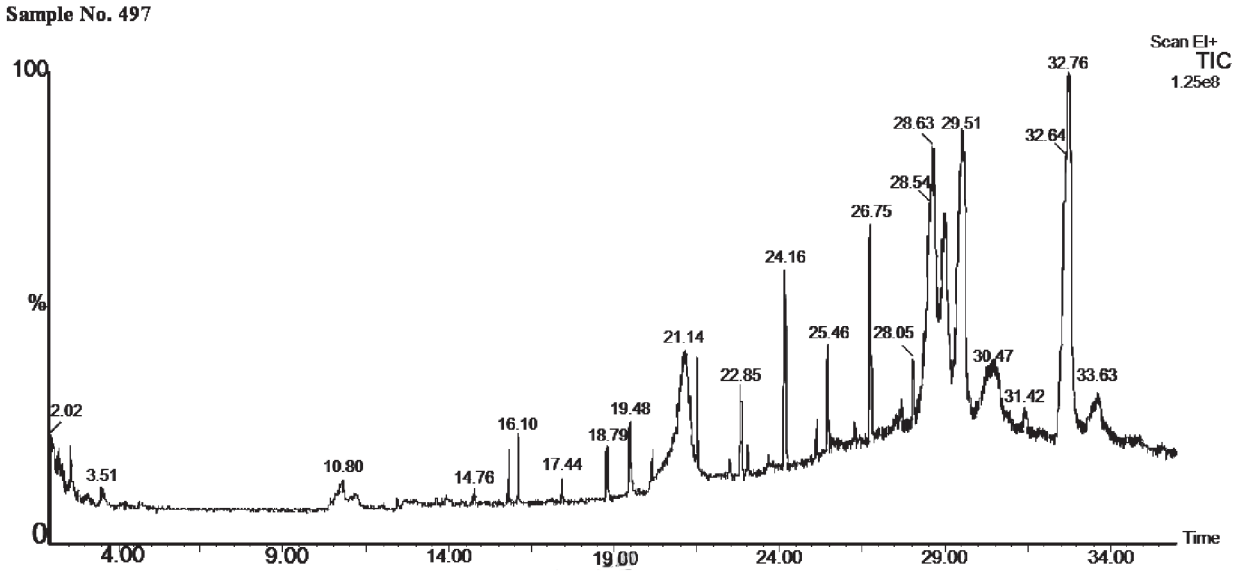

Fig. 2. GC-MS Chromatogram of *J. sambac* extract (soxhlet extraction) at 08.00 pm

Table 5. GC-MS analysis of *J. sambac* extract (soxhlet extraction) at 10.00 AM

RT	Name of the compound	Molecular Formula	MW	Peak Area (%)
2.23	2,6-Octadien-1-ol, 3,7 dimethyl-(Z)- (cis-Geraniol)	$C_{10}H_{18}O$	154	14.24
10.44	2-Aminononadecane	$C_{19}H_{41}N$	283	1.23
10.82	Cyclooctyl alcohol	$C_8H_{16}O$	128	0.57
12.45	1-Tetracosanol	$C_{24}H_{50}O$	354	1.51
13.92	1-Methyldodecylamine	$C_{13}H_{29}N$	199	0.38
14.76	2,4,6,8-Tetramethyl-1-undecene	$C_{15}H_{30}$	210	1.49
15.80	Octodrine	$C_8H_{19}N$	129	0.26
16.10	Heptadecane, 2-methyl-	$C_{18}H_{38}$	254	0.35
17.39	1-Hexacosanol	$C_{26}H_{54}O$	382	1.06
18.79	Heptadecane, 2,6,10,15-tetramethyl-	$C_{21}H_{44}$	296	0.31
19.49	Didodecyl phthalate	$C_{32}H_{54}O_4$	502	1.07
20.09	Decane, 2,3,5,8-tetramethyl-	$C_{14}H_30$	198	0.76
21.48	6H-Pyrazolo[1,2 a][1,2,4,5] tetrazine, hexahydro-2,3-dimethyl-	$C_7H_{16}N_4$	156	0.67
22.44	2-Nonen-1-ol	$C_9H_{18}O$	142	0.17
22.79	Octadecane, 6-methyl-	$C_{19}H_{40}$	268	0.76
23.01	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl- (Farnesol)	$C_{15}H_26O$	222	0.37
23.79	1-Eicosanol	$C_{20}H_{42}O$	298	0.09
24.13	Nonadecane, 2-methyl-	$C_{20}H_{42}$	282	1.54
25.40	Tetracontane, 3,5,24-trimethyl-	$C_{43}H_{88}$	604	0.86
26.67	Nonadecane	$C_{19}H_{40}$	268	17.41
27.95	Octadecane, 1-(ethenyloxy)-	$C_{20}H_{40}O$	296	0.94
28.67	1-Octadecyne	$C_{18}H_{34}$	250	2.53
29.47	Z,Z-2,5-Pentadecadien-1-ol	$C_{15}H_{28}O$	224	0.92
32.51	9-Octadecenoic acid (Z)-, phenylmethyl ester (Benzyl oleate)	$C_{25}H_{40}O_2$	372	24.04
33.60	Eicosanoic acid, phenylmethyl ester (Benzyl icosanoate)	$C_{27}H_{46}O_2$	402	26.47

Twenty eight constituents were identified as active principles in the jasmine samples taken at 8 pm. Major are, 9-Octadecenoic acid (Z)-, phenylmethyl ester (Benzyl oleate) (21.01%), 1-Octadecyne (15.35%) and Octadecanoic acid, phenylmethyl ester (Benzyl stearate) (14.04%). The jasmine volatile compounds responsible for its unique fragrance were released during this time.

The study on ideal harvesting stage for *Jasminum* sp. fresh flowers revealed that the E-nose instrument aids in identifying the ideal harvesting stage for fresh flower stage and suitable time for concrete extraction. This ultimately helps in industrial utility to identify the perfect stage and time for higher concrete recovery. Considering the bulk of the components eluted under these chromatographic conditions, and assuming these compounds possesses a response for fragrance exclusive for jasmine species. The identified constituents from jasmine concrete were responsible for their unique fragrance.

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Table 6. GC-MS analysis of *J. sambac* extract (soxhlet extraction) at 08.00 PM

RT	Name of the compound	Molecular Formula	MW	Peak Area (%)
2.21	2,6-Octadien-1-ol, 3,7 dimethyl-(Z)- (cis-Geraniol)	$C_{10}H_{18}O$	154	3.50
2.58	1-Octanol, 2,7-dimethyl-	$C_{10}H_{22}O$	158	3.01
3.51	Cyclopropyl carbinol	C_4H_8O	72	3.83
10.80	Cyclooctyl alcohol	$C_8H_{16}O$	128	1.44
14.76	2,4,6,8-Tetramethyl-1-undecene	$C_{15}H_{30}$	210	0.15
15.80	Octodrine	$C_8H_{19}N$	129	0.42
16.10	Heptadecane, 2-methyl-	$C_{18}H_{38}$	254	0.54
17.44	1-Hexacosanol	$C_{26}H_{54}O$	382	0.22
18.79	Heptadecane, 2,6,10,15-tetramethyl-	$C_{21}H_{44}$	296	0.42
19.48	Didodecyl phthalate	$C_{32}H_{54}O_4$	502	0.96
20.16	Decane, 2,3,5,8-tetramethyl-	$C_{14}H_30$	198	0.62
21.14	Octadecanoic acid, phenylmethyl ester (Benzyl stearate)	$C_{25}H_{42}O_2$	374	14.04
21.51	6H-Pyrazolo[1,2 a][1,2,4,5] tetrazine, hexahydro-2,3-dimethyl-	$C_7H_{16}N_4$	156	1.37
22.48	2-Nonen-1-ol	$C_9H_{18}O$	142	0.22
22.85	Octadecane, 6-methyl-	$C_{19}H_{40}$	268	0.93
23.03	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl- (Farnesol)	$C_{15}H_26O$	222	0.31
23.66	1-Eicosanol	$C_{20}H_{42}O$	298	0.33
24.16	Nonadecane, 2-methyl-	$C_{20}H_{42}$	282	2.00
25.46	Tetracontane, 3,5,24-trimethyl-	$C_{43}H_{88}$	604	1.08
26.75	Nonadecane	$C_{19}H_{40}$	268	2.41
28.05	Octadecane, 1-(ethenyloxy)-	$C_{20}H_{40}O$	296	0.86
28.63	1-Octadecyne	$C_{18}H_{34}$	250	15.35
28.99	Dodeca-1,6-dien-12-ol, 6,10-dimethyl-	$C_{14}H_{26}O$	210	7.83
29.51	Z,Z-2,5-Pentadecadien-1-ol	$C_{15}H_{28}O$	224	13.22
30.47	Heptadecanoic acid, heptadecyl ester	$C_{34}H_{68}O_2$	508	2.41
31.42	1,4-Dioxaspiro[4.5]decane, 8-(methylthio)-	$C_9H_{16}O_2S$	188	0.39
32.76	9-Octadecenoic acid (Z)-, phenylmethyl ester (Benzyl oleate)	$C_{25}H_{40}O_2$	372	21.01
33.63	Eicosanoic acid, phenylmethyl ester (Benzyl icosanoate)	$C_{27}H_{46}O_2$	402	1.13

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

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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 μmol m^{-2} s^{-1} PPFD) 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 μmol m^{-2} s^{-1} PPFD) 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-senescent (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.

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 ±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'

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** ± 0.5	126.7 ± 17.0	550.7** ± 26.6
Candle	2.4 ± 0.1	2.0 ± 0.2	16.5 ± 0.2	23.6** ± 0.2	429.3± 51.7	1138.7** ± 44.6
Collin	2.8 ± 0.2	2.1 ± 0.2	10.0 ± 1.5	18.2** ± 0.8	208.0 ± 31.7	713.3** ± 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* ± 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** ± 31.0
Rascal Green	9.5 ± 0.0	7.0** ± 0.5	9.8 ± 0.5	12.9** ± 0.1	218.7 ± 33.7	480.0** ± 31.2

Data are shown by the mean ± 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.

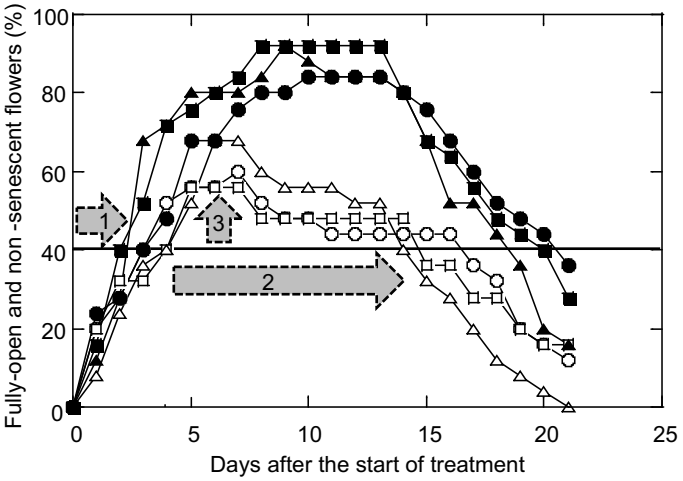

Fig. 1. Change in the percentage of fully-open and non-senescent flowers for cut flowers of 'Barbara' carnation treated with 2,4-PDCA. Bunches of cut flowers, each with 5 main flower stalks with 5 flowers (buds) on each stalk (25 flower buds in total), were treated continuously with 0 (control) or 2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

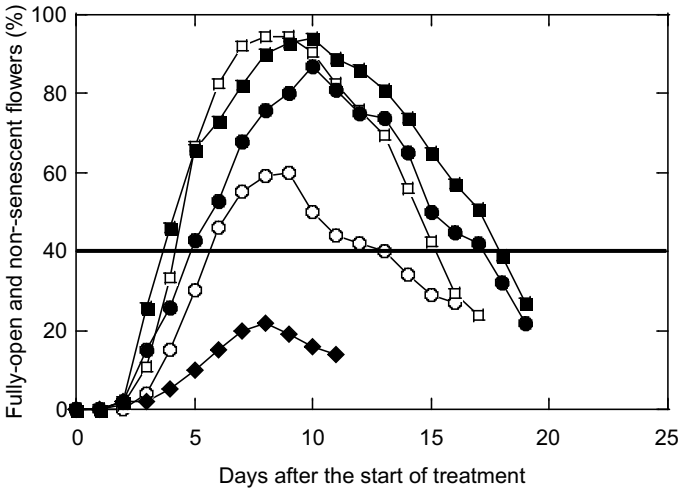

Fig. 2. Effects of pulse and continuous treatments with 2,4-PDCA on flower opening characteristics of 'Light Pink Barbara' carnation.

Bunches of cut flowers, similar to those described in the legend to Fig. 1, were treated with 2,4-PDCA at 0 (○), 5 (●), 10 (■) and 30 mM (◆) 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 (□). Change in the percentage of fully-open and non-senescent flowers is shown by the mean of 4 replicated samples.

(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. Fig. 2 shows the changes in the percentage of FONS flowers for cut 'LPB' flowers treated with 2,4-PDCA at 0, 5, 10 or 30 mM for 24 h, and thereafter left with their stalk end 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 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*.

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