REVIEW

Improvement of Flower Vase Life using Cross-Breeding Techniques in Carnation (*Dianthus caryophyllus* L.)

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Abstract

The vase life of cut flowers, or flower longevity, is one of the most important characteristics that determine their quality and their ability to satisfy consumer preferences. To improve the vase life of carnations, conventional cross-breeding techniques were used to develop many carnation lines with a long vase life. Two new cultivars, ‘Miracle Rouge’ and ‘Miracle Symphony’, with a genetically determined long vase life, were developed. They had vase lives of 17.7 to 20.7 days (3.2 to 3.6 times the vase length of the ‘White Sim’ cultivar) under standard conditions (23°C, 12-h photoperiod, 70% RH). The ethylene biosynthesis pathway in these cultivars was almost completely blocked during natural senescence, which was responsible for the long vase life. Changes in ethylene sensitivity with flower senescence were evaluated using a time-lapse video recording system, which provides a simple and accurate way of evaluating ethylene sensitivity. The video system revealed that the ethylene sensitivity of various cultivars including ‘Miracle Rouge’ and ‘Miracle Symphony’ after anthesis decreased with increasing age.

Disciplines: Horticulture / Plant breeding

Additional key words: ethylene production, ethylene sensitivity, flower longevity, flower senescence

Introduction

The vase life of cut flowers, or flower longevity, is one of the most important characteristics that determine their quality and their ability to satisfy consumer preferences, thus stimulating repeat purchasing. Carnation (*Dianthus caryophyllus* L.) is one of the main floricultural crops not only in Japan but worldwide. However, carnation flowers are highly sensitive to ethylene³⁶. Hence, ethylene is an important determinant of flower longevity, because it induces wilting of petals and autocatalytic ethylene production¹⁰.

Ethylene is a simple gaseous phytohormone with profound effects on many aspects of plant growth and development, including flower senescence and abscission. Senescence of carnation flowers is normally characterized by a climacteric-like pattern of ethylene production; that is, by a surge in ethylene production followed by a decline¹⁹. The increase in ethylene production is associated with the development of in-rolling flower petals and

subsequent wilting¹⁰.

Although the vase life of carnations is about 5 to 7 days in normal Sim-type cultivars, it can be extended by means of treatment with post-harvest chemicals. They are mainly grouped into two categories. One is inhibitors of ethylene biosynthesis, such as aminooxyacetic acid⁹, aminoethoxyvinyl glycine¹ and α -aminoisobutyric acid (AIB)^{21,22}. The other is inhibitors of ethylene action, such as silver thiosulfate (STS)³⁴, 2,5-norbornadiene^{32,35} and 1-methylcyclopropene²⁹. Of these, STS is widely used by commercial carnation producers to extend the vase life of cut flowers because of its outstanding effectiveness. STS is generally applied as a pretreatment solution to cut flowers. The persistence and mobility of STS allows very short pulse treatments. To cite one example, 0.5 mM STS pulse treatment for 2 hr significantly extended the vase life of normal carnation cultivars relative to the control²⁵. However, concerns about potential contamination of the environment from waste STS solutions have increased in recent years¹⁵. Therefore, alternative methods for improving the vase life of carnations are expected to develop.

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It would be desirable to genetically improve the vase life of carnation flowers, since the improved cultivars would require no chemical treatment to attain longer vase life. Therefore, a research breeding program was started by the National Institute of Floricultural Science (NIFS) in 1992 to improve the vase life of carnation flowers by means of conventional breeding techniques.

This paper reviews recent progress in the breeding of carnations by NIFS for long vase life.

Genetic variation of flower vase life among carnation cultivars

There is considerable genetic variability in post-harvest flower longevity among carnation cultivars. This was attributed to genetic variation in either ethylene synthesis or perception. Since the early 1990s, several commercial carnation cultivars ('Sandra', 'Chinera', 'Killer', 'Epomeo', 'Sandrosa', 'Roland', 'White Candle', etc.) with extended vase life have been reported^{4,19,20,25,30,37,40,41} (Table 1). These cultivars have a much longer vase life than most commercially grown standard carnations with climacteric ethylene production (e.g. 'White Sim'). Cultivars with low ethylene production during senescence ('Sandra', 'Killer', 'Sandrosa', 'Roland', 'White Candle', etc.) show neither petal in-rolling nor rapid wilting during senescence. Instead, these flowers fade and show necrosis and desiccation of the petals. Petal in-rolling at the onset of wilting is a well-known characteristic of ethyl-

ene-dependent senescence of normal carnation flowers. The flowers produce ethylene in a large amount during senescence with a climacteric pattern. By contrast, desiccation and browning of petals are the characteristics of ethylene-independent senescence of the variants with low ethylene production.

Another type of cultivar with long vase life has reduced sensitivity to ethylene ('Chinera' and 'Epomeo'). 'Chinera' and 'Epomeo' were considerably less sensitive to ethylene than 'White Sim'³⁷. This reduced sensitivity seems to explain the long vase life. However, the number and affinity of ethylene receptors were thought to be similar in 'Chinera' and the ethylene-sensitive 'White Sim'. Furthermore, ethylene treatment (2 $\mu\text{L}\cdot\text{L}^{-1}$ for 16 h at 23°C) for 'Chinera' flowers induced autocatalytic ethylene production without petal wilting (Onozaki et al., unpublished data). Therefore, the reduced wilting response of 'Chinera' to ethylene was thought to be regulated at a point beyond the receptor, presumably in the signal transduction chain³⁷. Woltering et al.³⁷ have shown that reduced ethylene sensitivity is heritable. Therefore, improvement of vase life of carnation by crossing and selection seems possible.

Selection and crossing of breeding materials based on vase life

Increased vase life of cut flowers is an important breeding target. To improve the vase life of carnation flowers, Onozaki et al.^{23,26} repeatedly crossed and selected promising progeny for four generations, from 1992 to 2004. The research-breeding program began in 1992 using six cultivars ('Pallas', 'Sandrosa', 'Candy', 'Tanga', 'White Sim', and 'Scania') as parental materials²³. The frequency distributions for vase life in the parental, first, second, third, and fourth generations were continuous normal distributions (Fig. 1). The frequencies of flowers with superior vase life (14 days or more) were 1.0% and 1.3% in the parental and first generations, respectively, but rose to 61.3% in the fourth generation. The proportion of flowers with inferior vase life (4 to 8 days) decreased markedly in the fourth generation. The mean vase life of the parental generation derived from crossing six cultivars was 7.4 days; in contrast, after four cycles of crossing and selection, vase life had improved to 14.7 days, a net increase of 7.3 days (Fig. 1). All selected lines with long vase life showed low ethylene production at senescence. In particular, selected fourth-generation lines showed extremely low ethylene production²⁶. Evaluation of the progeny by exposure to ethylene at 2 $\mu\text{L}\cdot\text{L}^{-1}$ concentration showed that two second-generation lines (64-13 and 64-54) were less sensitive to ethylene like

Table 1. Research on carnation cultivars with long vase life

Cultivar	Type	Reference
<i>Variants with low ethylene production</i>		
Sandra	standard	Wu et al. (1991a, b) ^{40,41}
Killer	standard	Serrano et al. (1991) ³⁰
Sandrosa	standard	Mayak & Tirosh (1993) ¹⁹
Roland	spray	Burchi et al. (1999) ⁴
White Candle	spray	Nukui et al. (2004) ²⁰
Cream Candle	spray	Nukui et al. (2004) ²⁰
Royal Green	spray	Nukui et al. (2004) ²⁰
Shion	spray	Nukui et al. (2004) ²⁰
Le Noir	spray	Nukui et al. (2004) ²⁰
Miracle Rouge	standard	Onozaki et al. (2006a) ²⁵
Miracle Symphony	standard	Onozaki et al. (2006a) ²⁵
<i>Variants with reduced ethylene sensitivity</i>		
Chinera	standard	Wu et al. (1991a, b) ^{40,41} Woltering et al. (1993) ³⁷
Epomeo	standard	Woltering et al. (1993) ³⁷

‘Chinera’²³, a cultivar that is known for its low ethylene sensitivity⁴¹ (Fig. 2).

Thus, many carnation lines with genetically long vase life could be developed using conventional cross-breeding techniques.

Evaluation methods for long vase life

Flower vase life is a difficult genetic character to assess accurately because it is influenced by growing conditions, developmental stage of the flowers at harvest and environmental conditions after harvest. Therefore, we carried out the selection of one population for vase life twice over 2 years to reduce undesirable nongenotypic variation. In the first year, about 30% of the seedlings were primary-selected for long vase life. In the second year, replicated tests were carried out after vegetative multiplication and selection to diminish the environmental variance, and about 20% of the population was further selected. This selection procedure is reliable in selecting lines with a genetically long vase life. In addition, we used standardized conditions (23°C, 12-h photoperiod, 70% RH) for evaluating the vase life to reduce post-harvest non-genetic variation in vase life from the start of this breeding program.

Breeding and characteristics of carnation Norin No. 1 ‘Miracle Rouge’ and No. 2 ‘Miracle Symphony’

Onozaki et al.²⁵ developed two new carnation cultivars, ‘Miracle Rouge’ and ‘Miracle Symphony’, that had vase lives of 17.7 to 20.7 days (3.2 to 3.6 times the vase life of a standard cultivar, ‘White Sim’) under standard conditions (23°C, 12-h photoperiod, 70% RH) (Table 2; Fig. 3). These cultivars were registered and released in November 2005 by the Ministry of Agriculture, Forestry and Fisheries of Japan. ‘Miracle Rouge’, a red standard-type cultivar, was selected from the third generation of these crosses, and ‘Miracle Symphony’, a white standard-type cultivar with red stripes, was selected from the second generation. Both showed high flower quality and adequate yields of cut flowers for commercial production, in addition to their long vase life.

Treatment with AIB or STS did not significantly prolong vase life in either cultivar²⁵. In addition, the petals and gynoecium of both cultivars produced only trace amounts of ethylene during natural senescence (Fig. 4). These results indicate that their ethylene biosynthesis pathway was almost completely blocked during natural senescence, and that this change was responsible for their improved vase life.

Fig. 1. Frequency distribution of vase life in parental, first, second, third and fourth generations

Values (%) in parentheses represent the proportion of seedlings with a vase life of 14.0 days or longer.

Table 2. Flower vase life and ethylene sensitivity (day 0) of carnation cultivars under standard conditions (23°C, 12-h photoperiod, 70%PH)

Cultivar	Expt. 1		Expt. 2		Response time to ethylene treatment (h)
	Vase life (days)	% ^{a)}	Vase life (days)	% ^{a)}	
White Sim	5.7 ± 0.3	100	5.6 ± 0.3	100	6.9 ± 0.3
Miracle Rouge	20.6 ± 0.7	361	17.7 ± 0.3	316	6.8 ± 0.2
Miracle Symphony	20.7 ± 0.9	363	17.9 ± 0.2	320	8.2 ± 0.2

Values of vase life are the means ± SE of the data for 10 flowers.

Values of response time to 10 $\mu\text{L}\cdot\text{L}^{-1}$ ethylene treatment are the means ± SE of 5 flowers.

a): The percentage of the value for the control cultivar, 'White Sim'.

Video evaluation of ethylene sensitivity after anthesis in carnation flowers

Differences in ethylene sensitivity among carnation cultivars were evaluated using a time-lapse video recording system²⁴. Using this system, breeders can "fast-forward" through the video until wilting symptoms are detected, which is faster and more efficient than having to manually check a large number of plants at frequent intervals. Measurement of the time until in-rolling of petals of 'White Sim', 'Nora', 'Chinera', and breeding line 64-54 flowers subjected to a range of 1 to 20 $\mu\text{L}\cdot\text{L}^{-1}$ ethylene showed that 10 $\mu\text{L}\cdot\text{L}^{-1}$ was the optimal concentration for sensitivity evaluation using the video system²⁴. With this system, clear differences were found in ethylene sensitivity among 10 cultivars and one line²⁴. Breeding line 64-54 had the longest ethylene response time (20.6 h to the start of petal in-rolling). Video monitoring thus appears to be a simple and accurate way of evaluating ethylene sensitivity.

Line 64-54 is the progeny of 'Candy' and 'Sandrosa'. 'Sandrosa' was sensitive to ethylene, with a response time of 6.2 h. 'Candy' had a response time of 11.6 h and showed significantly lower ethylene sensitivity than seven sensitive cultivars, including 'Sandrosa'²⁴. This result suggests that the genes conferring low ethylene sensitivity might be derived from 'Candy'. Further studies are needed to clarify the gene number and mode of inheritance of low ethylene sensitivity.

The video system was also used to study changes in the ethylene sensitivity of carnation flowers after anthesis. A shift in responsiveness to ethylene that was impossible to detect using previous methods could be detected using the video system. In the Sim-type carnation cultivars that were tested ('White Sim', 'Scania', 'U Conn Sim', and 'Nora'), ethylene sensitivity after anthesis decreased significantly with age in both early-cut (on day 0 (at the stage of outer petals horizontal)) and late-cut (left on plant for 3 or 6 days after anthesis and then cut) flowers. These

results clearly showed that the decline in ethylene sensitivity is caused by the increasing physiological age of the flowers²⁴. Several studies have examined the changes in ethylene sensitivity in carnation, using immature carnation flowers from the bud stage to anthesis^{2,5,14,18,38}. However, when these studies have been cited or reviewed, there has been a tendency to confuse the changes in sensitivity of immature flowers with those of mature flowers after anthesis. Hereafter, a clear distinction between the responses of these two stages should be made.

Furthermore, although 'Miracle Rouge' and 'Miracle Symphony' (which have long vase life) were sensitive to ethylene immediately after anthesis (Table 2), they became almost completely ethylene-insensitive or developed extremely low sensitivity by the end of senescence²⁶ (Fig. 5). This rapid decline in ethylene sensitivity during aging in 'Miracle Rouge' and 'Miracle Symphony' might have a profound effect on vase life, as would the negligible amount of ethylene production in both the petal and the gynoecium of these cultivars during natural senescence (Fig. 4).

Control of ethylene biosynthesis in carnation flowers

Ethylene is biosynthesized in senescing flower tissues through the following pathway: methionine → S-adenosyl-L-methionine → 1-aminocyclopropane-1-carboxylate (ACC) → ethylene. The penultimate and last steps are catalyzed by ACC synthase (ACS) and ACC oxidase (ACO), respectively. These steps involving ACS and ACO are generally considered to be rate-limiting reactions for ethylene biosynthesis. Both enzymes are well characterized and several genes encoding them have been cloned in plant species, including carnation. The climacteric increase in ethylene biosynthesis during carnation flower senescence was associated with a dramatic increase in the abundance of the mRNAs encoding ACS and ACO, while these mRNAs were undetectable in presenescent

Fig. 2. Carnation breeding lines with reduced ethylene sensitivity

Left: Ethylene-resistant lines (64-13 and 64-54) and 'China'. Right: Ethylene sensitive control. Flowers on day 0 were treated with ethylene at $2 \mu\text{L}\cdot\text{L}^{-1}$ for 16 hours.

Fig. 3. Variation in the vase life of control cultivars and 'Miracle Symphony'

A to D were photographed at 0, 6, 12, 18 days after harvest.

Left: 'White Sim'. Center: 'Miracle Symphony'. Right: 'Scania'.

Fig. 5. Effect of cut flower age at treatment time on ethylene sensitivity of 'Miracle Symphony' flowers

A, B and C were photographed at 0, 10, and 72 h after ethylene treatment began, respectively. Left: flower immediately exposed to $10 \mu\text{L}\cdot\text{L}^{-1}$ of ethylene after harvesting. Right: flower exposed to $10 \mu\text{L}\cdot\text{L}^{-1}$ of ethylene 18 days after cutting.

Fig. 4. Changes in ethylene production from the petals and gynoecia of four cultivars during senescence

Values represent means $\pm$ SE of the data for five replications.

---: 'Francisco', ---: 'Excerea',
.....: 'Miracle Rouge', —: 'Miracle Symphony'.

petals³⁹. Three ACS genes (*DC-ACS1*, *DC-ACS2* and *DC-ACS3*) and one ACO gene (*DC-ACO1*) have been identified in carnation and characterized from each part of carnation flowers^{11,13,27}. Expression of these genes is regulated in a tissue-specific manner during senescence. In carnation flowers, ethylene is first produced from the ovary followed by the styles and the petals³³.

Petal wilting in flower senescence is related to the expression of the cysteine proteinase (CPase) gene. Kosugi et al.¹⁶ reported that expression of the ACS and ACO genes is differently regulated from the expression of the CPase gene in petals of a transgenic carnation line transformed with an ACO cDNA in sense orientation.

Ethylene receptor genes in carnation flowers

The ethylene receptor gene, *ETR1*, was first isolated from *Arabidopsis thaliana* by the method of chromosome walking and shown to have sequence homology with bacterial two-component regulators⁷. The ethylene response pathway has been thoroughly studied, and many components of this pathway and the interactions among them have been discovered both in *Arabidopsis* and tomatoes. Double-mutant analysis has demonstrated that *ETR1* acts

upstream of all other signal transduction genes isolated by the triple response assay⁸. It is known that ethylene receptor genes make a family in plants. For example, five ethylene receptors exist in *Arabidopsis*. In carnation, three putative ethylene receptor genes, *DC-ERS1*, *DC-ERS2* and *DC-ETR1* have been identified. Shibuya et al.³¹ reported that *DC-ERS2* and *DC-ETR1* are ethylene receptor genes responsible for ethylene perception in carnation flower tissues during senescence.

Genetic engineering techniques versus conventional cross breeding

The other method to genetically improve the vase life of carnation flowers is genetic transformation. Several researchers have conducted studies on transgenic carnations that have long vase life (Table 3). Savin et al.²⁸ reported that the vase life of carnation flowers was extended by the introduction of an antisense ACO gene. Similarly, Kosugi et al.¹⁷ reported that cut flowers of a transgenic carnation line (the sACO-1 line), which was transformed by using a carnation ACO cDNA in the sense orientation, had a longer vase life than had flowers of the non-transformed plants. Then, Iwazaki et al.¹² generated 5 lines of 'Nora' carnation transformed with *DC-ACS1* cDNA in sense and antisense orientation. Cut flowers of all the transgenic lines obtained showed suppressed ethylene production during natural senescence as compared with flowers of non-transformed 'Nora' carnation. Bovy et al.³ reported that the vase life of carnations was also extended by the introduction of an *Arabidopsis etr1-1* gene, which is a mutated dominant ethylene insensitivity gene. These studies indicate that genetic engineering is a powerful tool for developing carnation with a long vase life. On the other hand, our new cultivars, 'Miracle Rouge' and 'Miracle Symphony', derived from conventional cross breeding, had vase lives of 17.7 to 20.7 days (3.2 to 3.6 times the vase life of the 'White Sim' cultivar) at 23°C²⁵. As carnation has a relatively short generation time (about 1 year), improvement by selection and crossing is not as time-consuming as in some other species such as tulip.

To my knowledge, the only transgenic floricultural crop currently sold commercially is a transgenic violet carnation named 'Moondust'. It needs many years before genetic transformants can be used successfully, as compared with conventional breeding. These include selection of an appropriate line, characterizing the line in terms of environmental risk assessment for the regulatory process and making sure any patented technology is covered by freedom to operate agreements⁶. A high cost of development due to securing licenses is not borne by conven-

Table 3. Research on transgenic carnations with altered ethylene synthesis or ethylene perception

Gene and promoter	Cultivar transformed	Transformant	Vase life (days)	Reference
ACC oxidase from carnation with MAC promoter	Scania, White Sim	#705, #2373B	8–9 days at 21°C	Savin et al. (1995) ²⁸
etr1-1 from <i>Arabidopsis</i> with its own promoter, CaMV 35S or FBP1	Rena	#7086, #7022, #8018, #8023, #9005, #9008	17–24 days at 20°C	Bovy et al. (1999) ³
ACC oxidase in a sense orientation with CaMV 35S promoter	Nora	sACO-1	9.5 days at 23°C	Kosugi et al. (2002) ¹⁷
ACC syntase in a sense orientation with CaMV 35S promoter	Nora	sACS-1	10 days at 23°C	Iwazaki et al. (2004) ¹²

tional breeding. Therefore, extending the vase life of carnation by means of conventional cross-breeding techniques appears to be a highly practical approach compared with more advanced genetic engineering methods.

Conclusion

The breeding of cultivars with genetically superior vase life appears to be an efficient approach to satisfying the consumer's quality expectations. A significant improvement of flower vase life was accomplished by the fourth generation based on conventional crossing techniques²⁶. Furthermore, two commercial cultivars with genetically long vase life ('Miracle Rouge' and 'Miracle Symphony') were developed in 2005²⁵. Results of our studies indicate that the flower vase life of carnations can be improved by crossing and selection.

One problem with these cultivars is that they are ethylene sensitive immediately after anthesis. The response time to 10 $\mu\text{L}\cdot\text{L}^{-1}$ ethylene of 'Miracle Rouge' and 'Miracle Symphony' was 6.8 and 8.2 h, respectively²⁵, whereas that of line 64-54 was 20.6 h²⁴. To resolve this problem, additional crosses are currently being made between selected lines with extremely long vase life and line 64-54 or other lines with low sensitivity to ethylene immediately after anthesis. Woltering et al.³⁷ have shown that reduced ethylene sensitivity is heritable, so it should be possible to breed ethylene-insensitive or less-sensitive lines with extremely long vase life by means of conventional cross-breeding techniques.

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