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Review

Breeding of carnations (*Dianthus caryophyllus* L.) for long vase life

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Carnation (*Dianthus caryophyllus* L.) is one of the main floricultural crops in Japan and worldwide. The vase life of cut ornamental flowers, including carnations, is important in determining their quality and consumers' preference. To improve the vase life of carnation flowers, my group started a breeding research program in 1992 using conventional cross-breeding techniques. We repeatedly crossed and selected promising offspring with long vase life for seven generations, from 1992 to 2008. In 2005, we developed two cultivars, 'Miracle Rouge' and 'Miracle Symphony', with genetically determined long vase lives of 17.7 to 20.7 days (3.2 to 3.6 times that of 'White Sim') under standard conditions (23°C, 70% RH, 12-h photoperiod). Line 532-6 showed an ultra-long vase life averaging 27.8 to 32.7 days (4.6 to 5.4 times that of 'White Sim'). We evaluated changes in ethylene sensitivity with flower senescence simply and accurately using a time-lapse video recorder. In 2010, we selected line 806-46b with both ultra-long vase life (27.1 days, 4.4 times that of 'White Sim') and ethylene resistance. Analyses using six cultivars and 123 selected lines from the 1st to the 7th generations revealed that the long vase life was strongly associated with a decrease in ethylene production.

Key Words: carnation, ethylene production, ethylene sensitivity, flower longevity, flower senescence.

Introduction

The vase life of cut flowers is one of the most important characteristics that determine their quality and their ability to satisfy consumers' preference, thus stimulating repeated purchase. The most common consumer request for cut flowers is long vase life (Ichimura 2013). Recently, several major Japanese supermarket chains have started to offer consumers a guaranteed vase life for some cut flowers (Ichimura 2013). For these reasons, genetic improvement of vase life is desirable.

The main breeding targets for ornamentals used to be visual qualities such as appearance, flower color, type, size, and plant form (Boxriker *et al.* 2017). Because vase life is a highly complex trait to measure, little improvement of flower longevity was attempted before the 1990s. Nowadays, vase life of cut flowers has become an important quality factor, and short-lived flowers have limited marketability and consumer appeal. Therefore, extended vase life is now an important breeding target. Inheritance, response to selection, or QTL analysis of vase life have been studied in gerbera (Wernett *et al.* 1996), Asiatic hybrid lilies (van der Meulen-Muisers *et al.* 1999), rose (Carvalho *et al.* 2015, Fanourakis *et al.* 2012), and chrysanthemum (van Geest *et al.* 2017).

Carnation (*Dianthus caryophyllus* L.) is one of the main floricultural crops in Japan and worldwide. At present, the world's major production areas are cool highlands with affordable labor, such as in Colombia and China (Onozaki 2006). The number of cut carnation flowers imported into Japan from these two countries has increased steadily each year. Of cut carnations sold in Japan, 55.8% were imported in 2015 (Onozaki 2016). Therefore, breeding of high-value-added or distinctive cultivars, such as long-vase-life cultivars, is required to allow Japanese growers to compete with imported carnations.

Carnation flowers are highly sensitive to ethylene (Woltering and van Doorn 1988). Exposure of fully open carnation flowers to ethylene induces autocatalytic ethylene production and wilting in petals (Halevy and Mayak 1981). Hence, ethylene is an important determinant of vase life of carnation.

Ethylene is an important plant hormone, involved in many aspects of plant growth and development, including flower senescence and abscission (Klee and Clark 2004). Senescence of carnation flowers is normally characterized by a climacteric surge in ethylene production followed by a decline (Mayak and Tirosh 1993). The increase in ethylene production causes the development of inrolling and subsequent wilting of petals (Halevy and Mayak 1981). Petal senescence is triggered by ethylene produced by the

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gynoecium during natural senescence (Satoh 2011, Satoh *et al.* 2005, Shibuya *et al.* 2000).

Although the vase life of ordinary carnations is about 5 to 7 days, it can be significantly extended by treatment with postharvest chemicals, which are categorized into two main groups. One group consists of inhibitors of ethylene production, such as aminooxyacetic acid (Fujino *et al.* 1980), aminoethoxyvinyl glycine (Baker *et al.* 1977), and α-aminoisobutyric acid (AIB) (Onozaki and Yamaguchi 1992, Onozaki *et al.* 1998). The other group includes inhibitors of ethylene action, such as silver thiosulfate (STS) (Veen 1979), 2,5-norbornadiene (Sisler *et al.* 1983, Wang and Woodson 1989), and 1-methylcyclopropene (Serek *et al.* 1995). The most widely used is STS, which is generally applied as a pretreatment solution to cut flowers by carnation growers. The persistence and mobility of STS allows very short pulse treatments. However, concerns about environmental contamination by waste STS solutions have been raised (Klee and Clark 2004), and STS is prohibited in several countries (Serek *et al.* 2007). Silver ions are very hard to remove from the environment, and at high concentrations can cause health problems if not handled properly (Chandler 2007). As chemical use poses both environmental and public health concerns, many current ethylene inhibitors may be banned in the near future (Scariot *et al.* 2014). In addition, the chemicals cost growers money to buy and apply. Therefore, alternative methods for improving vase life are needed.

It would be desirable to improve vase life genetically, since improved cultivars would require no chemical treatment. Therefore, a research breeding program was started by the NARO Institute of Vegetable and Floriculture Science in 1992 to improve the vase life of carnation flowers by means of conventional breeding techniques.

This paper reviews recent achievements in the breeding of carnations for long vase life.

Genetic variation in vase life among carnation cultivars

Considerable genetic variability in vase life of cut flowers among carnation cultivars is attributed to genetic variation in either ethylene synthesis or ethylene perception. Since the early 1990s, a number of commercial cultivars with extended vase life (**Table 1**) have been reported (Burchi *et al.* 1999, Ebrahimzadeh *et al.* 2011, Hotta *et al.* 2016, Mayak and Tirosh 1993, Nukui *et al.* 2004, Onozaki *et al.* 2006a, 2009, Satoh 2005, Serrano and Romojaro 1991, Woltering *et al.* 1993, Wu *et al.* 1991a, 1991b). These cultivars have a longer vase life than most commercially grown carnations with normal climacteric ethylene production (e.g., 'White Sim'). Cultivars with low ethylene production during senescence (e.g., 'Sandra', 'Killer', 'Sandrosa', 'Roland', 'White Candle') show neither petal inrolling nor rapid wilting during senescence. Instead, the flowers fade and show necrosis and desiccation. Petal inrolling at the onset of wilting is a well-known characteristic of ethylene-dependent senescence of normal carnation flowers (Iwazaki *et al.* 2004, Otsu *et al.* 2007, Satoh 2011), which produce ethylene in a large amount during senescence with a climacteric pattern. By contrast, desiccation and browning of petals are characteristics of ethylene-independent senescence by cultivars with low ethylene production.

Another type of cultivar with long vase life has reduced sensitivity to ethylene. 'Chinera' and 'Epomeo' are considerably less sensitive to ethylene than 'White Sim' (Woltering *et al.* 1993, Wu *et al.* 1991b). This reduced sensitivity seems to explain the long vase life. However, the number and affinity of ethylene receptors are similar in 'Chinera' and 'White Sim'. Therefore, the reduced wilting response of 'Chinera' to ethylene may be regulated at a point beyond the receptor, presumably in the signal transduction chain (Woltering *et al.* 1993). Woltering *et al.* (1993) have shown that reduced ethylene sensitivity is heritable, so it should be possible to breed ethylene-insensitive or less-sensitive lines

Table 1. Research on carnation cultivars with low ehylene production or reduced ethylene sensitivity

Cultivar	Type	Reference
Variants with low ethylene production		
Sandra	Standard	Wu *et al.* (1991a, 1991b)
Killer	Standard	Serrano and Romojaro (1991)
Sandrosa	Standard	Mayak and Tirosh (1993)
Roland	Spray	Burchi *et al.* (1999)
White Candle, Cream Candle, Royal Green, Shion, Le Noir	Spray	Nukui *et al.* (2004)
Light Cream Candle, Lester	Spray	Satoh (2005)
Miracle Rouge, Miracle Symphony	Standard	Onozaki *et al.* (2006a)
Polaris, Camille Pink, Chiffon, Bambino, Nina	Pot	Onozaki *et al.* (2009)
Baltico, Pilar	Standard	Ebrahimzadeh *et al.* (2011)
Kane Ainou 1-go	Spray	Hotta *et al.* (2016)
Variants with reduced ethylene sensitivity		
Chinera	Standard	Wu *et al.* (1991a, 1991b) Woltering *et al.* (1993)
Epomeo	Standard	Woltering *et al.* (1993)
Orenge Duo, Lemon Soft, Madeleine, Dreamin	Pot	Onozaki *et al.* (2009)

Breeding Science
Vol. 68 No. 1 BS

with extremely long vase life by means of conventional cross-breeding.

Crossing and selection based on vase life for seven generations

To improve the vase life of carnation flowers, my group repeatedly crossed and selected promising offspring with long vase life for seven generations, from 1992 to 2008, using conventional cross-breeding techniques (Onozaki *et al.* 2001, 2006b, 2011). We chose six commercial standard cultivars with large differences in vase life as initial breeding materials (**Fig. 1A**): four Mediterranean-type cultivars ('Pallas', 'Sandrosa', 'Candy', and 'Tanga') and two American 'Sim'-type cultivars ('White Sim' and 'Scania') (Onozaki *et al.* 2001). The first crosses were conducted in the spring of 1992.

In the breeding process, we selected lines with a long vase life in each generation to use as parents of the next generation (Onozaki *et al.* 2001, 2006b, 2011). Plants were not selected for ethylene production or sensitivity. (Note that "parental generation", "1st generation", … "6th generation" in our previous papers are referred to as "1st generation", "2nd generation", … "7th generation" here.)

The crossing and selection resulted in dramatic alterations in the vase life of each generation, and particularly in ethylene production of many lines (Onozaki *et al.* 2001, 2006b, 2011). The vase life in all generations showed continuous normal distributions (**Fig. 1B**). The mean vase life increased from 7.4 days in the 1st generation to 15.9 days in the 7th generation, a net increase of 8.5 days (**Fig. 1B**). The effect of crossing and selection did not stay constant, but varied by generation. The biggest genetic improvement occurred between the 4th and 5th generations, with an increase of 4.2 days. Thus, many carnation lines with genetically long vase life were developed using conventional cross-breeding techniques.

Methods for evaluation of long vase life

Vase life is a difficult genetic character to assess accurately because it is a highly complex trait to measure (Boxriker *et al.* 2017), and is influenced by growing conditions, the developmental stage of the flowers at harvest, and environmental conditions after harvest. As van Geest *et al.* (2017) explained, breeding for postharvest performance in ornamentals is challenging, since many different deteriorative processes determine vase life. Therefore, we carried out selection on each generation twice over 2 years to reduce environmental variation. In the first year, about 30% of the seedlings were selected. In the second year, following vegetative multiplication and selection to diminish the environmental variance, replicated tests were carried out, and about 20% of the population was further selected. This procedure is reliable in selecting lines with a genetically long vase life (**Fig. 2**). We used standard environmental conditions (23°C, 70% RH, 12-h photoperiod) for evaluating vase life to reduce postharvest non-genetic variation from the start of the program.

Breeding and characteristics of 'Miracle Rouge' and 'Miracle Symphony'

We developed two carnation cultivars, 'Miracle Rouge' and 'Miracle Symphony', with vase lives of 17.7 to 20.7 days (3.2 to 3.6 times that of 'White Sim') under standard conditions (**Fig. 3**) (Onozaki *et al.* 2006a). These cultivars were registered by the Ministry of Agriculture, Forestry and Fisheries of Japan and released in 2005. 'Miracle Rouge', a red standard-type cultivar, was selected from the 4th generation (**Fig. 1C**), and 'Miracle Symphony', a white standard-type cultivar with red stripes, was selected from the 3rd generation (**Fig. 1C**). Both showed high flower quality and adequate yields of cut flowers for commercial production, in addition to long vase life.

Treatment with AIB or STS did not significantly prolong vase life of either cultivar (Onozaki *et al.* 2006a). The petals and gynoecium of both cultivars produced only trace amounts of ethylene during natural senescence (**Fig. 4**). Instead, their long vase life was correlated with low expression of genes for 1-aminocyclopropane-1-carboxylic acid (ACC) synthase and ACC oxidase (*DC-ACS1*, *DC-ACS2*, and *DC-ACO1*) in the gynoecium and petals (Tanase *et al.* 2008). These results indicate that their ethylene biosynthesis pathway was almost completely blocked during natural senescence, and that this block was responsible for their improved vase life. However, their ethylene sensitivity is generally equal to that of normal, ethylene-sensitive 'Sim'-type cultivars (Onozaki *et al.* 2006a).

Selection of breeding line 532-6 with ultra-long vase life

The 7th-generation line 532-6 had the longest vase life among 4 cultivars and 18 lines (Onozaki *et al.* 2011), with means of 32.7 days in 2007 and 27.8 days in 2008, or 5.4 and 4.6 times, respectively, that of 'White Sim' (**Fig. 5A**). We defined this rare superior longevity as "ultra-long vase life". The petals of the other selected lines started browning from their edges, then slowly desiccated and faded. Those of line 532-6, however, showed no browning (**Fig. 5B**), and instead slowly desiccated and wrinkled.

'White Sim', 'U Conn Sim', 'Francesco', and other normal cultivars senesce by petal inrolling and rapid wilting, characteristics typical of ethylene-dependent senescence (Iwazaki *et al.* 2004, Otsu *et al.* 2007, Satoh 2011). On the other hand, 'Sandrosa', our selected lines, 'Miracle Rouge', and 'Miracle Symphony', with low ethylene production at senescence, did not show petal inrolling or rapid wilting at senescence, and instead faded and turned brown from the petal edges (**Fig. 5B**). Otsu *et al.* (2007) termed this "ethylene-independent senescence". Line 532-6 showed

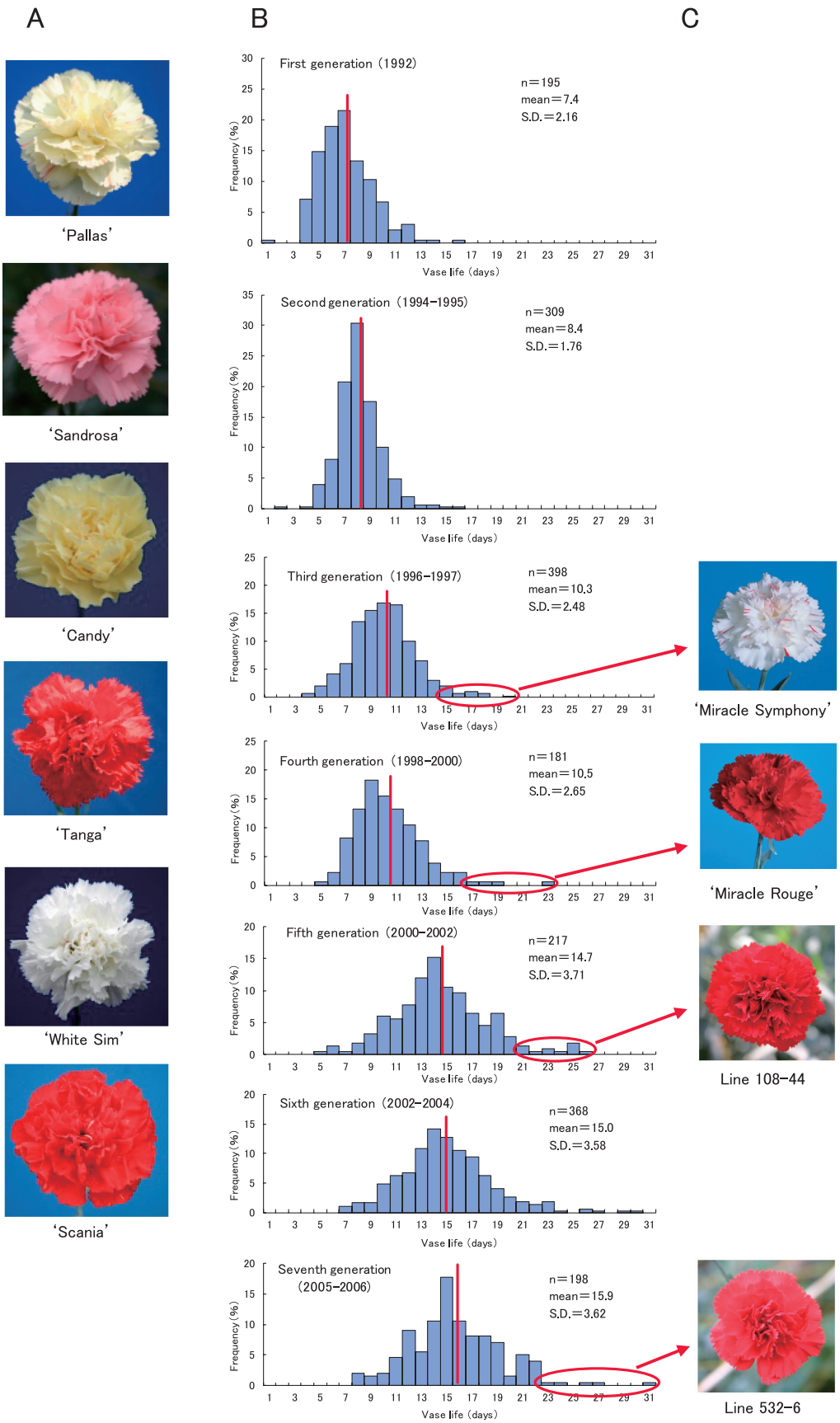

Fig. 1. (A) Six parental cultivars used as initial breeding materials. (B) Frequency distributions of vase life in 1st to 7th generations. Vertical bars represent means. (C) Selection of 'Miracle Symphony, 'Miracle Rouge', line 108-44, and line 532-6.

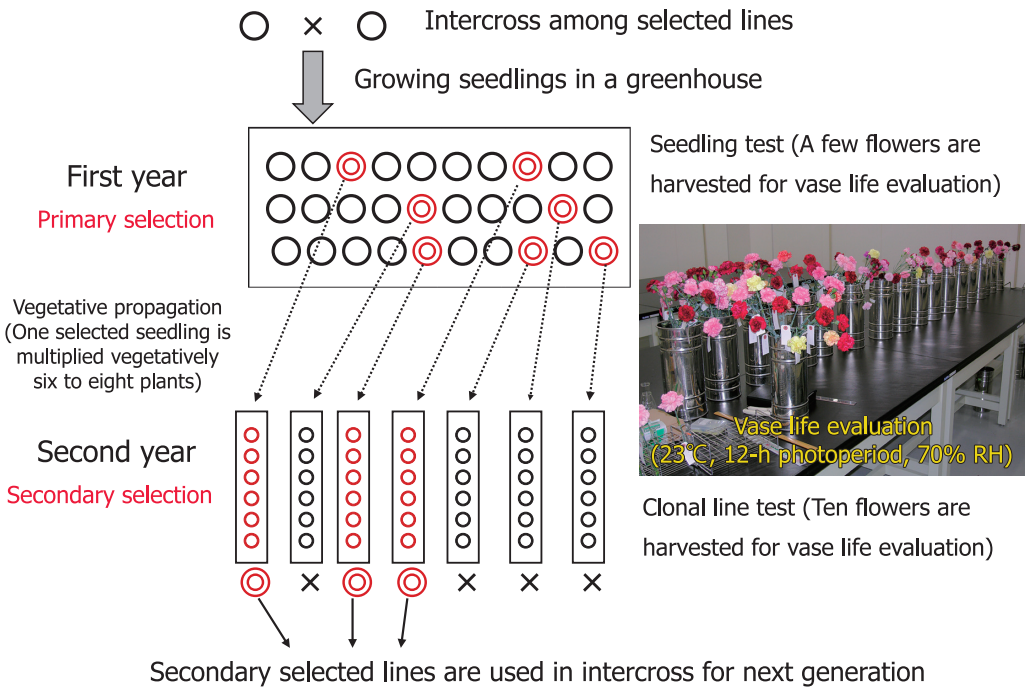

Fig. 2. Evaluation method and selection procedure for the improvement of vase life.

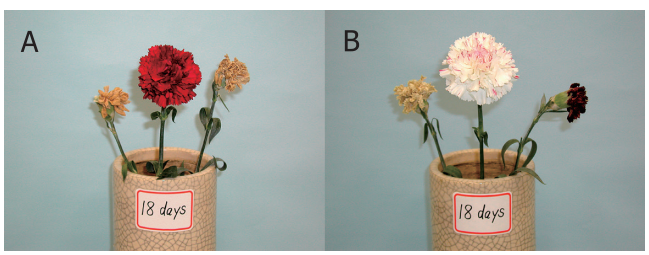

Fig. 3. Variation in the vase life of (A) 'Miracle Rouge', (B) 'Miracle Symphony', and control cultivars 18 days after harvest. The flowers were held in distilled water under standard conditions (23°C, 12-h photoperiod, 70% RH). A: Left to right, 'Nora', 'Miracle Rouge', 'White Sim'. B: Left to right, 'White Sim', 'Miracle Symphony', 'Scania'.

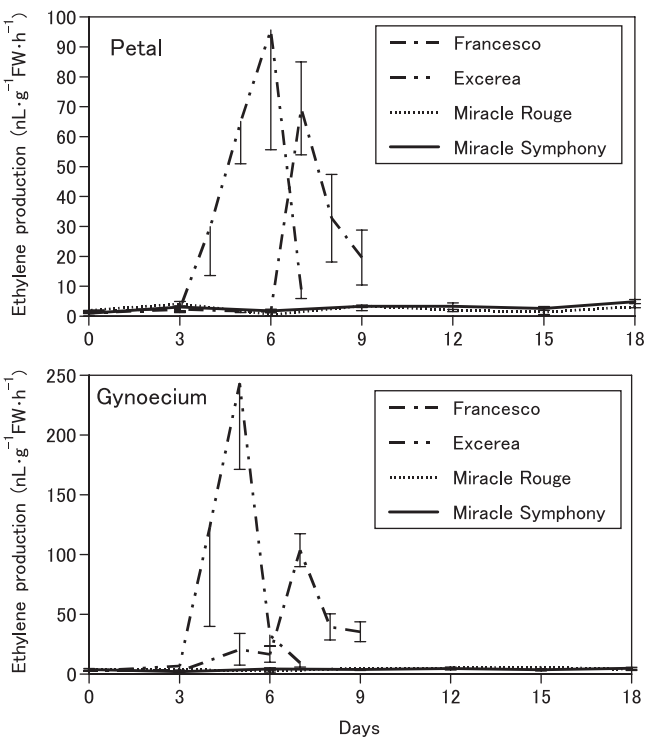

Fig. 4. Changes in ethylene production from petals and gynoecia of four cultivars during senescence. Values represent means ± SE of the data of five replications.

another senescence pattern, characterized by a lack of brownish discoloration of petals (**Fig. 5B**). This result indicates that the ultra-long vase life of line 532-6 results not only from extremely low ethylene production, but also probably from genes that regulate programmed cell death separate from those related to ethylene, possibly the senescence-related genes *DcCPIn* and *DcCP1* (Tanase *et al.* 2015).

Video evaluation of ethylene sensitivity after anthesis in carnation flowers

We developed a simple, accurate method for evaluating the ethylene sensitivity of carnation flowers using a time-lapse video recording system (Onozaki *et al.* 2004a) and concluded that 10 μL L^{-1} was the optimum concentration for sensitivity evaluation by the system. The system revealed clear differences in ethylene sensitivity among ten cultivars and one line: the selected 3rd-generation line 64-54 had lower ethylene sensitivity than the cultivars (**Fig. 6**) (Onozaki *et al.* 2004a). Using this system, breeders can "fast-forward" through the video until wilting symptoms are seen, which is faster and more efficient than having to look at a large

Fig. 5. Variation in the vase life of 7th-generation line 532-6 with ultra-long vase life and two control cultivars, 'Miracle Rouge' and 'White Sim' at (A) 25 days and (B) 26 days after harvest. A: Left to right, 'Miracle Rouge', 532-6, 'White Sim'. B: Left, 'Miracle Rouge'; right, 532-6.

number of flowers at frequent intervals.

Line 64-54 is the progeny of 'Candy' and 'Sandrosa'. 'Sandrosa' is sensitive to ethylene, with a response time of 6.2 h. 'Candy' has a response time of 11.6 h and is significantly less sensitive than seven sensitive cultivars, including 'Sandrosa' (Onozaki *et al.* 2004a). This result suggests that genes conferring low ethylene sensitivity might be derived from 'Candy'.

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: outer petals horizontal) and late-cut (cut 3 or 6 days after anthesis) flowers. These results clearly show that the decline in ethylene sensitivity is associated with the increasing physiological age of the flowers (Onozaki *et al.* 2004a).

Furthermore, although 'Miracle Rouge' and 'Miracle Symphony' (which have long vase life) were sensitive to ethylene immediately after anthesis, they became almost completely insensitive by the end of senescence (Onozaki *et al.* 2006a) (**Fig. 7**). This rapid decline in sensitivity during aging might be a key factor for long vase life, as would be the negligible amount of ethylene production by these cultivars during natural senescence (**Fig. 4**).

Impediments to breeding of long-life ethylene-resistant carnations

With the aid of the video evaluation system (Onozaki *et al.* 2004a), we demonstrated that the ethylene sensitivity of carnation can be reduced by conventional cross-breeding techniques (Onozaki *et al.* 2008) and developed 13 ethylene-resistant lines (**Fig. 6**) (Onozaki *et al.* 2008, 2011). All 13 lines have a yellow or orange tint in their flower ground colors, and their petals have pink or red stripes without exception (**Fig. 6**). These are expected to be useful for reducing the effects of exogenous ethylene that may occur during storage and transportation.

However, the mean vase life of these 13 ethylene-resistant lines ranged from 7.6 to 15.2 days under standard conditions, which was not as long as the values of the two ethylene-sensitive but long-vase-life cultivars 'Miracle Rouge' and 'Miracle Symphony' and other selected lines with extremely low ethylene production (e.g., line 532-6).

Unfortunately, the progeny of crosses between the ethylene-resistant lines tend to flower late (flower bud differentiation is delayed), making them less suitable for practical cultivation than early-flowering cultivars (Sparnaaij *et al.* 1990). We concluded that crosses between ethylene-resistant lines would not be effective at producing lines with both ethylene resistance and an extremely long vase life.

Selection of breeding line 806-46b with both ultra-long vase life and ethylene resistance

To select lines with both extremely long vase life and low ethylene sensitivity, we repeatedly crossed and selected promising progeny for three generations from 2003 to 2010. In 2010, we developed line 806-46b, with both ultra-long vase life (**Fig. 8A**) and ethylene resistance (**Fig. 8B**), out of 50 progeny derived from a cross between 606-65S and 609-63S (Onozaki *et al.* 2015). The mean vase life of line 806-46b was 27.1 days (4.4 times that of 'White Sim') under standard conditions, which was the longest vase life among 6 cultivars and 7 lines tested.

Lines 532-6 and 806-46b have an ultra-long vase life but different sensitivity to exogenous ethylene. The response

Fig. 6. Variation in sensitivity to ethylene (10 μL L^{-1}) among (upper left) 'Nora' (control), (lower left) 004-17 (highly sensitive), (upper right) 64-54 (resistant), and (lower right) 234-36S (resistant) at (A) 0 h, (B) 8 h, and (C) 48 h after treatment began.

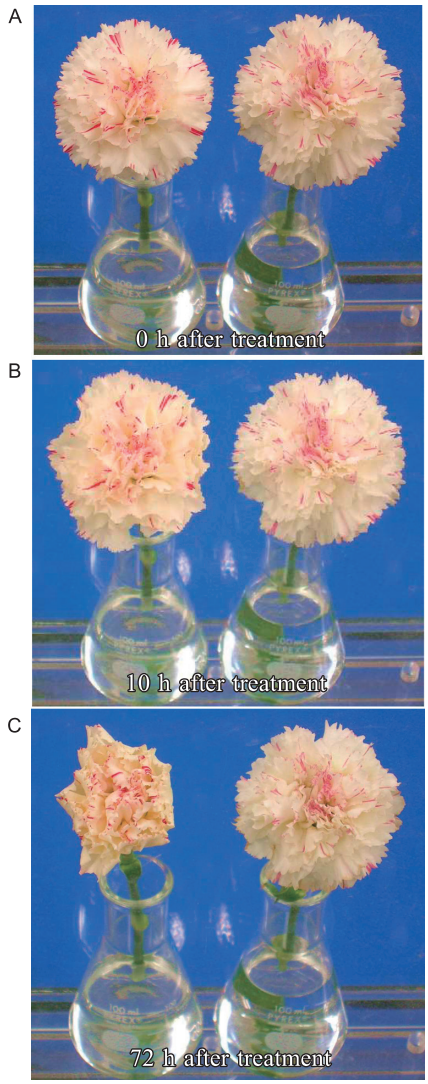

Fig. 7. Effect of cut flower age at treatment time on sensitivity to ethylene (10 µL L^{-1}) of 'Miracle Symphony' at (A) 0 h, (B) 10 h, and (C) 72 h after treatment began. Left: exposed immediately after harvest. Right: exposed 18 days after harvest.

time of line 806-46b to 10 µL L^{-1} ethylene was 21.8 h (**Fig. 8B**), whereas that of 'White Sim' was 5.8 h. Although line 532-6 has an ultra-long vase life and a low level of ethylene production at senescence, it is sensitive to exogenous ethylene application (Onozaki *et al.* 2011). In contrast, line 806-46b with an ultra-long vase life showed low ethylene sensitivity (**Fig. 8B**). These results show that the breeding of lines with both an extremely long vase life and ethylene resistance is possible using conventional cross-breeding techniques.

Line 806-46b did not show brownish discoloration of petal edges during senescence (**Fig. 8A**), which is typical of other selected lines with low ethylene production. Instead, its petals gradually lost surface turgor by moisture loss. This rare senescence pattern is shared by line 532-6 (**Fig. 5**). Line 806-46b showed extremely low ethylene production at

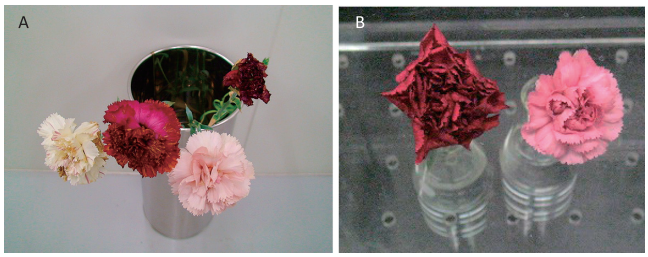

Fig. 8. (A) Variation in the vase life of line 806-46b with ultra-long vase life and three control cultivars, 35 days after harvest. The flowers were held in distilled water under standard conditions (23°C, 12-h photoperiod, 70% RH). Left to right: 'Miracle Symphony', 'Miracle Rouge', 806-46b, 'Excerea'. (B) Variation in sensitivity to ethylene (10 µL L^{-1}) between (left) 'Karen Rouge' (sensitive) and (right) 806-46b (resistant) at 12 h after treatment began.

senescence, as well as after treatment with ACC, an ethylene precursor. Treatment with exogenous ethylene induced ethylene production without wilting, indicating that autocatalytic ethylene production functions normally (Onozaki *et al.* 2015).

Thus, we successfully developed of line 806-46b with both an ultra-long vase life and ethylene resistance by conventional cross-breeding techniques. Line 806-46b would be useful in research on genes involved in ethylene synthesis and response to exogenous ethylene.

Relationship between ethylene production and vase life in breeding lines

We investigated vase life, ethylene production at natural senescence, ethylene production after ethylene treatment (autocatalytic ethylene biosynthesis), response time to ethylene treatment (ethylene sensitivity), and flower diameter in the six cultivars used as initial breeding materials and in 123 selected lines from the 1st to the 7th generations. We found large genetic variability in all five traits (Onozaki *et al.* 2018).

Ethylene production at natural senescence decreased markedly from the 1st to the 7th generations (**Fig. 9A**). In particular, all lines selected in the 4th to 7th generations produced very little ethylene. Ethylene production after ethylene treatment decreased gradually from the 1st to the 5th generation (**Fig. 9B**). The average ethylene production after ethylene treatment of the 5th to 7th generations was approximately the same (17.4, 15.1, and 18.2 nL g^{-1} FW h^{-1}, respectively). We found significant negative correlations of vase life with both ethylene production at natural senescence ($r = -0.63$**; **Fig. 9A**) and ethylene production after ethylene treatment ($r = -0.61$**; **Fig. 9B**).

However, there was no correlation between vase life and response time to ethylene treatment (ethylene sensitivity) (**Fig. 9C**). All cultivars and most lines showed response times of 6 to 16 h, and were classified as sensitive to moderately sensitive (Onozaki *et al.* 2008). Only one selected

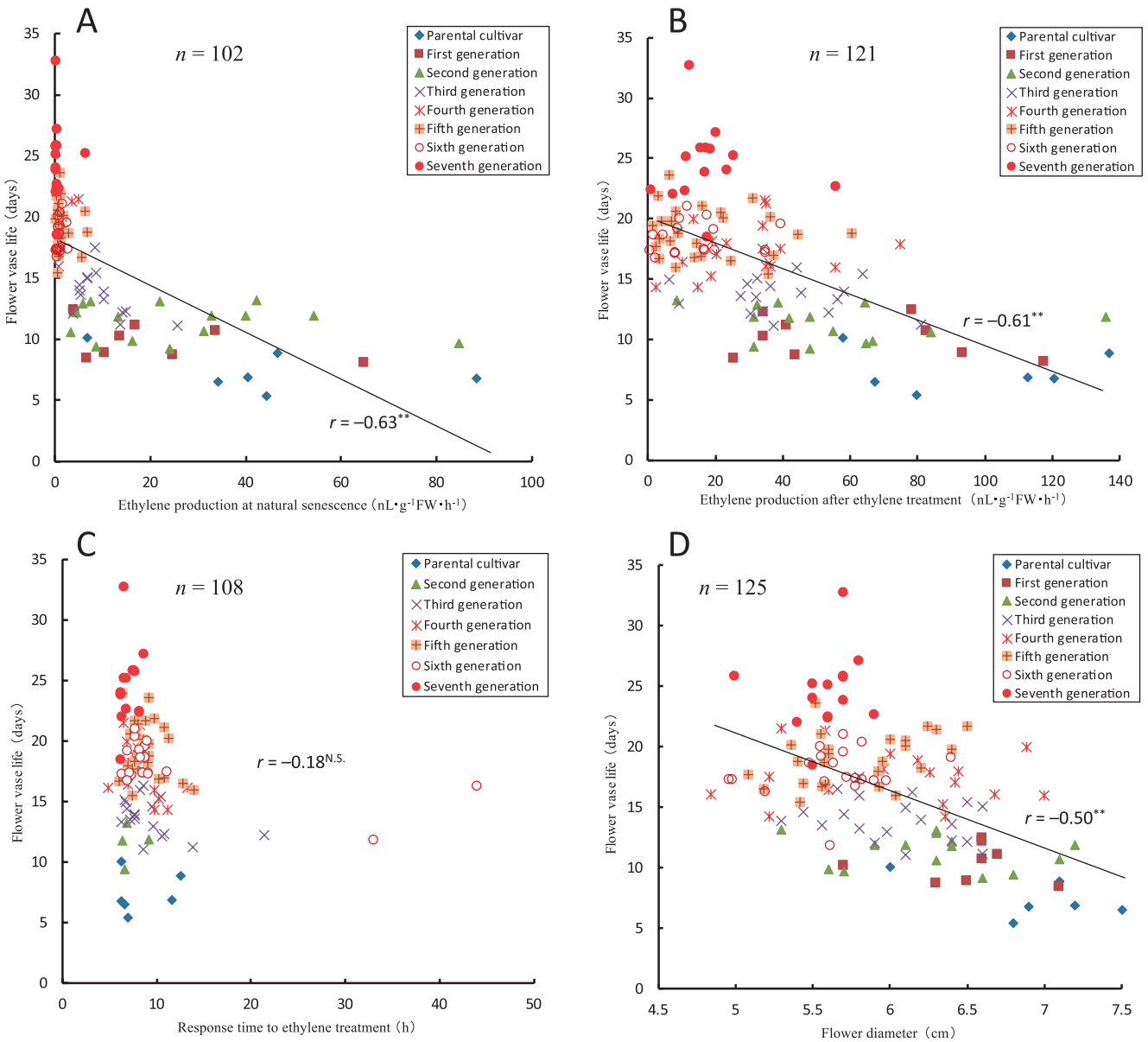

Fig. 9. Relationships between vase life and (A) ethylene production at natural senescence, (B) ethylene production after ethylene treatment, (C) response time to ethylene treatment, and (D) flower diameter. **Significant at 1% level; N.S.: not significant. In C, response time to ethylene treatment in 1st-generation lines was not measured, because we had not established our video evaluation method (Onozaki *et al.* 2004a) before 2001.

3rd-generation line (64-54) and two selected 6th-generation lines (203-42S and 204-41S) showed ethylene resistance, with response times of >16 h (**Fig. 9C**).

Thus, the long vase life of the selected lines was strongly associated with a decrease in ethylene production. Our results indicate that variation in vase life is due not to differences in ethylene sensitivity, but to differences in ethylene production (Onozaki *et al.* 2018).

'Sandrosa', which lacks a climacteric ethylene response (Mayak and Tirosh 1993), had the longest vase life and lowest ethylene production among the original six parental cultivars (Onozaki *et al.* 2001). It is interesting to note that almost all selected lines were descended from 'Sandrosa' (crossing data not shown). This result indicates that the low ethylene production in most selected lines was reduced by the introduction from 'Sandrosa' of genes related to low ethylene production, and that the trait of low ethylene production is heritable.

The mean vase life of 'Sandrosa' was 10.1 days (Onozaki *et al.* 2001). The proportion of flowers with a vase life superior to that of 'Sandrosa' (≥11 days) rose from only 6.7% in the 1st generation to 94.4% in the 7th generation (**Fig. 1B**). Thus, vase life showed a high rate of transgressive segregation in the later generations.

Although flower size is an important trait for commercial production, crossing and selection for flower vase life reduced flower diameter, with a significant negative correlation ($r = -0.50$**) between the two traits (**Fig. 9D**).

However, as this correlation might relate to inbreeding depression, further studies are needed to clarify the relationship. Carnation cultivars are developed to be highly heterozygous so as to avoid the effects of inbreeding depression. Sato *et al.* (2000) reported that inbreeding depression of carnation appears in the 3rd selfed generation (S_3), so it is almost impossible to produce S_4 seeds.

As flower diameter is an important characteristic in cut carnations, its extreme reduction is undesirable for commercial cultivars, especially the standard type (one flower per stem). To develop commercial cultivars with both long vase life and large flowers, we need to carry out selection and crossing with consideration of flower diameter. Another solution is to breed spray-type cultivars (multiple flowers per stem) with a long vase life, among which a smaller flower size is acceptable commercially.

Breeding of spray-type 'Kane Ainou 1-go' with long vase life

To breed spray-type cultivars, we have conducted joint research with the Aichi Agricultural Research Center since 2006. We developed 'Kane Ainou 1-go', a new spray-type carnation cultivar with a long vase life, in 2015, using the 5th-generation line 108-44 (**Fig. 1C**), a long-vase-life standard-type line with low ethylene production (Onozaki *et al.* 2006b), as a parent (Hotta *et al.* 2016). Flowers of 'Kane Ainou 1-go' had low ethylene production during senescence. The mean vase life was 19.2 to 21.3 days (about 3 times that of the spray carnation 'Silhouette') at 25°C and 60% RH under a 12-h photoperiod. The annual cut flower yield of this cultivar exceeded that of control cultivars, as it flowers earlier. The plant has a strong stem and large pale pink flowers (Hotta *et al.* 2016).

Conclusions and future perspectives

The breeding of cultivars with genetically superior vase life appears to be an efficient approach to satisfying consumers' quality expectations. The mean vase life was improved from 7.4 days in the 1st generation to 15.9 days in the 7th generation through conventional crossing techniques (**Fig. 1B**). Two cultivars, 'Miracle Rouge' and 'Miracle Symphony', with a genetically determined long vase life of 17.7 to 20.7 days under standard conditions, were released in 2005 (**Fig. 3**) (Onozaki *et al.* 2006a). Two lines, 532-6 and 806-46b, with an ultra-long vase life of over 27 days under standard conditions, were also developed (**Figs. 5**, **8**) (Onozaki *et al.* 2011, 2015). The results of our nearly two-decades-long breeding research revealed a close correlation between vase life and ethylene production in cut carnations (**Fig. 9A**, **9B**) (Onozaki *et al.* 2018), but we found no significant correlation between vase life and ethylene sensitivity (**Fig. 9C**).

DNA markers in carnation have been developed for resistance to bacterial wilt (Onozaki *et al.* 2004b, Yagi *et al.* 2012a) and flower type (Onozaki *et al.* 2006c, Yagi *et al.* 2014). In particular, a marker linked to bacterial wilt resistance is now being used in breeding to develop resistant commercial cultivars. QTL analyses for vase life have been made using our 4th-generation line 85-11 (Yagi *et al.* 2012b) and our breeding line 806-46b with both ultra-long vase life and ethylene resistance (Yagi *et al.* 2017). However, DNA markers tightly linked to vase life have not yet been developed. Breeding for long vase life using conventional breeding techniques requires a great deal of time and effort in the phenotypic evaluation of numerous seedlings. By combining conventional breeding techniques with the molecular genetics of flower vase life, we can develop more reliable methods for breeding carnations.

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