Introduction

Flower longevity is an important trait determining the quality of commercial flowers. Consumers appreciate long-lasting flowers and the distribution industry desires reduction of deterioration in the quality of flowers in the distribution chain. Several techniques have been developed to improve flower life for some cut flowers but not for many other flowers. An understanding of physiology and molecular biology of flower senescence is needed to efficiently improve flower longevity.

Flower longevity varies among plant species. For example, flowers of morning glory wilt within one day, whereas flowers of *Phalaenopsis* stay open several months. Flower longevity is considered to be closely linked with reproductive strategy of flowering plants, as it is an important factor in attracting pollinators (Pennell and Lamb 1997, Rogers 2006, Shibuya et al. 2016, van Doorn and Woltering 2008). Treatment with cycloheximide, which inhibits protein synthesis, delays petal senescence in several plants, supporting that petal senescence is an active process (Shibuya 2012). Ethylene plays a crucial role in flower senescence in some plant species. In several species that show ethylene-dependent flower senescence, genetic modification targeting genes for ethylene biosynthesis or signaling has improved flower longevity. Although little is known about regulatory mechanisms of petal senescence in flowers that show ethylene-independent senescence, a recent study of Japanese morning glory revealed that a NAC transcription factor, EPHEMERAL1 (EPH1), is a key regulator in ethylene-independent petal senescence. *EPH1* is induced in an age-dependent manner irrespective of ethylene signal, and suppression of *EPH1* expression dramatically delays petal senescence. In ethylene-dependent petal senescence, comprehensive transcriptome analyses revealed the involvement of transcription factors, a basic helix-loop-helix protein and a homeodomain-leucine zipper protein, in the transcriptional regulation of the ethylene biosynthesis enzymes. This review summarizes molecular aspects of flower senescence and discusses strategies to improve flower longevity by molecular breeding.

Key Words: ethylene, flower, programmed cell death, senescence, transcription factor.

Ethylene response of cut flowers

Patterns of flower senescence can be classified based on differences in how ethylene is involved: ethylene dependent and ethylene independent. In flowers of plant species showing ethylene-dependent senescence, an autocatalytic rise in endogenous ethylene production triggers petal senescence (Shibuya 2012, Woltering and van Doorn 1988). In general, inhibition of ethylene biosynthesis or perception delay flower senescence, and exogenous ethylene treatment accelerates senescence in these flowers. On the other hand, ethylene seems to have little effect on flower senescence in other plant species (Shibuya 2012, Woltering and van Doorn 2008).
1988). Flowers of species showing ethylene-independent senescence usually produce little ethylene during flower senescence. Treatment with ethylene inhibitors does not improve flower longevity and exogenous ethylene does not accelerate flower senescence. In addition to ethylene-dependent and ethylene-independent senescence, there are intermediate or mixed patterns of senescence (Shibuya 2012). For example, flowers of *Campanula* show ethylene-independent senescence in the absence of pollination; however, once pollinated, these flowers start producing ethylene, which causes accelerated petal senescence (Kato et al. 2002). In flowers of *Mirabilis jalapa* (four-o’clock), endogenous ethylene has little effect on petal senescence but application of exogenous ethylene accelerates it (Xu et al. 2007).

It would be useful to know which particular species of flowers respond to exogenous ethylene, because in many cases, endogenous ethylene is involved in the regulation of flower senescence in species that respond to exogenous ethylene. Responses to ethylene vary greatly among plant species. In carnation, 0.6 μL L⁻¹ ethylene induces visible petal senescence symptoms (inward rolling of petals) within 12 h (Wu et al. 1991), while in chrysanthemum, little effect is observed when flowers are treated with 1 μL L⁻¹ ethylene for more than ten days (Doi et al. 2003). Woltering and van Doorn (1988) evaluated ethylene sensitivity in 96 plant species by treating with 3 μL L⁻¹ ethylene for 22 to 24 h. In some species, however, longer exposure to ethylene has been reported to result in accelerated flower senescence. For example, daffodil was classified as a flower with very low ethylene sensitivity, but continuous treatment with 1 μL L⁻¹ ethylene hastens petal senescence (Hunter et al. 2004). Here, the ethylene response of cut flowers was classified based on the results reported in the literature and on the results of our studies (Kondo et al. 2017, Table 1). It should be noted that responses to ethylene vary even within species, but are specific to cultivars. Table 1 shows the results of tested cultivars in the literature. Furthermore, concentration of ethylene, time of treatment, experimental period, and evaluation of ethylene response vary among experiments. For the experiments reported in the papers or proceedings written in Japanese, I made notes on Table 1.

### Genes involved in ethylene biosynthesis

The ethylene biosynthetic pathway in plants has been characterized, and genes encoding key enzymes have been isolated (Kende 1993, Lin et al. 2009, Yang and Hoffman 1984). Ethylene is synthesized through the following pathway: S-adenosyl-l-methionine → 1-amino-cyclopropane-1-carboxylic acid (ACC) → ethylene. The last two reactions are catalyzed by ACC synthase and ACC oxidase.

**ACC synthase (ACS) and ACC oxidase (ACO)** are encoded by multigene families, and genes encoding these enzymes have been isolated from many ornamental plant species (Shibuya and Ichimura 2016). ACS genes have been isolated, for example, from carnation (Henskens et al. 1994, Jones and Woodson 1999, Park et al. 1992), geranium (Wang and Arteca 1995), *Phalaenopsis* (Bui and O’Neill 1998), petunia (Lindstrom et al. 1999), rose (Wang et al. 2004), snapdragon (Woltering et al. 2005), morning glory (Frankowski et al. 2009), tree peony (Zhou et al. 2013), and *Oncidium* (Shi and Liu 2016). ACO genes have been isolated, for example, from carnation (Tanase et al. 2012, Wang and Woodson 1991), *Phalaenopsis* (Nadeau et al. 1993), petunia (Tang et al. 1993), geranium (Clark et al. 1997), snapdragon (Woltering et al. 2005), tulip (Momonti et al. 2007), rose (Xue et al. 2008), tree peony (Zhou et al. 2013), and morning glory (Wilmowicz et al. 2014). ACS and ACO genes are differentially regulated in a spatial and temporal-specific manner. In carnation, for example, of the three ACS genes, DcACS1 is most abundant in petals while DcACS2 and DcACS3 are preferentially expressed in stamens (Jones and Woodson 1999). Differential expression of ACO genes has also been reported in petunia (Tang et al. 1994).

The regulatory mechanisms of ACS and ACO genes during flower senescence are still largely unknown. Recently, two transcription factors (TFs), homeodomain–leucine zipper (HD-Zip) and basic helix–loop–helix (bHLH), were reported to regulate these genes. PhHD-Zip, a HD-Zip TF gene, was up-regulated during petal senescence, and suppression of PhHD-Zip by virus-induced gene silencing significantly extended flower longevity in petunia (Chang et al. 2014). Silencing of PhHD-Zip reduced ethylene production and the abundance of transcripts of *ACO1*, *ACO4* and *ACS*. Furthermore, Yin et al. (2015) showed that PhFBH4, a bHLH TF, regulates petal senescence by modulating the ethylene biosynthesis pathway in petunia. Silencing of *PhFBH4* reduced and overexpression increased transcript abundance of *ACS1* and *ACO1*. The authors suggested that *ACS1* is a direct target of PhFBH4 since PhFBH4 physically interacts with a cis-element in the *ACS1* promoter. Further studies on these TFs will shed light on the transcriptional regulation of the ethylene biosynthesis pathway during petal senescence.

### Genes involved in ethylene signal transduction

Ethylene signaling is mediated by a complex multicomponent pathway (Lin et al. 2009). ETR1 (ETHYLENE RESPONSE1) has been identified as an ethylene receptor (Chang et al. 1993). Five ethylene receptor genes were cloned from *Arabidopsis thaliana* (Hua et al. 1995, Sakai et al. 1998) and these receptors have been shown to be negative regulators of ethylene responses (Hua and Meyerowitz 1998). The receptors act through CTR1 (CONSTITUTIVE TRIPLE RESPONSE1), which negatively regulates ethylene signaling (Huang et al. 2003, Kieber et al. 1993). Downstream of the receptor-CTR1 complex is EIN2 (ETHYLENE INSENSITIVE2), which positively regulates signaling (Alonso et al. 1999, Qiao et al. 2009, 2012).
Concentration of ethylene, time of treatment, and experimental period varied among experiments (for details, see references). For plant species examined by Kondo et al. (2017), treatment was with 10 µL L⁻¹ ethylene continuously at 23°C and response to ethylene (petal wilting and/or the abscission of flower parts) was evaluated every 24 h for 3 days. For Eustoma grandiflorum and Lathyrus odoratus examined by Shimizu-Yumoto and Ichimura (2006, 2009), ethylene was treated for 24 h and then kept in ethylene-free air at 23°C. Response to ethylene was evaluated based on the time from the end of the ethylene treatment to the time when petals wilt as described in Shimizu-Yumoto and Ichimura (2012). –, no cultivar name or unknown.

**Table 1. Ethylene response of cut flowers**

| Plant species that show accelerated flower senescence by exogenous ethylene treatment | Cultivar | Ethylene treatment | Reference |
|---|---|---|---|
| *Antirrhinum majus* (snapdragon) | Yellow Butterfly | 2, 10 µL L⁻¹, 48 h | Ichimura et al. (2008) |
| *Astrilbe* | – | 10 µL L⁻¹, continuous | Kondo et al. (2017) |
| *Bougainvillea* | Royal Daphne | 10 µL L⁻¹, continuous | Kondo et al. (2017) |
| *Calendula* | – | 10 µL L⁻¹, continuous | Kondo et al. (2017) |
| *Campanula medium* | Champion Pink | 2 µL L⁻¹, 48 h | Kato et al. (2002) |
| *Cattleya* | Pearl Harbor | 0.3, 3 µL L⁻¹, continuous | Goh et al. (1985) |
| *Cymbidium* | Angelica | 0.3, 3 µL L⁻¹, continuous | Goh et al. (1985) |
| *Delphinium hybrid* | Bellamosum | 10 µL L⁻¹, 24 h | Ichimura et al. (2009) |
| *Dendrobium hybrid* | Jaqelyn Thomas | 2 µL L⁻¹, 24 h | Porat et al. (1994) |
| *Dianthus caryophyllus* (carnation) | Sandra | 0.6 µL L⁻¹, 12 h | Wu et al. (1991) |

- Ethylene response of cut flowers

| Plant species that do not show accelerated flower senescence by exogenous ethylene treatment | Cultivar | Ethylene treatment | Reference |
|---|---|---|---|
| *Matthiola incana* (stock) | – | 1 µL L⁻¹, 48 h | Celikel and Reid (2002) |
| *Narcissus pseudonarcissus* (daffodil) | Dutch Master | 1 µL L⁻¹, continuous | Hunter et al. (2004) |
| *Oxypetalum caeruleum* (blue star) | Grand Canyon × Sparsholt | 3 µL L⁻¹, continuous | Goh et al. (1998) |
| *Rosa hybridra* (rose) | Sonia | 1 µL L⁻¹, 48 h | Ichimura et al. (2005) |
| *Spiraea cantoniensis* | – | 10 µL L⁻¹, continuous | Kondo et al. (2017) |
| *Spiraea thunbergii* | – | 10 µL L⁻¹, continuous | Kondo et al. (2017) |
| *Strelitzia reginae* | – | 10 µL L⁻¹, continuous | Kondo et al. (2017) |
| *Trachymene coerulea* (Didiscus caeruleus) | – | 10 µL L⁻¹, continuous | Kondo et al. (2017) |
| *Vanda* | Miss Joaquim | 0.3, 3 µL L⁻¹, continuous | Goh et al. (1985) |
| *Zantedeschia* (calla) | Wedding March | 10 µL L⁻¹, continuous | Kondo et al. (2017) |

- Ethylene response of cut flowers

| Plant species examined by Kondo et al. (2017) | Ethylene treatment | Reference |
|---|---|---|
| *Ammi majus* | 10 µL L⁻¹, continuous | Kondo et al. (2017) |
| *Bupleurum rotundifolium* | 10 µL L⁻¹, continuous | Kondo et al. (2017) |
| *Celosia* | 10 µL L⁻¹, continuous | Kondo et al. (2017) |
| *Dendranthema grandiflora* (chrysanthemum) | 1 µL L⁻¹, continuous | Doi et al. (2003) |
| *Dendrobium* | Jaqelyn Hawai | 0.3, 3 µL L⁻¹, continuous | Goh et al. (1985) |
| *Gladiolus sp.* | 1 µL L⁻¹, continuous | Serek et al. (1994) |
| *Lilium* (lily, Oriental hybrid) | 100 µL L⁻¹, 24 h | Elgar et al. (1999) |
| *Lilium longiflorum* | 100 µL L⁻¹, 24 h | Elgar et al. (1999) |
| *Oncidium* | 0.3, 3 µL L⁻¹, continuous | Goh et al. (1985) |
| *Tulipa hybridra* (tulip) | 3–5 µL L⁻¹, continuous | Sexton et al. (2000) |
| *Tulipa Kaufmanniana* (tulip) | 3–5 µL L⁻¹, continuous | Sexton et al. (2000) |
Toward the end of the signaling pathway, ethylene responses are mediated by TFs including EIN3 (ETHYLENE INSENSITIVE3) and ERF1 (ETHYLENE RESPONSE FACTOR1) (Chao et al. 1997, Solano et al. 1998).

Genes encoding ethylene receptors have been isolated from several ornamental plant species; for example, rose (Müller et al. 2000), geranium (Dervinis et al. 2000), carnation (Shibuya et al. 2002), Delphinium (Kuroda et al. 2003, Tanase and Ichimura 2006), chrysanthemum (Narumi et al. 2005), gladiolus (Arora et al. 2006), petunia (Wang and Kumar 2007), Oncidium (Huang et al. 2007b), and tree peony (Zhou et al. 2010). Genes have been reported that encode CTR1 in rose (Müller et al. 2002) and Delphinium (Kuroda et al. 2004), EIN2 in petunia (Shibuya et al. 2004) and carnation (Fu et al. 2011), and EIN3 in carnation (Iordachescu and Verlinden 2005, Waki et al. 2001), rose (Müller et al. 2003), petunia (Shibuya and Clark 2006), and tree peony (Zhou et al. 2010). In addition to components involved in an ethylene signal cascade, a MADS-box TF, FOREVER YOUNG FLOWER (FYF), acts as a repressor of flower senescence by repressing ethylene responses in Arabidopsis (Chen et al. 2011, 2015). The ectopic expression of Arabidopsis FYF causes both delayed senescence and delayed abscission of the floral organs in Arabidopsis (Chen et al. 2011). Recently, FYF was reported to negatively regulate ethylene response DNA-binding factors by activating an ethylene-responsive factor in the regulation of floral organ senescence and abscission (Chen et al. 2015).

Transgenic approaches to improve flower longevity by manipulating ethylene biosynthesis and responses

Since genes involved in ethylene biosynthesis and signal transduction have been isolated from several ornamental plants, flower longevity can be improved by transgenic techniques targeting those genes. In the 1990s, Savin et al. (1995) produced transgenic carnation suppressing ACO expression by an anti-sense method. In the transgenic carnation, ethylene production was reduced and petal senescence was clearly delayed. The vase life of untransformed carnation flowers was about 5 days from day of harvest to petal wilting, while flowers of transgenic plants had a vase life of 8 to 9 days at 21°C. After this report, transgenic plants with reduced ACS or ACO expression were shown to have prolonged flower longevity in petunia (Huang et al. 2007a) and torenia (Aida et al. 1998, Table 2). As with chemical approaches, inhibition of ethylene perception is a more efficient way to prolong flower life. The introduction of a mutated ethylene receptor gene, such as Arabidopsis etr1-1, is a particularly desirable strategy because even a single genetic manipulation may confer ethylene insensitivity in a variety of heterologous plant species (Wilkinson et al. 1997). This strategy has been applied to several ornamental crops, including Campanula (Sriskandarajah et al. 2007), carnation (Bovy et al. 1999), Kalanchoe (Sanikhani et al. 2008), Nemesia (Cui et al. 2004), petunia (Wilkinson et al. 1997), and torenia (Tanase et al. 2011), prolonging flower longevity (Table 2). For example, the longevity of nonpollinated flowers in wild-type petunia is 6.7 days on average, while flowers of transgenic plants harboring etr1-1 last 16.6 days on plants grown at day/night temperatures of 26/21°C (Gubrium et al. 2000). In addition to ethylene receptors, suppression of ethylene signaling components such as EIN2 and EIN3 has prolonged flower life in petunia (Shibuya et al. 2004, Shibuya and Clark 2006, Table 2). Besides ethylene biosynthetic enzymes and signaling components, the ectopic expression of Arabidopsis FYF has been reported to delay petal senescence by repressing ethylene responses in Eustoma grandiflorum (Chen et al. 2011). Suppression of PhHD-Zip and PhFBH4 also results in delayed petal senescence in petunia (Chang et al. 2014, Yin et al. 2015, Table 2). The longevity of nonpollinated wild-type flowers is about 7 days, while suppression of PhFBH4 by expressing the antisense PhFBH4 fragment extends flower longevity to about 9 days at day/night temperatures of 25/20°C (Yin et al. 2015).

It should be noted that in transgenic plants with reduced ethylene sensitivity, physiological side effects may limit their commercial use. For example, ethylene-insensitive transgenic petunia exhibits inhibited adventitious root formation and a high percentage of premature death (Clark et al. 1999, Shibuya et al. 2004). These negative side effects are likely due to constitutive ethylene insensitivity in transgenic plants, and the key to circumventing these undesirable side effects is to use a tissue-specific promoter. FLORAL BINDING PROTEIN1 (FBP1) is a floral organ identity gene of petunia that is expressed exclusively in petals and stamens (Angenent et al. 1992). The FBP1 promoter has been used to control the expression of the etr1-1 gene in transgenic carnation (Bovy et al. 1999), Campanula (Sriskandarajah et al. 2007), and Kalanchoe (Sanikhani et al. 2008) with limited side effects (Table 2). Recently, the InMYB1 promoter from Japanese morning glory was reported to function as a petal-specific promoter in a wide range of dicots including Eustoma, chrysanthemum, carnation, Japanese gentian and stock (Azuma et al. 2016). This promoter could also be used for the improvement of flower longevity similarly to the FBP1 promoter.

Roles of NAC TFs in the regulation of ethylene-independent petal senescence

In some plant species including lily, tulip, chrysanthemum, iris, and gladiolus, ethylene has little effect on the regulation of petal senescence (Woltering and van Doorn 1988, Table 1). Exogenous ethylene treatment does not accelerate petal senescence, and chemical inhibition of ethylene biosynthesis or perception does not delay senescence in these flowers. Thus, in these flowers, petal senescence has been considered to be regulated through an ethylene-independent pathway. Studies to identify genes that regulate PCD during petal senescence using differential screening and microarray
Table 2. Examples of transgenic ornamental plants with prolonged flower longevity

| Plant species                  | Gene construct          | Expression          | Reference                  |
|--------------------------------|-------------------------|---------------------|----------------------------|
| Carnation (Dianthus caryophyllus) | **ACO** (D. caryophyllus) | Silencing (Antisense) | Savin et al. (1995)         |
| ‘Scania’ and ‘White Sim’       | MAC promoter            |                     |                            |
| Carnation (Dianthus caryophyllus) | **ACO** (D. caryophyllus) | Silencing (Sense)   | Kosugi et al. (2000)        |
| ‘Nora’                         | CaMV 3S promoter        |                     |                            |
| Carnation (Dianthus caryophyllus) | **ACS** (D. caryophyllus) | Silencing (Sense)   | Iwazaki et al. (2004)       |
| ‘Nora’                         | CaMV 3S promoter        |                     |                            |
| Petunia (Petunia hybrida Hort. Vilm.-Andr.) | **AC5/ACO** (Brassica oleracea) | Silencing (Antisense) | Huang et al. (2007a)        |
| Torenia (Torenia fournieri)    | **ACO** (T. fournieri)   | Silencing (Sense, Antisense) | Aida et al. (1998)         |
| ‘Crown Mix’, ‘Crown Blue’, and ‘White’ | CaMV 3S promoter    |                     |                            |

Suppression of ethylene signaling

| Plant species                  | Gene construct          | Expression          | Reference                  |
|--------------------------------|-------------------------|---------------------|----------------------------|
| Campanula (Campanula carpatica) | **etr1-1** (A. thaliana) | Ectopic            | Sriskandarajah et al. (2007) |
| ‘Blue Uniform’                 | Petunia FBP1 promoter   |                     |                            |
| Carnation (Dianthus caryophyllus) | **etr1-1** (A. thaliana) | Ectopic            | Bovy et al. (1999)          |
| ‘Lena’                         | CaMV 3S/Petunia FBP1 promoter |                     | Sanikhani et al. (2008)    |
| Kalanchoe (Kalanchoe blossfeldiana) | **etr1-1** (A. thaliana) | Ectopic            | Cui et al. (2004)           |
| ‘Debbie’                       | Petunia FBP1 promoter   |                     | Wilkinson et al. (1997)     |
| Nemedia (Nemedia strumosa)     | Cm-ETRI/H69A (Cucumis melo) | Ectopic            | Shaw et al. (2002)          |
| genotype White                 | CaMV 3S promoter        |                     |                            |
| Petunia (Petunia hybrida)      | **etr1-1** (A. thaliana) | Ectopic            | Shibuya et al. (2004)       |
| ‘Mitchell Diploid’             | CaMV 3S promoter        |                     |                            |
| Petunia (Petunia hybrida Hort. Vilm.-Andr.) | **boers** (B. oleracea) | Ectopic            | Shibuya and Clark (2006)    |
| Petunia (Petunia hybrida)      | EIN2 (P. hybrida)       | Silencing (Sense, RNAi) | Shibuya et al. (2014)       |
| ‘Mitchell Diploid’             | CaMV 3S promoter        |                     |                            |
| Petunia (Petunia hybrida)      | EIL2 (P. hybrida)       | Silencing (Sense)   | Shibuya et al. (2014)       |
| ‘Mitchell Diploid’             | CaMV 3S promoter        |                     |                            |
| Torenia (Torenia fournieri)    | **Dc-ETRI** (D. caryophyllus) | Ectopic        | Tanase et al. (2011)        |
| ‘Crown Mix’                    | CaMV 3S promoter        |                     |                            |

Altered expression of transcription factors

| Plant species                  | Gene construct          | Expression          | Reference                  |
|--------------------------------|-------------------------|---------------------|----------------------------|
| Eustoma (Eustoma grandiflorum) | **FYF** (A. thaliana)   | Ectopic            | Chen et al. (2011)          |
| Japanese morning glory (Ipomoea nil) | **EPH1** (I. nil) | Silencing (RNAi)   | Shibuya et al. (2014)       |
| ‘Violet’                       | CaMV 3S promoter        |                     |                            |
| Petunia (Petunia hybrida)      | **PhHD-Zi** (P. hybrida) | Silencing (VIGS)   | Chang et al. (2014)         |
| ‘PrimeTime Blue’               | CaMV 3S promoter        |                     |                            |
| Petunia (Petunia hybrida)      | **PhFBH4** (P. hybrida)  | Silencing (VIGS, antisense) | Yin et al. (2015)          |
| ‘PrimeTime Blue’ and ‘Mitchell Diploid’ | CaMV 3S promoter |                     |                            |

analysis have identified upregulation or downregulation of numerous genes in several plant species, including Hemerocallis (Panavas et al. 1999) and Iris (van Doorn et al. 2003). However, no genes specific for cell death have yet been identified (van Doorn and Woltering 2008). The lack of effective transformation methods makes it difficult to determine the function of isolated genes in these plant species.

Petal senescence of Japanese morning glory ‘Violet’ is considered to be regulated independently of endogenous ethylene because chemical inhibition of ethylene biosynthesis or perception does not delay petal senescence (Shibuya 2012, Shinozaki et al. 2011, Yamada et al. 2006). Recently, EPHEMERAL1 (EPH1), a NAC (NAM/ATAF1,2/CUC2) TF, was shown to regulate petal senescence in ‘Violet’. EPH1 is expressed almost specifically in senescing petals but negligibly in vegetative tissues. Transgenic plants with suppressed EPH1 expression showed a great delay in petal senescence (Fig. 1, Shibuya et al. 2014). The transgenic plants grew normally and did not show negative side effects during cultivation in a growth chamber. The constitutive CaMV 35S promoter was used to express the EPH1 RNAi construct, but the expression patterns of the native EPH1 gene with quite specific expression in petals would likely result in negligible side effects.

The rise in abundance of EPH1 transcripts was not suppressed in petals treated with 1-methylcyclopropene, a specific inhibitor of ethylene action, or in petals of transgenic plants with reduced ethylene sensitivity due to suppression of EIN2 expression. This suggests that the expression of EPH1 is regulated independently of an endogenous ethylene signal. In the transgenic plants with suppressed EPH1 expression, expression of several PCD-related gene homologs,
including vacuolar processing enzyme and autophagy-related genes, were suppressed. In ethylene-independent petal senescence, a NAC TF such as EPH1 may be induced developmentally in an age-dependent manner irrespective of ethylene signal and upregulate PCD-related genes, resulting in petal senescence. In tulip, which shows ethylene-independent petal senescence, comparative analysis of gene expression revealed that several NAC TFs are upregulated in senescing inner tepals (Shibuya, unpublished). It is of particular interest whether NAC TFs play a similar role in other ethylene-independent species.

NAC TFs may also be involved in the regulation of ethylene-dependent petal senescence. The upregulation of NAC TFs in senescing petals has been reported in plants that show ethylene-dependent senescence, including Arabidopsis (Wagstaff et al. 2009), wallflower (Price et al. 2008), and petunia (Broderick et al. 2014). In petunia, expression of multiple NAC TF genes was downregulated in ethylene-insensitive petals in which expression of etr1-l was induced (Wang et al. 2013), suggesting that these NAC TF genes are regulated through an ethylene signal. In ethylene-dependent petal senescence, endogenous ethylene induced by pollination or stress may hasten the timing of upregulation of a NAC TF gene, resulting in accelerated petal senescence.

**Conclusion and future perspective**

The role of ethylene in petal senescence has been well characterized. Technically, it is possible to produce long-lasting flowers in plant species that show ethylene-dependent senescence by manipulating genes involved in ethylene biosynthesis or signaling. The introduction of a mutated ethylene receptor gene such as Arabidopsis etr1-l under the control of a petal-specific promoter would be the most effective way to improve flower longevity in these plants. In contrast, regulatory mechanisms of ethylene-independent petal senescence have been unclear. Recently, a NAC TF, EPH1, has been shown as a key regulator of ethylene-independent petal senescence through studies of Japanese morning glory. Since NAC TFs are also upregulated in senescing petals of ethylene-dependent species, NAC TFs may be a master regulator of PCD that integrates age-dependent (ethylene-independent) and ethylene-dependent signals. More studies are necessary to determine whether NAC TFs commonly regulate petal senescence in both ethylene-independent and ethylene-dependent species.

Molecular breeding of ornamental plants has entered a new era by the emergence of efficient genome editing systems such as CRISPR/Cas9. Although transgenic ornamental plants with prolonged flower longevity have been produced since the 1990s, there has been a lack of commercialization. A barrier could be the cost and public acceptance of genetically modified plants. Genome-editing techniques may change such a situation, because it is possible to make knockout mutants for targeted genes that do not harbor transgenes. This technique would be particularly efficient for a gene that plays a role only in a specific phenomenon, because knockout of the gene would not cause undesirable side effects. Information on genome sequences is accumulating in ornamental plant species including carnation (Yagi et al. 2014), orchid (Cai et al. 2015), petunia (Bombarely et al. 2016), Japanese morning glory (Hoshino et al. 2016), and sunflower (Badouin et al. 2017). Future work will reveal new gene targets of molecular breeding for improving flower longevity.

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**Fig. 1.** Time course of visible petal senescence in wild-type (WT) and transgenic plant lines with suppressed EPH1 expression (EPH1r-1 and EPH1r-3). The transgenic plants show approximately doubled flower longevity (Shibuya et al. 2014).
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