Review

Molecular aspects of flower senescence and strategies to improve flower longevity

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Flower longevity is one of the most important traits for ornamental plants. 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.

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 (Primack 1985). In some plant species, flower life is terminated by the abscission of flower parts in the presence or absence of petal senescence (van Doorn 2001). Here I use the term ‘flower senescence’ for phenomena that include both petal senescence and the abscission of flower parts. In this review, I mostly focus on petal senescence. Petal senescence is a type of programmed

cell death (PCD), which is a tightly regulated developmental process (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 and Ichimura 2016). As petal senescence is a genetically programmed developmental process, molecular breeding as well as classical breeding could improve flower longevity. Several reviews on flower senescence have been published (Rogers 2013, Scariot *et al.* 2014, Shahri and Tahir 2014, Shibuya and Ichimura 2016, Shibuya *et al.* 2016, van Doorn and Woltering 2008). In this review, I outline studies on physiology and molecular biology of petal senescence in the context of molecular breeding.

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

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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 $\mu\text{L L}^{-1}$ 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 $\mu\text{L L}^{-1}$ 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 $\mu\text{L L}^{-1}$ 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 $\mu\text{L L}^{-1}$ 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: L-methionine $\rightarrow$ S-adenosyl-L-methionine $\rightarrow$ 1-aminocyclopropane-1-carboxylic acid (ACC) $\rightarrow$ 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 (Momonoi *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, *DcACSI* is most abundant in petals while *DcACS2* and *DcACS3* are preferentially expressed in styles (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 *ACSI* and *ACO1*. The authors suggested that *ACSI* is a direct target of PhFBH4 since PhFBH4 physically interacts with a cis-element in the *ACSI* 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).

Table 1. Ethylene response of cut flowers

Plant species	Cultivar	Ethylene treatment	Reference
Plant species that show accelerated flower senescence by exogenous ethylene treatment			
<i>Antirrhinum majus</i> (snapdragon)	Yellow Butterfly	2, 10 $\mu\text{L L}^{-1}$, 48 h	Ichimura <i>et al.</i> (2008)
<i>Astilbe</i>	–	10 $\mu\text{L L}^{-1}$, continuous	Kondo <i>et al.</i> (2017)
<i>Bouvardia</i>	Royal Daphne	10 $\mu\text{L L}^{-1}$, continuous	Kondo <i>et al.</i> (2017)
<i>Calendula</i>	–	10 $\mu\text{L L}^{-1}$, continuous	Kondo <i>et al.</i> (2017)
<i>Campanula medium</i>	Champion Pink	2 $\mu\text{L L}^{-1}$, 48 h	Kato <i>et al.</i> (2002)
<i>Cattleya</i>	Pearl Harbor	0.3, 3 $\mu\text{L L}^{-1}$, continuous	Goh <i>et al.</i> (1985)
<i>Cymbidium</i>	Angelica	0.3, 3 $\mu\text{L L}^{-1}$, continuous	Goh <i>et al.</i> (1985)
<i>Delphinium</i> hybrid	Bellamosum	3 $\mu\text{L L}^{-1}$, 40 h	Ichimura <i>et al.</i> (2009)
<i>Dendrobium</i> hybrid	Jaquelyn Thomas	10 $\mu\text{L L}^{-1}$, 24 h	Porat <i>et al.</i> (1994)
<i>Dianthus caryophyllus</i> (carnation)	Sandra	2 $\mu\text{L L}^{-1}$, continuous	Wu <i>et al.</i> (1991)
	Chinera	0.6 $\mu\text{L L}^{-1}$, 12 h	
	White Sim		
<i>Eustoma grandiflorum</i>	Azuma-no-sakura	10 $\mu\text{L L}^{-1}$, 24 h	Shimizu-Yumoto and Ichimura (2009)
	Azuma-no-murasaki		
	Maite Sky		
	New Small Lady		
	Asuka-no-sazanami		
<i>Gentiana scabra</i>	Shinbisei	0.5, 2, 5, 10 $\mu\text{L L}^{-1}$, 24 h	Shimizu-Yumoto and Ichimura (2012)
<i>Lathyrus odoratus</i> (sweet pea)	Miranda	0.2, 2 $\mu\text{L L}^{-1}$, 24 h	Shimizu-Yumoto and Ichimura (2006)
<i>Lilium</i> (lily, Asiatic hybrid)	Cordelia	100 $\mu\text{L L}^{-1}$, 24 h	Elgar <i>et al.</i> (1999)
	Apeldoorn		
	Goldena		
	Mona		
<i>Matthiola incana</i> (stock)	–	1 $\mu\text{L L}^{-1}$, 48 h	Celikel and Reid (2002)
<i>Narcissus pseudonarcissus</i> (daffodil)	Dutch Master	1 $\mu\text{L L}^{-1}$, continuous	Hunter <i>et al.</i> (2004)
<i>Oxypetalum caeruleum</i> (blue star)	–	2, 10 $\mu\text{L L}^{-1}$, 24 h	Hiraya <i>et al.</i> (2002)
<i>Paphiopedilum</i>	Grand Canyon $\times$ Sparsholt	3 $\mu\text{L L}^{-1}$, continuous	Goh <i>et al.</i> (1985)
<i>Rosa hybrida</i> (rose)	Sonia	1 $\mu\text{L L}^{-1}$, 48 h	Ichimura <i>et al.</i> (2005)
	Delilah		
<i>Spiraea cantoniensis</i>	–	10 $\mu\text{L L}^{-1}$, continuous	Kondo <i>et al.</i> (2017)
<i>Spiraea thunbergii</i>	–	10 $\mu\text{L L}^{-1}$, continuous	Kondo <i>et al.</i> (2017)
<i>Strelitzia reginae</i>	–	10 $\mu\text{L L}^{-1}$, continuous	Kondo <i>et al.</i> (2017)
<i>Trachymene coerulea</i> (<i>Didiscus caeruleus</i>)	–	10 $\mu\text{L L}^{-1}$, continuous	Kondo <i>et al.</i> (2017)
<i>Vanda</i>	Miss Joaquim	0.3, 3 $\mu\text{L L}^{-1}$, continuous	Goh <i>et al.</i> (1985)
		3 $\mu\text{L L}^{-1}$, 20 h	
<i>Zantedeschia</i> (calla)	Wedding March	10 $\mu\text{L L}^{-1}$, continuous	Kondo <i>et al.</i> (2017)
	Captain Romance		
Plant species that do not show accelerated flower senescence by exogenous ethylene treatment			
<i>Ammi majus</i>	–	10 $\mu\text{L L}^{-1}$, continuous	Kondo <i>et al.</i> (2017)
<i>Bupleurum rotundifolium</i>	–	10 $\mu\text{L L}^{-1}$, continuous	Kondo <i>et al.</i> (2017)
<i>Celosia</i>	Sakata Pride	10 $\mu\text{L L}^{-1}$, continuous	Kondo <i>et al.</i> (2017)
	Castle Pink		
<i>Dendranthema grandiflora</i> (chrysanthemum)	Iwa-no-hakusen	1 $\mu\text{L L}^{-1}$, continuous	Doi <i>et al.</i> (2003)
	Seiun		
	Jinba		
	Seiko-ougon		
	Toku-meimon		
	Kin-meimon		
<i>Dendrobium</i>	Jaquelyn Hawaii	0.3, 3 $\mu\text{L L}^{-1}$, continuous	Goh <i>et al.</i> (1985)
		3 $\mu\text{L L}^{-1}$, 20 h	
<i>Gladiolus</i> sp.	–	1 $\mu\text{L L}^{-1}$, continuous	Serek <i>et al.</i> (1994)
<i>Lilium</i> (lily, Oriental hybrid)	Cassandra	100 $\mu\text{L L}^{-1}$, 24 h	Elgar <i>et al.</i> (1999)
	Stargazer		
<i>Lilium longiflorum</i> (lily)	Gelria	100 $\mu\text{L L}^{-1}$, 24 h	Elgar <i>et al.</i> (1999)
	Princess Gracia		
<i>Oncidium</i>	Carnival Costume	0.3, 3 $\mu\text{L L}^{-1}$, continuous	Goh <i>et al.</i> (1985)
	Summer Sprite		
<i>Tulipa hybrida</i> (tulip)	Golden Apeldoorn	3–5 $\mu\text{L L}^{-1}$, continuous	Sexton <i>et al.</i> (2000)
<i>Tulipa kaufmanniana</i> (tulip)	Shakespeare	3–5 $\mu\text{L L}^{-1}$, continuous	Sexton <i>et al.</i> (2000)

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 $\mu\text{L L}^{-1}$ 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.

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
Suppression of ethylene biosynthesis			
Carnation (<i>Dianthus caryophyllus</i>)	<i>ACO</i> (<i>D. caryophyllus</i>)	Silencing (Antisense)	Savin <i>et al.</i> (1995)
‘Scania’ and ‘White Sim’	MAC promoter		
Carnation (<i>Dianthus caryophyllus</i>)	<i>ACO</i> (<i>D. caryophyllus</i>)	Silencing (Sense)	Kosugi <i>et al.</i> (2000)
‘Nora’	CaMV 35S promoter		
Carnation (<i>Dianthus caryophyllus</i>)	<i>ACS</i> (<i>D. caryophyllus</i>)	Silencing (Sense)	Iwazaki <i>et al.</i> (2004)
‘Nora’	CaMV 35S promoter		
Petunia (<i>Petunia hybrida</i> Hort. Vilm.-Andr.)	<i>ACS/ACO</i> (<i>Brassica oleracea</i>)	Silencing (Antisense)	Huang <i>et al.</i> (2007a)
	CaMV 35S promoter		
Torenia (<i>Torenia fournieri</i>)	<i>ACO</i> (<i>T. fournieri</i>)	Silencing (Sense, Antisense)	Aida <i>et al.</i> (1998)
‘Crown Mix’, ‘Crown Blue’, and ‘White’	CaMV 35S promoter		
Suppression of ethylene signaling			
Campanula (<i>Campanula carpatica</i>)	<i>etr1-1</i> (<i>A. thaliana</i>)	Ectopic	Striskandarajah <i>et al.</i> (2007)
‘Blue Uniform’	Petunia <i>FBPI</i> promoter		
Carnation (<i>Dianthus caryophyllus</i>)	<i>etr1-1</i> (<i>A. thaliana</i>)	Ectopic	Bovy <i>et al.</i> (1999)
‘Lena’	CaMV 35S/Petunia <i>FBPI</i> promoter		
<i>Kalanchoe</i> (<i>Kalanchoe blossfeldiana</i>)	<i>etr1-1</i> (<i>A. thaliana</i>)	Ectopic	Sanikhani <i>et al.</i> (2008)
‘Debbie’	Petunia <i>FBPI</i> promoter		
<i>Nemesia</i> (<i>Nemesia strumosa</i>)	<i>Cm-ETR1/H69A</i> (<i>Cucumis melo</i>)	Ectopic	Cui <i>et al.</i> (2004)
genotype White	CaMV 35S promoter		
Petunia (<i>Petunia hybrida</i>)	<i>etr1-1</i> (<i>A. thaliana</i>)	Ectopic	Wilkinson <i>et al.</i> (1997)
‘Mitchell Diploid’	CaMV 35S promoter		
Petunia (<i>Petunia hybrida</i> Hort. Vilm.-Andr.)	<i>boers</i> (<i>B. oleracea</i>)	Ectopic	Shaw <i>et al.</i> (2002)
	CaMV 35S promoter		
Petunia (<i>Petunia hybrida</i>)	<i>EIN2</i> (<i>P. hybrida</i>)	Silencing (Sense, RNAi)	Shibuya <i>et al.</i> (2004)
‘Mitchell Diploid’	CaMV 35S promoter		
Petunia (<i>Petunia hybrida</i>)	<i>EIL2</i> (<i>P. hybrida</i>)	Silencing (Sense)	Shibuya and Clark (2006)
‘Mitchell Diploid’	CaMV 35S promoter		
Torenia (<i>Torenia fournieri</i>)	<i>Dc-ETR1nr</i> (<i>D. caryophyllus</i>)	Ectopic	Tanase <i>et al.</i> (2011)
‘Crown Mix’	CaMV 35S promoter		
Altered expression of transcription factors			
Eustoma (<i>Eustoma grandiflorum</i>)	<i>FYF</i> (<i>A. thaliana</i>)	Ectopic	Chen <i>et al.</i> (2011)
	CaMV 35S promoter		
Japanese morning glory (<i>Ipomoea nil</i>)	<i>EPH1</i> (<i>I. nil</i>)	Silencing (RNAi)	Shibuya <i>et al.</i> (2014)
‘Violet’	CaMV 35S promoter		
Petunia (<i>Petunia hybrida</i>)	<i>PhHD-Zi</i> (<i>P. hybrida</i>)	Silencing (VIGS)	Chang <i>et al.</i> (2014)
‘Primetype Blue’			
Petunia (<i>Petunia hybrida</i>)	<i>PhFBH4</i> (<i>P. hybrida</i>)	Silencing (VIGS, antisense)	Yin <i>et al.</i> (2015)
‘Primetype Blue’ and ‘Mitchell Diploid’			

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,

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).

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 *etr1-1* 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 etr1-1* 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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