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REVIEW

Flower senescence: some molecular aspects

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Abstract Some molecular aspects of flower senescence have been reviewed. The isolation, identification and characterization of different genes from various flowers (mainly from petals) associated with senescence have been discussed. The isolated genes were divided into different groups. A large proportion of genes have been found to be upregulated during flower senescence while some genes were also found to be downregulated indicating that there exists a complex interplay between the expression patterns of various genes. The genes involved in petal expansion are found to be upregulated during normal flower development from anthesis to open flower stage, but *XTH* (Xyloglucan endotransglucosylase hydrolase) is found to be involved in petal expansion as well as abscission. Cysteine proteases or the genes encoding cysteine proteases (assigned a central role in protein degradation) have been identified from various flower systems, but no cysteine protease has been identified from senescing *Mirabilis jalapa* flowers. In addition to proteases, the genes encoding ubiquitin (exhibiting proteasomal degradation by 26S proteasomes) have also been identified suggesting the two alternate pathways for protein degradation. Genes encoding specific nucleases have also been identified, but they displayed an early increase in transcript abundance before the senescence symptoms become evident and characterize the involvement of PCD during flower senescence. A range of transcription factors are described and their possible role in flower senescence has been discussed. A detailed description of genes involved in ethylene synthesis and the components involved in ethylene signaling have been presented.

Keywords Abscission · Cysteine proteases · Ethylene · Expansion · Senescence · PCD

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Introduction

Senescence, aging and death, conceived of in the past as inevitable, negative processes, are now considered an integral part of differentiation and development. Senescence involves a highly regulated gene expression and the presence of concerted mechanisms of cellular degradation (Yamada et al. 2007). The processes of senescence and senescence-induced PCD are regulated by a coordinated signaling pathway, which is consistent with the view that senescence involves PCD (Coupe et al. 2004). PCD is an active process that is regulated at both transcriptional and translational levels (Lawton et al. 1990; Nooden et al. 1997). Literature concerned with the physiology and biochemistry of flower senescence has been updated from time to time by various authors (Stead 1992; van Doorn and Stead 1997; Rubinstein 2000; van Doorn 2001; Zhou et al. 2005; Eason 2006; Rogers 2006; Tripathi and Tuteja 2007; van Doorn and Woltering 2008; Shahri and Tahir 2011). In our previous review (Shahri and Tahir 2011), we reported different strategies of flower senescence and some important events associated with it. The present review presents the information gathered from a number of recent research papers on isolation, characterization and identification of genes expressed during flower senescence with the intention to update the available literature on some molecular aspects of flower senescence, as in-depth understanding of the senescence and its regulation at molecular level is

essential for bringing the possible improvement in floricultural crops like cut flowers and ornamentals.

Genes associated with flower senescence: an overview

Genes associated with senescence have been isolated from a number of flowers exhibiting ethylene-sensitive, ethylene-insensitive or intermediate pattern of senescence (Lawton et al. 1990; Hunter et al. 2002; van Doorn et al. 2003; Breeze et al. 2004; Hoeberichts et al. 2007; Xu et al. 2007a). A large population of genes associated with flower senescence have been identified and isolated in *Narcissus*, *Alstroemeria*, *Iris*, *Mirabilis,* etc. (Channelière et al. 2002; Hunter et al. 2002; van Doorn et al. 2003; Breeze et al. 2004; Xu et al. 2007a). Some of the genes have been found to be upregulated while others downregulated during flower senescence. The genes upregulated during normal developmental petal senescence relate to remobilization of nutrients, and include proteases, nucleases, lipases and transporters (Hong et al. 2000; Wagstaff et al. 2002; Langston et al. 2005; Price et al. 2008). A general overview of some of the important genes or transcripts isolated from various flower systems is provided in Table 1. The expression pattern of these genes has been found to be spatially as well as temporally regulated. The spatial regulation is evidenced by a rose homolog of the *Arabidopsis APETALA3* gene *(jD10*) and a rose homolog of *Brassica P8* gene whose expression has been found to be more abundant in petals and stamens than other floral organs. An example of temporal regulation of gene expression during petal senescence is shown by the transcripts corresponding to two putative transcription factor genes (*eG04* and *lD10*) which were found to be abundantly expressed in old and senescing flowers than in petals of young flowers. Similar expression patterns have been found to be shown by the transcripts encoding a zinc-finger containing protein- *LSD1* (lesion simulating disease: *BoLSD1, BoLSD2*), Bax inhibitor (*BoBI-1*, *BoBI-2*) and serine palmitoyltransferase (*BoSPT1* and *BoSPT2*) whose mRNAs have also been found to increase during harvest-induced senescence in broccoli floret tissues Coupe et al. (2004). The broccoli LSD cDNAs (*BoLSD1* and *BoLSD2*) are reported to encode predicted proteins (193 amino acids long), with molecular weights of 20.3 and 20.5 kDa, respectively, whereas *BoBI-1* and *BoBI-2* encode a 247 amino acid protein (27.5 kDa) and a 246 amino acid protein (27.3 kDa), respectively. Structurally, both *BoBI-1* and *BoBI-2* proteins have been reported to contain six membrane-spanning domains and it has been suggested that the six putative transmembrane domains in the BI proteins might form ion-conducting channels in a similar manner to the mitochondria membrane pore-forming *Bcl-2* and Bax proteins (Lam et al. 2001). As far as the functional aspect of Bax inhibitor-1 (*BI-1*) is concerned, it is regarded as the most intensively characterized cell death suppressors conserved between plants and mammals (Hückelhoven 2004; Watanabe and Lam 2009) and has been reported to be endoplasmic reticulum (ER)-resident transmembrane protein (25–27 kDa with a hydrophobic tail at the C-terminus) that can interact with multiple partners to alter intracellular Ca^{2+} flux control and lipid dynamics. Like mammalian *BI-1*, plant *BI-1* genes have also been found to express in various tissue types (leaf, root, stem, flower, fruit, etc.) and their expression levels have been found to be usually enhanced during aging (senescence) and under stress conditions, suggesting that *BI-1* function is physiologically associated with cell death control and/or stress management (reviewed in Ishikawa et al. 2011). *BoSPT1* (603 bp) has been reported to encode a predicted protein of 121 amino acids while *BoSPT2* (573 bp) encodes a predicted protein of 103 amino acids. Yamada et al. (2007) isolated several senescence-associated genes (SAGs) from the petals of morning glory (*Ipomoea nil*) flowers. Two cell wall-related genes, one encoding an extensin (plant structural cell wall proteins implicated in growth and in disease resistance response) and one a caffeoyl-CoA-3-*O*-methyl transferase (involved in lignin production) have been found to be upregulated during early floral development while as a pectin acetyl asterase (implicated to be involved in cell wall degradation) has been found to be upregulated after flower opening.

Moreover, the identification of plant genes homologous to animal PCD (apoptosis) genes has been recently reported by Yamada et al. (2009) from the senescent petals of *Ipomoea nil* that included a Bax inhibitor-1 (*BI-1*), a vacuolar processing enzyme (VPE: homologous to caspases) and a monodehydroascorbate reductase [(MDAR: homologous to Apoptosis inducing factor (AIF)]. In addition, microarray screens and analyses of individual genes have revealed that a number of genes, generally considered to be stress-related, are also upregulated during petal senescence. These include metallothioneins, abscisic acid responsive genes and glutathione-*S*-transferases (Meyer et al. 1991; Channelière et al. 2002; Breeze et al. 2004; Price et al. 2008). In *Ipomoea*, genes-*In12*, *In15* and *In21* have been found to encode products related to stress responses (Yamada et al. 2007). Of the genes upregulated in wall flower petals specifically, 40 % have been found to encode chitinases, 23 % encode GSTs, 9 % are involved in reactive oxygen species (ROS)/stress responses, 9 % are involved in signaling, 6 % in remobilization/metabolism, 2 % in transcriptional regulation, 2 % in metal binding, and a further 9 % are of unknown function (Price et al. 2008). Of the GST genes identified in wall flower, two genes have been found to be most similar to *AtGSTF2* and *AtGSTF3* from *Arabidopsis*. Both *AtGSTF2* and *AtGSTF3* have a putative

Table 1 An overview of the genes involved in flower senescence

Source	Genes/transcripts/cDNAs isolated	Possible biological functions	References
Rose	*EF1α* (Elongation factor 1α), genes encoding metallothioneins, a receptor-like kinase, transcription factors (*eG04* and *lD10*), *GAPDH;* (Glyceraldehyde-3-phosphate dehydrogenase), a rose homolog of the *Arabidopsis APETALA3* gene *jD10* and a rose homolog of the *Brassica P8* gene	Protein and lipid turnover (protein synthesis), defense/stress, signal transduction, transcription, secondary metabolism (scent production), signaling role in programmed cell death or apoptosis and floral organ identity (petals)	Channelière et al. (2002)
Daffodil	Genes encoding serine and cysteine proteases	Proteolysis and remobilization	Hunter and Reid (2001), Hunter et al. (2002)
Iris	Sequences encoding Grap 2 and Cyclin D interacting protein, a MADS-domain transcription factor, a casein kinase and a nucleotide-gated ion channel-interacting protein	Regulation of flower senescence in Iris	van Doorn et al. (2003)
Mirabilis	Homologs of a range of transcription factors (Ring Zinc-finger protein) and proteases (upregulated genes)	Protein turn over and degradation and transcriptional regulation	Xu et al. (2007a)
	A homolog of *CCA1* (a 'clock gene' identified in *Arabidopsis thaliana*), a *Xa21* receptor-type protein kinase and an aspartyl protease. (downregulated genes)	Proteolysis and developmental control	
Alstroemeria	Partial cDNA of the senescence-related gene *Alstroemeria Defender Against Death 1 (ALSDAD1)*	Regulation of flower senescence	Wagstaff et al. (2003)
Broccoli	A zinc-finger containing protein- *LSD1* (lesion simulating disease: *BoLSD1, BoLSD2*), Bax inhibitor (*BoBI-1*, *BoBI-2*) and serine palmitoyltransferase (*BoSPT1* and *BoSPT2*)	Suppression of cell death, Regulation of sphingolipid signaling pathway Alter intracellular Ca^{2+} flux control and lipid dynamics Cell death control and/or stress management	Coupe et al. (2004)
Ipomoea nil	Two cell wall related genes (one encoding an extensin and the other a caffeoyl-CoA-3-*O*-methyl transferase), a pectin acetyl asterase, genes homologous to alcohol dehydrogenase and three cysteine proteases, a leucine-rich repeat receptor protein kinase and a 14-3-3 protein (a protein kinase). Genes encoding putative *SEC14* and *ataxin-2*	Growth and in disease resistance response Lignin production Cell wall degradation Remobilization of essential nutrients Signal transduction Golgi vesicle transport RNA metabolism	Yamada et al. (2007)
Ipomoea nil	Genes homologous to animal PCD genes [(Bax inhibitor-1 (*BI-1*), a vacuolar processing enzyme (VPE: homologous to caspases) and a monodehydroascorbate reductase [(MDAR: homologous to Apoptosis inducing factor (AIF)], vacuolar protein sorting 34 (*VPS34*) and *Arabidopsis* autophagy related proteins 4b and 8a (*ATG4b and ATG8a*)	Cell death suppression Vacuolar autophagy Protein turnover	Yamada et al. (2009)

ethylene-responsive enhancer element in their promoter sequences similar to that of a petal senescence-enhanced GST from carnation (Itzhaki et al. 1994). Both genes are members of the phi (ϕ) class of GSTs from *Arabidopsis* and have been suggested to function as glutathione peroxidases (Wagner et al. 2002). It has been postulated that GST activity may protect a senescing cell from lipid hydroperoxides prior to the actual cell death (Meyer et al. 1991). An important observation during *Alstroemeria* senescence is that the pattern of gene expression induced by ambient dehydration stress has been found to be similar to that seen during developmental senescence, whereas the pattern elicited by cold stress is different, as has been confirmed in the case of three genes: a metallothionein and two genes related to remobilization and proteolysis, respectively, indicating that some processes such as remobilization and ubiquitin-mediated proteolysis, associated in *Alstroemeria* are being activated by stress treatment (Wagstaff et al. 2010).

To sum up, it can be concluded that the genes upregulated during normal developmental petal senescence relate to remobilization of nutrients (proteases, nucleases, lipases and transporters), regulatory genes (transcription factors: NAC-domain transcription factors, Zinc-finger proteins), stress-related genes (Metallothioneins, Abscisic acid responsive genes, Glutathione-*S*-transferases), signal transduction genes (various classes of protein kinases: Xa21 receptor-like protein kinase, casein kinase, leucine-rich receptor kinase, 14-3-3 protein kinase), genes encoding different types of proteases (Cysteine proteases, Serine proteases, Aspartic proteases), 26S-proteasome machinery genes (involved in protein ubiquitination by 26S proteasomes), Cell wall degrading genes (Pectin acetyl transferase), Bax inhibitor genes, genes encoding Vacuolar processing enzymes (VPE) and genes related to RNA metabolism (Ataxin-2). Similarly, the genes that were found to be downregulated included genes encoding MADS-domain transcription factors, MYB transcription factors, gibberellin-induced protein, Cytochrome P_{450}, a homolog of 'clock gene' (CCA1), aspartyl protease (in senescing *Mirabilis* flowers) and Defender against Apoptotic Death (*ALSDAD1*). Thus, it is indicative of the fact that mechanism of flower senescence involves a continuous interplay of various genes that are differentially regulated in a spatio-temporal manner to bring the execution and advancement of events leading to senescence. Keeping in view the above summary, we will now discuss the various kinds of genes involved in flower senescence under the following headings.

Genes involved in cell wall expansion and abscission

Of the various genes involved in cell wall expansion, the genes encoding expansins are considered as primary regulators of cell enlargement in plants. Expansins are reported to be cell wall-located proteins and act in plant cell walls by disrupting non-covalent binding between matrix glycan cellulose microfibrils (Cosgrove 1999a, b, 2000b). Screening of the transcripts isolated from senescing carnation flowers has revealed the presence of at least three transcripts of which the most abundant one has been found to be strongly homologous to an *Arabidopsis thaliana* β-xylosidase gene (involved in senescence related to cell wall expansion; Goujon et al. 2003) while the two other transcripts homologous to expansin-encoding genes (involved in cell wall loosening during growth or disassembly; Cosgrove 2000a). Disassembly of the primary cell walls is regarded as an important process in the progression of chrysanthemum flower senescence (Elanchezhian and Srivastava 2001). While conducting the expression studies of two expansin genes (*DcExp1* and *DcExp2*; sharing 76 % identity with each other) in carnation flowers, Song et al. (2007) reported the expression of *DcExp2* in early flower development (as its expression decreased during senescence) while the expression of other transcript *DcExp1* has not been detected at any stage of flower development. It is therefore suggested that *DcExp2* might be involved in senescence progress of the cut carnation flowers at earlier stages and that *DcExp1* might have some other developmental role which needs to be ascertained. Similar studies conducted by Yamada et al. (2007) have also identified a gene, *In07* in *Ipomoea* that too encodes a putative extensin-like protein. Moreover, the expansin gene, *GgEXPA1* (Gibberellic acid responsive gene in gladiolus) has been reported to be expressed prominently during phases of active tepal expansion and cell elongation in stamen filaments, gynoecium styles and expanding leaves but not in tissues where expansion had ceased and senescence had been initiated (Azeez et al. 2010). Recently, four cDNAs encoding xyloglucan endotransglucosylase/hydrolase (XTH) (*DcXTH1-DcXTH4*) and three cDNAs encoding expansin genes (*DcEXPA1-DcEXPA3*) have been cloned and characterized from petals of opening carnation flowers of which two XTH genes (*DcXTH2* and *DcXTH3*) and two expansin genes (*DcEXPA1* and *DcEXPA2*) have been reported to be associated with petal growth and development during flower opening (Harada et al. 2011b). Moreover, the analysis of five transcripts (*RhCG1*, *RhCG2*, *RhCG4*, *RhCG6* and *RHAG1*) from two rose cultivars ('Black magic' and 'Maroussia') by Hajizadeh et al. (2011a) also revealed the presence of the transcripts that encode the products involved in cell wall expansion and degradation during senescence, e.g., *RhCG6* has been found to share 65 % sequence similarity with the gene encoding apple β-galactosidase protein. van Doorn et al. (2003) and O'Donoghue et al. (2009) have demonstrated that there occurs an increase in galactosidase transcript abundance

during *Iris hollandica* and *Petunia* petal senescence, that encodes an enzyme involved in cell wall degradation. Similarly *RhAG1* homolog has been found to share 30 % similarity to *Petunia* arabinogalactan protein. Arabinogalactan-proteins (AGPs) are cell wall proteoglycans containing a high proportion of carbohydrate (typically > 90 %), widely distributed in plant species and are located at the plasma membrane and secondary cell wall and in the media of cell cultures. It has been suggested that certain AGPs contribute in cell expansion (Shi et al. 2003), seed germination, in vitro root regeneration (van Hengel and Roberts 2003), and response to abscisic acid (Johnson et al. 2003; van Hengel and Roberts 2003).

In addition to the genes involved in cell expansion, a number of transcripts or genes involved in abscission of flowers or floral parts have been identified and characterized from different flower systems, e.g., five ethylene-responsive cDNAs have been isolated from *Rosa hybrida* and identified as an ethylene-induced cDNA homologous to a laccase gene (*RhLAC* gene). Three cDNAs have been isolated from petioles and two from pedicels. The gene has been found to encode a putative protein of 573 amino acids containing three conserved domains characteristic of the multicopper oxidase family and has been found to be highly induced in the leaf abscission zone of petioles and the bud abscission zone of floral bud pedicels, suggesting that *RhLAC* might play an important role in senescence and abscission in roses (Ahmadi et al. 2008). Similarly, the expression of two XTH genes (*RbXTH1* and *RbXTH2*; share 52 % amino acid identity and are conserved at the catalytic site) in *Rosa bourboniana* has been found to lead to petal abscission. Transcription of these genes has been found to be ethylene responsive, with the ethylene response being tissue-specific for *RbXTH1* but largely tissue-independent for *RbXTH2*. The Expression of these genes have been found to correlate with an increase in xyloglucan endotransglucosylase (XET) action in petal abscission zones of both ethylene-treated and field abscising flowers and it has been suggested that changes brought about by the XET action might allow easier accessibility of the wall to other hydrolytic enzymes, thereby accelerating abscission (Singh et al. 2011). Moreover, the promoter of *RbXTH1* has revealed the presence of the cis-element ATTTCAAA, present in the tomato ethylene-responsive E4 gene, the carnation ethylene-responsive *GST1* gene, and the rose cysteine protease promoter (Montgomery et al. 1993; Itzhaki et al. 1994; Tripathi et al. 2009). However, *RbXTH2* has not been found to contain any known ethylene-responsive elements, although sequences related to ATTTCAAA have been found, indicating that the ethylene-responsive expression in *RbXTH2* might be conferred by cis-elements other than the GCC box and the ATTTCAAA elements or by the modified ATTTCAAA. In *Arabidopsis*, the *BOP* (*BLADE-ON-PETIOLE2*) gene has been shown to play an essential role in floral abscission by specializing the abscission zone (AZ) anatomy. A homolog of *BOP* gene from tobacco, *NtBOP2* has been reported to be predominantly expressed at the base of the corolla in an ethylene-independent manner and that its antisense suppression has been found to cause a significant delay in corolla shedding (Wu et al. 2012).

From the above studies, it is evident that although the expression levels of expansin genes decline during senescence, they are important for the regulation of normal developmental program in different floral tissues eventually leading to the progression of senescence program. Furthermore, the role of XTH genes is dynamic, i.e., they are involved in cell wall expansion leading to opening of flowers and growth as well as in abscission of flowers and floral parts.

Genes encoding cysteine proteases and ubiquitin

The degradation of proteins is one of the hallmarks of senescence or PCD which is brought about by a variety of proteases and ubiquitin-mediated proteasomes. Of these proteases, cysteine proteases have been exclusively reported to be involved and thought to mediate remobilization of essential nutrients from senescing floral tissues. Genes encoding cysteine proteases have been shown to be induced during the onset of senescence in various flower systems as listed in Table 2. Of the various cysteine protease genes, some are known to act as developmental markers of senescence, e.g., *SAG12* in *Arabidopsis*, *BnSAG12-1* and *BnSAG12-2* in *Brassica napus*, *PhCP10* from *Petunia hybrida,* etc. (Noh and Amasino 1999; Jones et al. 2005). In almost all flowers systems, cysteine protease genes have been reported to be upregulated during senescence with the exception of three genes (*PhCP4*, *PhCP6* and *PhCP7*) from *P. hybrida* which are downregulated, implicating their role in protein turnover during normal developmental process (Jones et al. 2005). Moreover, the gene *PhCP6* has been found to be of particular interest because it was found to have homology to CysEP from castor bean and other KDEL-containing cysteine proteases. CysEP is localized with membrane-bound organelles called ricinosomes that are found at the beginning of PCD. Acidification of the ricinosomes during the later stages of cell death causes activation and release of CysEP following cleavage of the N-terminal propeptide and the C-terminal KDEL (Schmid et al. 1998, 2001). The recently characterized *RbCP1* gene from rose petals has been reported to encode a putative 37 kDa cysteine protease (357 amino acids) belonging to a typical papain type protease (having the presence of the CIA peptidase domain and the ERFNIN motif; Tripathi et al. 2009). Similarly in carnation, one of

Table 2 Genes encoding cysteine proteases

Source	Genes/transcripts/cDNAs isolated	Possible biological functions	References
Dianthus	*pDcCP1*	Remobilization of nutrients from the petals to the developing ovary	Jones et al. (1995)
Hemerocallis	*SEN10*	Hydrolysis of soluble proteins (indicating Petal PCD)	Valpuesta et al. (1995)
Broccoli	*BoCP2*	Dehydration responsive and postharvest protein degradation	Guerrero et al. (1998)
Arabidopsis thaliana	*SAG12*	Developmental markers of senescence	Noh and Amasino (1999)
Brassica napus	*BnSAG12-1* and *BnSAG12-2*		
Sandersonia	*PRT22*	Protein degradation	Eason et al. (2002)
Narcissus	*DAFSAG2*	Proteolysis and remobilization during later stages of senescence	Hunter et al. (2002)
Alstroemeria	*ALSCYP1*	Proteolysis	Wagstaff et al. (2002)
Petunia hybrida	9 genes (*PhCP2–PhCP10*)	Protein degradation and remobilization	Jones et al. (2005)
Ipomoea nil	*In15* and *In21*	Senescence-specific proteolysis	Yamada et al. (2007)
Rosa	*RbCP1*	Protein degradation and petal abscission	Tripathi et al. (2009)

Table 3 Genes encoding ubiquitin ligases

Source	Genes/transcripts/cDNAs isolated	Possible biological functions	References
Glycine	*XBAT32*	Development-induced PCD and ethylene synthesis/signaling	Kosslak et al. (1997)
Arabidopsis	*BHR1*	A part of the brassinosteroid response/pathogen response	Molnar et al. (2002)
	TED3/AtPex2p	Light signaling	Hu et al. (2002)
	ATL2	Plant defense	Serrano and Guzman (2004)
	XBAT31	Not clearly demonstrated	Nodzon et al. (2004)
	Xerico	Drought resistance and homeostasis of various plant hormones	Ko et al. (2006)
Rice	*XB3* (XA21 binding protein 3)	Pathogen-induced type of programmed cell death	Wang et al. (2006)
Mirabilis	*MjXB3*	Coordination of the senescence program	Xu et al. (2007b)

the identified cysteine protease gene has been found to display homology to a tobacco vacuolar processing enzyme (VPE: a caspase-like protein associated with senescence and virus-induced hypersensitive cell death: (Hatsugai et al. 2006; Hoeberichts et al. 2007). However, no cysteine protease has been isolated from senescing *Mirabilis Jalapa* flowers (Xu et al. 2007a).

In addition to cysteine endopeptidases, the genes encoding ubiquitin involved in proteasomal protein degradation have been identified from various flower systems, e.g., partial cDNA of ubiquitin (*ALSUQ1*) from *Alstroemeria*, a gene encoding polyubiquitin (an essential element in ubiquitin pathway) form *M. jalapa*, and transcripts from carnation homologous to genes encoding the components of the 26S proteosome machinery (*RPT6*, *RPN2*), a Ring finger protein and a U-box containing protein (Wagstaff et al. 2002; Hoeberichts et al. 2007; Xu et al. 2007a). The identification and upregulation of a Ring Zinc finger ankyrin protein (*MjXB3*) have also been reported from senescing *M. jalapa* flowers which share similarity to *XBAT31* and *XBAT32* of *A. thaliana and Glycine max,* respectively. Although the role of *XBAT31* has not been clearly demonstrated, *XBAT32* has been found to be expressed in root cortical cells during development-induced PCD and plays an important role in ethylene synthesis/signaling (Kosslak et al. 1997; Nodzon et al. 2004; Xu et al. 2007b; Prasad and Stone 2010). These ankyrin repeat RING domain-containing proteins are reported to have ubiquitin ligase activity (for which the RING domains are essential) and have been found to share high homology to that of E3-type binding proteins/ubiquitin ligases that targets proteins for proteolysis via ubiquitin pathway (Lorick et al. 1999; Schnell and Hicke 2003; Stone et al. 2005; Wang et al. 2006). These ubiquitin ligases are known to play diverse roles in plants as listed in Table 3. Of the different types of ubiquitin ligases, the *MjXB3* (isolated from *Mirabilis* flowers) has been fully characterized containing an open reading frame (ORF) of 1,341 bp. When compared to genes encoding Ring Zinc finger ankyrin proteins from other plant sources, high conservation of amino acids in the RING Zinc finger and ankyrin repeat domains and diversion beyond these domains has been deduced. Moreover, the

Table 4 Genes involved in nucleic acid degradation

Source	Genes/transcripts/proteins isolated	References
Hemerocallis	*DSA6*	Panavas et al. (1999)
Alstroemeria pelegrina	*DEAD/DEAH* box helicases	Breeze et al. (2004)
Petunia hybrida	*PhNUC1*	Langston et al. (2005)
Dianthus caryophyllus	*DcNUC1*	Narumi et al. (2006)
Rose cultivars ('Black magic' and 'Maroussia')	*RhCG1* and *RhCG2*	Hajizadeh et al. (2011a)
Tomato	*BFN1*	Farage-Barhom et al. (2008)

promoter sequence (2 kb) of *MjXB3* gene has been found to include putative binding sites for many DNA-binding proteins, including the bZIP, Myb homeodomain-leucine zipper (HD-Zip), MADS box, and WRKY transcription factors. The number of DNA-binding elements on the promoter has been found to be consistent with the network model of senescence control as has been suggested by He et al. (2001). *MJXB3* promoter has been found to be senescence-specific promoter in flowers as against *SAG12* of *Arabidopsis* which could not drive some GUS expression in fresh *Petunia* and carnation corollas. GUS expression under the control of the heterologous fragment (construct containing a 1 kb promoter region immediately upstream of the *MjXB3* gene) has been found to be specific to senescing *Petunia* and carnation flowers while no expression has been detected in three monocotyledonous flowers—day lily, daffodil and orchid *Dendrobium* (Xu et al. 2007b). On the other hand, the role of FOREVER YOUNG FLOWER (*FYF*; a MADS box gene in *Arabidopsis*) homologs in regulating flower senescence and abscission has been found to be highly conserved in both dicot and monocot plants, which is supported by the evidence that the ectopic expression of *OnFYF*, a FYF homolog from the Oncidium orchid (a monocot) delays flower senescence and abscission in transgenic *Arabidopsis* (Chen et al. 2011).

Thus, there has been upregulation of both cysteine proteases and 26S proteasome-mediated ubiquitin pathway during flower senescence suggesting the two alternative pathways of protein degradation (proteasomal as well as non-proteasomal). However, some flowers show upregulation of both cysteine endopeptidases as well as ubiquitin ligases during flower senescence (e.g., carnation, *Alstroemeria*) while others show only upregulation of ubiquitin ligases (e.g., *M. jalapa*). The role of cysteine proteases has been implicated in major protein degradation and remobilization besides abscission of flowers or floral parts as the ubiquitin genes have been found to only fluctuate during senescence. Of the various RING Zinc-finger ankyrin proteins (ubiquitin ligases), only *XBAT32* has been found to be involved in ethylene synthesis/signaling while *MjXB3* (isolated from *M. jalapa*) has no known function but thought to be involved in coordination of the senescence program.

Genes involved in nucleic acid degradation

Specific nuclease activities that can degrade both RNA and DNA have been reported to be induced in flower petals (Panavas et al. 1999; Xu and Hanson 2000; Hunter and Reid 2001). Some of the important genes or transcripts encoding nucleases during petal senescence are listed in Table 4. The *PhNUC1* has been found to be Co-dependent senescence-specific nuclease being expressed during the natural senescence of pollinated flowers and induced in non-senescing corollas by treatment with ethylene. Similar senescence-specific expression has been reported in a cDNA fragment, encoding a putative nuclease (*DcNUC1*). However, the activation of tomato *BFN1* has reported to occur well before the initiation of senescence (Farage-Barhom et al. 2008), thereby pointing out the early nuclear degradation (possibly involving PCD) as demonstrated by Hoeberichts et al. (2005) in flower petals of *Gypsophila.* Moreover, it has also been suggested that in *Alstroemeria petals*, PCD processes are initiated extremely early at a similar location on the petals to that observed for expression of the *BFN1* promoter in tomato (Wagstaff et al. 2003). Cloning of the *Arabidopsis BFN1* gene and sequencing of the corresponding polypeptide (protein) by Perez-amador et al. (2000) have revealed the similarity of the BFN1 protein to *DSA6* nuclease (involved in petal senescence; Panavas et al. 1999) and *ZEN1* nuclease (associated with PCD during tracheary element differentiation; Ito and Fukuda 2002). The regulation and expression pattern of *BFN1* has been analyzed by cloning its 2.3 kb portion of the 5′ promoter sequence and then by detecting its ability to activate the GUS reporter gene construct. The *BFN1* promoter has been specifically found to be capable of directing GUS expression in senescent leaves, differentiating xylem and abscission zones of petals in transgenic Arabidopsis and tomato plants. It has also been found active in other tissues, including developing anthers and seeds, and in floral organs after fertilization (Farage-Barhom et al. 2008). It has been suggested that *BFN1* might be involved in developmental PCD-related processes in Arabidopsis, as well as in senescence. Investigations on the intracellular localization of *BFN1* in transiently transformed tobacco protoplasts have

Table 5 Genes encoding transcription factors

Source	Genes/transcripts/proteins isolated	Possible biological functions	References
Dianthus	A homeodomain protein	Transcription factors	Waki et al. (2001)
	A MYB-like DNA-binding protein, a MYC protein, a MADS-box factor	Regulation of senescence but the exact role is unclear	Hoeberichts et al. (2007)
Arabidopsis	MADS box transcription factor	Delays petal senescence and abscission	Fang and Fernandez (2002)
Iris	MADS Box gene		van Doorn et al. (2003)
Arabidopsis	*AtNAP*	Leaf senescence	Guo and Gan (2006)
Mirabilis	*CCA1* and *F935* (Myb transcription factors)	Photoperiodic control, flower opening and maturation	Xu et al. (2007a)
	b-Zip and HD-Zip protein	Regulates osmotic and water relations of the opening and senescing flowers	
Dianthus	CEBP (Carnation ethylene-responsive element binding protein)	Ethylene signaling in carnation flower development and senescence	Iordachescu et al. (2009)
	ANAC092	Stress and senescence regulation	Balazadeh et al. (2010)
Alstroemeria	Myb, Lim, Hap5B and MADS box transcription factors	Stress and flower senescence regulation	Wagstaff et al. (2010)

revealed their initial localization in filamentous structures (being of ER origin) spread throughout the cytoplasm, which then clustered around the nuclei as the protoplasts senesced. In transgenic *Arabidopsis* plants, similar localization has been observed in young leaves and during late senescence, where *BFN1*-GFP construct has been found to be localized with fragmented nuclei in membrane-wrapped vesicles suggesting the existence of a dedicated compartment mediating nucleic acid degradation by *BFN1* in senescence and PCD processes (Farage-Barhom et al. 2011). Of the two transcripts (*RhCG1* and *RhCG2*; sharing homology to *Arabidopsis* DNA helicase gene) isolated from two rose cultivars ('Black magic' and 'Maroussia'), *RhCG2* has been found to be differentially expressed, i.e., upregulated in flowers of 'Black magic' and not in 'Maroussia'(Hajizadeh et al. 2011a). Similarly, Breeze et al. (2004) has also demonstrated the upregulation of *DEAD/DEAH* box helicases in *Alstroemeria pelegrina* during senescence.

In conclusion, degradation of nucleic acids by specific nucleases during flower senescence has been demonstrated in various flower systems. A number of cDNAs encoding such nucleases have been isolated and found to be expressed well before the initiation of senescence suggesting their role in programmed execution of flower senescence. *PhNUC1* has been found to be a cobalt-dependent senescence specific nuclease and both *PhNUC1* and *DcNUC1* have been found to be ethylene-responsive nucleases. The *BFN1* nuclease has been well characterized and its intracellular localization has also been investigated. The evidences so far have suggested that nuclear degradation by the nucleases occur well before the senescence symptoms become apparent and that they might play an important role in developmental PCD-related processes as well as in progress of senescence. The involvement of nucleases is also indicative of the fact that flower senescence involves PCD.

Genes encoding various transcription factors

The transcripts encoding a range of transcription factors have been isolated and found to be differentially regulated during development and senescence in various flower systems (Table 5), e.g., a homeodomain protein (a class of proteins generally representing transcription factors), MYB-like DNA-binding protein, MYC protein and Zinc-finger protein from *Dianthus* and *Mirabilis*, MADS Box genes from carnation, *Iris* and *Arabidopsis*, NAC domain transcription factors and CEBP "Carnation ethylene-responsive element binding protein" (Waki et al. 2001; Fang and Fernandez 2002; van Doorn et al. 2003; Hoeberichts et al. 2007; Iordachescu et al. 2009; Balazadeh et al. 2010). The *Iris* MADS box gene has been found to share 51 % identity with *RIN* (a MADS-box factor involved in developmental control of fruit ripening in tomato; Vrebalov et al. 2002) and that the corresponding translated fragment from carnation shares 34 % identity with tomato RIN and 55 % identity with the *Arabidopsis* pistillate protein (Hoeberichts et al. 2007). The importance of MADS box transcription factors in petal or flower senescence becomes evident by the fact that overexpression in *Arabidopsis* MADS box gene delays petal senescence and flower abscission (Fang and Fernandez 2002). However, the exact function of these genes in the regulation of flower senescence is as yet unclear. Kaufmann et al. (2009), while searching for the target genes of the MADS box transcription factor *SEPALLATA3* (*SEP3*; that plays an important role during flower development), observed binding of *SEP3* to two sites with

the *ANAC092* promoter (a NAC domain transcription factor), suggesting that it functions as an upstream regulator of the *NAC* gene. As far as *NAC* domain transcription factors are concerned, they represent a large fraction of the plant-specific family of transcription factors and senescence-regulated genes in many plants (Andersson et al. 2004; Guo et al. 2004; Lin and Wu 2004; Buchanan-Wollaston et al. 2005; Balazadeh et al. 2008, 2010) implicated in a wide range of processes, including tolerance to biotic and abiotic stress, and programmed cell death in xylem tracheids and vessels (Kubo et al. 2005). The expression of one of the NAC-domain TF genes (*ANAC092*) has been reported from partly or fully opened flowers and mature anthers too (Balazadeh et al. 2010). These NAC-domain transcription factors have been found to be differentially expressed with the downregulation in carnation flowers during natural senescence and upregulation in senescing *Arabidopsis* leaves (Guo and Gan 2006; Hoeberichts et al. 2007). Of the 36 EST sequences (representing at least 24 transcription regulating genes) identified in *Alstroemeria*, the largest group (8 genes) has been found to be represented by Zinc-finger proteins. Employing RT-PCR, it has been confirmed that the transcript levels of the C_2H_2-zinc finger transcription factor peaked at closed bud stage and at mid-senescent stage whereas the MADS box gene peaked at young bud stage and open flower stage (Wagstaff et al. 2010). Myb Transcription factor genes like *CCA1* and *F935* have been found to be downregulated genes, probably playing a role in the control of flower opening. However, it has not been fully demonstrated whether these genes are solely involved in directing floral opening and expansion, or whether reduction in their abundance permits the onset of flower senescence. Moreover, the homologs of b-Zip and HD-Zip proteins (Plant-specific transcription factors) have been found to be upregulated in senescing *Mirabilis* flowers, thought to be induced in response to the changing osmotic and water relations of the opening and senescing flowers in *Mirabilis Jalapa* (Xu et al. 2007a) and that HD-Zip transcription factor isolated from *Mirabilis* has been found to be a member of HD-ZIP-I family that also includes *Athb-7* and *Athb-12* transcription factors from *Arabidopsis thaliana* (Sessa et al. 1994; Lee and Chun 1998). Furthermore, a putative transcription factor CEBP (Carnation ethylene-responsive element binding protein; a nuclear-encoded chloroplast protein) has been identified and found to be involved in ethylene signaling and in the initial steps of carnation petal senescence. CEBP and EILs (EIN3-like proteins) have been shown to bind very similar promoter regions (Maxson and Woodson 1996; Solano et al. 1998) and the decrease in CEBP mRNA accumulation has been found to be accompanied by the sudden accumulation of *Dc-EIL3* during carnation petal development (Iordachescu and Verlinden 2005). The predicted CEBP protein (32 kDa) has been reported to contain two highly conserved RNA-binding motifs, *RNP-1* and *RNP-2*, an acidic region, a C-terminal nuclear localization signal and an N-terminal chloroplast transit peptide suggesting that it can locate both to the nucleus and chloroplast (Maxson and Woodson 1996; Iordachescu et al. 2009). Although the exact role of CEBP in chloroplast remains unclear, the similar proteins have been implicated to play a role in splicing and/or processing of chloroplast RNAs (Li and Sugiura 1990).

In conclusion, MADS-box transcription factors, MYB-like DNA-binding proteins, MYC protein and CEBP have been identified as downregulated genes whose transcript abundance peaked during initial stages of flower development (up to flower opening). The possible role of these genes in flower senescence is still unclear; however, they have been implicated to be involved in the initial steps of senescence process as there are evidences that overexpression of these transcription factors delays senescence. The upregulated transcription factors consist of HD-Zip proteins, B-Zip proteins and Zinc-finger proteins. NAC-domain transcription factor has been found to show differential expression in various tissue systems. It has been identified as downregulated gene in carnation petals, but found to be expressed in senescent leaves of *Arabidopsis*. Further *SEPALLATA3* (a MADS-box transcription factor) has been found to function as upstream regulator of NAC gene. The expression of these transcription factors is either controlled by developmental signals (*CCA1*) or induced in response to changing osmotic and water relations of the opening and senescing flowers (HD-Zip proteins).

Genes involved in ethylene synthesis (ACC synthase and ACC oxidase genes)

In ethylene-sensitive flower systems, ACC synthase and ACC oxidase are the key enzymes involved in ethylene biosynthesis. Several genes encoding 1-amino cyclopropane-1-caboxylate (ACC) synthase and ACC oxidase have been found to be upregulated during petal wilting in senescing carnation flowers. Initially, *CARACC3* has been cloned by Park et al. (1992), and found to be abundantly expressed in petals during natural and ethylene-induced flower senescence. Later, Henskens et al. (1994) isolated two cDNA clones encoding carnation ACC synthase. One of the clones has been found to be identical to *CARACC3* while the other clone (*CARAS1*) has been found to share only 66 % sequence similarity to *CARACC3* (in the amino acid sequence). *CARAS1* has been found to be more abundantly expressed in the styles rather than in the petals, thereby confirming the petal-specific nature of *CARACC3*. In the deduced amino acid sequence of the cDNA clone *CARAS1*, the amino acid residue tyr-215 (conserved residue among

many known aminotransferases and all known ACC synthetases) is replaced by Phe (Henskens et al. 1994; Zarembinsky and Theologis 1994). The residue has been thought to be involved in the binding of essential co-factor, Pyridoxal phosphate (Mehta et al. 1989). Henskens et al. (1994) has suggested that the gene might encode a non-functional enzyme; however, the positive correlation between *CARAS1* abundance and stylar ethylene production during aging is indicative of the fact that *CARAS1* does encode a functional enzyme (Have and Woltering, 1997). Ma et al. (2005) while studying the differential induction features of three ACS genes in roses found that *Rh-ACS2* is strongly induced by senescence. The tissue specificity of *Rh-ACS2* has been found to be quickly induced by ethylene in gynoecia (Xue et al. 2008). Similar observations have also been reported in carnation, where *CARAS1* (also named as *DCASC2*) showed a quicker and stronger response to ethylene treatment in gynoecia than in petals (Have and Woltering 1997). All these observations suggest that *Rh-ACS2*, a senescence-associated gene in rose petals, might play an important role in the induction of ethylene biosynthesis in gynoecia and in promoting the flower opening process. The genomic DNA structure of *DcACS1* has been successfully revealed in senescing carnation petals (*Dianthus caryophyllus* and *D. superbus*). The gene has been found to express in two different isoforms (*DcACS1a* and *DcACS1b*). Genomic PCR analysis of 32 carnation cultivars has shown that most cultivars have only *DcACS1a* while some have both *DcACS1a* and *DcACS1b*. Both the genes were found to have five-exon and four-intron structure. Nucleotide sequences of exons 1–3 in *DcACS1a* have been found to be completely identical to those in *DcACS1b*. However, substitution of several nucleotides has been found in exon4 and 5. Exon5 of *DcACS1b* has been found to be 18 nucleotides shorter than that of *DcACS1a*, causing shorter stretch of threonine residues characteristic to *DcACS1* gene. Introns 1–3 varied from 56 to 70 %, while the nucleotide sequence of Intron 4 has been shown to be completely identical in the two genes. Nucleotide sequence of 5′-UTR has been found to be conserved in *DcACS1a* and *DcACS1b*, but that of 3′-UTR was not. Moreover, *DcACS1* orthologous genes have been isolated *D. superbus* var. longicalycinus, designated as *DsuACS1a* and *DsuACS1b*. Exogenously applied ethylene has been found to induce autocatalytic ethylene production in petals of *D. superbus* var. longicalycinus with simultaneous accumulation of transcripts of *DsuACS1* (Harada et al. 2011a). ACC oxidase gene has been isolated from carnation (Wang and Woodson 1991). Constitutive expression of this gene has been reported in the styles but not in other floral organs (Woodson et al. 1992). Spanu et al. (1994) suggested that the post-translational regulation of ACC synthetase protein is achieved through phosphorylation and dephosphorylation of associated proteins.

To sum up, it can be concluded that ACC synthase and ACC oxidase genes (involved in ethylene biosynthesis) have been successfully isolated and characterized in various flower systems (carnation and rose) and their differential expression in different tissue systems has also been revealed. *CARACC3* gene from carnation has been found to have petal-specific expression, whereas *CARAS1* from carnation and *Rh-AS1* from rose have been found to express in gynoecia. The genomic DNA structure of *DcACS1* (both isoforms: *DcACS1a* and *DcACS1b*) has also been revealed and its orthologous genes have also been identified. The only ACC oxidase gene identified in carnation has been reported to be constitutively expressed in the styles and not in other floral organs.

Genes encoding ethylene receptors

The induction of petal senescence or abscission by ethylene or pollination is associated with transcriptional regulation of the ACS and ACO genes (Bui and ÒNeill 1998; Jones 2003; Fernández-Otero et al. 2006) and ethylene receptor genes (Shibuya et al. 2002; Kuroda et al. 2003, 2004). This induction is also accompanied with an increase of the *CTR* (Constitutive Triple Response) genes in some ornamental plant species (Müller et al. 2002; Kuroda et al. 2004). Several ethylene receptors (ETRs) are now known and the molecular mechanism underlying ethylene sensitivity in plants has been studied in plants like *Arabidopsis* (Bleecker and Schaller 1996). Analysis of the ethylene receptor genes in *Arabidopsis* has led to the identification of many *ETR1* and *ETR1*-like genes (Chang et al. 1993; Hua et al. 1995, 1998; Hua and Meyerowitz 1998; Sakai et al. 1998) encoding ETRs like *ETR1*, *ETR2*, *EIN4*, *ERS1* and *ERS2* reported to be transmembrane endoplasmic reticulum (ER) proteins with similarity to bacterial two-component histidine kinases. On the basis of their sequence similarity and structural features, these proteins have been classified into two subfamilies, i.e., ETR1-like subfamily (*ETR1* and *ERS1*) and ETR2-like subfamily (*ETR2*, *ERS2* and *EIN4*). *ETR1* and *ERS1* have three hydrophobic domains at the N-terminus and five consensus motifs (catalytic site subdomains typical of histidine kinases) found in bacterial histidine kinase, while *ETR2*, *EIN4* and *ERS2* have four hydrophobic domains at the N-terminus and lack most of the motifs in histidine kinases (Parkinson and Kofoid 1992; Hua et al. 1998; Klee 2002). Moreover, *ETR1*, *ETR2* and *EIN4* have been found to harbor the receiver domain [consisting of three residues (D, D and K), important for phosphorylation] that receives phosphate from the histidine kinase (transmitter) domain, while *ERS1* and ERS2 lack that domain. A detailed study on the structure of *ETR1* protein has revealed the presence of following components

Table 6 Genes encoding ethylene receptors

Source	Genes/transcripts/cDNAs isolated	References
Arabidopsis	*ETR1*and *ETR1*-like genes (*ETR2*, *EIN4*, *ERS1* and *ERS2*)	Chang et al. (1993), Wilkinson et al. (1995), Hua et al. (1995, 1998), Hua and Meyerowitz (1998), Sakai et al. (1998)
Lycopersicon esculentum	*NR* gene, *LeETR1*, *LeETR2*, *LeETR4* and *LeETR5*	Lashbrook et al. (1995), Tieman and Klee (1999)
Rumex palustris	*RP-ERS1*	Vriezen et al. (1997)
Cucumis melo	*Cm-ETR1* and *Cm-ERS1*	Sato-Nara et al. (1997, 1999)
Passiflora edulis	*PE-ETR1* and *PE-ERS1*	Mita et al. (1998)
Rosa hybrida	*RhETR1*, *RhETR2*, *RhETR3* and *RhETR5*	Müller et al. (2000a, b), Ma et al. (2006)
Dianthus caryophyllus	*DcERS1*, *DcERS2* and *DcETR1*	Shibuya et al. (2002)
Delphinium	*DlERS1*type1 and *DlERS1*type2, Dl-*ERS1-3* and *Dl-ERS2*	Kuroda et al. (2003, 2004), Tanase and Ichimura (2006)
Chrysanthemum	*DgERS1*	Narumi et al. (2005)
Oncidium	*OgERS1*	Huang et al. (2007)
Paeonia suffruticosa	*PsETR1-1*	Zhou et al. (2010)

(Schaller and Bleecker 1995; Kehoe and Grossman 1996; Aravind and Ponting 1997; Bleecker et al. 1998):

1. Three N-terminal hydrophobic domains capable of reversibly binding to ethylene.
2. Phytochrome-related T2L and R2L domains (homologous domains with the chromophore attachment domains of phytochrome photoreceptors).
3. A GAF domain (homologous domain found in photo-transducing proteins).
4. Two domains homologous to a histidine kinase.
5. A receiver of the bacterial two-component histidine kinase system.

As far as the functional aspect of ETR1 is concerned, it has been found to show high-affinity ethylene binding mediated by a copper ion associated with its ethylene-binding domain that binds to Cys^{85} residue (essential for both copper association and ethylene binding to the receptor; Rodriguez et al. 1999). Moreover, the isolation of *RAN1* gene and its role in delivering copper to *ETR1* to create a functional hormone ethylene receptor has also been demonstrated (Hirayama et al. 1999). *ETR1* homologs have also been isolated from other plants as listed in Table 6. Mutant alleles of *ETR1*, designated as *etr1-1*, *etr1-2*, *etr1-3* and *etr1-4* have been reported to cause ethylene insensitivity in plants. All of these mutations have been found to result from a single amino acid replacement (Ala^{31} to Val in etr1-3, Ile^{62} to Phe in etr1-4, Cys^{65} to Tyr in etr1-1, and Ala^{102} to Thr in etr1-2) in the three hydrophobic domains (Chang et al. 1993). Transformation of petunias with the mutated ethylene receptor gene (*etr1-1*) from *Arabidopsis* has been found to reduce ethylene sensitivity in flowers and thereby delay senescence (Wilkinson et al. 1997), while *Nicotiana sylvestris* plants expressing the dominant mutant ethylene receptor gene *ETR1-1* from *Arabidopsis* has been found to exhibit a substantial delay in both the onset and progression of leaf and flower senescence (Yang et al. 2008).

The expression pattern of various *ETR1* genes from different flower systems has revealed a differential expression as is evidenced by the higher expression of *RhETR1* in long-lasting miniature rose cultivar and that of *RhETR3* in short-lasting one, the constitutive expression of *RhETR5* (from cut roses) and *RhETR2* (from miniature roses) throughout flower development (Müller et al. 2000a, b, 2001; Ma et al. 2006; Tan et al. 2006) and by the higher expression of *Dg-ERS1* in petals of ethylene-sensitive chrysanthemum flowers (Narumi et al. 2005). Moreover, the *ETR* genes have been reported to show temporal regulation. A classical example of temporal regulation is provided by the carnation *ETR* genes (*Dc-ERS1*, *Dc-ERS2* and *Dc-ETR1*) of which *Dc-ERS2* has been found to be expressed at pre-opening stage while *Dc-ETR1* exhibited constitutive expression during senescence and that *Dc-ERS1* has not been detected throughout senescence (Shibuya et al. 2002). Similarly, the cDNAs (Dl-*ERS1-3* and *Dl-ERS2*) from *Delphinium* flowers have been found to exhibit constitutive levels during flower senescence while as the *Dl-ERS1* genes [*Dl-ERS1* (Delphinium little-strain *ERS1*) type-1 and *Dl-ERS1* type-2] have been shown to have increased expression prior to flower senescence following a decline thereafter (Kuroda et al. 2003; Tanase and Ichimura 2006). Likewise, the cDNA (*OgERS1*; phylogenetically related to ETRs from monocots) from Oncidium has been found to be abundantly expressed in roots and flower buds and to a lesser extent in pseudobulbs, leaves, and fully opened flowers (Huang et al. 2007). Similar studies in tree peony have also revealed the constitutive expression of *Ps-ETR1-1* as it

has been found to remain at a constant level throughout different opening stages (Zhou et al. 2010). As far as the effect of exogenous ethylene on the expression levels of ethylene receptor genes is concerned, it has been found to induce the expression of *RhETR1*, *RhETR2*, *RhETR3*, *DlERS1-3* and *DlERS2* on one hand and substantially inhibit the levels of *Ps-ETR1-1* mRNA on the other hand. Moreover, the expression levels of *Rh-ETR5*, *Dc-ERS2* and *Dc-ETR1* have been found to be ethylene independent. It has been speculated that the exogenous ethylene-independent expression pattern might result from the lower amount of ethylene produced during natural flower senescence than that of exogenous ethylene and that abscission of florets in *Delphinium* is caused by the elevated levels of ethylene receptor (*ERS1*), influenced by exogenous ethylene. Moreover in tree peony, there exists an inverse relationship between the level of ETRs and the sensitivity to ethylene, and the reduction in the amount of ethylene receptor proteins have been found to increase ethylene sensitivity of plant tissues. Therefore, the decrease in the level of *Ps-ETR1-1* mRNA in petals of ethylene-treated flowers has been suggested to increase the sensitivity of the petals to ethylene and hence accelerate their senescence (Shibuya et al. 2002; Kuroda et al. 2003, 2004; Ma et al. 2006; Tan et al. 2006).

In conclusion, it can be stated that the perception of ethylene during flower senescence is mediated by ethylene receptor genes (*ETR*s) which have been found to encode transmembrane ER proteins with similarity to bacterial two-component histidine kinase. A number of *ETR* genes have been identified and characterized from various flower systems. The *ETR1* gene from *Arabidopsis* has been reported to have hydrophobic regions capable of reversibly binding to ethylene with the involvement of copper ions associated with the domain. It has been postulated that *RAN1* delivers copper ion to *ETR1*. The differential expression of various ETRs have been found to be associated with varying longevity in miniature potted roses with the long-lasting cultivar expressing *ETR1*. From the available data, it may be speculated that there exists an inverse relationship between the level of ETRs and the sensitivity to ethylene in tree peony, but in *Delphinium*, it has been reported that abscission of florets is caused by elevated levels of ethylene receptor (*ERS1*) and that too influenced by exogenous ethylene.

Genes involved in ethylene signaling

Using molecular genetic approach, genes related to the ethylene signaling pathway have been isolated and characterized from a number of plants particularly in *Arabidopsis,* where the ethylene signaling pathway has been well characterized (Guzman and Ecker 1990; Kieber et al. 1993; Roman et al. 1995; Chao et al. 1997; Alonso et al. 1999). Perception of ethylene is brought about by a family of ETRs that in turn regulates the activity of *CTR1* (a negative regulator of the ethylene response pathway) whose protein sequence have been reported to share similarity to the Raf family of serine/threonine protein kinase thereby suggesting that it (*CTR1*) might act via Mitogen-activated protein (MAP) kinase cascade, since MAPKs have been have been implicated in coordinating stress responses, probably as the key factors in the PCD signal transduction pathway (Kieber et al. 1993; Mizoguchi et al. 1996; Waki et al. 2001). In *Delphinium*, the *DlCTR1*, encoding a polypeptide of 800 amino acids containing the expected serine/threonine kinase domain, the consensus ATP-binding site, and the serine/threonine kinase catalytic site has also been characterized (Kuroda et al. 2004). This is also confirmed by the analyses of two genes *In29* and *In42* (from senescing *Ipomoea nil* petals) encoding leucine-rich repeat transmembrane receptor protein kinase and a 14-3-3 protein kinase, respectively. The former has been implicated to play a role in signal transduction while the latter has been reported to play a role in processes such as progression through cell cycle, initiation and maintenance of DNA damage checkpoints, and prevention of apoptosis control in humans (Wilker and Yaffe 2004; Yamada et al. (2007). Hua and Meyerowitz (1998) have reported that the ETRs positively regulate *CTR1* in the absence of ethylene, and that ethylene binding cancels this interaction. In the absence of ethylene, therefore, an active form of *CTR1* inhibits downstream components and ethylene responses. In the presence of ethylene, CTR1 is inactive and then downstream components are activated and ethylene responses occur. *LeCTR2* (*TCTR2*) that encodes an *AtCTR1*-like kinase has been found to interact selectively with a subset of ETRs at the N-terminus while the C-terminus possesses kinase activity (Lin et al. 2008). It has been reported that ETRs are regulated at both the transcriptional and post-transcriptional levels while as *CTR1* is regulated mainly at the post-transcriptional level through association or dissociation with ETRs in the endoplasmic reticulum (Gao et al. 2003; Chen et al. 2005). *CTR1* genes have been identified and isolated from various plant systems as listed in Table 7. The expression analyses of these genes have also revealed their differential expression. Some of them have been reported to be expressed constitutively (*LeCTR2*, *RhCTR2*) while others (*LeCTR1*, *RhCTR1)* have been found to be upregulated during fruit ripening, flower opening, flower senescence and defense responses (Zegzouti et al. 1999; Alexander and Grierson 2002; Leclercq et al. 2002; Lin et al. 2008; Hajizadeh et al. 2011b). Similarly a homolog of *CTR1* (*Cup-CTR1*) from *Cucurbita pepo* has been found to be upregulated in male flowers only (Manzano et al. 2008).

Table 7 Genes involved in ethylene signaling

Source	Genes/transcripts/cDNAs isolated	References
Arabidopsis	*CTR1*	Kieber et al. (1993)
	At-ERFs	Nakano et al. (2006)
Lycopersicon esculentum	*LeCTR1-LeCTR4*	Lin et al. (1998)
	LeEIL1	Tieman et al. (2001)
Delphinium	*CTR1*	Kuroda et al. (2004)
Petunia hybrida	*Ph-EIL1*	Shibuya et al. (2004)
	PhERF1-PhERF13	Liu et al. (2011)
Ipomoea nil	*In29* and *In42*	Yamada et al. (2007)
Cucurbita pepo	*Cup-CTR1*	Manzano et al. (2008)
Nicotiana sylvestris	Ns-EIL1	Yang et al. (2008)
Paeonia suffruticosa	*Ps-EIN3-1*	Zhou et al. (2010)
Rosa hybrida	*RhCTR1* and *RhCTR2*	Hajizadeh et al. (2011b)
Dianthus caryophyllus	*DCEIN2*	Fu et al. (2011a)
	DCEBF1	Fu et al. (2011b)
	DcEIN3	Hoeberichts et al. (2003)
	Dc-EILs	Waki et al. (2001), Iordachescu and Verlinden (2005)
Citrus	*Cit ERF*	Yang et al. (2011)
Longan fruit	*DlHD2, DlERF1* and *DlERF2*	Kuang et al. (2012)

Based on double-mutant analysis, it is proposed that *CTR1* acts at or downstream from *ETR1*, *ERS1* and *EIN4*, and that *EIN2*, *EIN3*, *EIN5*, *EIN6* and *EIN7* act after *CTR1* (Hua et al. 1995; Roman et al. 1995). Chen et al. (2005) has reviewed that *CTR1* passes the signal to EIN2 (an integral membrane protein that acts as a positive regulator of ethylene pathway) through a series of *MAPK* cascades, and then to *EIN3/EILs* (transcriptional factors) that trigger the expression of downstream target genes such as *ERFs*. However, it has been recently demonstrated that CTR1 interacts and directly phosphorylates the cytosolic C-terminal domain of EIN2 in *Arabidopsis* (Li and Guo 2007; Ju et al. 2012). Although overexpression of the C-terminus of *EIN2* (an ER-localized membrane protein) has been reported to result in constitutive induction of a subset of ethylene responses and genes, it has been found to be inefficient in restoring ethylene sensitivity to an *ein2* null mutant (Alonso et al. 1999; Bisson et al. 2009). The regulation of *EIN2* has been found to be brought about by two F-box proteins—*ETP1* and *ETP2* (*EIN2-TARGETING PROTEIN*) that negatively regulate ethylene signaling as the presence of ethylene downregulates both *ETP1* and *ETP2* (which otherwise degrade *EIN2* in presence of ethylene) leading to accumulation of *EIN2* and consequently an ethylene response (Qiao et al. 2009). About 700 F-box genes have been reported in *Arabidopsis* which are known to mediate proteolysis via ubiquitin-mediated proteasomal degradation, e.g., *ORE9* required for initiation of *Arabidopsis* leaf senescence (Woo et al. 2001; Vierstra 2003). Recent studies in senescing carnation flowers have led to the identification of ethylene-dependent *DCEIN2* (3,828 bp ORF encoding 139.5 kDa protein of 1275 amino acids) encoding protein-containing 12 putative transmembrane domains close to the N-terminus similar to the *Arabidopsis EIN2*, *Petunia PhEIN2*, and tomato *SlEIN2* protein (Alonso et al. 1999; Fu et al. 2011a). Ethylene signaling downstream of *EIN2* has been found to be mediated by *EIN3* or *EIN3*-like *EIL* proteins (plant-specific transcription factors: Chao et al. 1997; Solano et al. 1998) that are regulated by two F-box proteins *EBF1* and *EBF2* (EIN3-binding F-box proteins) in a similar manner as *EIN2* regulation by *ETP1* and *ETP2* (Guo and Ecker 2003, 2004; Bishopp et al. 2006). The expression of *EBF2* has been found to be transcriptionally induced by *EIN3* that directly binds to the promoter of *EBF2*, thereby allowing a negative feedback regulation to desensitize ethylene signaling and that *EIN5*, a 5′–3′ exoribonuclease, is most likely involved in moderating *EBF1* and *EBF2* transcripts (Gagne et al. 2004; Olmendo et al. 2006; Konishi and Yanagisawa 2008). Recently, a carnation cDNA (*DCEBF1*; 1,878 bp) encoding EBF-like protein has been isolated whose expression has been reported to be enhanced by endogenous/exogenous ethylene, and inhibited by STS in petals and ovaries (Fu et al. 2011b). *EIN3* or *EIN3*-like proteins (*EIL1*, *EIL2*, *EIL3*, *EIL4* and *EIL5*; nuclear-localized transcription factors) have been found to be upregulated during senescence (Waki et al. 2001; Alonso et al. 2003; Hoeberichts et al. 2003; Yanagisawa et al. 2003; Shibuya et al. 2004; Iordachescu and Verlinden 2005; Zhou et al. 2010). As far as structural aspect of *Arabidopsis EIN3* protein is concerned, it has been found to harbor a highly

acidic domain at N-terminus, five small clusters of basic amino acids throughout the *EIN3* polypeptide, a proline rich domain, and an asparagine-rich domain at the C-terminus (Chao et al. 1997). Moreover, the *Ps-EIN3-1* (from tree peony) has been reported to be strongly inhibited by ethylene and that decrease has been attributed to the activation of some defense mechanisms, thereby decreasing the tissue sensitivity to ethylene (Lorenzo et al. 2003; Zhou et al. 2010). In carnation, the upregulation of ACC synthase and ACC oxidase genes has been linked to the upregulation of EIL genes and it has been suggested that a master-switch controlling the coordinated upregulation of numerous ethylene responsive genes is involved in the senescence of carnation flowers, of which *Dc-EIL3* might be part of. It has also been speculated that endogenous levels of soluble sugars in carnation act as a regulator of flower senescence by influencing *Dc-EIL3* gene expression (Hoeberichts et al. 2007). Tieman et al. (2001) have demonstrated that reduced *EIL* expression in tomato (*LeEIL1*) affects ethylene responses, including leaf epinasty, flower abscission, flower senescence and fruit ripening. However, the expression of *EIN3*-like gene (*EIL1*) in *Nicotiana sylvestris* plants has not been found to consistently alter the progression of senescence (Yang et al. 2008). Genetic analysis revealed that *EIL1* and *EIN3* cooperatively but differentially regulate a wide array of ethylene responses, with *EIL* mainly inhibiting leaf expansion and stem elongation in adult plants and *EIN3* largely regulating a multitude of ethylene responses in seedlings. When *EBF*1 and *EBF2* are disrupted, *EIL* and *EIN3* constitutively accumulate in the nucleus and remain unresponsive to exogenous ethylene application. Recently, it has been reported that *EIN2* is indispensable for mediating ethylene-induced *EIN3/EIL1* accumulation and *EBF1/2* degradation (An et al. 2010).

EIN3 or *EILs* have been found to induce the expression of other transcription factors, including the *ERFs* (ethylene responsive factors formerly known as ethylene-responsive element binding protein; *EREBP*) and *EDFs* (ethylene-responsive DNA binding factors) (Ohme-Takagi and Shinshi 1995; Suzuki et al. 1998; Li and Guo 2007), which is evident by the presence of ethylene responsive elements (EREs) in some senescence-related genes (*SR5*, *SR9* and *SR12*) and related transcription factors (Hunter and Reid 2001; Verlinden et al. 2002). ERFs are plant-specific *AP2/EREBP*-type transcription factors, characterized by the presence of a highly conserved DNA-binding domain (the *ERF* domain consisting of 58 or 59 amino acids) and regulate gene expression by binding specifically to the 11 bp GCC box of the ethylene responsive element of senescence-related genes (Ohme-Takagi and Shinshi 1995; Hao et al. 1998; Riechmann and Meyerowitz 1998; Yang et al. 2011). A number of *ERF* genes have been identified (as listed in Table 7) and classified into small groups on the basis of structural similarities, e.g., 12 groups (group I-X, VI-L and Xb-L) in *Arabidopsis* (Nakano et al. 2006). Similarly, Liu et al. (2011) has also characterized and classified the 13 ERFs from *Petunia* (*PhERF1-PhERF13*). Of the 13 *ERFs*, *PhERF2* and *PhERF3* have been shown to be associated with flower senescence. Yang et al. (2011) has identified a novel transcription factor (*Cit ERF*) in *ERF* family which has been suggested to play a variety of roles in some biological processes particularly fruit ripening and in enhancing different stress tolerances. It has been suggested that histone deacetylation plays an important role in epigenetic control of gene expression, e.g., *HD2*, a plant-specific histone deacetylase is able to mediate transcriptional repression in many biological processes. In longan fruit senescence, one histone deacetylase 2-like gene, *DlHD2*, and two ethylene-responsive factor-like genes, *DlERF1* and *DlERF2*, have been cloned and characterized. The application of nitric oxide has been found to delay fruit senescence (by enhancing the expression of *DlHD2* and suppressing the expression of *DlERF1* and *DlERF2*) indicating that a possible interaction between *DlHD2* and *DlERFs* in regulating longan fruit senescence. The direct interaction between *DlHD2* and *DlERF1* suggests that *DlHD2* might act with *DlERF*1 to regulate gene expression involved in longan fruit senescence (Kuang et al. 2012).

In conclusion, the ethylene signaling pathway has been fully elucidated in *Arabidopsis* but the accumulated data are to be fit into a more generalized model, so that it could be extended to the studies related to flower senescence. Although many studies involving identification, characterization and isolation of genes related to ethylene signaling have been made in various flower systems, a coherent picture is still not available that helps in understanding the proper execution and advancement of flower senescence mediated by ethylene. From the above discussion, it, however, becomes evident that ethylene signaling through *ETR1* involves the inactivation of *CTR1*, a negative regulator of ethylene response pathway regulated mainly at the post-transcriptional level, through MAPK cascade. Inactivation of *CTR1* activity has been found to activate downstream components (EIN2, EIN3/EILs, EIN5, EIN6, and EIN7) for the ethylene responses to occur. *CTR1* inactivation has been found to directly phosphorylate or indirectly activate *EIN2* through MAPK cascade whose function is in turn modulated by interaction with two F-box proteins (ETP1 and ETP2). Constitutive expression of *EIN2* leads to activation of *EIN3/EILs* which reach the adequate levels and attach to promoters allowing the expression of ethylene responsive genes. The function of *EIN3* or *EIL*s is modulated by two F-box proteins (EBF1 and EBF2) which in turn are regulated by *EIN5* (an exoribonuclease) to bring out the ethylene response. Thus, perception of ethylene or its signaling

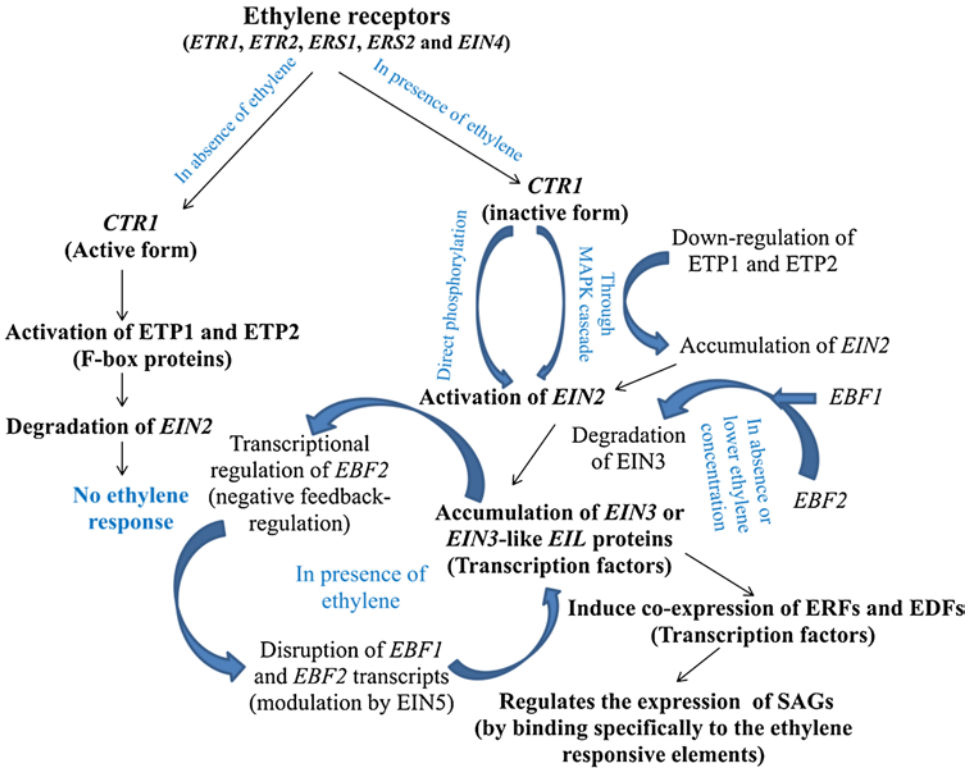

Fig. 1 Ethylene signaling in plants: components and signal transduction. Here ethylene is perceived by a set of ethylene receptors (ETRs) that transduces the signal to various downstream components for the regulation of gene expression. Abbreviations: *ETR* ethylene receptor, *CTR1* constitutive triple response, *MAPK* mitogen-activated protein kinase, *ETP* EIN2-targeting protein, *EBF* EIN3-binding F-box protein, *EIL* EIN3-like proteins, *ERF* ethylene response factors, *EDF* ethylene-responsive DNA-binding factors, *SAG* senescence-associated gene

involves an extensive cross-talk between various genes or their products (Fig. 1).

Future perspectives

The molecular and genomic revolutions have undoubtedly led to a revolution in the research being conducted in the field of plant senescence in general and flower senescence in particular. It is through molecular, mutational or transcriptomic approach that we have isolated and characterized numerous senescence-associated genes (genes coding for proteases, nucleases, transcription factors, ethylene biosynthesis and signaling, etc.). Moreover, the use of microarray technology, comprehensive transcriptomic sequencing projects and transgenic approaches will be of great help in bringing valuable information about the putative genes involved in flower senescence. This will provide us with a range of genes putatively involved in the implicated pathways leading to flower aging that may be blocked or induced to modify the progression of senescence. Although most of the genes or their corresponding proteins have been elucidated in detail; however, we are still far from developing an integrated picture of the executive mechanisms that control various aspects of senescence at molecular level that hinders our progress in addressing many open challenges regarding it. Thus, the major challenge for the researchers is to efficiently integrate the available information scattered in various flower systems into a flower senescence database (FSD) as has been developed for leaf senescence using bioinformatics approach. Moreover, the information gathered so far is based on studies conducted in a few model species like *Arabidopsis*, *Petunia, Mirabilis*, *Rosa*, *Alstroemeria,* etc.; therefore, another challenge in understanding the complex senescence regulation pathways is to extend this understanding to other species particularly the commercial ones (ornamentals) so that their vase life could be extended by exploiting the control points regulating flower senescence.

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