From models to ornamentals: how is flower senescence regulated?

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Abstract Floral senescence involves an ordered set of events coordinated at the plant, flower, organ and cellular level. This review assesses our current understanding of the input signals, signal transduction and cellular processes that regulate petal senescence and cell death. In many species a visible sign of petal senescence is wilting. This is accompanied by remobilization of nutrients from the flower to the developing ovary or to other parts of the plant. In other species, petals abscise while still turgid. Coordinating signals for floral senescence also vary across species. In some species ethylene acts as a central regulator, in others floral senescence is ethylene insensitive and other growth regulators are implicated. Due to the variability in this coordination and sequence of events across species, identifying suitable models to study petal senescence has been challenging, and the best candidates are reviewed. Transcriptomic studies provide an overview of the MAP kinases and transcription factors that are activated during petal senescence in several species including Arabidopsis. Our understanding of downstream regulators such as autophagy genes and proteases is also improving. This gives us insights into possible signalling cascades that regulate initiation of senescence and coordination of cell death processes. It also identifies the gaps in our knowledge such as the role of microRNAs. Finally future prospects for using all this information from model to non-model species to extend vase life in ornamental species is reviewed.

Keywords Arabidopsis · Autophagy · Ethylene · Petal senescence · Proteases · Transcription factors

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Introduction

Higher plants are radically different to animals in that the germ line is not set down during embryogenesis, but rather develops from somatic cells. This process is regulated by a combination of environmental and developmental cues that trigger the formation of a transient reproductive structure: the flower. In some species, the individual plant will go on to recreate a new germ line and new reproductive apparatus season after season until its death. However each individual flower has a lifespan which is usually a small fraction of the lifespan of the parent plant (Rogers 2006). The life of the flower typically terminates in senescence and ultimately a form of programmed cell death (PCD).

There has been much debate on the use of the terms "senescence" and "programmed cell death" (Thomas et al. 2003; van Doorn and Woltering 2004). In the context of petal senescence it seems appropriate to use these words almost interchangeably (Rogers 2006), in agreement with others in the field (van Doorn and Woltering 2008). This is because cell death, at least in some cells, starts very early during petal senescence (Wagstaff et al. 2003). However at an organ level, senescence implies a degree of remobilization of nutrients (Thomas et al. 2003) and cell death can also be seen as the terminal event, following the remobilization process.

Flowers are not the only plant organ to undergo senescence, however compared to leaves, the lifespan of flowers attached to the plant is less influenced by environmental factors. In many cases floral senescence is activated or greatly accelerated by pollination, and is thus tightly regulated by developmental signals (van Doorn and Woltering 2008; Rogers 2006). Petals have often been the focus of attention in floral senescence. They provide a relatively simple organ that consists of many similar cell types and

structures to those found in a leaf. Arguably petal senescence can therefore provide a useful model for studying the regulation of senescence in general, largely freed from environmental constraints.

This review starts by evaluating progress in identifying good models for studying petal senescence. It then focuses on those species in which most progress has been made to gauge our understanding of the key regulators of senescence, and prospects for regulating vase-life in ornamentals, particularly cut flowers.

The need for good models

Despite the many obvious advantages of studying petal senescence, our understanding of its regulation has been hampered by the lack of a good model species. This is due to several factors. Firstly two key features of petal senescence vary across species: sensitivity to ethylene and timing of abscission in relation to petal wilting. In many species floral senescence is coordinated by endogenous ethylene production often induced by pollination These include established models such as *Arabidopsis thaliana*, and many important ornamental species such as orchids (e.g. *Phalaenopsis* spp.), roses (*Rosa* spp.) and petunia (*Petunia* spp.) (Rogers 2006). In these species processes of remobilization, characteristic of senescence and leading to cell death, are coordinated by the ethylene signal. Furthermore, treatment with exogenous ethylene accelerates senescence, while treatment with inhibitors of ethylene synthesis or ethylene signalling, delay it (Stead and van Doorn 1994). In other species, however, little or no ethylene is produced in association with floral senescence. Furthermore floral senescence is insensitive to exogenous ethylene or ethylene inhibitors. This latter group also includes many important ornamental species such as lilies (*Lilium* spp.,) daffodils (*Narcissus pseudonarcissus)*, tulips (*Tulipa* spp.) and *Iris* (Woltering and van Doorn 1988).

Independently of whether or not floral senescence is ethylene-regulated, species also differ in the progression of petal senescence in relation to abscission. Thus in some species, such as rose and magnolia (*Magnolia grandiflora*), petals abscise while still turgid. In contrast, in other species such as *Iris* and petunia, petals wilt and deteriorate while still attached to the plant (van Doorn and Woltering 2008). Petal deterioration is associated with variable levels of nutrient remobilization followed by PCD (Verlinden 2003; Rogers 2006). This is often associated with increasing vacuole size, followed by vacuole rupture in a process that resembles autophagic cell death (e.g. in Japanese morning glory, *Ipomoea nil*; Shibuya et al. 2009a). In some species that abscise turgid petals, cell death markers are not apparent prior to abscission (Yamada et al. 2007). PCD

markers are however detected in other abscising species suggesting that the timing of PCD and abscission are independently regulated.

Thus identifying a single model that explains all the patterns of petal senescence is not attainable. However in other areas of plant development much has been achieved by focussing attention on Arabidopsis even though it cannot model all features of higher plant development. Unfortunately Arabidopsis has proven to be a somewhat limited model for studying detailed progression of petal senescence, although it has been used successfully to study petal abscission (Cho et al. 2008), and gross changes in petal senescence (Chen et al. 2011a). One of the key prerequisites of a good model for petal senescence is the ability to stage flowers based on key changes, usually associated to the development and maturation of the sexual organs. Unfortunately in Arabidopsis, although a developmental chart is available (Smyth et al. 1990) the detailed sequence of events is not as predictable as the chart would suggest. Furthermore Arabidopsis flowers abscise without substantial wilting, calling into question their role as a useful model for species whose petals wilt before abscission (Jones et al. 2009). Nevertheless, tools for Arabidopsis research outshine all other suitable models, and array data comparing young (stage 12) with older (stage 15) petals do provide the most complete dataset for the senescent petal transcriptome. The choice of these stages was validated by a clear up-regulation of the *SAG12* gene expression in stage 15 petals (Wagstaff et al. 2009). *SAG12* expression is an accepted marker for senescence in leaves and petals (Weaver et al. 1998). Its expression is closely linked to other petal senescence markers in wilting species where stages of senescence are better defined such as wallflowers (*Erysimum linifolium*; Price et al. 2008).

There are at least a couple of good alternative candidates as models for ethylene-sensitive petal senescence. Wallflowers are closely related to Arabidopsis and hence benefit from the use of cross-species microarrays. Approximately 80 % of genes tested cross-hybridised between wallflowers and Arabidopsis with very similar patterns of expression (Price et al. 2008). Wallflowers have the advantage over Arabidopsis of larger flowers with a range of petal colours, a predictable lifespan ending with wilting prior to abscission. Furthermore some varieties such as Bowles mauve are perennial (Price et al. 2008). However to date this species lacks many of the key tools required for an ideal model such as a transformation system, stocks of mutants and a genome sequence.

Petal senescence in petunia can be conveniently induced by pollination resulting in substantial wilting followed by abscission (Jones et al. 2009) thus allowing the study of both these processes. It boasts both transient and stable transformation systems (Conner et al. 2009; Chen et al. 2004),

increasingly complete genome and transcriptome sequences (Zenoni et al. 2011) and an increasing bank of available mutant lines. As yet these resources have not been fully exploited, but the future seems rosy for the study of ethylene-sensitive petal senescence.

Brassica species also have potential as models for petal senescence. Cross species arrays are available and have been tested for leaf transcripts (Trick et al. 2009). Software has also been developed for facilitating analysis of cross species arrays (Hammond et al. 2005) and tested across Arabidopsis and *Brassica oleracea*. Interest in *Brassica* species has also provided resources including genome sequencing projects, genetic maps and markers, and proteomic and metabolomic datasets (http://www.brassica.info/resources.php). However although *Brassica* species are used as ornamentals for their colourful foliage, they are not directly related to flower ornamentals and thus information gained from them would need to be translated to ornamental crop species.

Progress in finding good models for ethylene-insensitive petal senescence however is lagging behind. Ethylene sensitivity in petunia can be suppressed by expression of the *etr1-1* mutant allele, enabling the study of the processes that are essentially ethylene independent (Wilkinson et al. 1997). However, this is easily criticizable as a somewhat artificial system. Four o'clock (*Mirabilis jalapa*) is one of the few dicotyledonous species with apparently ethylene-independent petal senescence (Xu et al. 2007a). Although floral senescence is accelerated in this species by exogenous ethylene, little endogenous ethylene is produced during senescence and inhibitors of ethylene synthesis or action have little effect on its progression. However research on four o'clocks has not flourished even though a transformation system was established (Zaccai et al. 2007).

The lack of a good model has lead to the proliferation of papers studying floral senescence in a wide range of both monocotyledonous and dicotyledonous ornamental species (van Doorn and Woltering 2008; Rogers 2006), for which few tools and no complete genome sequences are available. However, this situation is rapidly improving with the advent of new much cheaper high throughput sequencing and the increasing use of virus induced gene silencing (VIGS; Edwards and Batley 2010; Senthil-Kumar and Mysore 2011).

Developmental and environmental signals

Although petal senescence is relatively unaffected by environmental signals there is an interaction between stress and senescence. Clearly the situation changes dramatically in cut flowers compared to those still attached to the plant. In petunias, growth at higher temperatures resulted in a shorter floral life span of unpollinated flowers attached to the plant (Gubrium et al. 2000). However, vase life of cut flowers was longer when plants had been grown at a higher compared to a lower temperature (Shvarts et al. 1997). In the cut flower *Alstroemeria* vase life was reduced by up to 20 % following stress treatments (Wagstaff et al. 2010) and was accompanied by substantial changes in gene expression.

Where comparisons have been made, longevity is generally reduced in cut flowers compared to those on the plant (e.g. in lilies: Arrom and Munné-Bosch (2010); carnation, *Dianthus caryophyllus* L.: van Staden and Dimalla 1980). In *Lilium* (*Lilium longiflorum* x Asiatic Hybrid var. Courier) antioxidants increased more sharply in the tepals of cut flowers although whether these might have a regulatory role is not known (Arrom and Munné-Bosch 2010). Furthermore changes in growth regulators differed between cut and uncut *Lilium* flowers (Arrom and Munné-Bosch 2012a). In particular levels of IAA and ABA were less in the outer petals of intact compared to cut flowers at anthesis. An interaction between environmental stress signals and plant growth regulators (PGRs) is also indicated by studies on transgenic petunias in which ethylene signalling has been down-regulated. In these flowers longevity is much more sensitive to growth at a higher temperature compared to wild type (Gubrium et al. 2000). More studies comparing global changes in transcription and metabolic status in stressed and unstressed and cut versus uncut flowers may reveal to what extent the regulatory signals and down-stream processes differ.

In species where multiple flowers are produced on the same stem, there is another important signal based on the interaction between the longevity of the less-developed and more-developed flowers. For example trimming younger flowers from *Alstroemeria* cymes increased longevity of the remaining flower (Chanasut et al. 2003). This is probably due to the important sink-source nutrient relationships within the inflorescence (Picchioni et al. 2007).

Nutrient status and energy depletion have been proposed as important regulators of floral senescence in cut flowers (Azad et al. 2008), and treatment with sucrose delayed floral senescence both in ethylene-sensitive (e.g. carnation: Hoeberichts et al. 2007) and ethylene-insensitive species (e.g. *Alstroemeria*: Chanasut et al. 2003; tulip: Azad et al. 2008; *Lilium*: Arrom and Munné-Bosch 2012a). In carnation (Hoeberichts et al. 2007) and wallflowers (Rogers, unpublished) sucrose treatment was very similar to treatment with the ethylene inhibitor STS indicating that it may be acting by repressing ethylene signal transduction. How the sucrose signal exerts its effects in ethylene-insensitive species remains to be determined. However, in *Lilium* at least, it affected the PGR balance (Arrom and Munné-Bosch 2012a) and thus may be acting via PGRs other than ethylene such as abscisic acid (ABA) or auxins.

Fig. 1 Pollination and/or age related processes, resulting in a reduction of cytokinins and rise in ABA, trigger up-regulation of ethylene biosynthetic genes and an increase in ethylene sensitivity. Ethylene-responsive TFs are activated directly or via a MAP kinase signalling cascade. Other TFs are activated by auxin and other signals including probably ROS. Protease transcription is up-regulated presumably by a combination of transcription factors. KDEL proteases are localised to the ER and then ricinosomes, VPEs are targeted to the vacuole where they may be involved in processing of downstream proteases, metacaspases are activated and contribute to cell death. Autophagy genes are also up-regulated and presumably result in the formation of auotophagosomes which later fuse with the vacuole
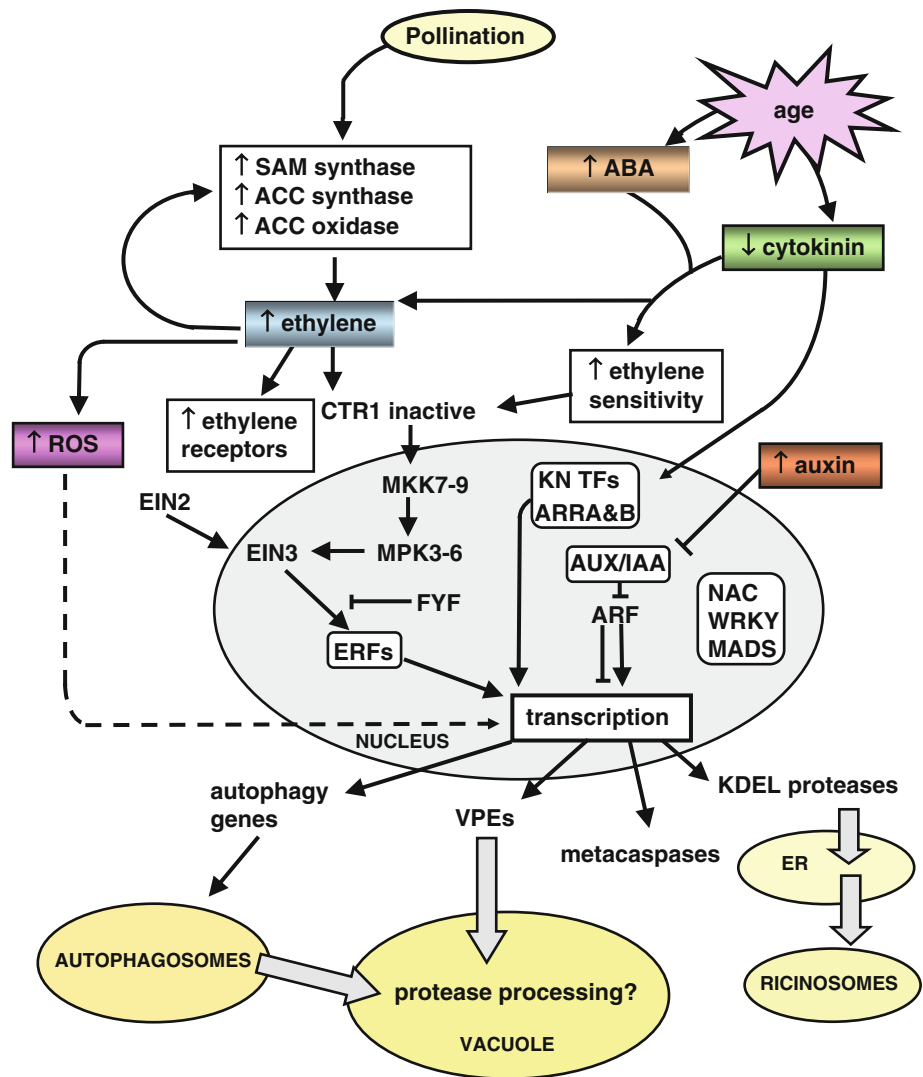
Plant growth regulators (PGRs)

In species with ethylene-sensitive floral senescence, pollination induces a burst of ethylene production associated with up-regulation of ethylene biosynthetic enzymes: S-adenosylmethionine (SAM) synthase, 1-aminocyclopropane-1-carboxylic acid (ACC) synthase and ACC oxidase (Jones 2004; Hoeberichts et al. 2007). Ethylene production is associated with pollen tube growth in the style and later with fertilisation (Hoekstra and Weges 1986; ten Have and Woltering 1997). This results in autocatalytic ethylene production in the petals (Woodson and Lawton 1988; Graham et al. 2012). In rose (Müller et al. 2000) but not carnation (Shibuya et al. 2002), petal senescence is associated with changes in expression levels of the ethylene receptor genes. However expression of a mutated ethylene receptor gene (*etr1-1*) resulted in delayed floral senescence in several species including petunia. Components of the

ethylene downstream signalling pathway (Yoo et al. 2009; Fig. 1) are expressed in petals and have also been targets for manipulation to delay flower senescence. Expression of *CTR1-like* genes in rose (*Rosa hybrida*) were up-regulated by exogenous ethylene (Ma et al. 2006), and expression of *Rh-CTR1* also increased during floral senescence (Müller et al. 2002). Mutation of *EIN2* delayed floral senescence in both petunia and Arabidopsis (Shibuya et al. 2004; Graham et al. 2012). *EIN3*-like gene expression increased in carnation during floral senescence and in response to ethylene (Iordachescu and Verlinden 2005), and its accumulation was delayed in sucrose-treated flowers. Down regulation (Tieman et al. 2001) or up-regulation (Yang et al. 2008) of tomato *EIN3*-like genes resulted in delayed or accelerated petal senescence respectively. This confirms a role for these genes too in ethylene-induced petal senescence.

In ethylene sensitive flowers this PGR clearly plays a decisive role in the initiation and coordination of

senescence and ultimately cell death. However, even in these species, other PGRs also play important roles. In several species studied there was a senescence-associated fall in cytokinins and rise in ABA levels (Borohov et al. 1976; Mayak and Halevy 1970; Chang et al. 2003; van Staden et al. 1987). Both these changes seem to be connected to ethylene signalling. In carnations sensitivity to ethylene was reduced by treatment with cytokinin and inhibition of cytokinin metabolism delayed senescence (Taverner et al. 2000). Furthermore treatment of carnation petals with cytokinins blocked the conversion of exogenously supplied ACC to ethylene (Mor et al. 1983). In transgenic petunia, expression of the *IPT* gene, resulting in overproduction of cytokinin, led to a decrease in ethylene sensitivity and a reduction in ABA accumulation (Chang et al. 2003). Blocking ethylene signalling in petunia expressing *etr1-1* abolished senescence-associated ABA accumulation in corollas (Jones et al. 2009) which is consistent with these results. Furthermore in carnation, exogenous ABA treatment accelerated senescence and increased both production of and sensitivity to ethylene (Ronen and Mayak 1981). However in hibiscus (*Hibiscus rosa-sinensis* L.;Trivellini et al. 2011) although exogenous ABA treatment accelerated flower senescence, it reduced ethylene biosynthesis and sensitivity. The complex relationship between ethylene, ABA and senescence in species with ethylene-sensitive floral senescence is therefore worthy of further investigation. There is a need for more physiological, transcriptomic and metabolomic studies in more species to make results more comparable and establish whether apparent species-specific differences are supported.

In ethylene insensitive cut flowers there is accumulating evidence that ABA may play an important role in petal senescence. Endogenous levels of ABA are found to rise in several species with ethylene insensitive floral senescence: e.g. daylily (*Hemerocallis hybrid*, cv. Stella d'Oro; Panavas et al. 1998), daffodil (Hunter et al. 2004a, b) and *Lilium* (Arrom and Munné-Bosch 2012a, b). Exogenous application of ABA to day lilies, resulted in an acceleration of natural senescence events including changes in transcriptional patterns (Panavas et al. 1998). Furthermore the effect could also be mimicked by treatment with sorbitol that induced an increase of endogenous ABA levels. Similar results were also obtained in daffodil (Hunter et al. 2004a) where it was further shown that the effects were ethylene-independent. However in daffodil, inhibitors of ABA biosynthesis did not delay senescence, although treatment with gibberellic acid (GA₃) did mitigate the effects of exogenous ABA. This suggests that the ABA might be acting via GA. In all three species, however, the rise in endogenous ABA levels occurred quite late. This suggests that although this PGR may have a role in coordination of the later stages of petal senescence it is not likely to be involved in the initiation of the senescence programme.

The role of other PGRs such as auxin, jasmonates (JA) or salicylic acid (SA) in petal senescence remains unclear (van Doorn and Woltering 2008). Endogenous auxin levels fell in *Lilium* post-anthesis, while SA increased and JA remained stable (Arrom and Munné-Bosch 2012b). However in wallflowers free IAA increased during petal senescence (Mohd Salleh et al. unpublished results). Furthermore, in senescent Arabidopsis petals auxin biosynthetic and responsive gene expression was strongly up-regulated (Wagstaff et al. 2009). Application of exogenous auxin also accelerated senescence and ethylene production in carnation petals (Wulster et al. 1982; van Staden 1995).

Endogenous levels of reactive oxygen species (ROS) and ROS-related enzyme activities have been measured in numerous flowers with both ethylene-sensitive or insensitive senescence programmes (Rogers 2012). In general ROS levels rise and antioxidant levels fall during both forms of petal senescence. However, changes appear to start too late for ROS to be a key initiator of petal senescence. Nevertheless, this does not exclude a signalling role for redox status and or the mitochondrion in the coordination of later events. More information on the intracellular localisation of ROS production, levels of different ROS moieties, and on ROS signal transduction is needed in order to be able to resolve their role in petal senescence with confidence.

## Signal transduction and transcription factors (TFs)

MAP kinases are central to signal transduction in many environmental and developmentally regulated cellular processes (Fiil et al. 2009), and senescence is no exception. The Arabidopsis genome contains 20 *MAPK*s, 10 *MAPKK*s, and more than 60 *MAPKKK*s (*MAPK* Group 2002). In leaf senescence MEKK1 bypasses the cascade by acting directly on the promoter of *WRKY53*, a senescence-associated TF (Miao et al. 2007). However MKK9 and MPK6 are also involved (Zhou et al. 2009). Less is known about MAPKs that might be involved in petal senescence. Expression of relatively few *MAPK* genes were up-or down regulated in Arabidopsis silique, leaf or petal senescence microarray analyses (Wagstaff et al. 2009) and none were specifically up or down-regulated in petal senescence. However expression of *MPK11* was up-regulated in both petal and silique senescence and may be an interesting target for further study. This *MAPK* is also involved in both biotic and abiotic stresses and responds to both ROS and ABA, providing a potential link between these processes. In wallflowers, expression of a homologue of *MKK9* was strongly up-regulated in both senescent leaves and petals (Price et al. 2008) and is also up-regulated in these tissues in Arabidopsis (Schmid et al.

2005). It is not surprising that at least some of the signal transduction mechanisms in leaf and petal are likely to be shared given the high levels of shared senescence-induced transcriptional changes in these tissues (Price et al. 2008; Wagstaff et al. 2009).

In ethylene-insensitive species *MAPK*-like transcripts have also been identified: in *Alstroemeria* a *MAP3Ka*-like transcript was up-regulated with tepal senescence (Wagstaff et al. 2010), although further work is needed to verify its expression pattern and function. Further work on MAP kinases involved in petal senescence is urgently needed.

Downstream of the MAP kinase cascade, TFs expressed during floral senescence have been identified in numerous EST, microarray, and SSH experiments including both ethylene-regulated species (e.g. wallflowers: Price et al. 2008; petunia: Liu et al. 2011; Arabidopsis: Wagstaff et al. 2009; carnation Hoeberichts et al. 2007) and ethylene-insensitive species (e.g. *Alstroemeria*: Breeze et al. 2004; *Iris*: van Doorn et al. 2003). A detailed analysis of the petal data from Wagstaff et al. (2009) revealed changes in the expression of 316 putative TFs of which 130 changed expression only in petal senescence and not in leaf or silique senescence. Forty-seven TF families were represented (as classified by Guo et al. 2005). The best represented was the *AP2-EREB* (ERF) family representing over 10 % of up-regulated TFs in petals (Fig. 2). Interesting differences are revealed by comparing TFs in three expression classes: (1) all those whose expression changed in petals (2) those whose expression was only up-regulated in petals but not in the other senescing tissues, and (3) those whose expression was up-regulated in all three senescing tissues (Fig. 2). Whereas the three most represented TF families up-regulated in class (1) were *AP2-EREBP* (*ERF*), *NAC* and *WRKY* accounting for almost 30 % of all the TFs expressed in this class, in class (2) the most represented families amongst TFs specifically up-regulated in petals were *AP2-EREBP*, homeobox (*HB*) and *AUX-IAA* (again accounting for about 30 % of the total). In contrast, in class (3) i.e. TFs up-regulated in all senescent tissues studied, the most represented families of up-regulated TFs were *NAC* (32 %), *WRKY* (18 %) and *bZIP* (11 %). Thus *AP2-EREBP*, *HB* and *AUX-IAA* TFs stand out as up-regulated TF classes that are most highly represented specifically in ethylene-regulated petal senescence and may be of significance in regulating specific senescence-associated events in this tissue. Although *WRKY* TFs were up-regulated in all three tissues, more family members were up-regulated in petals compared to leaves. Interestingly, *WRKY53* which has been identified as an important hub regulator in leaf senescence, integrating MeJA and SA signals (Balbi and Devoto 2008; Miao and Zentgraf 2007; Miao et al. 2007), was not up-regulated in petal senescence.
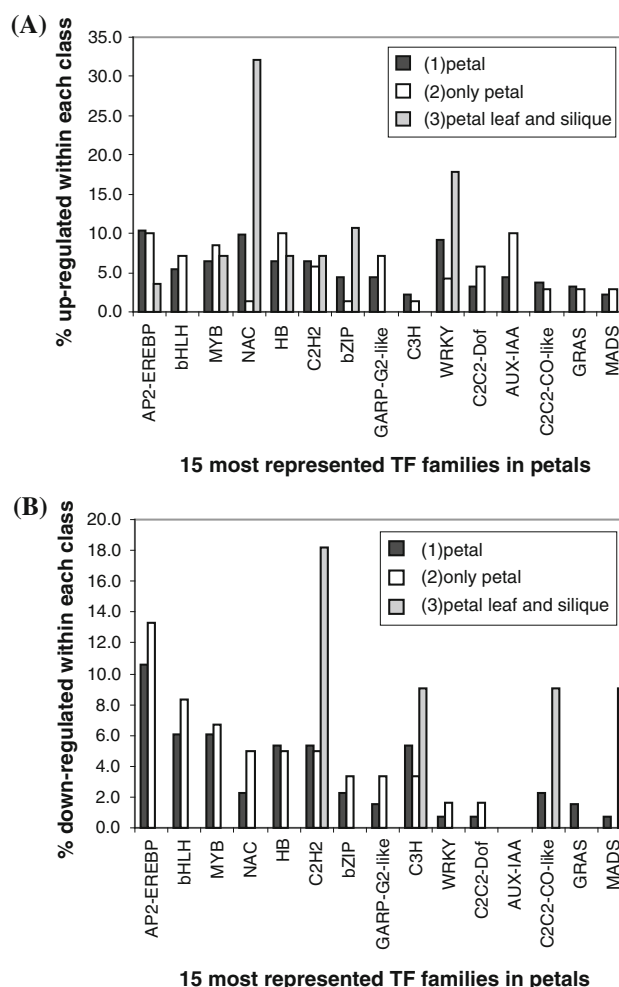
Fig. 2 Top 15 most represented transcription factor families expressed in *Arabidopsis* petals from the data of Wagstaff et al. (2009) whose expression was **a** up- or **b** down-regulated. (*1*) All TFs whose expression changed in petals (*2*) TFs whose expression only changed in petals (Stage 15 compared to stage 12) but not in senescent leaves (leaf 6: 6 weeks old/leaf 6: 4 weeks old) or senescent siliques (19-23/9-11 DAA), and (*3*) TFs whose expression changed in all three senescing tissues

The prevalence of the *AP2-EREB* TFs is not surprising given the established role of ethylene in these species. However the up-regulation of genes encoding AUX-IAA proteins raises again the issue of the role of auxin in petal senescence, which remains to be fully resolved in any species. AUX-IAA proteins are short-lived nuclear proteins forming part of an auxin co-receptor complex (Calderón Villalobos et al. 2012), that bind and repress auxin responsive factor (ARF) TFs. An auxin signal results in degradation of the AUX-IAA protein and de-repression of the ARFs.

Amongst the HB TFs up-regulated in petals was *KNAT1,* a member of the Class I *KNOX* family known to modulate cytokinin levels, and four members of the Class II *KNOX*

family most of which are of unknown function (Hay and Tsiantis 2009). A KNAT1 homologue was also up-regulated in senescent carnation petals (Hoeberichts et al. 2007) indicating that the involvement of these genes in the regulation of petal cytokinin levels may be common to different species. Furthermore expression of ARR genes, part of the cytokinin signal transduction chain (To and Kieber 2008), was altered in Arabidopsis senescent petals. Two of these (ARR1 and ARR2) are associated with the vascular tissue and, as noted by Wagstaff et al. (2009), may be important in retaining function of this tissue during senescence-associated nutrient remobilization.

Further work will be needed to establish the role of all these TFs through analysis of over-expression and knockout mutants, as well as screens to identify their downstream targets. Work on petal senescence in Arabidopsis will benefit greatly from systems biology approaches to identify hubs in the transcriptional networks regulating senescence and stress responses in leaves (Breeze et al. 2011). However, an analysis of individual TFs has revealed an important role in floral senescence for at least one MADS box TF: AGL42 (FOREVER YOUNG FLOWER; FYF). Chen et al. (2011a) showed that this gene acts as a repressor of ethylene-mediated floral abscission and senescence in Arabidopsis. It acts upstream of BOP2 and IDA, genes involved in floral abscission. It would be interesting to analyse the effects of FYF perturbation on the progression of petal senescence in more detail. It is important to try to clarify whether its role in abscission is distinct from its role in senescence, or whether the two are linked. It will also be interesting to establish whether homologues in other species play a similar role. An orchid (Oncidium sp.) homologue of FYF also delayed petal senescence/abscission when expressed in Arabidopsis (Chen et al. 2011b). This suggests that this gene may indeed be important in other species whose floral senescence is ethylene regulated. Whether it might also play a role in ethylene-independent senescence remains to be tested.

In petunia flowers, the ERF (Ethylene-responsive element binding factor) family of TFs was studied in detail (Liu et al. 2011). This TF family is one of the largest in plants with 122 members in Arabidopsis belonging to 12

groups (Nakano et al. 2006). Thirteen ERF TFs belonging to four of these groups were expressed in petunia corollas, showing distinct patterns of expression. Group VII ERFs, previously associated with fruit ripening and senescence (El-Sharkawy et al. 2009; Yin et al. 2010) were also associated with corolla senescence in petunia. Their expression was up-regulated with ethylene, down-regulated by STS, and up-regulated by MeJA and ABA supporting the link between ethylene and ABA responses. Expression of three of the ERFs, belonging to group IX were down-regulated during corolla senescence and with exogenous ABA. The authors hypothesise that the reduction in expression during senescence may be linked to the rise in endogenous ABA levels (Vardi and Mayak 1989). Expression of several ERFs was also up-regulated by SA treatment indicating an interaction between ethylene and SA signalling in petunia corolla senescence. However, surprisingly none of the ERFs responded to exogenous cytokinin treatment, despite a clear link between cytokinins and ethylene in petunia flowers (Chang et al. 2003).

In other species with ethylene-regulated petal senescence similar TFs have also been identified. In wallflowers expression of genes with homology to WRKY75 and two NAC TFs were up-regulated in both leaf and petal senescence (Price et al. 2008) as they were also in Arabidopsis. In carnation petal senescence, as in Arabidopsis, expression of IAA-AUX TFs (Hoeberichts et al. 2007) was also up-regulated suggesting a link to auxin signaling that is supported by changes in the endogenous levels of this PGR in senescing petals (see above).

Much less is known about the expression of TFs in species where petal senescence is ethylene-insensitive although transcriptomic analyses have revealed a few (Table 1). In Alstroemeria transcripts for 21 TFs were identified from the 2000 ESTs sequenced (Wagstaff et al. 2010). Of these, one C2-H2, one HMG-box, two zinc-finger, one LIM-domain and one homeodomain TF changed in expression during tepal senescence. Expression of several others was also affected by stress treatments indicating shared regulation between these processes. Transcriptomic analyses also identified TFs from daffodil (AP2/ERF and NAC families; Hunter et al. 2002) and Iris (HLH-Zip; van Doorn et al. 2003). It is interesting that WRKY TFs have not

Table 1 Transcription factors that are differentially expressed in senescent petals of species with ethylene-insensitive petal senescence programmes

Species	TF family	Reference
Alstroemeria	C2H2, HMG-box, Zinc finger, LIM domain, homeodomain	Breeze et al. (2004), Wagstaff et al. (2010)
Narcissus	AP2/ERF, NAC	Hunter et al. (2002)
Iris	HLH-Zip, MADS	Van Doorn et al. (2003)
Mirabilis jalapa	bZIP, C2H2, HD-Zip, JUMONJI, MADS, MYB	Xu et al. (2007a)

been identified from these species so far, although the promoter of a putative E3 ubiquitin ligase that was highly up-regulated in four o'clock senescent petals included putative binding sites for several TF classes including *WRKY* TFs (Xu et al. 2007b). Cheaper transcriptomic sequencing should help to establish whether *WRKY* TFs are indeed important in ethylene-independent floral senescence systems.

Another layer of regulation that is becoming increasingly studied is provided by microRNAs (miRNAs). These act as negative regulators and are involved in the regulation of many stages of plant development (Voinnet 2009) including senescence. The leaf-senescence *NAC* TF *ORE1* was found to be regulated by a complex trifurcate feed-forward mechanism involving a miRNA, *mi R164* (Kim et al. 2009), and *EIN2* from the ethylene-mediated senescence pathway. By negatively regulating *ORE1* expression, *miR164* may be fine tuning the timing of leaf senescence promoted by *EIN2*. *EIN2* then negatively regulates *miR164* as senescence progresses. In fact mathematical modelling showed that persistent rise in *EIN2* results in activation of *SAG12* supporting this fine tune control. *TCP* TFs are also miRNA regulated and participate in the regulation of leaf senescence (Schommer et al. 2008). Furthermore some miRNAs are highly conserved across species (Cuperus et al. 2011) providing opportunities for manipulation of developmental programmes through transformation. Thus a fruitful research area is likely to be an investigation of miRNAs regulating floral senescence. It is possible that in petals as well as leaves they may be involved in the fine tuning of senescence progression to orchestrate the complex remobilization processes and delaying cell death as required.

Downstream cell death regulation

Although cell death often starts before visible signs of petal deterioration are evident (Rogers 2006), it is clear from careful analyses of cellular structure (e.g. Wagstaff et al. 2003; Battelli et al. 2011) that cell death is not uniform across the petal. In many species mesophyll cells degenerate completely in older petals while the epidermal cells remain essentially intact. In *Iris* this differential timing coincides with a closure of plasmodesmata, interrupting the movement of nutrients, PGRs and other small molecules (van Doorn et al. 2003). This may result in effective starvation of the cells and lead to ATP depletion triggering cell death as discussed above.

Furthermore in several species such as *Iris, Lilium* and *Alstroemeria* (van Doorn et al. 2003; Battelli et al. 2011; Wagstaff et al. 2003) cell death starts at the petal margins and spreads inwards. The assumption is that this is part of a remobilization programme that favours the survival of the cells nearer the vascular tissue to enable transport of nutrients out of the petal. This presumes a long-range coordination of cellular responses, perhaps involving cytokinins as suggested by the up-regulation of the *ARR* genes discussed above.

At a cellular level petal PCD resembles closely autophagic (van Doorn and Woltering 2005; Shibuya et al. 2009a) or 'autolytic' (van Doorn 2011) cell death. This process is characterised by the formation of small vacuoles that fuse to merge with the central vacuole. The vacuole then ruptures releasing hydrolases which rapidly clear the remaining cytoplasm (van Doorn 2011). Auotophagosomes have been detected in petals (e.g. Japanese morning glory: Shibuya et al. 2009b) and genes encoding components of the conserved autophagic regulatory mechanism are expressed in the petals of several species (e.g. Arabidopsis: Wagstaff et al. 2009; Japanese morning glory: Shibuya et al. 2009b; *Alstroemeria*: Wagstaff et al. 2010). However to what extent the autophagic mechanism is a requirement for petal cell death is not clear. In Arabidopsis knockouts of autophagy genes, leaf senescence is accelerated, but it has been proposed that this might also be due to an indirect stress effect rather than an indication that autophagy delays senescence (van Doorn and Woltering 2008). A closer investigation of petal senescence phenotype in autophagy gene knockouts would be very useful.

In animal PCD a specific class of proteases, caspases play a key regulatory role both in the initiation and execution of cell death (Sanmartín et al. 2005). Caspase activity has been detected in a number of plant cell death systems (Bonneau et al. 2008), and recently in *Lilium* (*L. longiflorum*) petals (Battelli et al. 2011), however caspase genes with homology to those in animals have not been found in plant genomes. In plants, vacuolar processing enzymes (VPEs) are a family of proteases that share structural features with caspases and have caspase-1 activity (Rojo et al. 2004). They localise to the vacuole and are involved in maturation of hydrolases. In tobacco petals expression of a VPE gene increased 100-fold with age, and VPE genes are also up-regulated in senescent petals of carnation (Hoeberichts et al. 2007), daffodil (Hunter et al. 2002), Japanese morning glory (Yamada et al. 2009) and *L. longiflorum* (Rogers, unpublished). Furthermore of the four VPEs in Arabidopsis, expression of two (β and γ) was up-regulated in senescent petals (Wagstaff et al. 2009). Further work is clearly needed to determine whether perturbation of VPE expression affects petal senescence and to identify VPE substrates in petals. This will shed light on whether VPEs might be playing a regulatory role.

Metacaspase genes also form a large gene family in plants and resemble part of the caspase coding sequence (Tsiatsiani et al. 2011). Moreover, their expression in plants is associated with cell death processes (Coll et al.

2010). A metacaspase gene was expressed in petunia petals (Jones et al. 2009), but its role remains to be established. Expression of two Arabidopsis metacaspase genes (*AtMC9* and *AtNC3*) was up-regulated in senescent petals (Wagstaff et al. 2009) indicating that they may play a role in petal senescence. However as they comprise a large gene family unravelling their roles may take some time.

Petal senescence in many species is also associated with an increase in cysteine protease activity. In some species, such as *Iris* (Pak and van Doorn 2005) and *Sandersonia* (*Sandersonia aurantiaca*; Eason et al. 2002), treatment of flowers with inhibitors of cysteine proteases delayed visible senescence. This indicates a potential regulatory role for these proteases. In several species up-regulation of papain-family cysteine protease genes with a C-terminal KDEL sequence has been reported (E.g. *Sandersonia*, Eason et al. 2002; day lily, Valpuesta et al. 1995). Expression of some of these genes e.g. *Alstroemeria ALSCYP1* was up-regulated before visible signs of senescence (Wagstaff et al. 2002) suggesting that they might participate in a proteolytic cascade activating other down-stream proteases. The KDEL sequence anchors the proteases to the endoplasmic reticulum but they are also associated with ribosome studded vesicles known as ricinosomes (Schmid et al. 1999). Ricinosomes deliver their cargo of protease directly to the cytoplasm following vacuolar rupture and were also detected in senescing day lily petals (Schmid et al. 1999). However, they have not been reported in other species. Identifying the substrates for these early-expressed proteases and defining whether they are indeed released directly into the cytoplasm, are important goals to establish their role in petal cell death. In petunia, down-regulation of cysteine protease genes did not affect senescence progression indicating likely functional redundancy in this gene family (Jones et al. 2009) which will complicate their analysis by a genetic route.

Prospects for understanding and controlling senescence in ornamentals

Our understanding of the genes and processes regulating petal senescence in species where these are coordinated by ethylene is progressing fast. Although many of the pieces are still missing, a tentative model can be assembled to incorporate available data on the genes and processes involved, as discussed above (Fig. 1). Key tools are also becoming available in petunia for this to become a testable model for this group of species. The next step will then be to test and transfer this understanding to other species of commercial relevance and exploit control points to extend vase life. Already several different treatments are available for extending vase life in these species (Rogers and Stead 2011) involving down-regulation of ethylene responses

through chemical treatments. Furthermore, prospects seem good for refining chemical approaches e.g. through the use of nanosponges (Seglie et al. 2011). In addition an increasing number of these species can be transformed (Chandler and Tanaka 2007), and transgenic approaches for reducing ethylene signalling can be refined through the use of more specific promoters. Other targets may also be of interest such as *FYF* or hub TFs driven by tissue specific or senescence induced promoters such as the *SAG12* promoter (Chang et al. 2003).

Progress in understanding ethylene-independent floral senescence has lagged behind. Given that the PGR regulation in these species is likely to be very complex, there seems to be much more promise in the manipulation of the signalling and TF intermediaries. It will be crucial to determine whether information from the more tractable ethylene-regulated species can be directly transferred. This will depend on whether the key regulators are shared across the physiological groups. Comprehensive transcriptomic sequencing projects using new high throughput sequencing approaches will be very powerful especially in species such as *Lilium* where genome sizes of up to 127 000 Mb (Zonneveld et al. 2005) make genome sequencing projects unrealistic. This approach will provide a global view of gene expression changes crucially in up-stream signalling and TFs. This would then enable the use of systems approaches now available for leaf senescence in Arabidopsis. How this information is then used will depend on the species. A major challenge is to develop robust transformation protocols for a transgenic approach in these less tractable species. In some species, however, marker-assisted breeding programmes may be the most immediate way of exploiting the new information.

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