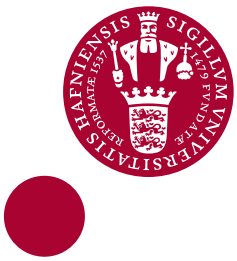

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REVIEW ARTICLE

Ethylene resistance in flowering ornamental plants – improvements and future perspectives

Andreas Olsen, Henrik Lütken, Josefine Nymark Hegelund and Renate Müller

Various strategies of plant breeding have been attempted in order to improve the ethylene resistance of flowering ornamental plants. These approaches span from conventional techniques such as simple cross-pollination to new breeding techniques which modify the plants genetically such as precise genome-editing. The main strategies target the ethylene pathway directly; others focus on changing the ethylene pathway indirectly via pathways that are known to be antagonistic to the ethylene pathway, e.g. increasing cytokinin levels. Many of the known elements of the ethylene pathway have been addressed experimentally with the aim of modulating the overall response of the plant to ethylene. Elements of the ethylene pathway that appear particularly promising in this respect include ethylene receptors as ETR1, and transcription factors such as EIN3. Both direct and indirect approaches seem to be successful, nevertheless, although genetic transformation using recombinant DNA has the ability to save much time in the breeding process, they are not readily used by breeders yet. This is primarily due to legislative issues, economic issues, difficulties of implementing this technology in some ornamental plants, as well as how these techniques are publically perceived, particularly in Europe. Recently, newer and more precise genome-editing techniques have become available and they are already being implemented in some crops. New breeding techniques may help change the current situation and pave the way toward a legal and public acceptance if products of these technologies are indistinguishable from plants obtained by conventional techniques.

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INTRODUCTION

The production of potted ornamental plants and cut flowers is a growing industry with annual turnover in the tens of billions of dollars,[1] with the European Union being the single largest producer and consumer.[2] Due to the competitive nature of the ornamental plant industry, research and development of new products and improvement of quality in existing products have become essential. The quality of flowering ornamentals, often judged by the longevity of their flowers, is an extremely important parameter in assessing quality.[3] During the postharvest period, plants will likely experience stress as a result of poor lighting, temperature, and suboptimal humidity or watering.[4,5] This stress often leads to visual symptoms such as wilting, color change, and abscission of various plant parts including flowers, petals, and buds. In climacteric plants, stress triggers the production of the phytohormone ethylene, which quickly deteriorates plants visually. Ethylene of exogenous origin can also affect the plants in closed spaces[6] and plants that have been exposed to ethylene will often no longer be sellable.

In recent years, the biological significance of ethylene in ornamental production, its signaling pathway within the plant, and methods to alleviate its consequence to the aesthetic value of ornamental plants have been extensively reviewed.[7–13] However, many reviews only consider a limited number of plant species, or focus on a particular perspective for solving the problem in only one or two of the levels of the ethylene pathway. In order to acquire an overview of the problems associated with ethylene-induced quality losses in ornamental plant production, recent research and advances in the field of ethylene biology and breeding techniques must be considered. The present review aims to compare results from past approaches in order to discuss future breeding strategies. It will also point out avenues not yet explored in the area of ornamental plant breeding which may be essential for reducing ethylene responses that remains a decisive factor for high-quality production of many ornamental plants.

REGULATION OF THE ETHYLENE PATHWAY

The ethylene biosynthesis and signaling pathway can be presented in a linear model (Figure 1). Ethylene is essential for many processes in the plant and thus, there is a constant, low ethylene production (Figure 1a).[14] Under certain conditions, however, ethylene biosynthesis and sensitivity increase in specific tissues and this triggers the ethylene signaling pathway (Figure 1b).[15] This initially starts as an increase in expression of some of the enzymes responsible for ethylene biosynthesis[16–18] which leads to higher ethylene production[19] that may amplify itself in an autocatalytic fashion in some cases.[20]

Recently, it was shown that different ACS homologs of *Dianthus caryophyllus*[18] and *Dianthus superbus*[21] were expressed in association with either the basal or the climacteric phase. In *Petunia*[22] and *Paeonia suffruticosa*,[23] the same was observed for certain ACS and ACO homologs. It therefore seems that these enzymes, central for ethylene biosynthesis (Figure 1), may hold the key for the ability of the plant to transition from basal to climacteric ethylene production (Figure 1).[24] Furthermore in *Arabidopsis*, ACS activity was demonstrated to be affected by the formation of hetero- and homo-dimers,[24,25] and phosphorylation by the mitogen-activated protein kinase (MAPK) MPK6, which itself is expressed in response to stress.[26] Recently, a similar role for MPK6 was demonstrated in *Rosa hybrida*.[27]

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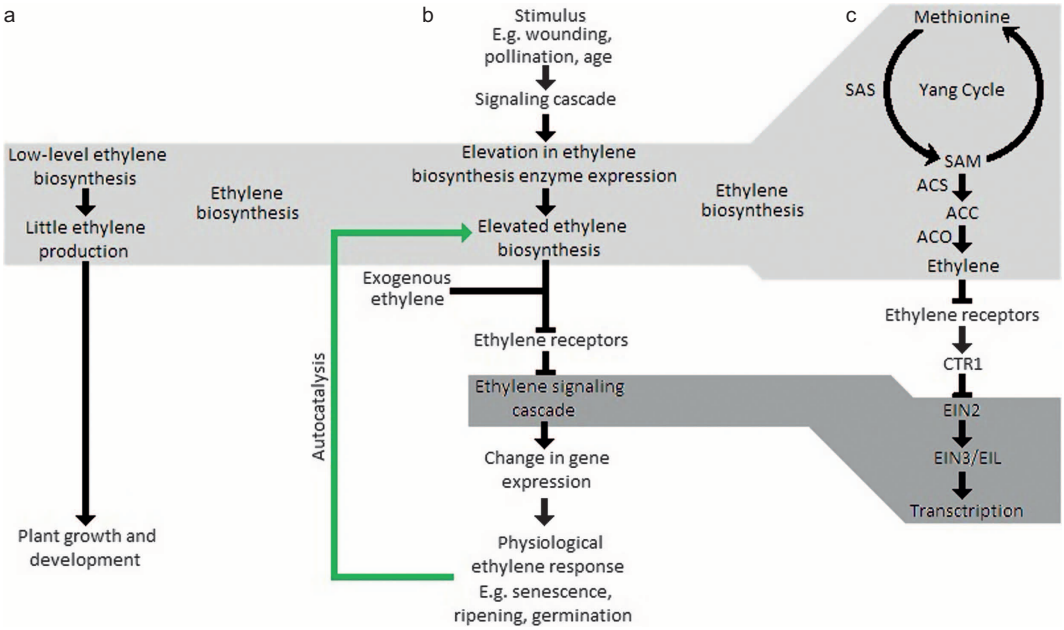

Figure 1. Simplified ethylene pathway. (**a**) Basal production of ethylene in the flowers during development before senescence. (**b**) The ethylene pathway upon triggering. The stimulus is translated to elevated ethylene synthesis producing higher levels of ethylene which inactivates the receptors initiating the signaling cascade which changes gene expression and finally induces physiological processes in the flower which may include the initiation of an autocatalytic loop.[14] (**c**) Simplified molecular events of the ethylene pathway. Methionine is enzymatically converted to S-adenosyl-L-methionine (SAM) by SAM synthase (SAS). SAM is partially converted back to methionine via several steps, but it also produces 1-aminocyclopropane-1-carboxylic acid (ACC) by ACC synthase (ACS). ACC is transformed to ethylene by ACC oxidase (ACO). Ethylene binds to receptors and stops their signal to CONSTITUTIVE TRIPLE RESPONSE1 (CTR1), which then stops its suppressing signal to ETHYLENE INSENSITIVE2 (EIN2). The released EIN2 is then cleaved and part of it is transported into the nucleus where activation of the ETHYLENE INSENSITIVE3/ETHYLENE INSENSITIVE3-LIKE (EIN3/EIL) transcription factor family occurs. This initiates a transcription cascade by activation of ETHYLENE RESPONSE FACTORs (ERFs) which eventually leads to differential gene expression and a physiological response.[15]

The function of ethylene receptors too, is subject to modification from various proteins such as RESPONSIVE TO ANTAGONIST1 (RAN1),[28,29] REVERSION TO ETHYLENE SENSITIVITY1 (RTE1),[30–32] and their own histidine autokinase activity.[33] Receptors often act simultaneously, and in physical association with each other,[34,35] but as with the ethylene biosynthesis enzymes, different receptor homologs are expressed depending on the developmental stage of the flower, as has been documented in *D. caryophyllus*[36], *R. hybrida*,[37] *Oncidium*,[38] *Delphinium*,[39] and *Pelargonium hortonum*.[40]

The function of EIN2 can be inhibited not only by CTR1 but also ETHYLENE RESISTANT/ETHYLENE RECEPTOR1 (ETR1)[41] and EIN2 TARGETING PROTEIN1 (ETP1) and ETP2. *ETP1* and *ETP2* were down-regulated in the presence of ethylene,[42,43] but activated *CTR1* in the absence of ethylene.[44] Unlike most of the other genes of the ethylene pathway, *EIN2* has no homologs and no functional overlap with other genes.[45–47] There is some indication that *EIN2* expression is influenced by auxin and abscisic acid (ABA),[48] which means that *EIN2* could be a point of crosstalk for several different pathways. Interestingly, increase in EIN2 protein levels has been shown to be concomitant with expression of the transcription factor ORESARA1 (ORE1) in *Arabidopsis*, associated with age-induced programmed cell death. ORE1 is suppressed by miRNA164, which declines with cell age.[49] In *R. hybrida* flowers exposed to ethylene, different miRNAs including miRNA164, exhibited a change in expression level in petals,[50] indicating a possible new level for expression control of ethylene genes.

EIN3/EIL homologs also change in the transition of the plant tissue to the climacteric phase. In *D. caryophyllus*, *DcEIL3* increased[51] and *DcEIL1* decreased[52] in expression as flower development occurred. Tanase *et al.*[53] on the other hand documented that *DcEIL3* expression did not change with age in petals, but *DcEIL4* was expressed less in older flowers. In *P. suffruticosa*, *PsEIL1* accumulated from the flower opening stage to senescence, while *PsEIL2* and *PsEIL3* decreased after flower opening.[54] *PsEIL2* and *PsEIL3* mRNA levels increased in response to exogenous ethylene, while *PsEIL1* was unaffected by this treatment and plants treated with 1-methylcyclopropene (1-MCP) and exposed to ethylene, exhibited a decrease in *PsEIL3* expression.[54] In *Oncidium gardneri*, *Og*EIL1 and *Og*EIL2 were constitutively expressed in flower buds, but when the buds were exposed to ethylene, *OgEIL1* clearly peaked in expression relative to *OgEIL2*.[55] Collectively, these genes present numerous targets for modifying ethylene regulation molecularly.

REDUCING ETHYLENE-PROMOTED SENESCENCE

Approaches addressing the problem of ethylene-induced senescence can be broadly divided into two main groups: interventions directly addressing the ethylene pathway and those indirectly targeting it as described below. Because the ethylene pathway is fundamentally integrated into plant metabolism, changing a seemingly unrelated pathway of the plant can result in some effect on the ethylene pathway. Techniques to combat senescence such as changes in the gas composition of the atmosphere or temperature of storage places for plants have also been examined and gave mixed results.[56–58] These techniques, although successful to a point, lead to unsustainable production of ornamental plants because they extend the time needed for production and cost in terms of consumption of electricity, water, personnel, and specialized equipment. Silver thiosulfate (STS) and 1-MCP have been proven to be powerful inhibitors of ethylene responses and STS is commonly used in ornamental plant production. However, silver is harmful to humans and the environment and thus its use should be

avoided. Plant breeding on the other hand strives to improve the intrinsic quality of ornamental plants and thereby produce sustainable products.[59]

TARGETING THE ETHYLENE PATHWAY DIRECTLY

Cross-pollination coupled with ethylene screening

The simple strategy of choosing individuals in a population that display superior flower ethylene tolerance and crossing these individuals with each other, will result in progeny with a lower ethylene sensitivity than the original population, if the trait is heritable.[60] Success in such breeding has been reported in *Begonia*[61] and *D. caryophyllus*.[62] It has long been known that there are vast differences among cultivars of *D. caryophyllus* in their ethylene biosynthetic ability and affinities of receptors to ethylene.[63] By using only simple pollination, Onozaki and coworkers[62] achieved a significant improvement in the longevity of *D. caryophyllus* flowers from 1992 to 2004. Their original material had flower longevity of 5.7 days,[64,65] but through repeated crossing, the sixth generation had increased its mean of flower life to 15.9 days.[62] An investigation into the cause of this increase, revealed that the ethylene biosynthesis enzyme genes *DcACS1*, *DcACS2*, and *DcACO1* were all expressed in extremely low levels in cultivars with a good longevity and ethylene production was as low.[66,67]

This approach involves screening for ethylene insensitivity and will only be applicable if a population exhibits significant differences in response between individuals. The number of ornamental flower cultures showing such diversity is, however, very large. Differences in longevity of flowers have been noted for *Paeonia*,[68] *Delospermum*, *Campanula*, *Sedum*, *Cephalaria*, *Lobelia*, *Armeria*, *Primula*, *Penstemon*,[69] *Lisianthus*, *Trachelium*, *Zinnia*,[70] *Potentilla*, *Lysimachia*, *Veronica*, *Chelone,*[71] and *Rosa*[11,72,73] which also exhibited varying ethylene production levels.[73–76] Similar observations has been made for *Pelargonium*,[77] where heritability has also been documented,[78] as it has been for *Impatiens walleriana*,[79] *Antirrhinum majus*,[80–82] *Dianthus barbatus*,[83] and *Petunia*.[84] In many ornamental plant genera, cultivar-specific variation in ethylene sensitivity has been demonstrated including *Phalaenopsis*[85,86] and *Kalanchoë*.[87]

Hybridization

Many of the abovementioned genera are typically represented by hybrids in ornamental plant production. Hybridization is achieved by interspecific cross-pollination and it thus produces extremely heterogeneous progeny, which may be good for producing cultivars with higher ethylene tolerance. A well-documented example of this is found in Australian waxflower breeding where *Chamelaucium* species were hybridized with *Verticordia plumosa*, forming more ethylene-insensitive plants.[88] Hybrids of *Leptospermum* species[89] and *Grevillea*[90] similarly have been reported to have longer longevity than the parental species, which may be correlated with higher ethylene tolerance as both genera are climacteric. This aspect, however, has not yet been investigated.

The major disadvantage of both intra- and interspecific cross-pollination is that it cannot be used directly for improving existing cultivars. Considerable back-crossing to the original plant may be necessary.[91] This problem can be solved to some extent by employing marker-assisted selection, if reliable markers are found[92] and have been used successfully in ornamentals such as *D. caryophyllus* with regards to bacterial wilt resistance.[93] Hybridization can be more demanding as manipulation of the style as well as embryo rescue or ovule and ovary culture may be necessary in order to yield any progeny, as exemplified in *Kalanchoë* species hybridization.[94]

Conventional mutagenesis

Techniques that increase genetic variability where no natural variation exists are found in conventional breeding with the use of mutagenic chemicals or radiation.[95] Such breeding strives to change the cultivar in a certain qualitative aspect and leaves its agronomical traits unchanged, making it easier for producers to handle, and saves time that would otherwise be spent on back-crossing.[71,96] However, as it is impossible to control where the mutation occurs in the genome, many plants will have to be screened before a plant exhibiting any change in the relevant trait is found.[97] Targeting Induced Local Lesions In Genomes (TILLING) can be used to save time that would otherwise be needed for phenotyping the plants and has been used successfully in the field of ethylene-response improvement. Dahmani-Mardas *et al.*[98] produced *Cucumis melo* with a knockout mutation in the *CmACO1* gene which displayed a longer shelf life and better firmness than none-mutated plants. TILLING is readily applicable to ornamental plants as is exemplified in *Petunia*.[99]

Genetic transformation

Genetic transformation is the transfer of foreign or native genes and promoters to a target genome by *Agrobacterium*-mediated transformation, particle bombardment, or infiltration. *Agrobacterium tumefaciens*-mediated transformation with recombinant DNA has been used in various ways to modify ethylene biosynthesis and signaling. These approaches have improved understanding of ethylene biosynthesis and signaling; however, their use in commercial breeding is limited and this technique's products are considered genetically modified.[59,100]

Antisense or sense transformations of *ACS* and *ACO* genes have been successfully attempted in various ornamental species including *D. caryophyllus*,[101–113] *Torenia*,[104] *Petunia*,[22,105] and *Begonia*.[60,106] All transformed plants exhibited longer shelf life, presumably due to lower production of ethylene. Klee *et al.*[107] conducted an alternative modification of the biosynthesis pathway with the removal of ACC by the addition of the enzyme ACC deaminase, found in bacteria, to *Solanum lycopersicum* which significantly delayed ripening of the fruit. However, even though plants with lower ethylene production have a longer flower life, their quality can still be negatively affected by exogenous ethylene.

Transformations of *Petunia* with the *Atert1-1* mutated gene from *Arabidopsis* using CaMV 35S constitutive promoter, resulted in plants with considerably higher ethylene tolerance but also with severely hampered growth.[108] Succeeding studies used a flower-specific FBP1 promoter in *Kalanchoë*,[109] *Campanula*,[110,111] and *D. caryophyllus*,[112,113] which provided a better flower longevity without other developmental effects, since the promoter ensured expression solely in flowers. Transformations of mutated genes other than *Atetr1-1* have also been studied in *Nemesia strumosa*[114] and *Torenia fournieri*. In both cases, ethylene insensitivity was increased, but not as much as with *Atert1-1* (Table 1).[8]

Other promoters such as FBP3,[115] P_{SAG-12},[116] FS19, and FS26[8] that are solely associated with flower tissue, also have potential to be used in this context and other promoters are still being investigated.[117] Chemically inducible promotor systems are also within the reach of today's technology, and are exemplified by the DEX-inducible system, demonstrated in *Petunia hybrida*[118] (Table 1), and more famously, the ethanol-inducible system[119] which has been successfully used in different crops.[120] Inducible systems seem not to affect the plant adversely,[121] but the inducing chemical still needs to be applied to the plant in due time, which makes such a system commercially less appealing.

Great care needs to be exercised when choosing a promoter, as a study by Cobb *et al.*[122] demonstrated that *Petunia* transformed with *Atetr1-1* did not have delay in senescence under the flower-specific APETALA3 (AP3) promoter, as this promoter only drives expression in buds and young flowers, but not in mature flowers (Table 1).

Overexpressing *PhEIN2* in *Petunia* using the CaMV 35S promoter has also been attempted. This produced plants with significant

Table 1. Transformation of ornamental plants modifying ethylene receptors

Promoter	Gene	Plant species	*Agrobacterium tumefaciens* strain	Plasmid	Reference
CaMV 35S	*Atetr1-1*	*P. hybrida*	ABI	pMON11063	108
Petunia FBP1 CaMV 35S	*Atetr1-1*	*D. caryophyllus*	AGL0	pBEO210	
				pBEO220	112
AP3	*Atetr1-1*	*P. hybrida*	?	?	116
CaMV 35S	*Brassica oleracea ers* (*boers*)	*P. hybrida*	LBA4404	pBOERS4421	123
CaMV 35S	*CmETR1/H69A*	*N. strumosa*	GV2260	pBICm-ETR1/H69A	113
CaMV 35S	*CmERS1/H70A*	*Lotus japonicus*	*Mesorhizobium loti*, MAFF303099	pGD499	124
Petunia FBP1	*Atetr1-1*	*Kalanchoe blossfeldiana*	AGL0	pBEO210	109
Petunia FBP1	*Atetr1-1*	*Oncidium*, *Odontoglossum*	EHA105 and LBA4404	pBEO210	125
Petunia FBP1	*Atetr1-1*	*Campanula carpatica*	AGL0	pBEO210	110
CaMV 35S	*DcETR1nr*	*T. fournieri*	?	pBIDc-ETR1nr	53
GVG DEX-inducible	*Atetr1-1*	*P. hybrida*	LBA4404	pTA7001	118

delays in petal senescence in response to exogenous ethylene or pollination, as well as inhibited root development and a shorter life span.[126] When this silencing transformation was combined with the *Atetr1-1* mutation, ethylene was inhibited even more,[127] demonstrating the possibility of increasing insensitivity by stacking transformations of several components simultaneously.

Petunia plants transformed with a sense *PhEIN3* exhibited delayed petal senescence, but no increase of flower longevity,[8,127] which may possibly be due to the redundancy found among EIN3/EIL members. *S. lycopersicum* was transformed with a construct silencing EIN3 BINDING F-BOX1 (EBF1) and EBF2, which rapidly target EIN3/EILs for degradation that significantly increased the rate of senescence in *S. lycopersicum*.[45] This presents an opportunity for future research, potentially by overexpression of EBFs.

ERFs are a sizable and diverse group of genes, many of which are up-regulated in response to ethylene, but have largely been overlooked by researchers so far. Chang *et al.*[128] observed that by silencing the ERF *Petunia* transcription factor homeodomain-leucine zipper protein (PhHDZip), *PhACS* and *PhACO* were decreased in expression in transformed flowers and led to an increase of flower longevity by 20%.

Genome-editing technologies

During the last decades, several innovative biotechnologies have been developed which appear to have potential in breeding toward ethylene tolerance.[100,129,130] These technologies are based on engineered nucleases that cleave DNA in a sequence-specific manner, thus enabling targeted genome-editing. Modifying or inactivating specific gene function is possible due to sequence-specific DNA binding domains or RNA sequences. The oldest of these techniques is zinc finger nucleases (ZNFs), which relies on an engineered endonucleases that are able to attach to a specific target sequence and induce a double-strand break. This is perceived by the cell, which repairs the break by mechanisms which may cause sequence alterations or introduce small templates.[131,132] A newer alternative to ZNFs is transcription activator-like effector nucleases (TALENs), which functions in much the same manner as ZNFs. Recently, however, the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein9 (CRISPR/Cas9) system has been developed. The CRISPR/Cas9 system functions via a mechanism similar to RNA interference, which can recognize and cleave foreign DNA.[100] Both TALEN and CRISP/Cas9 have been demonstrated to be functional for targeted mutagenesis in *S. lycopersicum*,[133,134] however, no modifications of the ethylene pathway have yet been conducted. It is important to mention that all these methods rely on a technology in order to deliver the construct to the genome of the plant, and are therefore still limited to plants where a genetic transformation, regeneration or virus-based delivery system is reliable. In order to bypass negative effects on growth of plants due to defective ethylene signaling, the genes that should be targeted are those which have homologs that are clearly associated with the climacteric phase. Using this approach, the pathway should function normally under growth and development. Therefore, certain homologs of ACS, ACO, ethylene receptors, and EIN3/EIL may present good targets for knockouts.

TARGETING THE ETHYLENE PATHWAY INDIRECTLY

Hormonal interaction

Application of cytokinins such as kinetin and zeatin to petals of *D. caryophyllus* delayed the conversion of ACC to ethylene.[135] Cytokinin oxidase/dehydrogenase, responsible for cytokinin degradation, was up-regulated during senescence in *D. caryophyllus*[136] and *Petunia*,[137] and when it was inhibited in *D. caryophyllus* petals, the senescence phase was prevented.[138] This knowledge has been applied by transformations with isopentenyl transferase, important for the synthesis of many cytokinins, under the control of senescence-associated promoter P_{SAG12}. Transformed *Petunia* plants exhibited elevated levels of cytokinins in flowers and significant delay in senescence and ethylene production as well as higher tolerance for exogenous ethylene.[139] Similarly in *Rosa*, ethylene sensitivity decreased in leaves, but flowers were not studied.[140]

Exogenous application of hormones and other substances have also been shown to decrease the expression of genes of the ethylene pathway including ABA, which inhibited *ACS* and *ACO* in *Hibiscus*[141] and *D. caryophyllus*.[142] Moreover, nitric oxide down-regulated the activity of *Rh*ACO and lowered the production of ethylene resulting in longer shelf life of *R. hybrida* flowers[143] and glucose down-regulated *PsACS1* in *P. suffruticosa* flowers.[144] Other sugars have also been shown to increase flower longevity.[136,145–148] These studies demonstrate that there is a vast potential in exploring new ways to achieve products of higher quality.

Senescence-related and non-ethylene pathway genes

Exploration of different genes which is seemingly not connected to the ethylene pathway has also been pursued. There are three main strategies in this area. The first is identification of genes that are highly expressed in young tissue but not during senescence, and constitutively overexpressing those. The second approach goes the other way and starts with the identification of genes highly expressed in senescing tissue, and silencing their expression. The third tactic is targeting protein synthesis.

The first strategy can be exemplified by the gene *FOREVER YOUNG FLOWER (FYF)* from *Arabidopsis*, which was highly expressed in young flowers but not in old flowers. Transformation using this gene under constitutive expression in *S. lycopersicum* resulted in

down-regulation of various *ACS* and *ACO* homologs. The same transformation in *Eustoma grandiflorum* caused a delay in senescence and down-regulation of *ERFs*.[149] Furthermore, when combined with *etr1*, *ein2*, or *ctr1* mutations, it further enhanced flower longevity in *Arabidopsis*.[149]

Investigating a MADS-domain transcriptional regulator, AGAMOUS-LIKE-15 (AGL15), Fernandez *et al*.[150] noted its accumulation in young tissue and developing organs. Constitutive overexpression of *AGL15* increased the longevity of petals significantly in *Arabidopsis*[151] and caused a repression of *GmACO1* in *Glycine max*.[152] It is now established that AGL15 has similar activity as AGL18, both serving as repressors of early flowering and in this way leading to better longevity if still expressed when flowering does occur.[153] Another MADS-domain transcriptional regulator that has been recognized to be associated with young flowers of *Phalaenopsis equestris* is PeMADS6. *Arabidopsis* plants transformed with a construct overexpressing *PeMADS6* exhibited flower longevity up to four times longer than wild type plants.[154]

Examples from the second strategy of silencing senescence-related genes, include *ACTIN-RELATED PROTEIN 4* (*ARP4*), a chromatin modification gene, that has been silenced using RNAi in *Arabidopsis*,[155] which resulted in longer flower life. The gene *MjXB3*, coding for a RING zinc finger ankyrin repeat protein, has been demonstrated to be highly expressed in senescing flowers of *Mirabilis jalapa*, *P. hybrida*, and *D. caryophyllus*. Using virus-induced gene silencing for *MjXB3* in *P. hybrida*, Xu *et al*.[156] demonstrated that flower longevity was extended by two days, corresponding to a 20% increase compared to wild type flowers.

The third strategy of targeting protein synthesis owes its inspiration to ethylene-insensitive flowers,[13] where complete inhibition of protein synthesis in flowers increases flower longevity.[157] The relevance of this to climacteric flowers has been attested by silencing *PBB2* (coding for the beta subunit of the 26S proteasome) using an inducible system in *Petunia*.[158] Mature cut flowers that were induced lasted considerably longer than uninduced flowers. Reid and Jiang[13] demonstrated the same concept by silencing one of the ribosomal subunit genes (*RPL2*), again using an inducible system. Once more, the longevity of cut *Petunia* flowers placed in water with the inducing agent was much better than those uninduced. This approach, however, still relies on the application of an inducer, and is not realistic for use in commercial potted-plant production. For the cut-flower industry, however, there may be great potential.

Natural transformation

Transformation with the naturally occurring soil bacterium *Agrobacterium rhizogenes* has been termed natural transformation[159] due to the fact that no recombinant DNA is used and the infection is a natural process. Authorities in Denmark have confirmed that naturally transformed plants are not covered by the GMO legislation in Europe.[59] Using a wild strain of *A. rhizogenes*, Lütken *et al*.[59,160] transformed *Kalanchoë* plants and observed that plants which contained *rol*-genes from *A. rhizogenes* exhibited significantly increased postharvest quality,[159] an ability which was maintained through two generations along with the *rol*-genes.[161] The mechanism behind this response is yet unknown and many open reading frames of the plasmids in question still remain uninvestigated. Changes in longevity of flowers transformed with *rol*-genes may be due to altered hormone homeostasis and/or sugar metabolism and transport.[159]

DISCUSSION

Strategies targeting the ethylene pathway indirectly seem to be as effective as those which target it directly. Of the indirect strategies, ectopic overexpression of genes associated with juvenile tissue, such as *FYF* or *AGL15*, seems to be particularly promising, as is the prospect of overproduction of hormones that are antagonistic to ethylene, such as cytokinins and ABA. Strategies that focus on the ethylene pathway directly have been more numerous and have targeted nearly all known elements of the ethylene pathway. Research comprising flower-specific promotor sequences in genetic transformations seems to be especially propitious in providing the ability to express the transferred gene only in flowers or even more specifically, only in senescing flowers. Silencing several genes simultaneously in the flowers of plants as in *Petunia*,[119] may be the ultimate answer, and is a feature that is well within the grasp of current technology and methodology. Various combinations of overexpression of certain genes associated with development and silencing genes associated with senescence may very well be a worthwhile strategy as well.

It is remarkable that both conventional techniques such as simple cross-pollination and genetic transformation using recombinant DNA succeeded in breeding ornamental plants which have higher tolerance for ethylene and thus higher intrinsic quality. *Agrobacterium*-mediated transformation, where reliable, has the advantage that breeding can be much faster when transferring specific target genes. Breeding based on genetic transformation is more precise and can easily cross incompatibility boundaries in comparison with conventional crossing.[91] This is a particularly important advantage in ornamental plants that have long development cycles such as orchids,[162] however, in fast growing plants, this advantage is less substantial.[60]

Although conventional and genetic transformation are not mutually exclusive, the fact remains that ornamental plant products that are genetically modified are conspicuously missing on the market, especially in Europe, with only a handful of products sold worldwide.[163] The basic reason for this is the status of such product as genetically modified organisms (GMOs). Europe, the biggest consumer, producer and breeder of ornamentals has some of the most comprehensive and strict legislation concerning GMOs.[1] At the moment, the approval of GMO products is conducted on a case-by-case basis and is both expensive and time-consuming.[164] Moreover, the technology and training required for producing GMOs are themselves expensive, which brings a substantial economic burden.[60] Producers not operating in Europe also face difficulties as GMO legislation may be quite different from country to country.[165] However, the emergence of new genome-editing techniques presents new challenges particularly for European legislation in the area of GMOs, which could lead the way to broader acceptance of new breeding techniques and their products.

Techniques such as ZFN, TALENs, and CRISPR/Cas9 that can be used to achieve precise mutagenesis and silencing or overexpressing genes are now readily available, and can be used in such a way that does not introduce foreign elements to the genome of the final product.[127,166] The question of whether the products of these techniques are considered GMOs or not has not yet been settled, and jurisdictions that consider the methods alone rather than the actual properties of the final plant product may end up with ineffective legislation that cannot be enforced, as pointed out in Seeds of Change.[167] Certainly such techniques have the ability to greatly reduce the time, and thus the bulk of the cost which is associated with plant breeding. The first commercially available plant derived from a genome-editing technique, a herbicide-resistant oilseed rape, has already been announced.[168] There are still technical difficulties associated with the actual transformation step for many ornamental plants, which can only be overcome by further research. For this reason, it is must be of the utmost importance for any plant breeding company to devote some of its resources to research in such new breeding techniques.

CONCLUSIONS

Strategies targeting the ethylene pathway directly seem to have as much potential as those targeting indirect components, and very

diverse species of ornamental plants suggest great potential in ethylene tolerance breeding. Although conventional breeding techniques are slower than newer breeding techniques, they remain the most used in ornamental plant breeding for higher ethylene tolerance. This is most probably due to the legal status of genetic modification approaches. However, as newer precise genome-editing techniques become available, it is very likely that the products of such techniques will be accepted worldwide. The reason for this is that such plants would be indistinguishable from plants derived by the use of conventional breeding techniques. It is therefore recommendable for ornamental plant breeders to begin the implementation of such new breeding technologies, as they have great to potential to lead to superior plant products needed by the ornamental plant industry.

COMPETING INTERESTS

The authors have no conflicts to declare.

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