

Genetic regulation of ethylene perception and signal transduction related to flower senescence

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Abstract

The plant hormone ethylene is involved in the regulation of a number of physiological and developmental processes and its role as a regulator of flower senescence is of particular economic importance in floriculture. The present review focuses on genetic control of flower senescence, particularly at the level of ethylene perception. Research in a number of flowering plants has indicated that flower senescence is the result of a complex regulation of both ethylene biosynthesis and perception. In the present review, we outline current knowledge of ethylene signal transduction in the model plant *Arabidopsis* and in economically important ornamental flowers. The various members of the ethylene receptor families in carnation and rose are differentially regulated during flower senescence and provide evidence that regulation of receptor gene expression can modulate sensitivity and influence the response of the flowers to ethylene. Several approaches to decrease ethylene sensitivity and improve postharvest quality have been reported, such as the use of antisense RNA for ethylene biosynthesis genes or transformation with a gene coding for a mutant receptor that does not bind ethylene and which constitutively suppresses the normal ethylene responses. Further basic research on the mechanism of ethylene signal transduction will probably reveal further possibilities for biotechnological modifications that might complement traditional breeding methods to improve postharvest quality.

Key words: CTR, *Dianthus caryophyllus*, ethylene, EIN, ETR, flower senescence, postharvest, receptors, *Rosa hybrida*.

Introduction

The plant hormone ethylene is involved in the regulation of a number of physiological and developmental processes such as growth, seed germination, flower initiation, senescence of leaves and flowers, organ abscission, fruit ripening, adventitious root formation, pathogen attack and stress responses¹. The role of ethylene as a regulator of fruit ripening and flower senescence is of particular economic importance in horticulture, especially in the flower industry. The present review focuses on the genetic control of flower senescence. Findings in *Arabidopsis* are presented to give an overview of the present knowledge in the field, which is an essential starting point for research approaches aiming in genetic modification to improve postharvest quality. The elucidation of ethylene biosynthesis, perception and signal transduction pathways may suggest targets to selectively block those processes in flower development and senescence that are detrimental. Biotechnological interventions to abrogate ethylene synthesis or to reduce sensitivity to ethylene, preferably confined to specific tissues, may result in improved postharvest performance for those horticultural crops whose senescence processes are regulated by ethylene². Ethylene biosynthesis is well characterized and the genes coding for the enzymes involved have been isolated from a large number of plant species (Figure 1). Ethylene is synthesized from methionine through the intermediates S-adenosyl methionine (SAM) and the cyclic amino acid 1-aminocyclopropane-1-carboxylic acid (ACC). The enzyme converting methionine to SAM is SAM synthetase, while ACC synthase converts SAM to ACC and S-adenosyl methanethiol. ACC is oxidized to ethylene, HCN and CO₂ by ACC oxidase, with methanethiol being

reincorporated into methionine via the Yang cycle. The genes encoding ACC synthase and ACC oxidase have been cloned from a variety of plant species and have been shown to be members of multigene families in all species investigated to date³. The two reactions catalysed by ACC synthase and ACC oxidase are generally accepted as being rate-limiting for ethylene production^{4,5}. A wide variety of external and internal stimuli have been shown to induce the expression of the ethylene biosynthetic genes and, in addition, posttranscriptional regulation seems to play an important role in regulation of ethylene biosynthesis⁶. Under some conditions ACC may be converted into N-malonyl ACC by ACC N-malonyltransferase³ or into 1-(gg-L-glutamylamino) cyclopropane-1-carboxylic acid (GACC)⁷. These processes are also thought to play a role in the control of ethylene biosynthesis. Research on carnation^{8,13}, geranium^{14,15}, orchids^{16,17}, *Petunia*^{18,19}, and rose^{20,23} has indicated that flower senescence is the result of a complex regulation of both ethylene biosynthesis and perception. This review is mainly concerned with the regulation of the genes of ethylene perception and signal transduction during flower senescence. Much progress has been made in the elucidation of the ethylene signal transduction pathway from molecular studies using the model plant *Arabidopsis*. Present knowledge of ethylene receptor genes and other genes involved in the ethylene signal transduction pathway in *Arabidopsis* is summarised in this review and used as a basis for discussion of recent findings related to the genetic regulation of flower senescence. Finally, various approaches for improvement of the postharvest quality in ornamentals by genetic transformation are discussed. Strategies to modify the ethylene biosynthesis pathway or ethylene perception are reviewed and

discussed in relation to the current standard model for ethylene signal transduction.

The Ethylene Receptor Gene Family

In *Arabidopsis*, ethylene response mutants have been identified by screening for alteration of the so-called “triple response”, which refers to morphological changes in dark grown seedlings in response to ethylene, viz. larger radius and shorter length of hypocotyl, exaggeration of curvature of apical hook and horizontal growth. The ethylene-insensitive mutants, e.g. *etr1-1*, do not exhibit a triple response²⁴. The gene *ETR1* encodes a protein similar at the amino acid level to bacterial two-component sensor response-regulator systems, which acts early in the signal transduction pathway as an ethylene receptor using the phosphotransfer mechanism established for bacterial sensors and receivers^{25,29}. *ETR1* has three main domains (Figure 2), viz. in the N→C direction a sensor domain with putative membrane-spanning regions, an His kinase domain and a receiver domain^{25,30}.

To date, five members of the putative ethylene receptor gene family in *Arabidopsis*, belonging to two subfamilies, have been described (Figure 3). Subfamily 1 consists of *ETR1* and *ERS1*, and subfamily 2 of *ETR2*, *EIN4*, and *ERS2*. Members of the same subfamily have higher amino acid sequence similarity to each other than to members of the other subfamily and each subfamily has distinct conserved intron positions that are not shared between the two subfamilies. Subfamily 1 receptors have three putative membrane-spanning regions in the N-terminus and a well-conserved His-kinase domain, whereas subfamily 2 receptors have four putative membrane-spanning regions and lack some of the His-kinase hallmarks^{25,26,31,32}. The division in subfamilies is not related to the presence or absence of the receiver domain, because the two members without the receiver domain (*ERS1* and *ERS2*) belong to different subfamilies^{33,34}. Some mutations in the membrane-spanning receptor domains result in dominant ethylene insensitivity^{34,35}, indicating that these proteins are likely to function as ethylene receptors. Loss of function single mutants in each of the *Arabidopsis* *ETR1*, *ERS1*, *ETR2*, *EIN4*, and *ERS2* genes did not exhibit defects in ethylene responses. However, mutants in three or four of these genes have constitutive ethylene phenotypes in the absence of ethylene, indicating that these genes collectively negatively regulate ethylene responses³⁴.

In tomato, a similar family of two-component ethylene receptors has been characterized, from which six putative receptor genes have been cloned, *LeETR1*-*LeETR6*³⁷⁻⁴³. *LeETR3* was identified as the gene *NR* associated with the Never-Ripe phenotype⁴⁴. Predicted protein sequences of *LeETR1* and *LeETR2* are highly homologous to *Arabidopsis* *ETR1*, while *NR* exhibits similarity to *Arabidopsis* *ERS1*, which also lacks the response regulator domain⁴¹. Ethylene receptors have been shown to act as negative regulators of ethylene responses in *Arabidopsis* and tomato^{36,45,46}. Simultaneous loss-of-function mutations in four tomato receptor genes resulted in constitutive ethylene responses such as leaf epinasty and accelerated flower senescence⁴⁵. Furthermore, transgenic tomato plants overexpressing one receptor gene showed an ethylene insensitive phenotype⁴⁶. These results indicate an inverse relationship between the level of ethylene receptors and the sensitivity to ethylene. A reduction in the amount of

ethylene receptor protein should thus result in increased ethylene sensitivity.

From Ethylene Perception to Response: Genes involved in Signal Transduction

Several molecules involved in the transduction of ethylene responses downstream of the receptors have been identified in *Arabidopsis*⁴⁷ (Figure 2). *CTR1* encodes a putative protein kinase with homology to the mammalian Raf protein kinase (MAPKKK) family. Loss-of-function *CTR1* mutants result in a constitutive ethylene-responsive phenotype in the absence of ethylene, indicating that *CTR1* negatively regulates the ethylene-response pathway^{2,48}. The fact that *CTR1* acts downstream from the ethylene receptors has been further confirmed by epistasis analysis, and *CTR1* may be part of a MAP kinase cascade^{2,49}. Yeast two-hybrid and *in vitro* binding assays indicate that C-terminal regions of both *ETR1* and another ethylene receptor *ERS1* interact directly with *CTR1*⁵⁰. *EIN2* and *EIN3* are proteins acting downstream of the *CTR* proteins. *EIN2* acts downstream to *CTR*, and possibly to other unidentified members of a MAP kinase pathway, and upstream to *EIN3*^{31,47}. *EIN2* is an integral membrane protein with 12 putative membrane-spanning domains in the amino-terminal part of the protein, which exhibits sequence similarity to a mammalian family of metal-ion transporters⁵¹. The gene *Ethylene-Insensitive3* (*EIN3*) is genetically epistatic to *ETR1*, *CTR1* and *EIN2* and functions downstream to these in the signalling pathway. As for *ETR1*, *EIN3* mutants show severely decreased ethylene response⁵². In *Arabidopsis*, three other genes, encoding the *EIN3*-related products *EIL1*, *EIL2* and *EIL3*, have been characterized. *EIN3* and *EIL1* were shown to be localized exclusively in the nucleus when they were expressed as a fusion protein with the GUS reporter protein in *Arabidopsis* protoplasts, suggesting that these proteins are transcription factors⁵². The primary target of *EIN3* seems to be the gene *Ethylene-Responsive-Factor1* (*ERF1*), an early ethylene-responsive gene encoding a GCC-box-binding protein⁵³. *ERF1*, like *EIN3*, is a positive regulator of ethylene signalling.

Ethylene Receptor Gene Expression during Flower Senescence

The existence of ethylene receptors with homology to the ethylene receptor family in *Arabidopsis* has been described for the ornamentals carnation^{11,54,55}, rose^{20,21}, and geranium⁵⁶. In carnations, three ethylene receptor genes *DC-ERS1*⁵⁴, *DC-ERS2*¹¹ and *DC-ETR1*⁵⁵ have been identified so far (Figure 3). Northern analysis during flower senescence revealed that *DC-ERS2* and *DC-ETR1* mRNAs were present in considerable amounts in petals, ovaries and styles of the flower at the fully-opened stage. In the petals the level of *DC-ERS2* mRNA decreased as flower senescence progressed, whereas it increased slightly in the ovaries and was unchanged in styles throughout senescence. Levels of *DC-ERS2* and *DC-ETR1* mRNAs in petals decreased inversely with the increasing ethylene production by the flowers, while *DC-ERS1* mRNA was not detectable at any time. Exogenously applied ethylene did not affect the levels of *DC-ERS2* and *DC-ETR1* mRNAs in the petals, indicating that *DC-ERS2* and *DC-ETR1* genes are not subject to positive regulation by ethylene. In addition, the level of these transcripts decreased independently of ethylene production in the petals

of flowers treated with 1,1-dimethyl-4-(phenylsulphonyl) semicarbazide (DPSS), which blocks ethylene production. It remains to be resolved whether the decrease in the levels of these mRNAs was caused by down-regulation of the expression of the genes or by stimulation of mRNA degradation. The results in *Dianthus* indicate that *DC-ERS2* and *DC-ETR1* are ethylene receptor genes responsible for ethylene perception and that their expression during flower senescence is regulated in a tissue-specific manner and independently of ethylene. According to the negative regulator model, the decrease in the level of *DC-ERS2* mRNA in petals may cause an increase in sensitivity to ethylene and hence accelerated petal wilting in carnation flowers during senescence¹¹.

In miniature roses, fragments of rose ethylene receptor genes have been isolated and characterisation of the genes suggests that in *R. hybrida* an ethylene receptor gene family with 2 subfamilies exists, as in *Arabidopsis* (Figure 3). Fragments of four rose ethylene receptor genes, termed *RhETR1-4*, have been cloned^{20,21}. *RhETR1* and *RhETR4* exhibit high amino acid similarity to *AtERS1*, while *RhETR2* is more similar to *AtETR1*. Three of the receptors belong to subfamily 1, whereas *RhETR3* belongs to subfamily 2. To analyze the observed differences in flower longevity and ethylene sensitivity in miniature roses, northern analysis was conducted for *RhETR1-3* during flower senescence after treatment with ethylene and ABA. The relative transcript abundance in the flowers varied significantly during development and after hormone treatment, but there was detectable expression of all three genes at all flower stages, and after ABA and ethylene treatments. Exposure to low ethylene concentrations resulted in an upregulation of *RhETR1* and *RhETR3* in flowers of both of the cultivars analyzed. It was assumed that cultivar differences in flower sensitivity to ethylene may partly be due to differences in receptor expression levels during flower development^{20,21}.

While *RhETR2* expression varied little during flower development and in response to ethylene and ABA treatment, *RhETR1* and *RhETR3* exhibited differential expression during flower development and appeared to be rate-limiting for ethylene perception and determinants of flower longevity. Expression of *RhETR1* was distinctly higher in the cultivar 'Bronze', with short flower life, than in the long-lasting 'Vanilla'. While expression of *RhETR1* preceded the ethylene production by the flowers, abundance of the *RhETR3* transcript increased during flower senescence in 'Bronze', indicating that the ethylene response system in rose flowers is composed of multiple receptor types with overlapping patterns of expression. In 'Vanilla', a cultivar which has excellent flower longevity despite moderate ethylene production^{20,21,57,58}, expression of *RhETR1* and *RhETR3* was reduced. These results indicate that differences in flower life among rose cultivars – in an ethylene free environment and in response to exogenous ethylene – may be due to differences in receptor expression levels. These results do not conform to the standard model of ethylene signal transduction³¹, which predicts that a reduction in the level of receptors would result in increased ethylene sensitivity, while an increase in receptor numbers would result in decreased sensitivity. In geranium two ethylene receptor genes, *PhETR1* and *PhETR2*, have been isolated⁵⁶, and the deduced amino acid sequences of the proteins were 78% and 79% identical, respectively, to *ETR1* from *Arabidopsis thaliana* (Figure 3). *PhETR1* and *PhETR2* are members of a multigene family and are expressed at moderate levels in leaves, pedicels, sepals,

pistils and petals, and at very low levels in roots. *PhETR1* and *PhETR2* mRNAs are expressed in geranium florets long before these are receptive to pollination, and the transcript levels remain constant throughout floral development. RNA levels for *PhETR1* and *PhETR2* in pistils and receptacles were unaffected by self-pollination or by treatment with ethylene that induces petal abscission. The results indicate that the amount of *PhETR1* and *PhETR2* mRNA is not indicative of the level of sensitivity of geranium florets to ethylene. Control of ethylene induced petal abscission in geranium florets may be mediated by another uncharacterized member of the *PhETR* gene family, at the post transcriptional level, or via a downstream component of the signal transduction pathway⁵⁶.

Characterization of Genes for CTR and EIN3-Homologues during Flower Senescence

Two partial *Dianthus* cDNAs encoding CTR homologues have been identified (*DC-CTR1*, Acc.no. AF261147 and *DC-CTR2*, Acc.No. AF261148), but to date no investigation of the expression of these genes has been published. In *Rosa hybrida* two CTR gene homologues have been isolated and characterised, and their expression during flower senescence and in response to ethylene has been examined. *RhCTR1* (GenBank Acc.no. AF 271206; 66% amino acid identity to *Arabidopsis CTR*) levels were upregulated in senescing flowers, while *RhCTR2* (Acc. no. AY029067, 87% amino acid identity to AtEDR1 and 90% identity to LeCTR2) was constitutively expressed during flower senescence. Expression of both genes was increased in response to ethylene, which may indicate a role for these genes in ethylene sensitivity and postharvest performance²². However, assuming that flowers become more ethylene sensitive during senescence, the negative regulator model predicts that CTR expression will decrease as a function of developmental stage and in response to exogenous ethylene. In *Dianthus caryophyllus* a cDNA clone encoding a putative EIN3-like protein, termed *DC-EIL1* (Acc.no. AF2661654), was isolated. The amino acid sequence is 49% and 52% identical with the corresponding regions of *Arabidopsis* EIN3 and tobacco TEIL, respectively¹³. Northern blot analysis revealed that the *DC-EIL1* transcript level decreased in flower tissue, especially in petals, during natural senescence and in response to ethylene and ABA treatment. Since ethylene production increases during natural senescence and after the application of ABA, and exogenous ethylene lowered the mRNA level, it is highly probable that ethylene causes the decrease in mRNA. Thus, the findings in carnations are in contrast to the findings that ethylene does affect the levels of EIN3 transcripts in *Arabidopsis*⁵² or of TEIL transcripts in tobacco⁵⁹. Since EIN3 and its homologues have been shown to act as positive regulators of ethylene responses⁵², the observation in *Dianthus* is the opposite of what might be expected according to the negative regulator model¹³.

In *Rosa hybrida*, a cDNA fragment encoding part of an EIN3 transcription factor homologue, termed *RhEIN3* (Acc. no. AY052825), was isolated. The deduced protein has 83% and 88% identity to the corresponding regions of *Arabidopsis* EIN3 and EIL1, respectively. To investigate *RhEIN3* transcript abundance during flower development, expression studies were conducted in the miniature rose cultivars 'Bronze' and 'Vanilla' at 3 stages of flower development, viz. the bud, the open flower, and the incipient senescence stage. 'Bronze' has short flower life and high ethylene production, while 'Vanilla' has a long

flower life and moderate ethylene production during flower senescence. For the open flower stage, changes in expression of *RhEIN3* after ethylene and ABA treatment were investigated. The gene *RhEIN3* was constitutively and stably expressed during flower development in both cultivars and in response to ethylene and ABA. The constitutive pattern of *RhEIN3* expression could not be correlated with the previously observed cultivar differences in flower life or ethylene sensitivity in flowers or leaves of miniature roses²³. These findings are in accordance with previous findings in *Arabidopsis*⁵² and tobacco⁵⁹ that ethylene does not affect the level of *EIN3* mRNA, and suggest that control of ethylene sensitivity occurs upstream of *EIN3* and its homologues.

Inhibition of Ethylene Biosynthesis or Transformation with *etr1-1* to improve Postharvest Quality

Several studies on the effects of decreased ACC oxidase activity in ornamentals have been performed. Transgenic carnation plants were produced by introducing an antisense carnation ACC oxidase gene under the control of a constitutive promoter⁶⁰. Flowers from one transformant from each of the cultivars 'Scania' and 'White Sim' exhibited lowered climacteric ethylene production and distinctly delayed flower senescence. The vase life of untransformed carnations is about five days, while flowers of the transgenic carnations had a vase life of eight to nine days⁶⁰. In Christmas begonia (*Begonia x cheimantha* Everett) antisense ACC oxidase expression resulted in transgenic plants with improved shelf life^{61,62}. The transgenic lines had a longevity similar to that of non-transformed control plants that had been sprayed with STS (silver thiosulphate) to prevent the action of ethylene. Plants in which ethylene biosynthesis is inhibited, either by chemicals or by genetic modification, remain sensitive to exogenous ethylene. It would therefore be expected that a strategy aiming to blocking ethylene perception is more effective to increase flower life in ornamentals, since this should protect against exogenous as well as endogenous ethylene. Several cases of genetic transformation of horticultural crops with the ethylene-insensitive receptor gene *etr1-1* have been reported. Bovy et al.⁸ found that expression of the *Arabidopsis etr1-1* gene in transgenic carnations delayed flower senescence, resulting in a significant increase in vase life. In about half of the transgenic plants obtained, flower senescence was delayed by at least 6 days relative to control flowers, with a maximum delay of 16 days, a 3-fold increase in vase life. These flowers did not show the phenotype typical of ethylene dependent carnation flower senescence. In *etr1-1*-transgenic carnations flowers, expression of the *ACO1* gene was down-regulated, indicating that the autocatalytic induction of ethylene biosynthesis was absent due to dominant ethylene insensitivity. The delay in senescence observed in *etr1-1* transgenic flowers was longer than in flowers pretreated with inhibitors of either ethylene biosynthesis (AOA) or ethylene receptors (silver thiosulfate)^{8,63,64}.

Agrobacterium-mediated transformation of tomato and *Petunia* plants with the *Arabidopsis etr1-1* mutant gene under control of the constitutive CaMV 35S promoter resulted in ethylene insensitive plants¹⁸. Flower senescence in non-pollinated and self-pollinated flowers was delayed by a week for transgenic ethylene-insensitive *Petunia* compared to untransformed plants. Other aspects of horticultural performance were also altered. Time from seed sowing to first

flower anthesis was decreased by a week, fruit set percentage was slightly lowered and fruit ripening was delayed by up to 7 days in ethylene-insensitive plants compared to untransformed *Petunia*¹⁹. Moreover, ethylene-insensitive plants showed reduced adventitious root formation and produced fewer commercially acceptable rooted cuttings than the untransformed plants^{15,19}. Recently, *Petunia* has been transformed with *boers*, a mutated allele of *BOERS*, an ethylene receptor gene of *Brassica oleracea*⁶⁵. Flowers of transgenic plants remained un wilted and retained pigmentation longer than those of untransformed controls, consistent with observations for *etr1-1* transgenic carnations⁸ and *Petunia*^{18,19}. Furthermore, flowers were unaffected by exogenous ethylene. Excised shoots of transformed plants released more ethylene than the wild type, consistent with findings of Wilkinson et al.¹⁸ Unfortunately, *boers*-transformed plants exhibited diminished disease resistance, perhaps as a consequence of the lowered ethylene sensitivity. This disadvantage might be overcome by the use of flower-specific promoters to confine transgene expression⁶⁵.

Also in *Petunia*, Cobb et al.⁶⁶ have used binary vectors containing the *etr1-1* gene under the control of flower-specific promoters, a floral binding protein (FBP1) promoter or an apetala-3 (AP3) promoter. Of the plants screened so far, 73% of the FBP1-plants and 32% of the AP3-plants have flower life double that of non-transgenic *Petunia* flowers. Some of the FBP1-transgenic plants had turgid fully-opened flowers for up to 14 days, comparing favourably with the non-transgenic controls with an average flower life of 3 days.

The Model of Ethylene Signal Transduction and Future Prospects

Ethylene sensitive flowers offer unique model systems for studying biological responses mediated by the plant hormone ethylene. Regulation of receptor gene expression could function either to increase ethylene sensitivity by reducing ethylene receptor abundance, or decrease sensitivity by increasing receptor expression. However, in numerous ethylene responses examined, increases in receptor gene expression have been observed, e.g. in response to stress, during senescence or in response to exogenous ethylene^{20,21,41,42,46,67,68}. Results obtained by studying genes involved in floral ethylene perception and signal transduction have sometimes been conflicting. Payton et al.³⁹ reported that the tomato receptor gene *tETR* (identical to *NR*) in whole flowers was developmentally regulated during flower development in tomato, with maximal mRNA accumulation occurring during early senescence. Lashbrook et al.⁴¹ have, in contrast, shown that *NR* transcript abundance does not alter greatly during floral development in petals, styles, ovaries, anthers, sepals and pedicels. Tiemann and Klee⁴² have shown that transcripts from the two other tomato receptor genes *LeETR4* and *LeETR5* are expressed in flower buds and accumulate in mature flowers. More generally, in tomato plants decreased receptor gene expression has never been reported during any ethylene response, either in flowers or other organs⁴³. Klee and Tieman⁴³ have suggested that ethylene receptor gene expression may serve a dampening role, slowing down ethylene responses once they have been initiated. Conflicting results have also been obtained in ethylene receptor expression studies in *Dianthus* and rose flowers during development and senescence. In *Dianthus* there appears to be an inverse relationship between the level of ethylene receptor expression

and sensitivity to ethylene, with a reduction in the amount of ethylene receptor proteins increasing ethylene sensitivity¹¹. This is what the standard model predicts. In roses, however, a parallel relationship appears to exist between the level of ethylene receptor transcript and flower longevity^{20,21}. As was found to be the case in tomato flowers^{39,42}, the expression of *RhETR3*, one of the four ethylene receptor genes in rose, increased in senescing flowers. Similarly, ethylene receptor gene abundance was high in a rose cultivar with short floral life, while it remained at low levels in another cultivar with long flower life. Ethylene treatment clearly increased receptor gene expression in rose flowers, while exogenously applied ethylene did not affect receptor gene level in *Dianthus* or geranium⁵⁶. However, when results from different investigations into receptor expression are compared, it should be borne in mind that different ethylene concentrations (0.1-10 $\mu\text{L}\times\text{L}^{-1}$) and different exposure times have been used. A long exposure time at a low ethylene concentration may induce more distinct changes in ethylene receptor expression than short exposure to high ethylene concentrations. In addition, flowers on intact plants, as used for the *R. hybrida* studies²⁰⁻²³, may react differently to exogenous ethylene treatments than excised flowers, as used for carnation^{11,13}. It might be that whole plants, due to their many leaves, can absorb higher amounts ethylene than excised flowers.

According to the standard model of ethylene signal transduction (Figure 2) the ethylene receptors (*ETR1* and its homologues) activate CTR1 in the absence of ethylene, and that active CTR1 inhibits, probably via intermediates, the action of EIN2. EIN3 and its homologues have been suggested to be positive regulators of the ethylene response. In contrast, the receptors and CTR1 negatively regulate the ethylene signal. The model thus implies that an increase in the level of receptors or CTR1, or a decrease in the level of EIN3, should result in decreased ethylene sensitivity, whereas a decrease in the level of receptors or CTR1, or an increase in the level of EIN3, should result in increased ethylene sensitivity. Under the physiologically reasonable assumption that ethylene sensitivity increases during flower senescence and in response to ethylene, a decrease in the level of receptors or CTR1, or an increase in the level of *EIN3*, might therefore be expected. This was not the case in rose where the transcript levels of CTR1, its homologue CTR2, and also EIN3 were investigated in senescing flowers^{20,21}. Transcription of the two *CTR*-genes was constitutive during senescence and increased in response to exogenous ethylene. Conversely, the transcription factor *RhEIN3* was found to be constitutively expressed in senescing flowers and in response to ethylene and ABA treatment.

The biological explanation for the multiplicity of ethylene receptors in plants is currently unknown, but it may be that individual receptors maintain a distinct functional identity via the capacity to respond differentially to developmental hormonal cues⁴¹. This explanation may also be applicable in rose flowers, where ethylene increased expression of *RhETR1*^{20,21} and *RhETR3* in both cultivars, while only *RhETR2* was constitutively expressed in the cultivar 'Vanilla'. In *Arabidopsis* leaves, transcript levels of *ERS1*, *ETR2* and *ERS2* genes were upregulated by ethylene, while the expression of *ETR1* and *EIN4* was not affected by ethylene treatment³⁴. In addition to the requirement for ethylene perception, the responsiveness of flowers to ethylene has been shown to be significantly altered during flower development^{12,69}. Differential regulation of

expression of the receptor gene family by ethylene may provide a mechanism to achieve differential sensitivities even for the same response under different conditions. In roses, increased receptor transcript levels after ABA treatment suggest the involvement of ABA in the senescence process. It is not known whether ABA-promoted flower senescence is due to increased ethylene production or to a change in tissue sensitivity to ethylene⁷⁰. Additionally it can be assumed that changes in responsiveness to ethylene are likely to be controlled by a number of internal and external factors, including hormone levels, carbohydrate status, environmental stresses and pollination signals^{12,71,72}. Alternate strategies to decrease ethylene sensitivity other than by transformation with the gene for a receptor such as *etr1-1* might be feasible. If the standard-model is correct (which would imply a need to explain those observations that do not seem to be in accordance with it), then up-regulation of receptor level, up-regulation of *CTR1* levels, or down-regulation of *EIN3* might conceivably lead to ethylene-resistant plants or plants in which selected organs are ethylene-insensitive. It is likely that the continuing basic research into the mechanisms of ethylene signal transduction will reveal additional ways in which these goals may be achieved.

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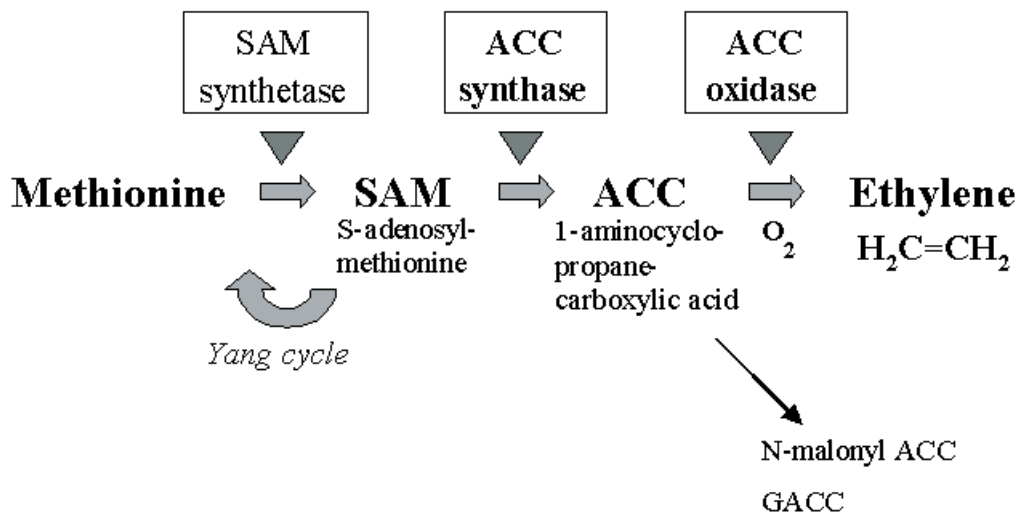


Figure 1. Scheme of the ethylene biosynthesis.

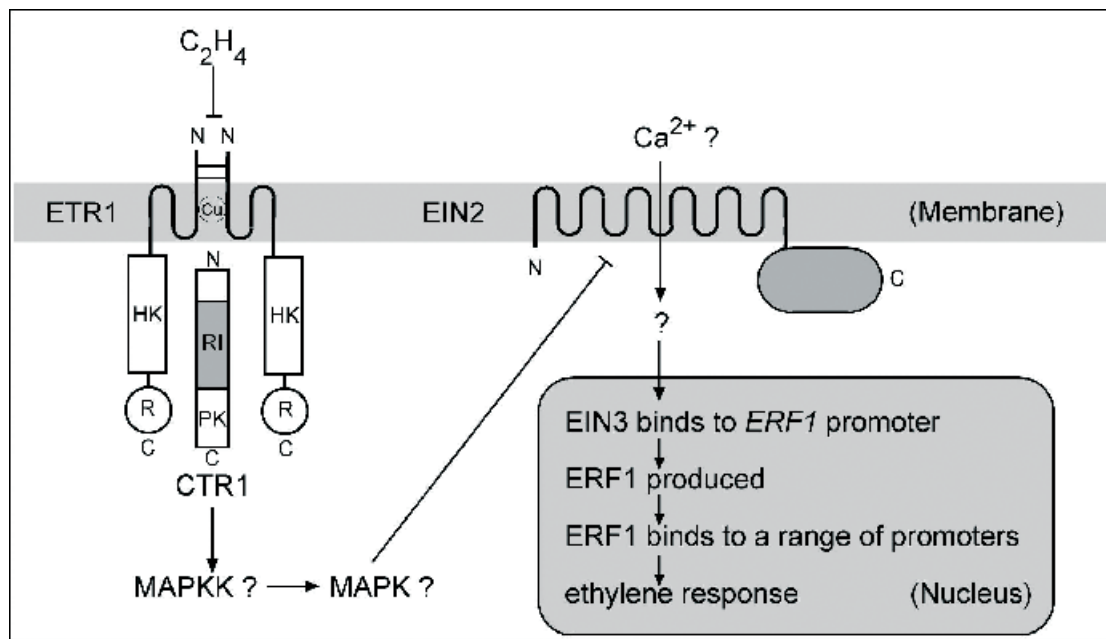


Figure 2. Scheme of the gene ETR1 and the signal transduction pathway.

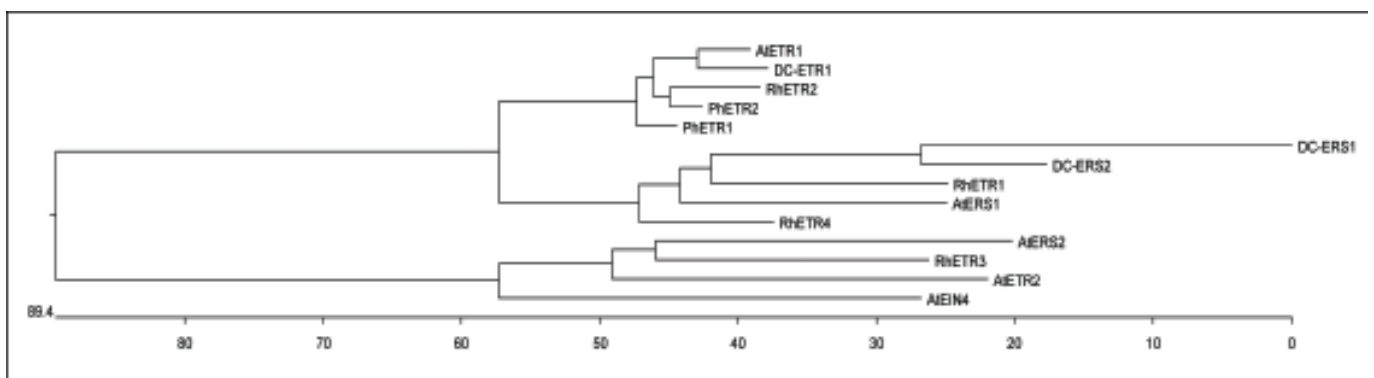


Figure 3. Scheme of the members of the putative ethylene receptor gene family in *Arabidopsis*.