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Role of 1-aminocyclopropane-1-carboxylate (ACC) Synthases Genes and Genes Involved in Ethylene Signal Transduction in Rose Flower Senescence

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ABSTRACT

This review focuses on the expression patterns of genes involved in ethylene perception (ethylene receptors and signal transduction) and ethylene biosynthesis. Special emphasis is placed on 1-aminocyclopropane-1-carboxylate synthases (ACS) and their involvement in rose flower petal senescence. The role of ethylene in rose flower opening and petal senescence, including some cultivar-dependent variations, is well documented. Three full-length and a number of partial length ACS genes from *Rosa hybrida* L. have been analyzed and compared; and their differential expression patterns are consistent with the presence of an ACS multi-gene family in roses. As more full-length ACS genes from the *Rosa* multi-gene family are identified, a better picture of their differential regulation will emerge. These findings may allow for specific genetic manipulation of ACS gene(s) to enhance the vase-life of rose cut flowers and allow growers to reduce their losses during transportation.

Keywords: ACC oxidase, ACC synthases, ethylene receptors, flower petal senescence, signal transduction
Abbreviations: **ACC**, 1-aminocyclopropane-1-carboxylate; **ACS**, ACC synthases; S-**AdoMet**, S-adenosyl-methionine; **CTR**, Constitutive Triple Response; **EIN**, Ethylene Insensitive; **ERF**, Ethylene Responsive Transcription Factor; **ERS**, Ethylene Response Sensor; **ETR**, Ethylene Response; **Raf**, a family of serine/threonine kinases first discovered in Rat fibroblast

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INTRODUCTION

For centuries roses have been prized by humans for their beautiful flowers. There is great variability for petal colors and fragrances, and roses continue to be one of the most important crops in the floriculture industry (reviewed in Gudin 2000). They are used as cut flowers, potted plants, and garden plants. With an annual retail sale value of well-over $10 billion [extrapolated from a value of one billion euros for cut roses alone in 1999 in United States and European markets (Pemberton 2003)], cut roses/rose plants etc represent an important cash crop for growers. The genus *Rosa* is diverse and includes roughly 200 species and over 18,000 cultivars (Gudin 2000).

Additionally, rose petals serve as a source of fragrance in the cosmetic industry and as a natural flavoring agent in the food industry. Rose oil (or more popularly known as *rose attar* in the Middle East and India) is commercially the most valuable material derived from roses (5 ml sells for about $50), including rose water. There is hardly a household in the Middle East and India where rose attar is not used in day-to-day life. Roses and rose petals are also used widely as offerings in the temples and Gurdwaras and in a wide variety of ceremonial occasions in India and many other cultures. Rose petals (sometimes collected from the temples) are converted into a rose petal preserve or jam by mixing with honey or sugar which is popularly known as *gulukand* (derived from the word *gulaab*, which is the name for rose) in India. Many varieties of *gulukands* are available in the markets; their fragrance and taste vary with the variety of roses used. In the market place these formulations bring very high prices. They form a part of the herbal medicine system widely practiced in India (known as Ayurvedic and Yunani systems of medicine). Because of their high commercial and aesthetic value, it is not surprising that roses have been subjected to intensive cross-breeding for centuries to develop new cultivars with attractive flower colors, desirable flower forms, better fragrance, and longer vase life (Krüssmann 1981; Gudin 2003).

The purpose of colorful flowers in plants is to attract pollinators; once pollination is completed plants shed or senesce their petals. Many different forms of visual changes have been observed in flowers that include petal senescence or abscission and changes in floral scent emission. These

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changes appear to be mediated by the phytohormone ethylene (van Doorn 1997, 2002). In some species, e.g. *Pelargonium* spp., petals abscise rapidly following pollination (Clark *et al*. 1997; van Doorn and Stead 1997; Fan *et al*. 2007) and in others, such as carnation, orchid, and petunia, flower petals wilt within several days of pollination and this response is reproduced upon their exposure to exogenous ethylene (Reid *et al.* 1989; O'Neil *et al.* 1993; van Doorn 1997; Bui *et al*. 1998; van Doorn 2002). The commercial value of flowers decreases dramatically after the first sign of flower wilting because it serves to indicate limited vase life. Therefore, approaches that slow the rate of flower senescence are important to growers and have a great deal of potential to increase market value of cut flowers. Since ethylene appears to play a critical role in flower senescence, it has been a major focus of research during the past 10-15 years (reviewed in van Doorn 2002; Tanaka *et al*. 2005). These studies have largely focused on the regulation of ethylene biosynthetic pathways and, to a more limited extent, on the ethylene perception and signal transduction pathway as well (reviewed in Wang *et al*. 2004; Tanaka *et al*. 2005; Shibaya and Clark 2006). In the present review we focus on the studies being carried out on *Rosa* cultivars.

The plant hormone, ethylene, plays an important role in flower senescence and is also involved in plant growth, development, and stress related processes including fruit ripening (reviewed in Sato and Theologis 1989; Matto and Suttle 1991; Abeles *et al*. 1992; Zarembinski and Theologis 1994; Bleecker and Kende 2000; van Doorn 2002). Ethylene synthesis in plants starts with the conversion of L-methionine into S-adenosyl-methionine (S-AdoMet) by the enzyme S-adenosyl-methionine synthase. S-Ado-Met is transformed into 1-aminocylcopropane-1-carboxylate (ACC) by ACC synthase (ACS) which is then converted into ethylene by ACC oxidase (reviewed in Yang and Hoffman 1984; Kende 1989) (**Fig. 1**). ACS catalyses the rate limiting step in the ethylene biosynthetic pathway (reviewed in Yang and Hoffman 1984; Bleecker Kende 2000) and is primarily regulated at the level of transcription (reviewed in Rottmann *et al*. 1991; Bailey *et al*. 1992; Liang *et al*. 1992; Bleecker and Kende 2000; Chang and Bleecker 2004; Chen *et al*. 2005). ACS is induced by a variety of factors which include germination, seedling growth, flowering, fruit ripening, and organ senescence and is also induced by a variety of external factors including wounding, viral or fungal infection, chilling, and exposure to some chemicals (reviewed in Matto and Suttle 1991; Rottmann *et al*. 1991; Bailey *et al*. 1992; Liang *et al*. 1992; Montgomery *et al.* 1993; Bleecker and Kende 2000; Chang and Bleecker 2004; Chen *et al*. 2005; Fan *et al.* 2007). Ethylene induction requires *de novo* synthesis of this enzyme (Rothmann *et al*. 1991; Liang *et al*. 1992). Due to the critical role of ACS in ethylene synthesis, ACS genes have been cloned from a wide variety of plant sources (reviewed in Matto and Suttle 1991; Bleecker and Kende 2000; Wang *et al.* 2004; Chang and Bleecker 2004; Chen *et al*. 2005; Fan *et al.* 2007). These studies have led to the conclusion that ACSs are encoded by a multigene family in which each gene is differentially regulated in response to internal and external signals (reviewed in Huang *et* al. 1991; Matto and Suttle 1991; Rottmann *et al*. 1991; Bailey *et al*. 1992; Liang *et al*. 1992; Itzhaki *et al.* 1994; Bleecker and Kende 2000; Chang and Bleecker 2004; Wang *et al.* 2004; Chen *et al*. 2005; Fan *et al*. 2007).

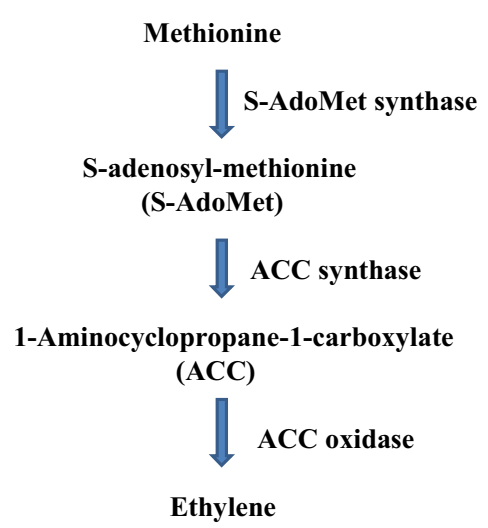

Fig. 1 Ethylene biosynthetic pathway. Based on Yang and Hoffman 1988.

ETHYLENE PERCEPTION

In addition to regulated synthesis, ethylene in the cell is perceived via a signal transduction pathway involving ethylene specific receptors. In the past decade a number of genes which form part of the components of the signal transduction pathway have been identified in *Arabidopsis thaliana* (L.) Heynh. using screening of mutants in the triple response to ethylene in dark grown seedlings (**Fig. 2**; reviewed in Guo and Ecker 2004; Klee 2004; Chen *et al*. 2005). Analysis of these mutants suggests that ethylene is perceived by a family of membrane localized receptors. One of these genes, called ethylene response gene (*ETR1*), codes a histidine kinase with homology to a sensor protein of the bacterial two component system (Parkinson and Kofoid 1992; Chang *et al*. 1993). Expression of *ETR1* in yeast has shown that it binds ethylene, thus supporting the notion that *ETR1* functions as an ethylene receptor. Since this initial discovery, a total of five ethylene receptor genes have been cloned from *Arabidopsis*. They are *ETR1*, *ETR2*, ethylene response sensor 1 (*ERS1*), *ERS2*, and ethylene insensitive 4 (*EIN4*) (Hua *et al*. 1995; Hua *et al*. 1998; Sakai *et al*. 1998).

An analysis of the loss-of-function mutants in *ETR1* has shown that receptors are negative regulators of the ethylene response (Hua and Meyeronitz 1998). These receptors appear to function through constitutive triple response1 gene (*CTR1*) which encodes a protein with sequence homology to the *Raf* family of serine/threonine protein kinase genes (first described in *Ra*t *f*ibroblasts) (reviwed in Guo and Ecker 2004). Loss-of-function mutations in *CTR1* lead to a constitutive ethylene response, suggesting that *CTR1* is a negative regulator of ethylene response (Kieber *et al*. 1993; Huang *et al*. 2003). *EIN2* is the next component in the path-

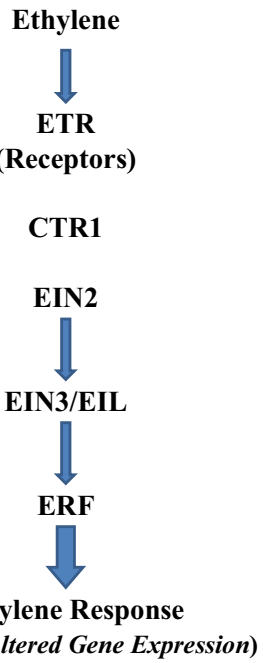

Fig. 2 Ethylene signaling pathway. Based on Chen *et al.* 2005.

way. *EIN2* is a single copy gene and loss-of-function mutation in this gene results in loss of ethylene response. *EIN2* codes for an integral membrane protein with homology to Nramp m*etal* transporter (Chen and Bleecker 1995; Roman *et al*. 1995). Two other proteins called *EIN3* and Ethylene Response Factor (*ERF1*), localized in the nucleus (Chao *et al*. 1997), have been identified and participate downstream in the pathway. The primary target of *EIN3* seems to be the *ERF1* gene promoter (Solano *et al*. 1998). Recent studies show that levels of *EIN3* protein increase rapidly in response to ethylene, and the protein is rapidly degraded in the absence of ethylene (Guo and Ecker 2003). These results suggest that *EIN3* and *ERF1* are transcription factors that function as positive regulators in ethylene response (**Fig. 2**).

Role of ethylene perception genes in rose flower senescence

Using either probes or primers developed from *Arabidopsis* genes involved in signal transduction pathway, several research groups have isolated components of the ethylene signal pathway from a number of ornamental plant species, such as ethylene receptors from roses (Müller 2000a, 2000b, 2002, 2003), carnations (Shibuya *et al*. 2002), and *Pelargonium* (Dervinis *et al*. 2000). Similarly, the *EIN2* homolog from petunia and the *EIN3* homolog from carnation (Waki *et al*. 2001), rose (Müller *et al*. 2003), and petunia (Ciardi *et al*. 2003) have been described. These studies suggest that the ethylene signal transduction pathway is, by-and-large, conserved in a variety of ornamental plant species.

In miniature potted roses, two cultivars that exhibit different post-harvest characteristics were used by Müller *et al*. (2000a) to examine the role of ethylene receptor gene expression in determining differences in flower longevity. 'Vanilla' has long lasting flowers and was compared with 'Bronze', which has a shorter flower life, for levels of *RhETR1* (similar to *AtERS1)* transcripts. Results showed that expression levels of *RhETR1* were distinctly higher in 'Bronze', suggesting that modulation of receptor levels may contribute to ethylene perception and sensitivity. Thus, short flower life in 'Bronze' expressing high levels of *RhETR1* transcript may contribute to sensitivity to ethylene due to an increased number of receptors resulting in rapid flower senescence (Müller *et al*. 2000a, 2000b).

Further expression analysis of *RhETR2* and *RhETR3* (*ETR2* clusters with *AtETR1* and *RhETR3* shows high sequence similarity to *AtETR2* and *AtERS2*) revealed that they are differentially expressed in 'Vanilla' and 'Bronze'. *RhETR2* is constitutively expressed throughout flower development in both cultivars, whereas *RhETR3* expression increased in senescing flowers of short flower life 'Bronze', but remained at low levels in the long-lasting flowers of 'Vanilla' (Müller *et al*. 2000a, 2000b).

These studies on the differences in ethylene receptor gene expression in these two cultivars were further extended to other genes involved in the ethylene signal transduction pathway, including two CTR-like kinases that interact with the ethylene receptor and *EIN3* transcription factor that serves as a positive response regulator of ethylene (see **Fig. 2**). There was no apparent change in expression levels of *EIN3* transcripts, which suggests stable constitutive expression of this gene in the two cultivars (Müller *et al*. 2003). On the other hand, two homologs of *CTR* genes from *Rosa*, called *RhCTR1* and *RhCTR2* isolated from petals, showed clear differences in the levels of transcripts. While *RhCTR2* was constitutively expressed during flower development in both cultivars, *RhCTR1* expression increased during flower senescence and was more rapid and increased to higher levels in 'Bronze' than 'Vanilla' (Müller *et al*. 2000a, 2000b). These studies suggest that increased ethylene synthesis during senescence resulted in enhanced expression of *RhCTR1*.

In separate studies, Ma *et al.* (2006) and Tan *et al.* (2006) also examined the role of the ethylene signal transduction pathway in the sensitivity of two cut rose cultivars to ethylene perception, namely 'Samantha' and 'Kardinal'. In 'Samantha', ethylene up-regulated the expression of ethylene receptor gene (*ETR*), but expression in 'Kardinal' was not affected. *ETR* is considered to be a negative regulator in the signal transduction pathway. Therefore, up-regulation of *ETR* after ethylene treatment, down regulates to ethylene sensitivity in 'Samantha', but not in 'Kardinal'. Ethylene showed no significant change in the expression of the other two genes that operate downstream of *ETR*, namely *CTR* and *EIN3* (Tan *et al*. 2006).

Similarly, in a more extensive study on the expression of genes involved in ethylene perception in 'Samantha', Ma *et al*. (2006) observed up-regulation in ethylene receptor genes *RhETR1* and *RhETR3* and, similarly, expression of *RhCTR1* and *RhCTR2* was enhanced by ethylene. There was no change in expression of transcription factor genes *EIN3-1* and *EIN3-2*. These results also suggest that ethylene regulates the opening of rose flowers through expression of two ethylene receptor genes (*RhETR1* and *RhETR3*) and two *CTR* genes (*RhCTR1* and *RhCTR2*).

When it comes to ethylene perception and the signal transduction pathway, the studies of Müller *et al.* (2000, 2003), Ma *et al*. (2006), and Tan *et al*. (2006) point to several important areas: 1) Ethylene receptor involvement in flower petal senescence is important and is cultivar dependent with variable cultivar sensitivity. 2) They point to the need for additional work, because results in some instances are contradictory. While it makes sense in the studies with the miniature potted roses of short- and long-lived flower cultivars, the results with 'Samantha' and 'Kardinal' on exposure to exogenous ethylene are quite the opposite from what one would expect. These apparent contradictions may be due to our lack of sufficient understanding of the ethylene signaling process. Further research would be helpful in providing better rationale of these differences.

Expression patterns of genes involved in ethylene biosynthesis and their relationship to rose flower senescence

The opening and senescence of many types of flowers including carnation, petunia, orchid, and roses appear to be correlated closely to ethylene production (Woltering and van Doorn 1988). Roses are generally considered ethylene sensitive (Reid *et al*. 1989) but this sensitivity can also be cultivar dependent (Yamamoto *et al*. 1994). In the past five to seven years, researchers have started to examine the role of ethylene in rose flower opening and in flower senescence. In a first report on the cloning of ACC synthase cDNA from 'Kardinal' (Wang *et al.* 2004), we described stages of rose flower opening from Stage 1, tightly closed bud; Stage 2, partially opened bud; Stage 3, completely opened bud (sepals have moved away from petals); Stages 4-6, partially opened, but in progressive stages of flower opening; to Stage 7, opened flower; and finally Stage 8, fully opened flowers showing anthers with petals showing visual signs of senescence (**Fig. 3**). Based on research published since these researchers have, by and large, used these stages to study the role of exogenous ethylene or the involvement of ethylene biosynthetic gene expression on rose flowering and flower petal senescence (Ma *et al*. 2005, 2006; Tan *et al*. 2006). Besides the short-lived 'Bronze' and the long-lived 'Vanilla' among miniature roses (Müller *et al*. 2000), cut roses 'Kardinal' and 'Samantha' have served as model systems among the roses for studies on the role of ethylene in flower opening/senescence (Wang *et al*. 2004; Ma *et al*. 2005, 2006; Tan *et al*. 2006). Using highly sensitive RT-PCR to measure transcript levels, Wang *et al*. (2004) showed that the expression of *RKacc7*, now designated as *RhACS1*, correlated with the opening and senescence of flower petals (**Fig. 4**). We noted that this gene was also expressed at significant levels in sepals that surround the petals at the bud stage (Wang *et al*. 2004) and at Stage 3 these sepals move away from the bud (**Fig. 3**). Therefore, it is likely that ethylene released by sepals may not only start the process of

Fig. 3 Stages of rose flowering. *Stage 1* unopened bud, *Stage 2* partially opened bud, *Stage 3* completely opened bud, *Stages 4-6* partially opened bud in progressive stages of flowering, *Stage 7* opened flower, and *Stage 8* fully-opened flower showing anthers. Reprinted from **Wang D, Fan J, Ranu RS** (2004) Cloning and expression of 1-aminocyclopropane-1-carboxylate synthase cDNA from rose (*Rosa x hybrida*). *Plant Cell Reports* **22**, 422-429, with kind permission of Springer Science+Business Media, ©2004.

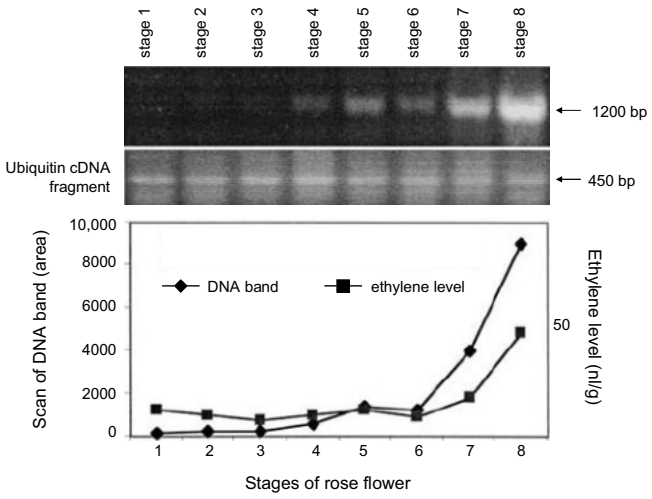

Fig. 4 Expression of RKacc7 during various stages of flowering. Transcripts were assayed by RT-PCR. Experimental details have been described in Wang *et al*. (2004). *Top panel* shows transcript levels at *Stages 1-8*. These flower stages are shown and described in detail in **Fig. 3**. *Middle panel* shows constitutively expressed ubiquitin gene as an internal control. *Bottom panel*: the DNA band area was measured and plotted as a graphic representation of ACC synthase transcript levels. Ethylene levels were determined by gas chromatography (Hewlett Packard model 5890). Rose petals were kept in 15-ml sealed tubes for 2 h or 4 h at 22°C, and aliquots were removed to determine ethylene. The area under the ethylene peak from triplicate samples is plotted. Reprinted from **Wang D, Fan J, Ranu RS** (2004) Cloning and expression of 1-aminocyclopropane-1-carboxylate synthase cDNA from rose (*Rosa x hybrida*). *Plant Cell Reports* **22**, 422-429, with kind permission of Springer Science+Business Media, ©2004.

moving sepals away from tightly closed flower petals, but also provide a trigger for the induction of ACC synthase in petals. This may not only start the process of flower opening, but may also be later responsible for flower senescence as it peaks when visible symptoms of flower petal senescence are detected (Wang *et al*. 2004) (**Figs. 3, 4**).

Using the same basic approach, Ma *et al*. (2005, 2006) investigated the differential response of flower opening in 'Samantha' and 'Kardinal' to exogenous ethylene. While exogenous ethylene promoted flower opening in 'Samantha', it inhibited flower opening in 'Kardinal'. Ethylene treatment increased production of ACC and activities of ACC synthase and ACC oxidase in petals at an earlier stage (Stage 3) in 'Samantha'; and in 'Kardinal' it increased much more dramatically but peaked in a later stage (Stage 4). Analysis of transcript levels of *RhACS1* (our nomenclature or *RhACS3* by Ma *et al*. 2005) assayed by Northern blot showed that expression of *RhACS1* induced by ethylene correlated with the activities ACS synthase and ethylene production in both cultivars, which is consistent with our findings (Wang *et al*. 2004). There was a much more dramatic accumulation of *RhACS1* mRNA in 'Kardinal' than in 'Samantha'.

In addition to *RhACS1 (RhACS3)*, two more *RhACS* genes were described by Ma *et al*. 2005. They are called *RhACS1* and *RhACS2*, though they represent only partial sequences. To avoid confusion in this review, we shall designate *RhACS1* and *RhACS2* of Ma *et al*. (2005) as *RhACS2* and *RhACS3*, respectively, reserving *RhACS1* for the first full-length ACS gene (Wang *et al*. 2004). Using specific probes generated from these sequences, Northern blot analyses by Ma *et al*. (2005) showed that there were no detectable levels of *RhACS2* transcript in the control, but ethylene treatment induced the accumulation of *RhACS2* transcript in Stages 4 and 5 in both cultivars. Further, wounding induced the accumulation of *RhACS2* transcript dramatically and strongly inhibited the expression of *RhACS1*, but had no detectable effect on *RhACS3* expression. These results are consistent with the differential regulation of expression of these *RhACS* genes in the two *Rosa* cultivars suggesting clearly that *RhACS1* may be involved in flower opening and flower senescence/wilting, whereas *RhACS2* is involved in wounding and wilting. Wilting may be con-

sidered a form of cell death, which may signal the induction of *RhACS2*. From the nature of induction of ACS genes in wilting, it appears that *RhACS1* and *RhACS2* may act in a cooperative fashion resulting in dramatic enhancement of ethylene production.

In a similar study, Pan *et al*. (2005) observed a direct correlation between ethylene production, ACC synthase activity, and ACC synthase transcript accumulation with flower development (opening) and, to a degree, flower petal senescence. Similarly, ACC levels changed with ethylene production. This study did not identify the cultivar of *Rosa hybrida* L., therefore, it is difficult to compare these results directly with the results observed in 'Samantha' and 'Kardinal' even though they are in general agreement with studies of Wang *et al*. (2004) and Ma *et al*. (2005, 2006).

Müller *et al*. (2000b) examined transcript levels of the genes involved in ethylene biosynthesis in miniature potted roses of long ('Vanilla') and short lived ('Bronze') flowers and observed significant differences. The abundance of ACC oxidase transcript increased during latter stages of flower development in petals of both cultivars, but it was significantly higher in 'Bronze' than 'Vanilla'. On the other hand, the ACC synthase transcripts increased during flower senescence in 'Vanilla', but remained at a low but constant level in 'Bronze' even though flowers senesced faster in 'Bronze' suggesting that levels of ACC synthase did not restrict ethylene production. These results, taken together, point to differences both in the synthesis of ethylene and sensitivity to ethylene perception due to increases in the number of ethylene receptors in 'Bronze' versus 'Vanilla' (Müller *et al*. 2000).

A REVIEW OF ROSE ACS GENES

In a previous paper our group described cloning and the sequences of three full-length ACS genes from 'Kardinal' (Ranu *et al.* 2008) and a number of groups have also published partial genemic/cDNA sequences (Müller *et al.* 2000b; Ma *et al.* 2005; Mibus and Serek *et al.* 2005) or full-length cDNA sequences (Wang *et al.* 2004) from *Rosa* (**Table 1**). As a part of this review, a comparison of ACS genes described by all groups and deposited with Genbank was performed to determine the similarities and differences between these rose ACS genes. From these analyses, several points emerge. Our *RhACS1* (EF584008) shares 100% amino acid sequence homology with a previously described cDNA (RKacc7, AY378152) and 100% sequence identity with a fragment of cDNA (AY0803738) described by Ma *et al*. (2005), suggesting that they are the same gene. These sequences of Ma *et al.* (2005) are derived from the last exon of our *RhACS1*. Our other two genes, *RhACS12* and *RhACS17*, share 85 to 95% amino acid sequence homologies. All three full-length genes are clearly different based on differences in sequence length in each of the introns in these genes, in addition to the differences in the amino acid sequence. Moreover, promoter segments encompassing over 1000 bp upstream of the start codon show little sequence similarities (Ranu *et al.* 2008). The four gene fragments isolated by Mibus and Serek (2005) appear to be derived from the first and second exons covering amino acids 93-225 and their amino acid sequence identity varied from 39 to 46% when compared with *RhACS1*, *RhACS12* and *RhACS17*, suggesting that they are derived from different genes. Similarly, the other two genes described by Ma *et al*. (2005) show 42-46% sequence identity with our genes and appear to be derived from the second/third exon sequences. An overall picture that emerges from these analyses is that these represent a related, yet divergent multi-gene family. If we take into consideration all of these genes that are cataloged in Genbank, it constitutes a total of 9 genes. In the case of diploid *Arabidopsis*, a total of 12 different ACS genes (*AtACS1-12*) have been described (http://www.arabidopsis.org/); though not all of them are functional since gene *AtACS3* is most likely a pseudogene and *AtACS1* encodes a nonfunctional ACS (Tsuchisaka and Theologis 2004). Moreover, data from diploid *Arabidopsis* suggests that these isoforms of ACS genes are located on different chromosomes (http://www.arabidopsis.org/). Since many *R. hybrida* cultivars are tetraploid and generated through complex interspecific hybridization, it is likely they carry multiple copies of ACS genes. Based on the logic of this argument a number closer to 15 to 20 is within the range expected. In the course of time, independent evolutionary events would result in the divergence of these genes among the ACS gene family. This is clearly reflected in the observed gene structure differences within the three full-length ACS genes and their promoters (Ranu *et al.* 2008). This observation is also consistent with our previous results showing multiple DNA bands from Southern blots of genomic DNA using a full-length cDNA probe from 'Kardinal' (Wang *et al*. 2004).

Table 1 *Rosa hybrida* complete and partial sequences of putative ACC synthase genes from the data of several laboratories.

Gene name (Ranu Lab)	Genomic size	mRNA size	Accession Number[a]
RhACS1	Full length	1,750 bp	AY378152 EF584008
RhACS12	Full length		EF584009
RhACS17	Full length		EF584010
(Serek Lab)			
RhACS2	1346 bp	492 bp	AY525066
RhACS3	658 bp	378 bp	AY525067
RhACS4	937 bp	474 bp	AY525068
RhACS5	523 bp	375 bp	AY525069
(Gao Lab)			
RhACS1			AY061946
RhACS2			AY803737
RhACS3	Same as*RhACS1*		AY803738

[a] The ACS genes of *R .hybrida* in NCBI Genbank database (http://www.ncbi.nlm.nih.gov)

In the future we will not only have complete sequences of additional ACS genes, but also full-length sequences of genes for which only partial sequences are currently known. Further studies on their expression patterns will provide a more complete picture of their role and expression under a variety of biotic and abiotic conditions. These findings may allow for specific genetic manipulation of ACS gene(s) to enhance the vase-life of rose cut flowers and also allow growers to reduce losses during transportation.

CONCLUDING REMARKS

In this review we have examined the relationship of the three full-length ACS genes from *R. hybrida* with other ACS genes described by several groups. In addition, we have attempted to summarize the studies which suggest a strong correlation between the role of ethylene in rose flower opening and petal senescence. These studies provide evidence that both the enhanced expression of at least one (at this point in time) ACS gene (*RhACS1*) and perhaps two (*RhACS2*) are required for flower opening and senescence. Also, a limited increased expression of ACC oxidase may be a contributing factor. Enhanced ethylene production is also coupled with increased ethylene perception which is suggested by the induction of some ethylene receptor genes (*ETR*), including those involved in the signal transduction pathway. Some of these changes are also cultivar dependent. These studies also point to differences between the effects of exposure of flowers to exogenous ethylene and endogenous ethylene produced as a result of increased induction of ACS genes in various cultivars, e.g. 'Samantha' is much more sensitive to exogenous ethylene than 'Kardinal'. Exposure of 'Kardinal' to ethylene inhibits flower opening, whereas exogenous ethylene appears to enhance flower opening in 'Samantha' suggesting a direct involvement of endogenous ethylene in flower opening in 'Kardinal'.

As we move further into the Genomics Era and have the

complete sequence of the *Rosa* genome, it will be possible to develop genetic manipulation/modification programs that will be able to develop rose plants with individual as well as multiple desirable characteristics that would be difficult to do through traditional breeding alone. For instance, roses with a blue petal hue have recently been developed through genetic manipulation of the flavonoid biosynthetic pathway (resulting in the accumulation of delphinidin) (Katsumoto *et al.* 2007). Breeders can expect better tools that will allow specifically targeted breeding strategies (Ranu 2009) called designer breeding. This will save a lot of time for rose breeders as well as growers. A full picture of all the genes involved in ethylene biosynthesis and ethylene perception as well as signal transduction and their expression patterns will emerge. Growing bodies of information generated through this research can then be used for the benefit of breeders, growers, and consumers.

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