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1-Aminocyclopropane-1-carboxylate (ACC) Synthases of *Rosa hybrida*: Analysis of Genomic Gene Structure and the *Cis*-Acting Regulatory Elements in their Promoters

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ABSTRACT

The phytohormone ethylene is involved in the modulation of a variety of growth and developmental processes in plants, including fruit ripening. Many forms of visual changes observed in rose flowers, including flower opening, petal senescence and changes in floral scent emission are correlated to ethylene levels in flowers. As 1-aminocyclopropane-1-carboxylate (ACC) synthase (ACS) is one of the key regulatory enzymes in ethylene biosynthesis, ACS genes have been intensively investigated. Here we describe the structure of three full-length ACS genomic clones from *Rosa hybrida* cv. 'Kardinal'. These genes contain four exons and three introns and share sequence homologies with other plant ACSs with typical features that are characteristic of all ACSs. Plants selectively activate genes via interaction between transcription factor(s) and their specific binding motifs located in the genes' promoters. To identify/analyze *cis*-acting elements/motifs located in promoters of ACS genes, we have taken a computational approach using PLACE and AGRIS databases on the assumption that commonalities of *cis*-regulatory elements in the promoters are related in each gene to their expression in response to a particular signal. The resulting ethylene related *cis*-elements have been identified. The relative positions of these common regulatory elements vary among these promoters suggesting that protein-protein interactions among transcription factors may be another factor(s) in determining differential gene regulation. In future, as more full-length ACS genes from the *Rosa* multi-gene family are identified, a better picture of their differential regulation will emerge. This knowledge may allow the development of new rose cultivars with desirable characteristics through genetic manipulations/modifications.

Keywords: *cis*-acting element/sequence motif, ethylene, flower petal senescence, receptors, signal transduction, transcription factor
Abbreviations: ABA, abscisic acid; **ACC**, 1-aminocyclopropane-1-carboxylate; **ACS**, ACC synthase; **AGRIS**, Arabidopsis Gene Regulatory Information Server; **PLACE**, PLAnt *Cis*-acting Regulatory DNA Element; **RT-PCR**, Reverse Transcriptase-Polymerase Chain Reaction; **SA**, salicylic acid

INTRODUCTION

The plant hormone ethylene is primarily responsible for the senescence process (Woltering and van Doorn 1988; Abeles *et al.* 1992; O'Neil *et al.* 1993; van Doorn 2002) and is also involved in fruit ripening, plant growth, and development (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). In the cell, ethylene synthesis is initiated by the conversion of L-methionine into *S*-adenosyl-L-methionine, which is transformed into 1-aminocyclopropane-1-carboxylate (ACC) by ACC synthase (ACS). ACC is converted into ethylene by ACC oxidase (reviewed in Yang and Hoffman 1984; Kende 1989) (**Fig. 1**). ACS catalyzes the first rate-limiting step in the biosynthesis of ethylene and is thus considered to be the key regulatory enzyme (reviewed in Yang and Hoffman 1984; Bleecker and Kende 2000). ACSs in many plants have been shown to be encoded by a multi-gene family in which each gene is differentially regulated in response to internal and external signals (reviewed in Yip *et al*. 1990; Rottmann *et al*. 1991; Liang *et al*. 1992; Zarembinski and Theologis 1993; Bleecker and Kende 2000; Tsuchisaka and Theologis 2004; Chen *et al*. 2005). The expression of ACS genes is primarily regulated at the transcriptional level (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).

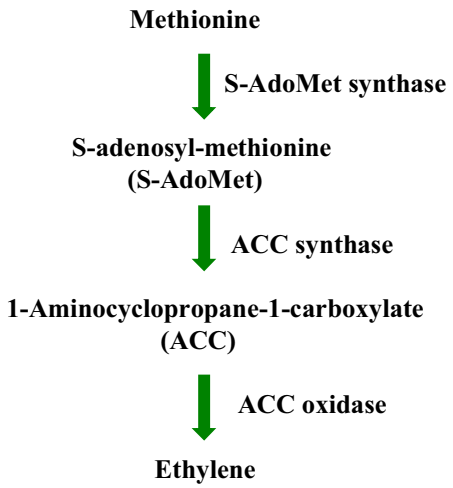

Fig. 1 Ethylene biosynthetic p

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ACS genes are differentially regulated by numerous growth, developmental and external stimuli (reviewed in Rottmann *et al*. 1991; Liang *et al*. 1992; Bleecker and Kende 2000; Chang and Bleecker 2004; Fan *et al*. 2004; Tsuchiasaka and Theologis 2004; Wang *et al*. 2004; Chen

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et al. 2005). Therefore, elucidation of control mechanisms that uncover important regulatory elements will provide significant information about how these genes are expressed in response to various internal and external cues. As a continuation of our work on ACS genes in *Rosa,* in the current study we have identified and isolated several full-length ACS gene members from *Rosa hybrida* cv. 'Kardinal' and carried out promoter analysis on the *cis*-acting regulatory elements responsible for the controlled expression of ACS genes. We have searched for motifs in the non-coding promoter region 5′ to the translational start site/codon of these ACS genes. Our basic assumption is that important regulatory motifs found in *Arabidopsis* that are potential binding sites of transcription factors are likely to be conserved in genes with similar expression patterns in other plant species. By unmasking regulatory elements in the promoter region of gene members belonging to the ACS multi-gene family, we hypothesized that different plant systems showing similar spatial and temporal expression patterns in response to ethylene may also have common regulatory elements in the promoter region.

Here, we report the characterization of three full-length genomic clones of ACS genes of rose screened from a genomic library of *R. hybrida* 'Kardinal'. The complete nucleotide sequences of the genes, *RhACS1*, *RhACS12*, and *RhACS17*, have been determined, including about a thousand base pairs (bp) upstream from the start codon. All these genes contain four exons and three introns and share sequence homologies with other ACS genes from various plants (reviewed in Matto and Suttle 1991; Abeles *et al*. 1992; Zarembinski and Theologis 1994; Bleecker and Kende 2000; van Doorn 2002). The genes also contain the conserved amino acid residues and the substrate and pyridoxal 5′-phosphate binding sites that are characteristic of all ACSs (reviewed in Yip *et al*. 1990; Huang *et al.* 1991; Zarembinski and Theologis 1994; Bleecker and Kende 2000; Chang and Bleecker 2004).

Also, we have taken a computational approach in which the promoter region encompassing about 1000 bp from the 5′-upstream sequences from the start codon of the three *R. hybrida* ACS genes have been analyzed and compared them with the complete list of *Arabidopsis* ACS gene family using database comparisons and weight matrices to identify *cis*-acting elements/motifs that may contribute to gene regulation. Our overall analyses show the presence of common regulatory elements which include general transcription factors binding elements, such as several TATA boxes, CAAT boxes (Le Gourrierec *et al.* 1999), and others in which ethylene is an important factor in gene regulation such as GCC GCC, core of GCC-box that functions as ethylene-responsive element and is found in many pathogen-responsive genes (Ohme-Takagi and Shinshi 1995; Brown *et al*. 2003; Chakravarthy *et al*. 2003)(see results section). We also note that in spite of the presence of many common *cis*-acting elements in promoters of the three *Rosa* ACS genes, their relative position in each promoter varies, suggesting, therefore, that besides binding of respective transcription factors to these elements, protein-protein interactions between transcription factors may be another important factor in determining differential regulation of the ACS multi-gene family members.

MATERIALS AND METHODS

Material

DNA primers used in this study were synthesized at Macromolecular Resources, Colorado State University, Fort Collins, CO.

Plant material

Rosa hybrida commercial variety, 'Kardinal', widely used in the cut flower industry, was chosen in this study. Flower petals and young leaves were harvested, immediately frozen in liquid nitrogen, and stored at -70°C until used.

Preparation of genomic library

Genomic DNA library was prepared from DNA extracted from young, healthy leaves essentially according to Guillemaut and Marechal-Drouard (1992). Partial digestion of DNA with *Sau*3AI was prepared and size fractionated by sucrose density gradient [10-40% in 10 mM Tris-HCL (pH 8.0), 10 mM NaCl and 1 mM EDTA] centrifugation at 22,000 rpm for 22 h at 20°C in a Beckman Ultracentrifuge SW40 rotor. Fractions in the molecular weight range of 10-25 Kb were used for construction of a library into the compatible *Bam*HI site of the Lambda (λ) Dash II replacement vector essentially according to the instructions provided by Stratagene, La Jolla, California. Wild-type λ bacteriophage with the active *red* and *gam* genes are unable to grow on the host-strain of *E. coli* containing P2 lysogen [XL1-Blue MRA (P_2)]. Therefore, wild type lambda phage cannot grow on XL1-BlueMRA(P_2) but recombinant phage containing genomic DNA fragments of about 20 Kb will grow and were used for selection of recombinant phages. Genomic DNA predigested with alkaline phosphatase (to prevent self-ligation) was ligated into compatible (predigested) *Bam*HI λ Dash II arms at 4°C for 24 h. Gigpack II Gold λ packaging extract was used for *in vitro* packaging of the recombinant λ DNA essentially according to instructions of Stratagene. The phage library was screened with a probe prepared from a full-length Rose ACC synthase cDNA (RKacc7) described previously (Wang *et al*. 2004). After three cycles of screening, a total of 22 different clones were isolated.

Characterization of DNA inserts and recombinant phage DNA preparation

Size of insert in the recombinant λ phage genomic clones was determined after digestion with *Not*I which results in the release of the insert. Results showed that the insert in each clone had an average molecular weight of 20 Kb (results not shown). Recombinant phage DNA from the clones was prepared with the Qiagen Midi Kit Qiagen, Inc. 27220 Turnberry lane, Velencia, CA 91355) using confluent plate lysis method for phage growth.

Table 1 List of primers used to sequence ACS clones.

Primer	Sequence 5'- 3'
1035F	CCTTCAAATCCTGGTGGC
1523R	CTGTAAGAGCGAATTAACCC
22-1140-9	CTTCGTCTCGGGTCCGGGTC
P1	AAGGTATGCACCTAGGTCG
P2	AACTCAACCTGCAAATTGCC
12-21 P3	CACCCAGTATTGCATCCCT
17-40 P3	CAAGTCCAAAGCTAACTACC
Rose 12	ACGGAGCCGAGAACGAG
cDNA-723	CTCTGTGAACTTTACTCTGG
cDNA1604	GAACCGGGATGGTTCCG
M13R-1727	GACCCTCAGACTGTGAC
639-656	GCTCGCTGAACAGCAAGC
1-1122-103R	GTCTCGGGTCCGGGTC
1-1513-529R	AGACCCAACCTCTCCCC
1035F	CCTTCAAATCCTGGTGGC
Race-end1	GCCACCAGGATTTGAAGG
Race-7F	GGGTTAATTCGCTCTTACAG
M13R	GCGGATAACAATTTCACACAGG
M13F	GTAAAACGACGGCCAGT
rose 7a	GGTGATAATCAGGCCCAC
rose 7b	CCCTGATTTGATACATGCTG
rose 7c	GCCGTATTATGTCGGTAC
rose 12R	CTCGTTCTCGGCTCCGTCAT
rose12	ACGGAGCCGAGAACGAG
rose 13	ACCCAACTCGTCGTACGGATC
rose 13r	CTAGGCATGCTGCTCAACCCA
rose 15a	CGGGCCATAAACTGATATA
rose 14	CTCCGGGRTCAGCCAAGCAAAA
ARA1	CCARCTCAAYTCTCTATCYAATCTG
ARA2	CTGATTTTCWGCYAGACCCATTTG

DNA sequencing

Based on restriction digestion analyses of the 22 clones after digestion with restriction enzymes: *Not*I, *Eco*RI, *Bam*HI, *Hind*III, *Xba*I, *Xho*I and *Sal*I, three clones were selected for DNA sequence work. These clones are *RhACS1, RhACS12,* and *RhACS17*. The clones were sequenced by direct PCR based sequencing initially using primers from RKacc7 cDNA (Forward primer 1035F, 5′-CC TTCAAATCCTGGTGGC and reverse primer 1532R, 5′-CTGTAA GAGCGAATTAACCC). To obtain additional sequences in both directions (Fan *et al.* 1996; Sanger *et al.* 1977; Ranu 1996) further sequencing was developed through a combination of sub-cloning of restriction fragments or carried out by genomic walking; by designing additional primers based on new gene sequence information. In this fashion, sequences covering about 1000 bases upstream of the translational start site (into the promoter region) and 60-1000 bases towards the 3′-end of each gene were obtained. DNA sequencing was performed either by the Macromolecular Resources at Colorado State University, Fort Collins, Colorado or by the Macrogen Sequence Resources Seoul, South Korea. All of the primers designed to sequence these clones are listed in **Table 1**.

Other procedures

The following procedures have been described in our previous reports on rose and geranium ACC synthases (Wang *et al*. 2004; Fan *et al*. 2007); construction of cDNA library; Southern blot analysis; preparation of [^{32}P] labeled ACC synthase probe; screening of genomic library for ACC synthase genes; assay of ACC synthase transcripts by RT-PCR; and, ethylene measurement by gas chromatography. Other methods have been described by Sambrook *et al*. (1989).

RESULTS AND DISCUSSION

Characterization of *Rosa* ACC synthase genomic clones

In a previous study, this laboratory described the complete sequence of an ACS cDNA (RKacc7; Genbank #AY378152) from 'Kardinal' petals and showed that the expression levels of this gene correlate with the opening and senescence of rose blooms and flower petals (Wang *et al*. 2004). Also, our Southern blot analysis of the genomic DNA probed with full-length RKacc7 probe at high and low stringency showed multiple DNA bands which suggested that a related-multi-gene family may encode for ACC synthases in rose as well (Wang *et al*. 2004). Screening of a 'Kardinal' λ bacteriophage genomic library with the full-length RKacc7 cDNA probe led to the isolation of an additional three clones whose make-up on restriction enzyme analysis including the amplification of a 500 base pairs (bp) fragment corresponding to the last exon of *RhACS1* (RKacc7) with primers 1035F and 1532R (**Table 1**) suggested that they contain complete gene body and promoter sequences. These clones were sequenced; they are *RhACS1*, *RhACS12* and *RhACS17*.

An analysis of the sequences of these three clones revealed that they all contain three introns and four exons (**Figs. 2-4**). The complete sequence of *RhACS1* is presented in **Fig. 2** and the four exons of this gene show complete sequence homology with RKacc7 cDNA (Genbank # AY378152). The sequence also covers 1715 bp upstream of the start codon and 983 bp downstream of the stop codon (Genbank #EF584008) with a region encoding 480 amino acids. The three introns vary in length from 80 (#3) to 121(#1) and 145 (#2). **Fig. 3** shows the complete sequence of the second clone, *RhACS12* (Genbank #EF584009). Besides the exons encoding 481 amino acids and the three introns, the sequences cover 1448 bp upstream of the start codon and 63 bp downstream of the termination codon. In this case, intron length varies from 80 (#3) to 212 (#1) and 768 (#2). The complete sequence of the third clone, *RhACS17*, is presented in **Fig. 4** (Genbank #EF584010) and covers 1061 bp upstream of the start codon and 57 bp downstream of the stop codon with exons encoding 488 amino acids. The intron length varies from 72 (#3), 86 (#3), and 818 (#2).

In all cases a consensus dinucleotide representing AG/GU slice site is located at the boundary of each exon-intron junction. Each gene contains the conserved GISKDLSLPG FRV peptide sequence that is common to all ACS genes with conserved lysine (K) residue in the active site that binds pyridoxal phosphate and S-AdoMet (Yip *et al*. 1990; Zarembinski and Theologis 1994; Eliot and Kirsch 2004).

Overall, based on differences in the three intron lengths, sequence differences in promoter segments and in the 3′-end untranslated region, the three ACS genes are clearly different. As with other ACC synthases they share amino acid sequence homologies with each other that varies from 87 to 95% using RhACS1 as 100%.

Promoter analysis

One of the challenges in biology today lies in the identification of sequence elements/motifs that are involved in regulation of gene activity. Plants selectively activate genes via the interaction between sequence specific motifs located in the promoter region and their corresponding transcription factors. Transcription of a gene is not only shaped by the transcription factor(s) and their interaction with a specific motif (Le Gourrierec *et al.* 1999), but also by remodeling of chromatin in preparation for gene expression (Wasserman and Sandelin 2004). Thus, characterization of *cis*-acting elements in promoter sequences may provide important clues in linking their role with gene activity and in determining expression patterns that link a multitude of genes (Lenhard *et al*. 2003). Compared to the number of genes, very few promoters have been well characterized (Cazzonelii *et al*. 2005), due primarily to the complex nature of interactions that take place between large numbers of *cis*-acting elements and transcription factors. It appears that transcription factors function in networks along with other regulatory proteins, which in turn modulate expression of other regulatory genes. These tight regulation patterns allow a specific signal to initiate and modulate coordinated expression of a set of genes important in the plant response to internal and external cues.

The mechanism(s) of regulation of differential expression of ACS genes in the ACS multi-gene family is of great interest. This multi-gene family can also serve as a model system in identifying regulatory elements that regulate the differential expression of individual members of the other gene families to numerous internal and external cues. It is fair to assume that the commonality of *cis*-regulatory elements in the promoters is related to expression of genes in response to a particular signal.

Several resources are available to search for *cis*-regulatory motifs in a promoter sequence, such as PLACE (PLAnt Cis-acting regulatory DNA Element at http://www.dna.affrc.go.jp/PLACE/signalscan.html), a *cis*-regulatory element database for plants and AGRIS (Arabidopsis Gene Regulatory Information Server at http://arabidopsis.med.ohio-state.edu/) which integrates data from a variety of sources, such as AtTFDB and AtcisDB databases (Davuluri *et al*. 2003). In our analysis we have considered that genes with similar expression patterns would or are likely to contain common motifs in their promoter regions and a common set of transcription factors are likely to control these genes. In this regard, we note that ethylene response *cis*-elements and the proteins that interact with these elements have been identified for the tomato *E4* gene (Montgomery *et al*. 1993; Coupe and Deikman 1997), the carnation senescence-related glutathione-*S*-transferase gene (Itzhaki *et al*. 1994) and tobacco defense genes (Ohme-Takagi and Shinshi 1995). Thus, in our analysis of the promoter regions of the three *RhACS* genes, we have taken a computational approach using database comparisons to identify binding motifs. The 5′-upstream sequence encompassing about 1000 bp from the start codon of *RhACS1*, *RhACS7*, and *RhACS17* was

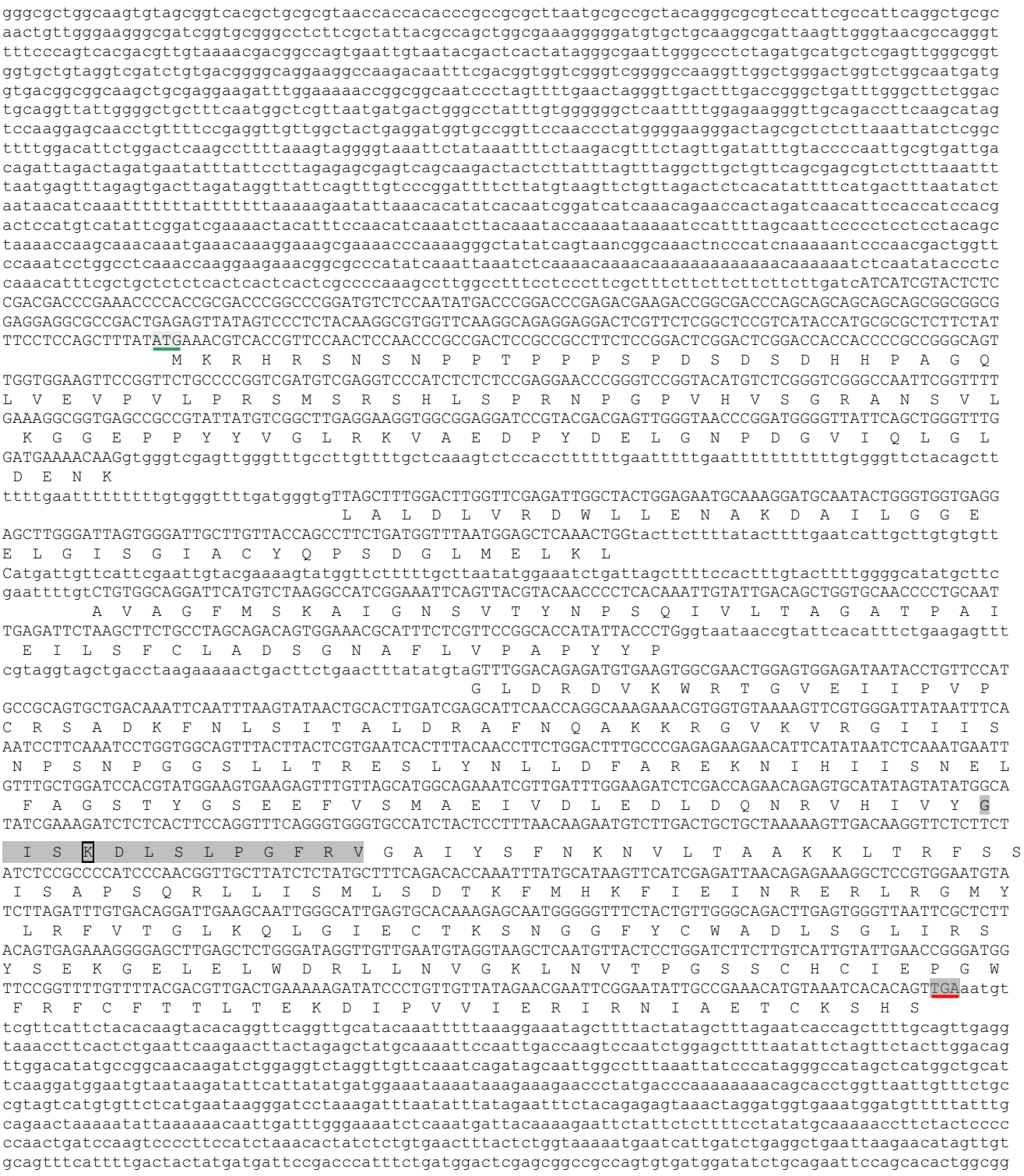

Fig. 2 Complete DNA sequence of *RhACS1* and its flanking 5′ and 3′ regions. Coding regions are shown in uppercase letters and noncoding regions in lowercase letters. The derived amino acid sequence is presented in the one-letter code below the DNA sequence. The putative start site is underlined in green and stop codon is underlined in red. The conserved amino acid residues characteristic of ACS genes are highlighted in grey and the lysine that binds pyridoxal 5'-phosphate and S-AdoMet residue is boxed.

input into the PLACE data website (http://www.dna.affrc.go.jp/PLACE/signalscan.html) and compared with those of other ACS genes from *Arabidopsis*. The resulting ethylene related elements were selected to be putative binding motifs that may be involved in ACS regulation. The motifs identified have common (or shared) sequences with 100% sequence homology across the three *R. hybrida* genes. We point out in these analyses no attempt is being made to present an exhaustive details of all the *cis*-acting elements.

The first point to emerge from these studies is that in spite of a great deal of sequence homologies in the ACS (coding sequences) protein sequences (varying from 87-95%), the promoter regions showed no clear cut sequence conservation. In fact, the prevailing message is a high level of variability. Based on the response of many genes to ethylene, we identified the following *cis*-acting elements that may be involved in the expression of these genes:

1. GCCGCC, core of GCC-box found in many pathogen-responsive genes and functions as ethylene-responsive element (Ohme-Takagi and Shinshi 1995; Brown *et al*. 2003; Chakravarthy *et al*. 2003).

2. TGACG "ASF-1 binding site" ASF-1 binds to two

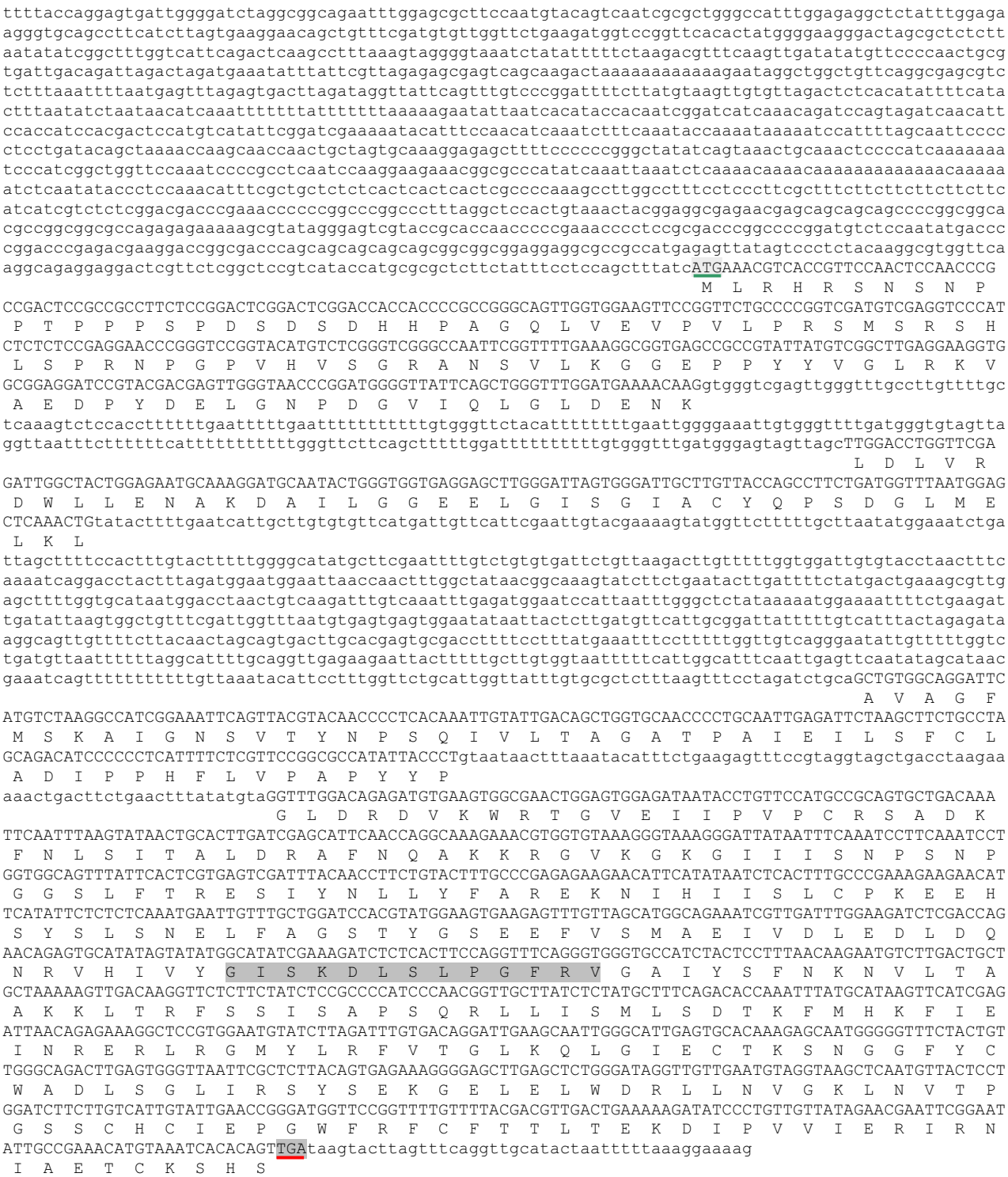

Fig. 3 Complete DNA sequence of *RhACS12* and its flanking 5′ and 3′ regions. Coding regions are shown in uppercase letters and noncoding regions in lowercase letters. The derived amino acid sequence is presented in the one-letter code below the DNA sequence. The putative start site is underlined in green and stop codon is underlined in red. The conserved amino acid residues characteristic of ACS genes are highlighted in grey and the lysine that binds pyridoxal 5'-phosphate and S-AdoMet residue is boxed.

TGACG motifs found in promoters that are involved in transcriptional activation of several genes by auxin or salicylic acid, light response and abiotic and biotic stress activity (Terzaghi and Cashmore 1995; Despres *et al*. 2003).

3. TGTCTC site or ARF (auxin response factor) binding site (Ulmasov *et al*. 1999; Harper *et al*. 2000; Hagen and Guilfoyle 2002; Goda *et al*. 2004; Nemhauser *et al*. 2004; Inukai *et al*. 2005; Nag *et al*. 2005).

4. NGATT "ARR1-binding element" response regulator and functions as a transcriptional activator (Sakai *et al*. 2000; Ross *et al*. 2004).

5. VCGCGB "CGCG box" multiple CGCG elements found in promoters- involved in multiple signaling pathways in plants (Yang and Poovaiah 2002).

6. GATAA or "I box" conserved sequence upstream of light-regulated genes (Terzaghi and Cashmore 1995; Jiao *et al.* 2005).

7. CCGAAA "LTR-1" the low temperature response element (Dunn *et al*. 1998).

8. WAACCA MYB recognition site involved in dehydration response (Grotewold *et al.* 1994; Abe *et al*. 2003; Simpson *et al.* 2003).

9. AACGG "myb core" activator (Planchais *et al*. 2002).

10. TTGAC "W-box" recognized by salicylic acid (SA)-induced WRKY DNA binding proteins and act as negative regulatory elements (Eulgem *et al*. 1999, 2000;

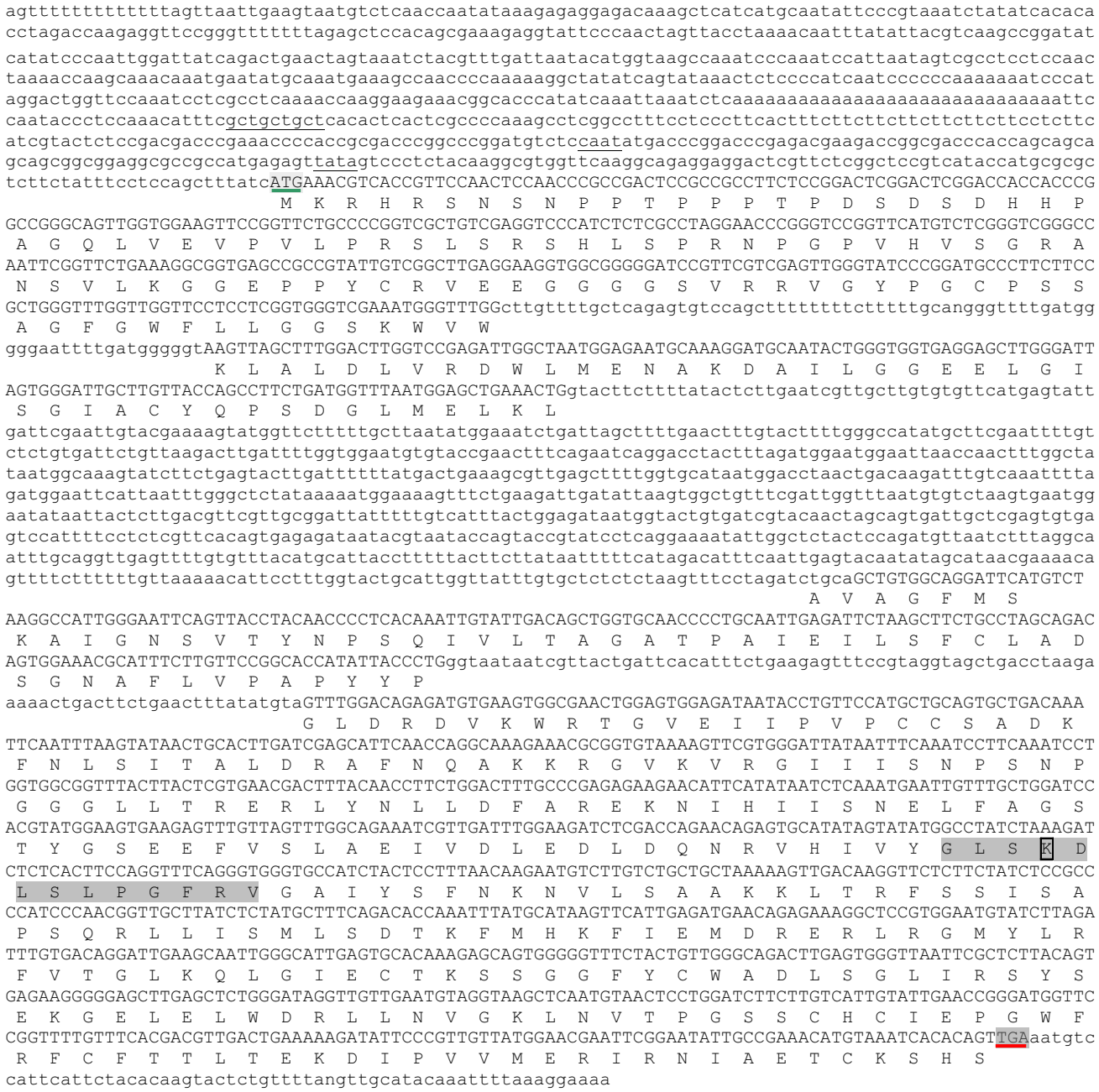

Fig. 4 Complete DNA sequence of *RhACS17* and its flanking 5′ and 3′ regions. Coding regions are in uppercase letters and noncoding regions are in lowercase letters. The derived amino acid sequence is presented in the one-letter code below the DNA sequence. The putative start site is underlined in green and stop codon is underlined in red. The conserved amino acid residues characteristic of ACS genes are highlighted in grey and the conserved lysine that binds pyridoxal 5'-phosphate and S-AdoMet residue is boxed.

Maleck *et al*. 2000; Yu *et al*. 2001).

11. GATA and SORL1 (GGGCC) that are involved in light regulated genes (Lam and Chua 1989; Gilmartin *et al.* 1990; Teakle *et al.* 2002; Hudson and Quail 2003; Reyes *et al* 2004).

12. E box element (CANNTG) that appears to be involved in a variety of environmentally influenced genes (Szopa *et al* 2003).

13. Finally, the Anaero1consensus (AAACAAA) involved in anaerobic response and also found in about 13 anaerobic genes involved in fermentation pathway (Zarembinski and Theologis 1993; Mohanty *et al.* 2005). In addition several TATA and CAAT boxes that are present in many promoters and are associated with the binding of general transcription factors, are also present in these promoters. These results are presented in **Figs. 5-7**. Further, we note that even though the promoters of these genes contain these common *cis*-acting elements, their relative location within the promoter sequences in each gene vary greatly suggesting, therefore, that besides binding of respective transcription factors to these elements, protein-protein interactions between transcription factors may be another important factor in determining differential regulation of the ACS multi-gene family members.

These findings of various and multiple *cis*-regulatory elements in the three promoters suggest a complex molecular response(s) may be involved in various environmental and biotic stresses. They appear to require mediation through the interaction of multiple complex regulatory systems of gene expression and signal transduction requiring *cis*-acting elements and *trans*-acting factors with potential for cross-talk (between different systems) in which ethylene may play a role. Further validation of these identified *cis*-regulatory elements would be necessary. These correlative results can, however, serve as a guide for a hypothesis-driven experimental research design to determine the mechanisms of transcriptional gene regulation in which ethylene may be involved.

ACS genes from a variety of plant sources have been

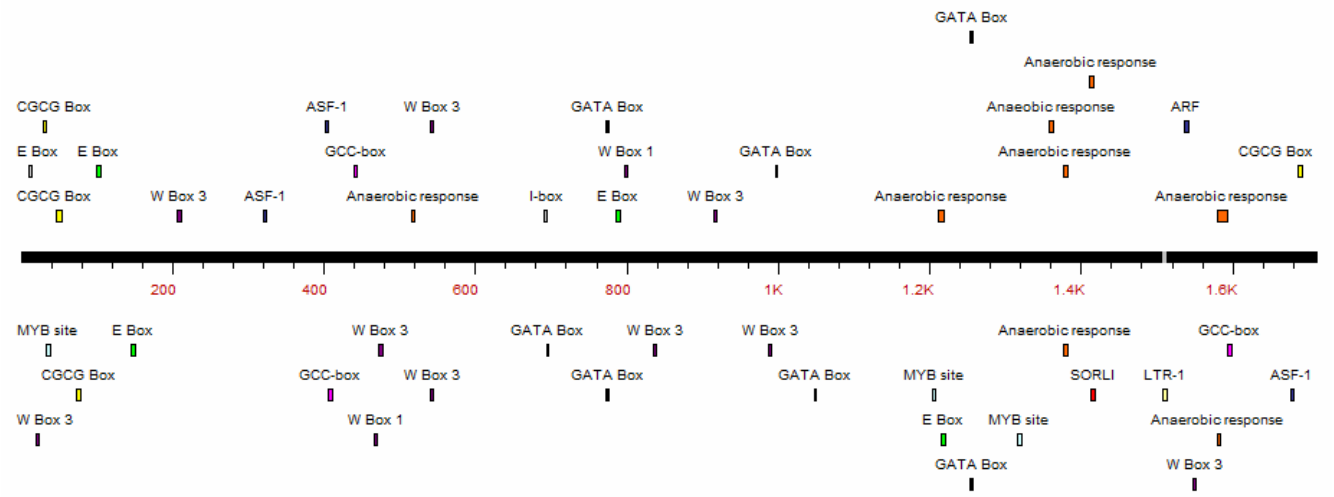

Fig. 5 The upstream promoter region of *RhACS1*, showing common ethylene-related response elements. The following promoter elements have been identified to relate to ethylene response and signaling: ARF (Auxin Response Factor); ASF-1 binding site, Anaerobic response element, CGCG Box, E Box, GCC-box, GATA box, I-Box , MYB site, LTR-1, W Box 1; W Box 3. Refer to text for description of each element. The elements were identified using PLACE and constructed using DS gene software. See the results section for details. The start codon would be immediately to the right of the thick solid line with nucleotide position shown in red.

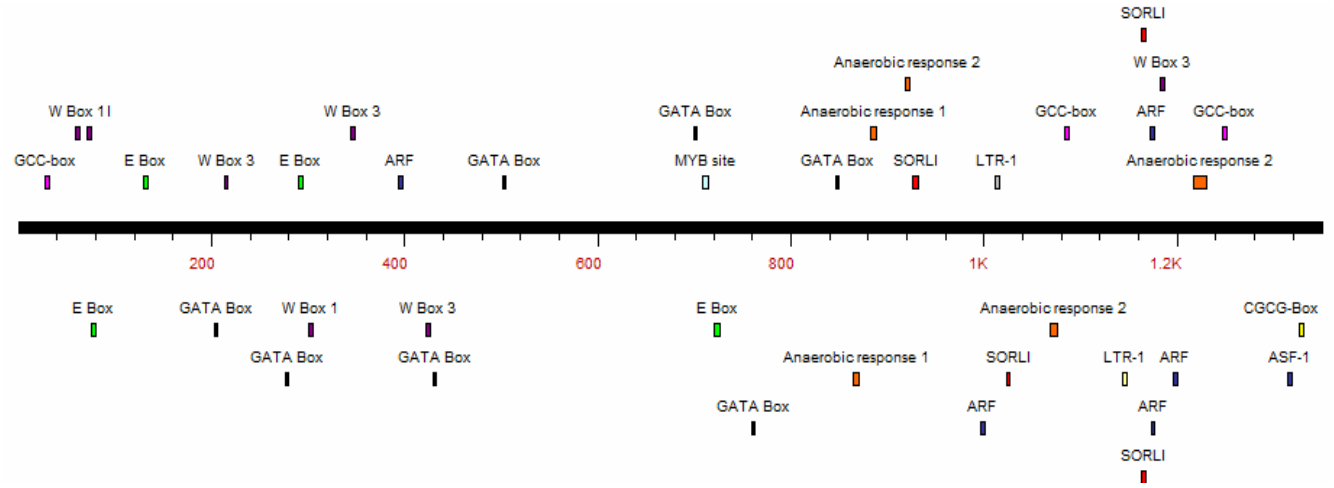

Fig. 6 The upstream promoter region of *RhACS12* showing common ethylene-related response elements. Other details are presented in **Fig. 5** and results section.

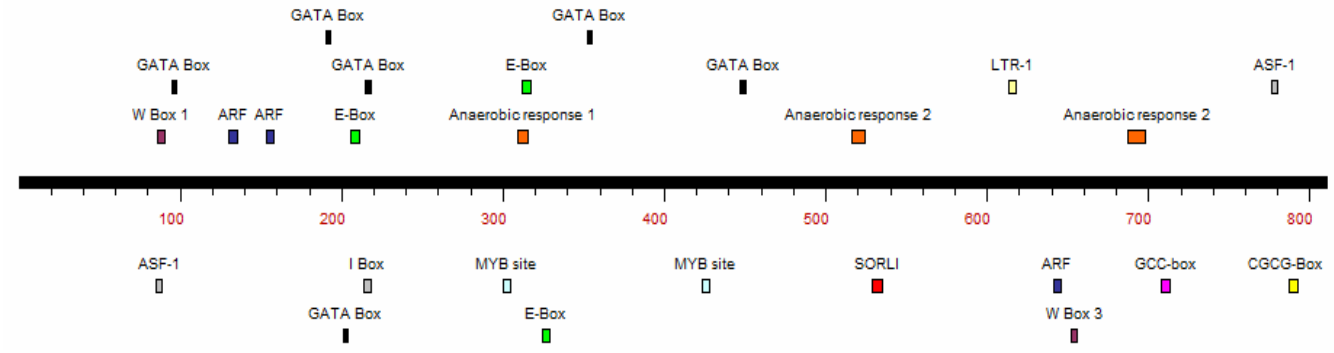

Fig. 7 The upstream promoter region of *RhACS17* showing common ethylene-related response elements. Other details are presented in **Fig. 5** and results section.

isolated (reviewed in Chang and Bleecker 2004) and have been shown to be encoded by a multi-gene family in which each gene is differentially expressed in response to internal and external stimuli e.g. in the case of *Arabidopsis* there are twelve ACS genes (Tsuchisaka and Theologis 2004; http://www.arabidopsis.org/). In the present study on *Rosa* ACS genes, we have described three full-length genes. Several groups have published partial sequences of a number of ACS genes including a segment of cDNA (Müller *et al.* 2000; Ma *et al.* 2005; Mibus and Serek 2005). Since all these isolates differ from each other, an overall picture emerging from these findings is that, as in other plants, we are dealing with a related but divergent multi-gene family. This observation is also consistent with our previous results from Southern blot analysis of the genomic DNA probed with RKacc7 full-length cDNA (Wang et al. 2004) and from the limited data available on the expression pattern of some of the genes (Wang *et al.* 2004; Ma *et al.* 2005). We note that a more complete comparative analysis of the *Rosa* ACS gene is presented in the next paper (Ranu *et al.* 2008 in press). In future, as a full complement of full-length ACS genes from the *Rosa* multi-gene family is identified, a better

picture of their differential regulation will emerge. This knowledge may be used to develop new rose cultivars with desirable characteristics through genetic manipulations or modifications.

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