J. Plant Biotechnology(2002) *Vol. 4(1). pp. 7~15*

Journal of Plant Biotechnology

Identification of Fruit-specific cDNAs in a Ripened Inodorus Melon Using Differential Screening and the Characterization of an Abscisic Acid Responsive Gene Homologue

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Key words: *Cucumis melo.* var kuwata, differential screen ing, fruit ripening, gene expression, promoter

Abstract

Eight cDNAs corresponding to fruit-specific genes were isolated from ripened melon through differential screening. Sequence comparison indicated that six of these cDNAs encoded proteins were previously characterized into aminocyclopropane-1-carboxylate (ACC) oxidase, abscisic acid, stress and ripening inducible *(ASR)* gene, RING-H2 zinc finger protein, pyruvate decarboxylase, or polyubiquitin. *RFS2* and *RFS5* were the same clone encoding polyubiquitin. The other cDNAs showed no significant homology with known protein sequences. The *ASR* homologue (*Asr1*) gene was further characterized on the cDNA and genomic structure. The deduced amino acid sequence had similar characteristics to other plant ASR. The *Asr1* genomic DNA consisted of 2 exons and 1 intron, which is similar to the structure of other plants *ASR* genes. The promoter region of the *Asr1* gene contained several putative functional *cis*-elements such as an abscisic acid responsive element (ABRE), an ethylene responsive element (ERE), a G-box or DPBF-1 and 2, Myb binding sites, a low temperature responsive element (LTRE) and a metal responsive element (MRE). The findings imply that these elements may play important roles in the response to plant hormones and environmental stresses in the process of fruit development. The results of this study suggest that the expressions of fruit-specific and ripening-related cDNAs are closely associated with the stress response.

Introduction

Since fruits provide essential nutrients including vitamins, minerals, and functional materials such as caro tenoids to human, their economical importance and the demand are increasing. It is well-known fact that the nutritional content in fruits changes during fruit ripening, and thus the research on fruits has been focused mostly on ripening (Seymour et al., 1993). Recently, there have been some attempts to manipulate the nutrition and quality, especially characteristics associated with ripening (Romer et al., 2000; Muir et al., 2000). The fruit specific and/or ripening-associated genes and their promoters have been utilized for the genetic engineering of fruits (Hadfield et al., 2000).

Fruit ripening is known to be a complex developmental process, characterized by the differentiation of chloroplast into chromoplast. The ripening process involves many biochemical and physiological changes, including the breakdown of chlorophyll, degradation of the cell wall, conversion of starch to sugars, alteration in pigment biosynthesis, and accumulation of flavor, aromatic compounds, polyunsaturated lipids, and lipid peroxides (Giovannoni, 2001). These changes make the fruits edible and desirable to seed dispersing organisms, and render

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Received Jan. 20, 2002; accepted Feb. 15, 2002.

fruits valuable. Studies with both differentially expressed genes and their mutants with impaired ripening have indicated that fruit ripening is a genetically programmed organ differentiation (Giovannoni, 2001).

According to respiration and ethylene biosynthesis rates, the developmental stages of fruits are divided into two major classes, climacteric and non-climacteric. Both types of fruits have not only unique appearances including hormonal regulated responses and cell wall metabolism, but also have common changes in biochemical and physiological aspects by sharing of common genes and genetic regulatory elements. Genes showing differential expression patterns during fruit ripening have been isolated from a number of climacteric fruits and non-climacteric fruits (Giovannoni, 2001). It is known that many ripening-regulated genes are fruit-specific genes (Hadfield et al., 2000).

Muskmelon is one of the most economically important vegetable crops cultivated in many parts of the tropic and subtropic regions. Muskmelon is a polymorphic species and has been further classified into two groups, reticulatus and inodorus group (Seymour et al., 1993). The reticulatus group includes the netted or rough-skinned melons and the inodorus group includes the smooth-skinned types, which show different developmental patterns associated with fruit ripening. While reticulates group is growing until the time of abscission, inodorus group develops more rapidly and then ceases to grow until it ripens. The differences suggest that there are discrete molecular mechanisms specific to unique fruit development traits that differentiate each type. Until now, most studies on fruit ripening of muskmelons have been concentrated on reticulatus melon, probably from the difference in favorable cultivars between the West and the Orient. Thus, there is a need to identify additional genes involved in melon ripening from inodorus types.

In reticulatus melon, genes involved in the aroma formation (Shalit et al., 2001), ethylene metabolism and signal transduction (Balaque et al., 1993; Ayub et al., 1996; Sato-Nara et al., 1999; Ishiki et al., 2000), carotenoid biosynthesis (Karvouni et al., 1995), and cell wall disassembly (Hadfield et al., 1998; Rose et al., 1998) have been characterized in molecular levels. Recently, cDNAs which are up-regulated by ripening were cloned by differential screening from the Charentais melon, a reticulatus type (Aggelis et al., 1997; Hadfield et al., 2000) as an alternative model system for climacteric fruits. Thus, the studies with inodorus melon in molecular levels can lead to an expansion of the information on the fruit ripening in climacteric fruits. Furthermore, the isolation of ripening-regulated genes and promoters may provide a key for modification of fruit quality and nutrient content by genetic engineering.

In this study, the fruit-specific cDNAs by differential screening from a ripened inodorus melon cultivar (*Cucumis melo.* var kuwata) were identified. A sequence analysis and comparison indicated that the fruit-specific genes in inodorus melon might be closely related in their functions during fruit ripening.

Materials and Methods

Plant materials

Oriental melon (*Cucumis melo.* var kuwata), cultivated at the Songju Fruit Vegetable Experiment Station, Korea, was used throughout this study. Oriental melon fruit was subsequently harvested at specified ripening stages (Rose et al., 1998): IG, attaining full size between 14-21 DAP (days after pollination); MG, full size between 21-26 DAP; RP, ripe fruit suitable for commercial harvest; OR, overripe fruit. Tissues including leaves, stems, and roots were sampled at mature ripe (RP) stage.

Construction of a cDNA library and differential screening

Total RNA was extracted from ripened melon fruits using the hot phenol RNA isolation procedure (Verwoerd et al., 1989). Poly(A)$^+$ RNA was isolated by PolyATtract mRNA Isolation System III (Promega). A melon fruit cDNA library was constructed by using the Zap-cDNA synthesis and Gigapack II Gold cloning kits (Stratagene) according to the manufacturer's instructions. Duplicate plaque lifts, about 10,000 pfu per 150 mm plate, were made of a dilution of the cDNA library. The library was screened with the radiolabeled cDNA probes, as described below, by standard plaque lift methods (Sambrook and Russell, 2000). After prehybridization for 1-2 hr at 42°C in 30% formamide, 5 × Denhardt's solution, 5 SSPE, and 100 μg/mL denatured salmon sperm DNA, filters (Hybond-N membranes from Amersham Pharmacia Biotech) were washed twice in 2 × SSC and 0.05% SDS for 15 min at 42°C and twice in 0.2 × SSC and 0.1% SDS for 15 min at 68°C.

Duplicate filters from ripe melon cDNA library were first hybridized to cDNA probes synthesized from 1 g poly(A)$^+$ RNA either from ripe fruits or preripe fruits. The clones were isolated on the basis of their ability for preferential hybridization to the probe of ripe fruits. Primary isolates were put through second round screens either from the probe of ripe fruits or leaf. After the secondary screen, 8 clones remained positive and the phagemid from each clone was *in vivo* excised from the lambda-ZAP vector according to the manufacturer's instructions (Stratagene).

The cDNA probe was labeled with [α-^{32}P]dCTP by using the Prime-a-Gene labeling system (Promega).

Nucleotide sequencing

Nucleotide sequencing using the dideoxy chain termination method (Sanger et al., 1977) was done using universal primer of T3 promoter and T7 promoter, and custom-made (DNA International) oligonucleotide primers with an automated DNA sequencer (ABI 377, Perlkin Elmer/ABI). The partial length sequences were compared with all known translated DNA sequences at the National Center for Biotechnology Information using the BLASTX algorithm. Another computer analyses for the nucleotide and amino acid sequences were done by PCGENE software (IntelliGenetics Inc., Release 6.60).

Northern blot analyses

In an orthern blot analysis, the total RNA was isolated from fruits in four developmental stages (IG, MG, RP, and OR), and from leaves, stems, roots, and plancentas containing seeds of melon. RNA was fractionated on a denaturing agarose (1.2%) gel, and then transferred to the nylon (Hybond-N) membrane. Filters were prehybridized at 42°C for 1-2 hr in 50% formamide, 5 × SSPE, 5 × Denhardt's solution, 0.1% SDS, and 100 μg/mL denatured salmon sperm DNA. The hybridization to the labeled probe with [α-^{32}P]dCTP and random primer was done overnight in a hybridization buffer. The filters were washed twice at room temperature for 10 min in 2 × SSC and 0.1% SDS, once at 65°C for 15 min in 1 × SSC and 0.1% SDS, and twice at 65°C for 15 min in 0.1 × SSC and 0.1% SDS.

Construction and screening of a genomic library

Genomic DNA was isolated from strawberry leaves, by the method of Dellaporta (1983). The DNA was digested partially with *Sau*3AI, ligated with the ZAP Express *Bam*HI-Predigested Vector (Stratagene), and packaged into GigapackIII gold cloning kits (Stratagene), according to the manufacturer's instruction. *In vivo* excision of pBK-CMV phagemid vector was done in the *Escherichia coli* XLOLR strain. The library was screened with a radiolabeled *Asr1* cDNA probe, labeled with [α-^{32}P]dCTP, by standard plaque lift methods (Sambrook and Russell, 2000). The screening was done as described in differential screening.

Results and Discussion

Differential screening of ripening-associated and fruit-specific cDNA clones

Through differential screening of a ripe melon fruit cDNA library, using radiolabeleld cDNA prepared from mRNA of preripe (IG) fruits and ripe (RP) fruits as probes, twenty putative positive ripening up-regulated clones were isolated. To avoid the isolation of cDNAs encoding seed proteins, the placenta/seed was removed from fruits. The pericarp and peel of fruits were used as experimental sample. To select cDNAs specifically expressed in fruits, clones from the primary screen were pooled and gone though another round of differential screening using radiolabeled cDNA generated from transcripts of RP fruits and leaves as probes. As a result, eight clones remained positive and each plasmid was partially sequenced (Figure 1 and Table 1). Database searches performed using partial length sequences showed that six of eight cDNAs are very similar to sequences in the database (Table 1). That is, six clones have known functions of pyruvate decarboxylase, ACC oxidase, and putative stress-induced proteins including RING-H2 zinc finger protein, *ASR* genes, and polyubiquitin. Two clones, *RFS2* and *RFS5*, indicated the same

Table 1. Ripening up-regulated and fruit-specific cDNAs from an inodorus melon.

RFS Clone No.	GenBank Accession No.	Homology (E value[a])	Proposed Function	Related Sequence Acession No.
RFS1		ACC oxidase (2e-63)	Ethylene biosynthesis	X69935, D31727, X95551
RFS2, RFS5	AF436850	Polyubiquitin (7e-88)	Protein degradation	X98063, X73156
RFS3	BI975269	None		
RFS4	AF426403, AF426404	ASR (3e-55)		
RFS6	AF436851	RING-H2 zinc finger protein (4e-44)	Stress response	U52865, AF093141 U76610
RFS7	AF436852	Pyruvate decarboxylase (2e-60)	Defense response	AF211532, AB026654
RFS8	NA[b]	None	Fruit flavor biosynthesis	AF193791, U71121, AL031804

[a]This value was calculated by BLASTX program.
[b]Not accessible

clone, which results from high expression level as shown in Figure 2. The others showed no significant homology with known protein sequences. Although the results showed different genes from Hadfield et al. (2000), except for ACC oxidase, it remains to be tested whether other seven clones are specific in inodorus melon group or in reticulatus.

The cDNA clones described above were used to probe northern blots prepared with RNA from fruits and leaves (Figure 2). Generally, the blots showed that the mRNA is strongly expressed in fruits and weakly in leaves, which supports that our differential screening has been well performed. The transcripts of RFS3, 4, 6, and 7 were detected only in fruits, not in leaves.

A. RFS1
CAAAAACAAAACCTCGATACCAAAGAGTTCTTTTTTCTTTACTTGCACAAACCAAATCTTGTATCTACAAAA
AGAAATGGCTGTCTTTCCTATCATCAACTTGGAAAACATCAATGGTGATGGTAGAGCTAAGATATTGGAGCA
AATTGAAGATGCCTGCCAAAATTGGGGTTTCTTTGAGTTGGTGAACCATGGGATCCCACATGAGTTTTTGGA
CATGGTGGAGAAGATGACAAGAGATCATTACAAGAAATGTATGGAAGAGAGGTTTAAGGAGACTGTGCTGAG
CAAAGGCTTAGAGGCTGCACAAGCTGAAGTTAATGATATGGATTGGGAAAGCACCTTTTTCTTACGCCATCT
ACCTGAATCAAACATCTCCCAGATGTCTGATCTCGACGAGGAGTATAAGAAAATTATGAAGGAATTTGCGAA
GAAATTGGAGAATCTTGCTGAGGAGTTGTTGGACCTGCTATGTGAGAATCTTGGGTTGGA

B. RFS8
CCAATTCACCGAGGTNATTTGAAGGAGGGTCNCCCCCAANAATNTTTTTGAANAAGAAANGNGACTNNTTTA
ACCAAGGGGCTCCTTNTTTTTGGGGNGGGATTAAATTNNGAAATNCCNNAANCCGNCCCCNCNAAAAAAAAA
GCCCCNATTTTCCGGNGGGNGTTTAAAACCTCTTGGGGGGN

Figure 1. Nucleotide sequence of RFS1 (A) and RFS8 (B). The sequence was determined by use of universal primer of T7 promoter.

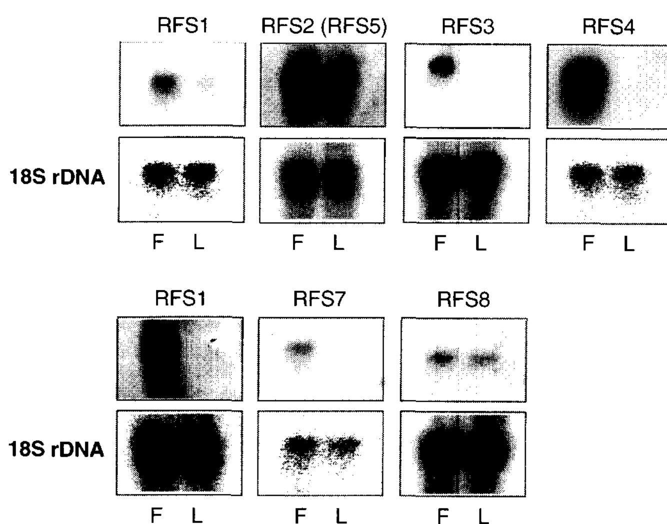

Figure 2. Northern blot analysis of cDNA clones in fruits (F) and leaf (L). Total RNA (10 μg/lane) was separated on a 1.2% formaldehyde gel, transferred to Hybond-N membrane (Amersham Pharmacia Biotech), and hybridized with cDNA probe. The same blot was hybridized with an 18S rDNA probe.

Features of the putative ripening-related and fruit-specific cDNA

Six clones similar to known genes were found to be characterized into ripening-regulated or stress responsive genes, as described below:

RFS1 encoding ACC oxidase has been previously characterized in melon and shown to be expressed abundantly during fruit ripening (Balaque et al., 1993; Ayub et al., 1996). Also, it is consistent with the result of Hadfield et al. (2000) who isolated a cDNA encoding ACC oxidase as a ripening-regulated cDNA in Charentais melon. ACC oxidase catalyzes the final step in ethylene biosynthesis. Ethylene is a key hormone in ripening of climacteric fruits including tomato and melon.

The protein deduced from the *RFS2* or *RFS5* sequence is related to polyubiquitin proteins, which mark proteins for degradation (Leverson et al., 2000). It has been reported that polyubiquitin is involved in response to stresses such as treatment with heat, injury, oxidative stress such as ozone and ethylene (Garbarino et al., 1992; Genschik et al., 1994; Wegener et al., 1997). Fruit ripening in melon was accompanied by a large rise in ethylene production, which may be induced by the up-regulation of gene expression in ACC oxidase (Martnez-Madrid et al., 1999). Also, a plant cell receives oxidative stresses from byproducts such as superoxide radical, hydrogen peroxide, and lipid oxides produced during fruit ripening (Allen, 1995; Kim and Chung, 1998). From these results, it is suggested that the function of polyubiquitin in melon is part of a fruit ripening.

The deduced amino acid sequence of *RFS4* cDNA (*Asr1*) showed that it is closely related to plant ASR proteins. It has been reported that the expression of *ASR* gene results from several stresses including cold, osmotic pressure, abscisic acid treatment, and in the process of fruit ripening (Gilad et al., 1997a; Padmanabhan et al., 1997; Vaidyanathan et al., 1999). A number of gene encoding ASR have been isolated and characterized from several plants including rice (Vaidyanathan et al., 1999), potatoes (Silhavy et al., 1995; Schneider et al., 1997), citruses (Canel et al., 1995), pines (Chang et al., 1996; Padmanabhan et al., 1997), apricots (Mbeguie-A-Mbeguie et al., 1997), lilies (Wang et al., 1998; Chang and Wang, 1999), tomatoes (Amitai-Zeigerson et al., 1994; Amitai-Zeigerson et al., 1995; Rossi et al., 1996; Gilad et al., 1997a; Gilad et al., 1997b), and pears (Itai et al., 2000). The detailed information on ASR including melon *Asr1* was described as below.

The deduced amino acid sequence of the *RFS6* cDNA clone showed high similarity with RING-H2 zinc finger protein of tobacco (GenBank accession no. AF211532) and

Arabidopsis (accession no. AB026654, AC005724), which is an *Avr9/Cf-9* rapidly elicited gene and may be involved in the defense response triggered by pathogen attack in plants (Salinas-Mondragon et al., 1999; Durrant et al., 2000). It was also induced in leaves in response to other stresses such as wounding, flooding of the apoplast in plants, and production of reactive oxygen species (Durrant et al., 2000). As described above, fruit ripening accompanies the accumulation of reactive oxygen species. Further more, a recent report indicated that the RING-H2 finger mediates the polyubiquitination described above, by direct interaction with ubiquitin-conjugating enzyme (Leverson, 2000).

The putative protein derived from the *RFS7* cDNA sequence has a similar sequence with pyruvate decarboxylase of strawberry (accession no. AF193791) and *Arabidop sis* (accession no. U71121, AL031804, AL161582), which catalyzes the formation of acetaldehyde, a precursor for the formation of ethyl esters important for fruit flavor. The expression profile of strawberry pyruvate decarboxylase cDNA showed differential expression according fruit development (Aharoni et al., 2000).

The isolation of stress responsive genes as fruit specific and ripening-regulated genes suggests that the process of stress response and fruit ripening may share common elements, which is supported by the results of *Asr1* genomic structure analysis, as described below.

Sequence comparison of the RFS4 cDNA (*Asr1*) with other plant ASR gene

As shown Figure 2, the *Asr1* transcripts are expressed fruit-specific and most strongly out of isolated eight clones, but these are not detected in leaves. To obtain the information on the relationship between fruit ripening and gene expression, the *Asr1* gene was analysed.

Firstly, a cDNA encoding Asr1. Full-length cDNA was isolated by RACE (rapid amplification of cDNA ends) using 5′ Full RACE core set (Takara) (data not shown). The longest RACE product was sequenced and added into partial cDNA clone isolated from differential screening. The length of the *Asr1* cDNA (accession no. AF426403) is 518 bp containing a 339 bp coding region, which has an uninterrupted open reading frame deriving 12,901 Da (112 amino acids) polypeptide with an isoelectric point of 6.23. Comparison of the hydrophobicity profile predicted a highly hydrophilic protein which is likely to be a soluble protein. The deduced amino acid sequences were compared between melon and other plants (Figure 3). The sequences contain a conserved residue, a glycine in the rectangular of Figure 3, which were predicted a myristoylation site as described above. However, a serine residue predicted as a phosphorylation site in melon Asr1 protein was not conserved in other plants ASR protein. The existence of a nuclear targeting signal (KKESKEEEKE AEGKKHHH) at the carboxyl terminus suggests that the *Asr1* protein is a nuclear protein (Varagona and Raikhel, 1994). Recently, it was reported that plant ASR protein is a nuclear-localized protein and a putative transcription factor (Gilad et al., 1997a). The homology appears to be at two regions, a short region of about 20 amino acids at the amino terminus and a longer region of about 90 amino acids at the carboxyl terminus in polypeptides (Figure 3, Box 1 and 2). The spacing sequence is variable in both length and amino acids composition. The phylogenetic dendrogram indicated that the sequence of Asr1 has the highest homology with potato and tomato (Figure 4). Generally, the phylogenetic relatedness is closer to each other in isoforms of same plant species than inter-species.

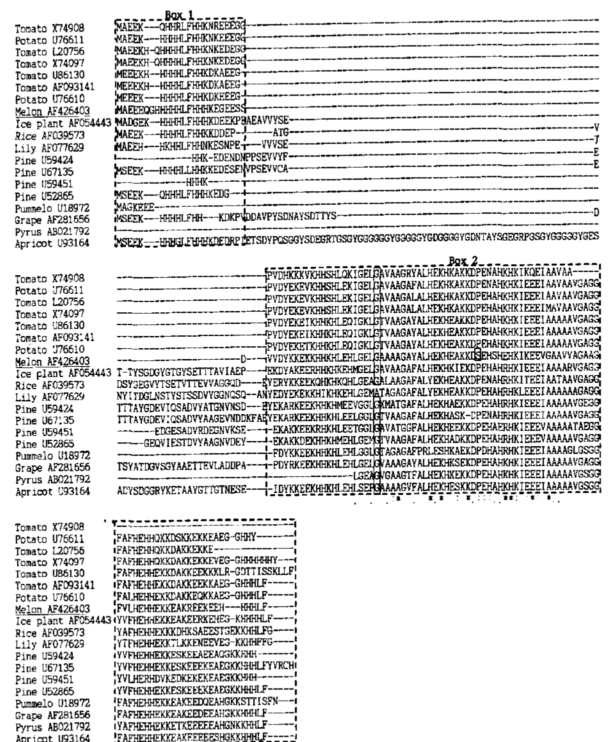

Figure 3. Comparison of the deduced amino acid sequences of the *ASR* gene from various species. The gene identities are described by plant names and GenBank accession numbers. The predicted amino acid sequences of the plants *ASR* gene were aligned using the Clustal X (version 1.64b) and manually adjusted to obtain optimal alignment. Gaps are marked with dashes. The conserved amino acid residues are marked with asterisks. A glycine residue of putative myr istoylation site and a serine residue of putative phosphoryla tion site are boxed.

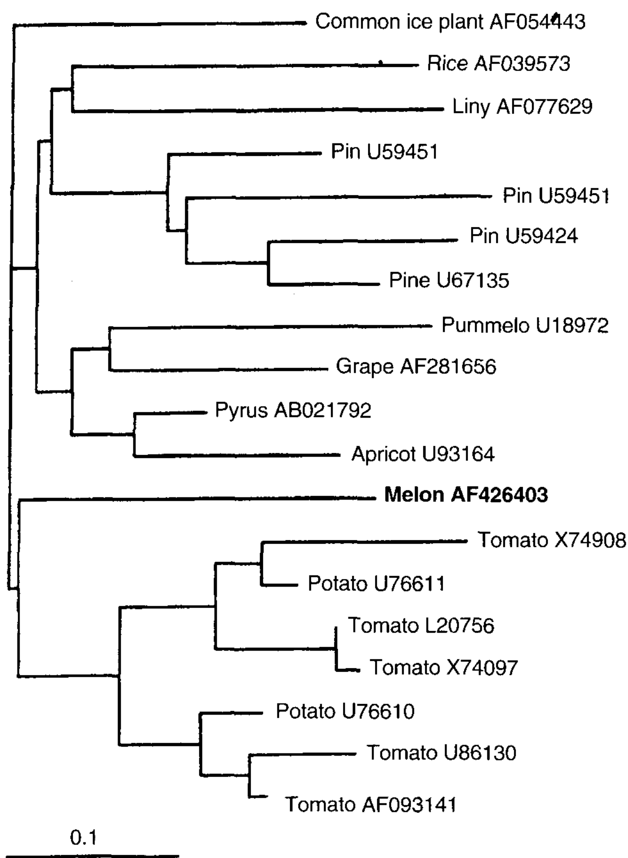

Figure 4. Phylogenetic relationship of the melon Asr1 to other various plants ASR. Phylogenetic analysis is based on the deduced amino acid sequences of *ASR* from various species, which the cDNA sequences was retrieved from GenBank. The tree was generated by Clustal X (version 1.64b) and TreeView (version 1.6.1).

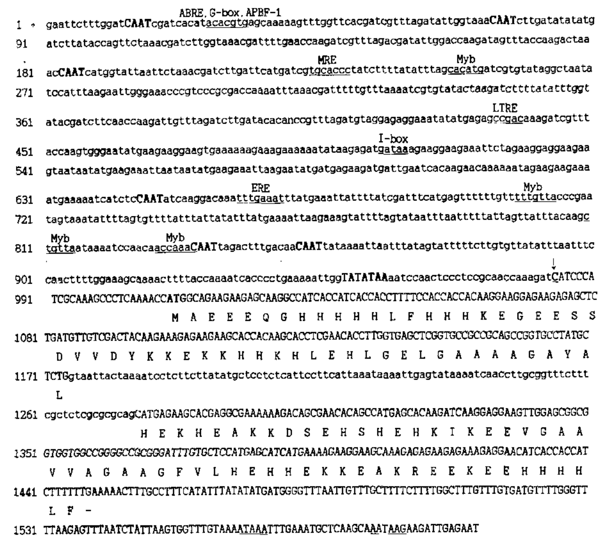

Figure 5. Nucleotide sequence and deduced amino acid sequence for the *Asr1* genomic clone of melon. Uppercase letters represent exons, and lowercase letters indicate an intron, and 5'-upstream regions. TATA and CAAT boxes are represented by uppercase letters in bold. The potential promoter elements are represented both by underlines and letters above: ABRE, abscisic acid responsive element; ERE, ethylene responsive element; MRE, metal responsive element; LTRE, low-temperature responsive element; G-box, I-box, and Myb, other cis-elements. The other underlined letters indicate the poly(A) signal. The transcription start site is represented by uppercase letter (gatcAT) in bold with arrow.

Genomic structure of *Asr1* cDNA clones

To elucidate the relationship between fruit ripening and gene expression in inodorus melon, we isolate a genomic DNA, containing the *Asr1* gene, of about 10 kbp in length from melon genomic library through hybridization with a cDNA encoding Asr1 was isolated. The genomic DNA was digested with *Eco*RI, and then the fragments were inserted into pBluescript SK(+) vector. The fragment containing an *Asr1* genomic region was partially seq uenced. Sequence analysis and restriction mapping showed that the sequencing region of 1,602 bp in length contains an *Asr1* genomic region (accession no. AF426404). The restriction map and structure of the genomic DNA are shown in Figure 5.

By comparison of the gene sequence with cDNA, the exon/intron organization was identified. The isolated *Asr1* gene was composed of two exons interrupted by one intron (Figure 5), which is similar to the structure of tomatoes (Amitai-Zeigerson et al., 1994; Gilad et al., 1997a; Gilad et al., 1997b) and potatoes (Schneider et al., 1997). The splice site of the intron followed the GT-AG rule for eukaryotic mRNAs. Putative polyadenylation signals were found at two positions 15621567 (AATAAA) and 15851590 (AATAAG). The transcription start site was inferred from RACE results to isolate full-length cDNA clone encoding Asr1 (Figure 5).

Several potential regulatory elements were found between 1 and 983. A putative TATA box (TATATAA) was identified at position 950. Six putative CAAT box motifs were dispersed throughout the upstream region. In addition to the ubiquitous elements, the *Asr1* promoter region contained sequences similar to regulatory *cis*-elements found in other plant genes. A potential ABRE (abscisic acid responsive element) (ACACGTG) is found at position 27 bp, which is overlapped with G-box element and DPBF-1 and 2, a class of bZIP transcription factors, binding core sequence (CACGTG). The overlapping observations were found in other genes (Busk and Pagès, 1998). It is presumed that this site is preferred to the G-box element or DPBF-1 and 2, because the level of abscisic acid contents was decreased in the development of fruit

(Martnez-Madrid et al., 1999). Four putative Myb binding sites with several different functions were found at four positions: 247 bp (CACATG) involved in response to dehydration and in abscisic acid induction, 708 bp (TTT-GTTA) forming gibberellin response complex, 820 bp (CTGTTA) involved in response to water stress and in regulation of flavonoid biosynthesis, and 829 bp (ACCAAAC) involved in regulation of phenylpropanoid biosynthesis. An LTRE (low temperature responsive element) (CCGAC) and an MRE (metal responsive element) (TGCACCC) were located at position 435 bp and 224 bp, respectively. Also, since ethylene is a major regulator of fruit ripening in melon, it is expected that the ethylene-responsive element (ERE) exists in the *Asr1* promoter region (Montgomery et al., 1993). As expected in this study, an ERE (TTTGAAAT) was found at position 662 bp in reverse orientation, which suggests that the *Asr1* gene is an ethylene-regulated gene. Also, a putative I-box element (GATAA) was located at position 506 bp, which is conserved sequence of light-regulated genes in both monocots and dicots (Terzaghi and Cashmore, 1995). The existence of several stress responsive and ripening-regulating elements such as Myb, LTRE, MRE, ABRE, ERE, and G-box supported that *Asr1* gene is a stress-inducible and ripening-related gene. While the large amount of ethylene accumulates during fruit ripening of melon, the level of abscisic acid decreases (Martínez-Madrid et al., 1999). Thus, ERE is important for the regulation of fruit ripening, but not for ABRE. From these observations, it is proposed in this study that the regulation of expression in the development of fruit occurred primarily by ethylene and stresses, which supports that the stress response and ripening were closely associated. The multiple promoter elements suggest that the gene may be regulated by a number of stimuli in non-fruit tissues, which its expression is tested in vegetative tissues when exposed to stress.

In summary, the eight clones showing differential expression and functional relatedness in an inodorus melon were isolated under the study. Out of these clones, the *Asr1* gene showed strongest expression in fruit specifically. The results suggest that the *Asr1* mRNA is expressed in response to several stresses and hormones, and the protein is a nuclear protein and DNA binding protein. The *Asr1* promoter showing fruit dominant and specific expression may be useful for genetic engineering to endow valuable quality and nutrient contents in melon fruits. By use of this promoter, the manipulation of sugar content in melon fruits is being attempted.

Further analysis by interactions of putative *cis*-elements with a transcription factor will elucidate function of Asr1 protein in response to hormones and stresses. Also, the studies on localization of Asr1 protein by protoplast culture and the binding of Asr1 protein with DNA may be helpful in functional cellular metabolism.

Acknowledgements

This work was supported by the Brain Korea 21 Project in 2002 and by research grants from Ministry of Agriculture and Forestry in Korea.

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