GENE NOTE

Characterization of an abscisic acid responsive gene homologue from *Cucumis melo*

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Abstract

A cDNA and genomic DNA encoding an abscisic acid responsive gene (ASR) homologue (Asr1) was isolated from an inodorus melon, *Cucumis melo* var. kuwata, cDNA and genomic library. The *Asr1* gene showed the strongest fruit-specific expression and differential expression profiles during fruit development, which were expressed from a low copy gene. The promoter region of the *Asr1* gene contained several putative functional cis-elements, which may be involved in the response to plant hormones and environmental stresses. These results suggest that *Asr1* may play an important role in the regulation of melon fruit ripening.

Key words: ASR, *Cucumis melo* var. kuwata, fruit ripening.

Fruit ripening is known to be a complex developmental process that 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 the accumulation of flavour, aromatic compounds, and lipid peroxides (Giovannoni, 2001). Studies with both differentially expressed genes and mutants with impaired ripening have indicated that fruit ripening is a genetically programmed process (Giovannoni, 2001). Muskmelon is one of the most economically important vegetable crops which is cultivated in many parts of the tropical and subtropical regions of the world. It has been classified into two groups; the reticulatus and inodorus groups (Seymour et al., 1993). Cultivars of the inodorus melon group are the Orient and the smooth-skinned types. This report investigates whether the ASR homologue may play an important role in fruit ripening of inodorus melons.

A cDNA (RFS4) encoding *Asr1*, a fruit ripening-related gene, was isolated through differential screening using a ripe fruit cDNA library of *C. melo* var. kuwata, an inodorus melon. The length of the *Asr1* cDNA (Accession No. AF426403) is 518 bp. The *Asr1* cDNA contains a 339 bp coding region, which encodes a 13 kDa polypeptide with an isoelectric point of 6.23. It lacks tryptophan and cysteine residues. The *Asr1* polypeptide has a putative nuclear targeting signal (KKESEKEKEAEKGGKHCHH) at the carboxyl terminus. The motif search predicted the presence of one cAMP- and cGMP-dependent protein kinase phosphorylation site and one myristoylation site. It was reported that myristoylation plays a role in membrane binding of proteins and that the residues facilitate membrane interaction. The existence of a nuclear targeting signal, a phosphorylation site and a myristoylation site suggests that melon *Asr1* protein is a nuclear protein. It was reported recently that plant ASR protein is a nuclear localized protein, has a Zn²⁺-dependent DNA-binding activity in tomato (Gilad et al., 1997), and is a putative transcription factor in grape berries (Accession No. AF281656). The phylogenetic dendrogram indicated that plant ASR can be categorized into four main groups and the sequence of *Asr1* has the highest homology with tomato and common ice plant (Accession No. AF054443) (Fig. 1). The genomic blot pattern probed with *Asr1* cDNA showed one or two bands, which indicated that the *Asr1* gene is present as a small gene family in the melon genome (data not shown).

Northern blot analysis of the *Asr1* gene showed that the transcripts of the melon *Asr1* were detected only in fruit tissues of the seed/placenta and pericarp, but not in leaves, stems, and roots (Fig. 2). During fruit development, the transcripts of *Asr1* showed differential accumulation profiles, and the highest accumulation level was at the RP stage. The transcripts increased to their maximum at the RP stage, but slightly decreased as the fruits matured. These results indicate that expression of the *Asr1* gene is regulated in a tissue- and developmental-specific manner at the transcript level. While plant ASR mRNA was commonly accumulated during fruit development (Canel et al., 1995; Gilad et al., 1997; Vaidyanathan et al., 1999; Itai et al., 2000), the patterns of melon *Asr1* expression were slightly different from other plant ASR for different tissue types. In other plants, ASR transcripts and protein were detected in most tissues under unstressed conditions with different levels of expression. It has also been reported that expression of the plant ASR gene results from several stresses including cold, osmotic pressure, abscisic acid treatment, and in the process of fruit ripening (Gilad et al., 1997; Padmanabhan et al., 1997; Vaidyanathan et al., 1999). Thus it is potentially for stress-regulated expression of *Asr1* throughout the plant.

In order to obtain more information on the regulatory mechanism of expression, *Asr1* genomic DNA (Accession No. AF426404) containing a promoter region, was isolated from a melon genomic library. In addition to the ubiquitous elements including TATA and
CAAT boxes, the Asr1 promoter region contained sequences similar to the regulatory cis-elements found in other plant genes. A potential abscisic acid responsive element (ABRE) was found at position ±984 from the transcriptional start site, overlapped with a G-box element and DPBF-1 and 2, a class of bZIP transcription factors, binding core sequence. An ethylene-responsive element (ERE) was located at position ±662 in the reverse orientation, which suggests that the Asr1 gene is an ethylene-regulated gene. Four putative Myb binding sites were found at four positions ±155, ±164, ±760, and ±737. In addition, a low temperature responsive element (LTRE) and a metal responsive element (MRE) were located at positions ±549 and ±760, respectively, between ERE and ABRE. The existence of several stress responsive and ripening-regulating elements such as Myb, LTRE, MRE, ABRE, ERE, and G-box support the conclusion that the Asr1 gene is a stress-inducible and ripening-related gene.

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References