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Molecular cloning and functional characterization of two apple *S*-adenosylmethionine decarboxylase genes and their different expression in fruit development, cell growth and stress responses

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Abstract

Two full-length *S*-adenosylmethionine decarboxylase (SAMDC) cDNAs, *MdSAMDC1* and *MdSAMDC2*, were isolated from apple [*Malus sylvestris* (L.) Mill. var. *domestica* (Borkh.) Mansf.]. Both cDNAs encoded tiny and small ORFs in addition to the SAMDC ORFs, and genomic sequences of *MdSAMDC1* and *MdSAMDC2* contained two or three introns in the 5′ upstream regions, respectively. Yeast complementation experiment indicated that two *MdSAMDCs* encoded functional proteins, and that the tiny and small ORFs possibly repressed their translation efficiency. RNA gel blot analysis showed that *MdSAMDC1* were differentially regulated in fruits depending on the developmental stage and in cell suspension during the culture period, but *MdSAMDC2* did not. In contrast, *MdSAMDC2* was positively induced by cold and salt stresses, but *MdSAMDC1* was not. These results suggest that *MdSAMDC1* is mainly involved in fruit development and cell growth while *MdSAMDC2* in stress responses, compared with their respective counterpart.

Keywords: Apple (*Malus sylvestris* var. *domestica*); Extreme temperature stress; Fruit development; *S*-adenosylmethionine decarboxylase; Salt stress

1. Introduction

Polyamines are a group of small positively charged molecules that are produced ubiquitously in living cells and are involved in many cellular processes such as DNA replication, transcription, translation and cell proliferation. In plants, polyamines have been reported to play a crucial role in morphogenesis, embryogenesis, early fruit development and senescence (Galston and Sawhney, 1990; Pandey et al., 2000) and in the elicitation of resistance or tolerance responses to some biotic and abiotic stresses (Bouchereau et al., 1999).

S-adenosylmethionine decarboxylase (SAMDC), a pyruvoyl-dependent enzyme producing the aminopropyl group for spermidine and spermine, is one of the rate-limiting enzymes in polyamine biosynthesis. Genes encoding SAMDC have been cloned from several organisms including yeast (Kashiwagi et al., 1990) and human (Pajunen et al., 1988). Due to the important role of polyamines in plant development, there has been growing research interest on the function of *SAMDC* genes in many plant species. Recently, *SAMDC* cDNAs have been isolated from a variety of plant species such as potato (Mad Arif et al., 1994), pea (Marco and Carrasco, 2002), soybean (Tian et al., 2004), rice (Pillai and Akiyama, 2004) and so on. Generally,

Abbreviations: BA, N^6-benzylaminopurine; DAF, days after flowering; 2,4-D, 2,4-dichlorophenoxyacetic acid; DIG, digoxigenin-dUTP; EtdBr, ethidium bromide; IBA, 3-indolebutyric acid; ORF, open reading frame; SAMDC, *S*-adenosylmethionine decarboxylase.

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SAMDC cDNAs showed a temporal and spatial expression pattern, suggesting that *SAMDC* functions in a developmental and organ/tissue specific manner. Investigation indicated that *SAMDC* transcript initially produced a proenzyme that was then cleaved at a serine residue via an autocatalytic mechanism to form a functional protein complex containing α and β subunits (Xiong et al., 1997). Furthermore, the translation efficiency of SAMDC proenzyme was negatively regulated by tiny and small open reading frames (ORFs) located in the upstream regions of the encoding ORF (Hanfrey et al., 2002). On the other hand, results from transformation experiments with *SAMDC* genes indicated that a variety of plants showed enhanced resistance or tolerance to abiotic stresses including ozone, salt and drought (Roy and Wu, 2002), as well as biotic stresses such as fungal wilts (Waie and Rajam, 2003). In addition to stress responses, transgenic potato with antisense *SAMDC* cDNA showed a reduction in the level of *SAMDC* transcripts with modified phenotypes (Kumar et al., 1996). Moreover, tomato plants transformed with a *SAMDC* gene exhibited enhanced fruit phytonutrient content, fruit juice quality and vine life (Mehta et al., 2002). Thus, several lines of evidence suggest an important role of SAMDC not only in plant developmental and physiological processes, but also in eliciting plant responses to environmental stresses.

There is, however, a dearth of knowledge on the physiological function of *SAMDC* in fruit trees because only a peach *SAMDC* gene has been isolated so far (Ziosi et al., 2003). Therefore, understanding the function of *SAMDC* in apple, one of the most economically important fruit trees world-wide, may provide novel tools to overcome constraints for the breeding of apples with desirable agronomic traits including fruit quality characteristics, plant architectural types for easy orchard management and the manipulation of tolerance to biotic and abiotic stresses. In this study, to gain insights into molecular features of *SAMDC*, two full-length *SAMDC* cDNAs, *MdSAMDC1* and *MdSAMDC2*, were cloned by screening an apple shoot cDNA library and functionally characterized by yeast complementation. RNA gel blot analysis suggested that *MdSAMDC1* and *MdSAMDC2* were differentially regulated in developmental or physiological processes and in their response to extreme temperature and salt stresses.

2. Materials and methods

2.1. Plant materials

Flower buds, young and mature leaves of the apple cultivar 'Orin' (*Malus sylvestris* var. *domestica*) were collected in the experimental orchard of National Institute of Fruit Tree Science (Tsukuba, Japan). Fruits were collected at 19, 61, 103, 145 and 174 days after full bloom (DAF), from the same cultivar 'Orin' at the Apple Research Center, National Institute of Fruit Tree Science (Morioka, Japan). Fresh leaves, flowers and fruit flesh were immediately frozen in liquid nitrogen and stored at −80 °C for RNA extraction as described by Wan and Wilkins (1994).

Apple callus was induced from young fruits and maintained at 1-month intervals on callus subculture medium containing MS-salt (Murashige and Skoog, 1962), Nitsch organic component (Nitsch and Nitsch, 1969), 3% sucrose, 4.5 μM 2,4-dichlorophenoxyacetic acid (2,4-D), 1 μM N^6-benzylaminopurine (BA) and 0.8% agar in the dark at 25 °C. For suspension culture, the callus was transferred into the liquid subculture medium and placed on a rotary shaker at approximately 120 rpm in the dark at 25 °C. The cell suspension was subcultured three times at 2-week intervals before being used for growth experiment.

2.2. cDNA cloning

Total RNA was isolated from apple shoots with RNAqueous-Midi™ (Ambion). One microgram of total RNA was used to synthesize first-strand cDNA with a kit (Amersham Pharmacia Biotech). A partial *SAMDC* cDNA fragment was obtained by RT-PCR using an amplification profile containing 1 cycle of 10 min at 94 °C; 30 cycles of 30 s at 94 °C, 30 s at 45 °C, and 90 s at 72 °C and 1 cycle of 10 min at 72 °C. Degenerate primers: 5′-GAY TCN TAT GTN CTN TCN GAG TCN AG-3′ (upstream) and 5′-CT NGC RTA RCT GAA NCC RTC YTC NGG-3′ (downstream) were designed based on the conserved regions of plant SAMDC amino acid sequences DSYVLSESS and PEDGFSYAS, respectively. The amplified fragment was sequenced and compared with the *SAMDC* cDNAs of other plants to confirm whether it was a *SAMDC* homologue. The confirmed fragment was labeled with digoxigenin-dUTP (DIG) by PCR (Roche Diagnostics) and used as a probe to screen an apple shoot cDNA library as described by Zhang et al. (2003). Positive plaques were excised as pBluescript clones following the manufacturer's instructions (Stratagene). The cDNA clones were sequenced on both strands using the BigDye™ Terminator Cycle Sequencing Ready Reaction kit (PE Biosystems). DNA sequences were analyzed using the GCG software package (Genetics Computer Group) and GENETYX-MAC 10.1 (Software Development).

2.3. In vitro translation and Western blot analysis of *MdSAMDC2*

ORF encoding SAMDC in the *MdSAMDC2* was amplified by PCR with primers specific to *Nco*I (upstream primer 5′-CCA TGG CTG TAC CGG TCT CT-3′) and *Sma*I (downstream primer 5′-CCC GGG GAT CTT TGC CAT AC-3′) sites. The amplified PCR product was ligated into pIVEX2.3d expression vector (Roche Diagnostics) with His-tag. After in vitro translation using *Escherichia coli* lysate according to the instruction