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Cloning of two isoforms of soluble acid invertase of Japanese pear and their expression during fruit development

Kunio Yamada, Takuya Kojima, Nancy Bantog, Tetsuji Shimoda, Hitoshi Mori, Katsuhiro Shiratake, Shohei Yamaki*

Graduate School of Bioagricultural Sciences, Nagoya University, Chikusa, Nagoya 464-8601, Japan

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Summary

Soluble acid invertase (S-AIV; EC 3.2.1.26) in Japanese pear fruit has an important role in accumulating hexoses during fruit enlargement and regulates the sucrose-to-hexose ratio in the vacuole. Full-length cDNA of *PsS-AIV1* and *PsS-AIV2* isoforms were cloned from Japanese pear fruit and their amino acid sequences share 40% identity; *PsS-AIV1* was confirmed to code S-AIV isozyme purified previously. The roles of *PsS-AIV1* and *PsS-AIV2* genes throughout fruit development and in sugar composition were investigated by semi-quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) analysis using specific primers of their transcripts. *PsS-AIV1* transcript had a maximum level at 34 days after full bloom (DAFB) and decreased rapidly during fruit development; *PsS-AIV2* transcript increased gradually during fruit growth from 34 DAFB, had its maximum level at 79 DAFB and remained high until 107 DAFB at active fruit enlargement. The activity of S-AIV was highest at 34 DAFB, decreased during fruit growth until 66 DAFB, remained almost the same during early fruit enlargement until 79 DAFB and then decreased again. Soluble sugars fructose and glucose began accumulating predominantly during fruit enlargement from 66 DAFB; sucrose began increasing rapidly during fruit maturation from 121 DAFB. High expression of *PsS-AIV1* transcript and high enzyme activity in the young fruit stage seems to have an important role in supplying a lot of substrate for energy needed for cell division and

Abbreviations: DAFB, days after full bloom; PRF, Protein Research Foundation; RACE, rapid amplification of cDNA ends; RT-PCR, reverse transcriptase-polymerase chain reaction; S-AIV, soluble acid invertase

*Corresponding author. Tel./fax: +81 52 789 4025.
E-mail address: yamaki@agr.nagoya-u.ac.jp (S. Yamaki).

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growth by hydrolyzing sucrose to hexoses. Increasing *PsS-AIV2* expression during fruit enlargement may lead to rapid cell expansion through increased osmotic pressure by accumulation of a large amount of hexose in the vacuole.

Introduction

Invertase (β-fructosidase, EC 3.2.1.26), which converts sucrose into glucose and fructose, is common in many higher plants. They include acid invertase and neutral invertase, which have different optimum pH range for their activity. A lot of research has been conducted about the acid invertase, though the function of neutral invertase, which is thought to exist in cytosol, is not known well. The acid invertase is present in the vacuole (soluble form) and in the cell wall (bound form). The bound form in the cell wall has a role in the unloading of sucrose translocated from the phloem to the apoplast, resulting in sugar uptake by the sink tissues (Roitsch and Gonzalez, 2004). Soluble acid invertase (S-AIV) has an important biological function relating to sucrose metabolism and presumably hydrolyzes sucrose to supply hexoses necessary for cell growth and development (Tymowska and Kreis, 1998; Tang et al., 1999). In grape berry, S-AIV cDNAs *GIN1* and *GIN2* have been cloned (Davies and Robinson, 1996). The expression of *GIN1* and *GIN2* in berries, which is higher in early berry development, declines greatly at the start of hexose accumulation (after the veraison period). Although vacuolar invertases participate in hexose accumulation in grape berries, expression of the genes and synthesis of the enzymes may precede the onset of hexose accumulation (Davies and Robinson, 1996). In carrot, S-AIV has two isoforms, SI and SII (Sturm et al., 1995). The *SI* gene is expressed in primary root and leaves at the early growth stage, and *SII* is expressed prominently up to the middle stage of tap root enlargement. Therefore, the *SI* gene may have a role in the supply of hexoses during cell division, and the *SII* gene may contribute to sugar accumulation and regulation of sugar composition in the vacuole (Sturm et al., 1995). However, the growth of tomato plants and the development of tomato fruit transformed by the *S-AIV* antisense gene are normal, and therefore, the highest activity of S-AIV at the later developmental stages in tomato fruit is not essential for the development of fruit and the sink strength (Ohyama et al., 1995). The S-AIV activity is correlated closely with the ratio of sucrose to hexose in sink organs of tomato fruit (Ohyama et al., 1995), potato tubers (Zrenner et al., 1996) and carrot root (Tang et al., 1999) transformed by the antisense gene of S-AIV. Sucrose hydrolysis by S-AIV in the vacuole is important for the cell expansion by regulating the osmotic pressure (Klann et al., 1996; Tymowska and Kreis, 1998).

Cell division in Japanese pear fruit stops during the early development stage, the fruit enlarges by accumulating hexoses in the vacuole, and then it accumulates a lot of sucrose during fruit maturation (Moriguchi et al., 1992). S-AIV activity is high in young fruit and decreases during fruit maturation (Moriguchi et al., 1992; Tanase and Yamaki, 2000). During fruit maturation, S-AIV seems to have important roles in supplying hexoses at the young stage and regulating the sucrose-to-hexoses ratio in the vacuole at mature stage. Perhaps, the presence of isozymes, which are usually expressed differentially during development, is needed for S-AIV to carry out appropriately its various roles. Isozymes AIV1 and AIV2 of S-AIV have been purified from Japanese pear fruit (Hashizume et al., 2003).

This study aimed to isolate the cDNA clones of S-AIV from Japanese pear fruit and to investigate their roles in fruit development by analyzing the expression of their transcripts, enzyme activity and sugar content.

Materials and methods

Plant material

Japanese pear fruit (*Pyrus pyrifolia* Nakai cv. 'Hosui') was harvested in Nomura's orchard at Anjou, Aichi-pref., Japan, at 34, 50, 66, 79, 93, 107, 121, 131, 139, and 147 days after full bloom (DAFB) in 2004. The fruit flesh collected at each stage was frozen in liquid nitrogen and was stored at $-80\,^{\circ}C$ until used for analysis.

Determination of partial amino acid sequence of S-AIV

An 80 kDa polypeptide for S-AIV1 and a mixture of 80, 52 and 34 kDa polypeptides obtained by using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) for S-AIV2 were digested with