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GENE NOTE

Putative *PIP1* genes isolated from apple: expression analyses during fruit development and under osmotic stress

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Abstract

To gain insight into the function of plasma membrane intrinsic protein (*PIP*) genes in apple, two genes, *MdPIP1a* and *MdPIP1b*, were isolated. *MdPIP1* expression was in accordance with the volume increase during fruit development, which is a loading process of water and solutes. In addition, the expression of *MdPIP1* was up-regulated in the stems by osmotic stress. These results indicate that *MdPIP1* may play important roles not only in fruit expansion, but also in maintaining water homeostasis under stress conditions.

Key words: Apple, fruit expansion, gene expression, osmotic stress, plasma membrane intrinsic protein (PIP).

Most fruits contain 80–95% water on a fresh weight basis. Rapid expansion of fruit is achieved by accumulating a large amount of water during the developmental processes (Coombe, 1976). Water deficiency makes fruits reduce in size and even fail to expand so as to drop, while excessive water supply decreases sugar content and results in poor taste. A dramatic change in water condition also leads to unfavourable fruit quality. For example, irrigation or rain following a long time of drought usually incurs fruit splitting. All these factors will greatly reduce the market value of fruits and bring economic loss to farmers. In addition, the plant stem dedicates itself to fruit yield and quality by transporting water and nutrients. Water homeostasis in this organ is important for the proper maintenance of this function. Fruit trees as perennial plants have to survive in the located environment for a long time once they have been planted. Therefore, many environmental factors can affect the water status. Recently, it has been documented that aquaporins are crucial to controlling water homeostasis in plants (Chrispeels and Agree, 1994). Investigations on the role of plasma membrane intrinsic protein (*PIP*) genes in regulating the accumulation of water during fruit development and under stress conditions should be valuable.

To isolate a partial *PIP* cDNA, RT-PCR was performed. The primer pairs 5′-GAC TAC AA(G/A) GA(G/A) CCA CCA CC-3′ (forward) and 5′-AGT GCA GCT CCA ATG AAG G-3′ (reverse) were designed for first PCR and the primer pairs 5′-ATC TC(T/C) GGT GGI CAC (A/G)TI AAC CC-3′ (forward) and 5′-G(T/G)C C(G/A/C)(G/A) CCC A(G/A)(T/A) AIA (C/T)CC AGT GG-3′ (reverse) for nested PCR. The PCR product was subcloned into the pCR®2.1 vector. After confirming the homology by searching the DNA databank, the partial *PIP* fragment was used as a probe to screen the cDNA library from apple shoots. As a result, two full-length cDNAs (*MdPIP1a*, AB100869 and *MdPIP1b*, AB100870) were isolated and identified as *PIP1* members. Due to the high identity of *MdPIP1a* and *MdPIP1b*, it was difficult to make specific probes to distinguish them. Hence DIG-labelled *MdPIP1a* was used as a probe for expression analysis. Northern blotting using total RNA from different apple tissues detected two different mRNA bands in all tissues tested, which might arise from the expression of a homologous clone such as *MdPIP1b* or additional RNA species as yet unknown (Fig. 1A). Among non-fruit tissues, *MdPIP1* showed the highest expression level in shoots and the second-highest level in young leaves, while the hybridization signal was just a trace in mature leaves (Fig. 1A). Among fruit tissues, young fruits at 19 d after full bloom (DAF) showed *MdPIP1* expression at a low level, while it was expressed strongly in apple fruits at later stages, from 61–174 DAF (Fig. 1A). Thus *MdPIP1* expression levels were in accordance with the patterns of fruit volume increase (Fig. 1B).

To understand whether and how the *MdPIP1* gene in the stem is expressed in response to various conditions of water supply, shoot cuts were treated by dipping in PEG solutions. The expression of *MdPIP1* in osmotic treatment by –2 and –4 MPa PEG solutions was up-regulated in 6 h compared with that in the reference solution. A similar trend was observed with a prolonged treatment time (Fig. 2). *MdPIP1* gene expression in the reference solution showed almost the same levels for 12 h, but its level increased at 24 h.

Cell expansion in fruits occurs mainly by water uptake accompanied by the transport of sugars and other solutes into vacuoles (Coombe, 1976). Therefore, water channels on the vacuole membrane are considered to be important for this phenomenon. Tonoplast intrinsic protein (*TIP*) genes were isolated and assayed during fruit development in pear (Shiratake *et al*., 2001) and peach (Sugaya *et al*., 2001). However, *γTIP* mRNA accumulation failed to be detected at the cell-expanding stage in both fruits. In this study, up-regulated expression of *MdPIP1* coincided with the fruit cell-expanding stage, suggesting the involvement of *MdPIP1* in water absorption during fruit expansion.

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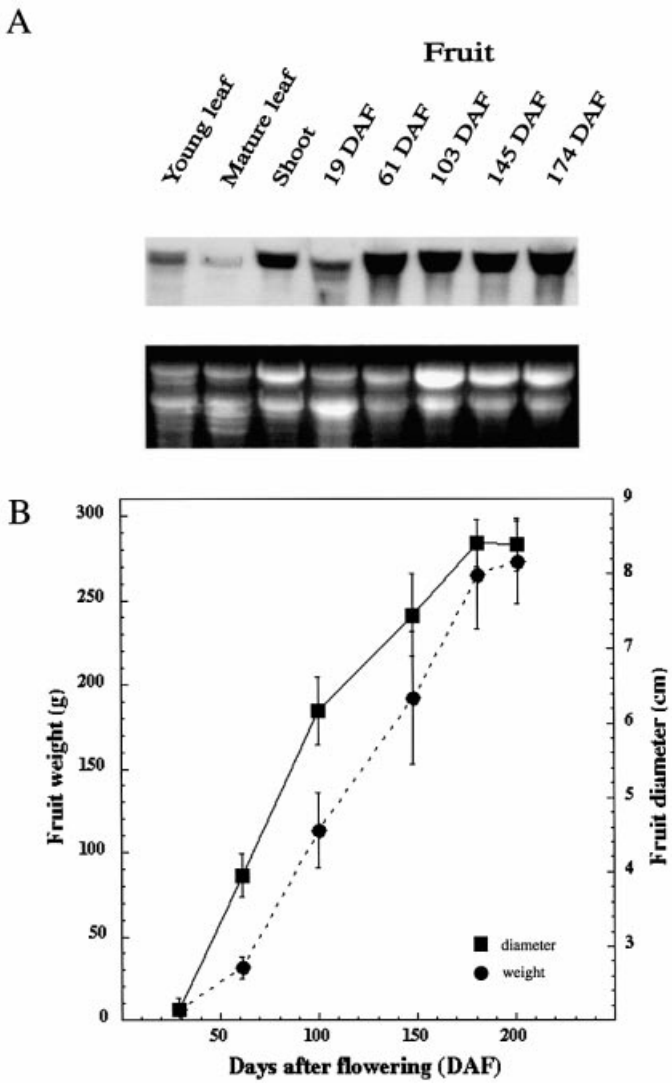

Fig. 1. (A) Northern blot analysis of *MdPIP1* in leaves, shoots and fruits. Total RNA was isolated from leaves, shoots and fruit tissues and was hybridized with DIG-labelled *MdPIP1a*. (B) Seasonal changes in apple fruit weight and diameter. Data at each stage are the mean of at least 10 replicates. Vertical bars represent mean ±SE.

The regulation of *PIP* upon osmotic stress varies with plant organs and species. Under water stress, the expression of *PIP* is down-regulated in rice roots and leaves (Li *et al*., 2000) and in the common ice plant (Yamada *et al*., 1995), but up-regulated in rice stems (Malz and Sauter, 1999) and in rape seed (Gao *et al*., 1999) as in the present result in apple stems. Despite the controversial results on *PIP* that expression may be, in part, due to the complex functions of this gene family in different cell types and at different development stages (Yamada *et al*., 1995), the present results still indicate that the expression of *MdPIP1* is regulated in response to various conditions of water supply through intracellular water transport in apple stems.

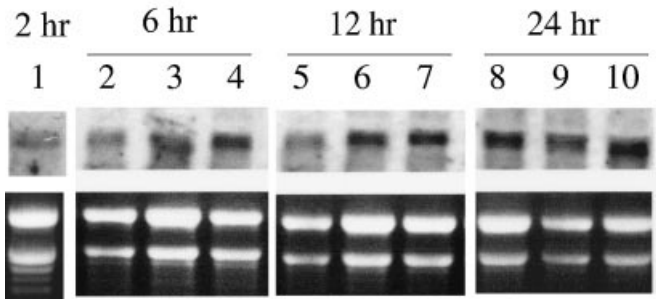

Fig. 2. Northern blot analysis of *MdPIP1* in stem under osmotic treatments. Semi-hard apple shoots were cut, and their cut ends were immersed immediately into a reference solution [10 mM KCl, 1.5 mM $CaCl_2$, 5 mM HEPES (pH 6.0)] for 2 h at 25 °C. After 2 h (lane 1), the shoots were transferred in the same way into reference solution (lanes 2, 5 and 8), –2 MPa (lanes 3, 6 and 9) or –4 MPa (lanes 4, 7 and 10) osmotic treatment solution for 6, 12 and 24 h at 25 °C. Osmotic solutions were prepared according to Michel and Kaufman (1973). After treatment, the leaves were picked off. Total RNA was isolated from stems and was hybridized with DIG-labelled *MdPIP1a*.

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