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Cloning and expression analysis of *PpSUT2* encoding a sucrose transporter in pear

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ABSTRACT. A 1794-bp cDNA fragment was amplified from mRNA isolated from pear (*Pyrus pyrifolia* NaKai. Cuiguan) leaves by using primers based on the sequences generated during the analysis of the pear transcriptome. The 597-amino acid sequence encoded by the cDNA was compared with the sequences in GenBank, and it was found to be similar to that of members of the sucrose-proton co-transporter family. The hydrophobic protein, which was predicted to have 11 transmembrane domains, was designated as *PpSUT2*. Real-time fluorescent quantitative polymerase chain reaction analysis indicated the accumulation of *PpSUT2* mRNA throughout the plant, with the highest levels in the buds. Analysis of the expression of *PpSUT2* during fruit development showed that the abundance of its transcripts increased at the end of April and then decreased to the lowest level at the end of July. Subcellular localization studies with the pCXDG vector as a probe demonstrated that *PpSUT2* localized to cell membranes. An expression vector was constructed by inserting the *PpSUT2* cDNA into pET32(a), and the vector was expressed in *Escherichia coli* (strain BL21) after induction with 1 mM isopropyl β-D-1-thiogalactopyranoside at 25°C. Analysis using sodium dodecyl

sulfate-polyacrylamide gel electrophoresis identified the induction of a 71-kDa protein. Further analysis indicated that PpSUT2 might be not directly involved in sucrose transport, instead, functioning as a sucrose sensor on the cytoplasmic membrane.

Key words: Sucrose transporter; *PpSUT2*; Subcellular localization; Real-time fluorescent quantitative polymerase chain reaction; Prokaryotic expression

