MOLECULAR GENETICS CHARACTERIZATION OF SUGAR TRANSPORTERS AND RELATED GENES DURING FLOWERING, GRAIN FILLING AND SEED GERMINATION IN RICE (Oryza sativa L.).

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Ph.D.(BIOTECHNOLOGY)

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ABSTRACT

The characterization of sugar transporters and related genes during flowering, grain filling, and seed germination has been investigated in rice. The partial cDNA of OsSUT2 (Oryza sativa L. sucrose transporter 2) was further cloned to complete the full-length of cDNA. The deduced amino acid sequences and topology of OsSUT2 and additional sugar transporter OsMST5 (Oryza sativa L. monosaccharide transporter 5) were similar to the sequences and topology of other plant sucrose and monosaccharide transporters, respectively. The functional sugar transport activity of these OsSUT2 and OsMST5 cDNAs were characterized by heterologous expression analysis. The growth of the mutant yeast strain, Saccharomyces cerevisiae SUSY7 co-expressed with OsSUT2 on a media containing sucrose as a sole carbon, indicated that OsSUT2 is an encoding functional sucrose transporter. Additionally, the ability of S. cerevisiae strain LBY416 co-expressed with OsMST5 cDNA to transport some monosaccharides, indicated that OsMST5 is a functional hexose transporter. Moreover the increased transport rate of yeast cells with OsMST5 cDNA, when ethanol as an electron donor was added, suggested that OsMST5 was an energy-dependent symporter. The transcriptions of OsSUT2 and OsMST5 were exclusively detectable before the pollinating stage, suggesting the association of these sugar transporters in import of sucrose and hexose is derived from sucrose breakdown, to promote pollen development at the early stage. In addition to the expression of a sugar transporter during flowering, other related genes were monitored by ESTs microarray analysis. The hybridization of fluorescence Cy5 dye-labeled poly A+ RNA isolated from designed tissues at 0, 3, 6, 10, and 14 DAP with target cDNAs containing 9,000 rice ESTs revealed the expression profile of a number of genes specific to early ovule and endosperm development. An interesting clone detected during early ovule development stage was selected and further cloned and characterized. The full-length cDNA of that candidate named OsORFX was cloned. The open reading frame of rice OsORFX cDNA composed of 549 bp encoding for 183 amino acids with a calculated molecular weight about 19.5 kDa. The predicted genomic OsORFX composed of 884 bp; 4 exons and 3 introns located on chromosome 2. The deduced amino acid sequence showed about 50% identity to predicted amino acid sequence of LeORFX from a tomato. The transcription of this gene was highly detected in seeds at 0 DAP.

Furthermore, the role of OsHXK II (Oryza sativa L. hexose kinase II) on rice seed germination has been studied. The hexokinase II knockout rice was isolated by means of reverse genetic approach. The abolish of transcription of OsHXK II was examined by RT-PCR. The persistence of OsHXK II knockout lines seedling to elongate in a sugar solution under anaerobic conditions, which may suggest the role of OsHXK II as a sugar sensor in rice embryos required additional experiments to confirm.

KEY WORDS : FLOWERING / GRAIN FILLING / SUGAR TRANSPORTER / MICROARRAY / RICE

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