

MOLECULAR AND FUNCTIONAL CHARACTERIZATION OF THE RICE HEXOKINASE GENE FAMILY

Jung-Il Cho, Seho Ko, Sang-Kyu Lee, Nayeon Ryoo,
Jun Ok Lee, Youn-Hyung Lee, Seong Hee Bhoo,
Tae-Ryong Hahn, Jong-Seong Jeon

Plant Metabolism Research Center & Graduate School of Biotechnology,
Kyung Hee University, Suwon 449-701, Korea

Hexokinase not only catalyzes the ATP-dependent conversion of hexoses to hexose 6-phosphates but also acts as a sugar sensor that perceives the levels and phosphorylation status of sugar to transmit this information to the nucleus through a signal transduction pathway. To investigate the role of hexokinase for rice development, we isolated cDNA clones showing amino acid similarity to the plant hexokinase proteins from a search of rice sequence databases, and analyzed gene structure, chromosomal localization, evolutionary relationships, temporal and spatial expression patterns and regulation by sugars such as sucrose, glucose and fructose. Rice hexokinase genes have a very conserved gene structure and mapped on chromosome 1, 5 and 7. The phylogenetic tree shows that *OsHXK2* has a high similarity to *AtHXK1* and *AtHXK2*. Profile analyses revealed that *OsHXK2* to *OsHXK9* are expressed ubiquitously in various organs whereas *OsHXK3* was flower-specific, especially pollen-specific. Sugars induced the accumulation of several *OsHXK* transcripts in excised leaves and immature seeds, while the level of *OsHXK7* was significantly down-regulated. We confirmed the hexokinase activity of these clones using the strategy of functional complementation of a yeast triple mutant (*hvk1, hvk2, glk1*). Interestingly, *OsHXK4* was not able to complement the yeast mutant because of the presence of the chloroplast transit peptide, but the yeast mutant regained the hexokinase activity when the chloroplast transit peptide was removed from *OsHXK4*. We confirmed that *OsHXK4* is a chloroplast stromal enzyme using transgenic callus and plants transformed with an *OsHXK4*-GFP fusion construct.

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