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Isolation of phosphate starvation induced genes by SSH

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Objectives

Our ultimate goal is to isolate many rice genes induced by phosphate starvation with SSH technique.

Material and Methods

Suppression subtractive hybridization (SSH) were used to isolate rice gene that specifically induced by phosphate starvation. SSH were performed by using PCR-Select™ cDNA Subtraction kit (Clontech, USA) according to the manufacturer's instructions. Phosphate starvation treated rice calli and phosphate supplement treated rice calli were used as tester and driver, respectively. Tester and driver cDNAs are hybridized, and the hybrid sequences are then removed. Consequently, the remaining unhybridized cDNAs represent genes that are expressed in the tester, but are absent from the driver mRNA.

Results and Discussion

Functional analysis was first achieved by grouping phosphate starvation responsive genes according to the predicted functions of their proteins. Individual genes were assigned to sixteen different functional categories, which were automatically derived using MIPS A. thaliana database (MATDB) at the server of Munich Information Center for Protein Sequences (MIPS). Percentage of functional classification are like that metabolism, protein FATE are 7%, energy, cell cycle and DNA processing, transcription, protein synthesis, cellular transport, cellular communication, cellular organization are 3%, cell rescue are 4%, cell fate, development, transport facilitation are 1%, subcellular localization are 10%, classification not yet are 6%, and unclassified proteins are 42%.

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