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Global Identification of *ANTH* Genes Involved in Rice Pollen Germination and Functional Characterization of a Key Member, OsANTH3

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[Introduction]

In pollen germination process, the secretory vesicles deliver cell wall and plasma membrane materials, and excessive materials are sequestered via endocytosis. AP180 N-terminal homology (ANTH) domain-containing proteins function as adaptive regulators for clathrin-mediated endocytosis in eukaryotic systems. However, The role or even the existence of the *ANTH* gene family in monocot plants is not well understood. Our study will contribute to a comprehensive understanding of the ANTH family in rice and their role in late pollen development.

[Materials and Methods]

The plant material used two japonica rice (Dongjin and Nipponbare), together with mutant using T-DNA insertion and CRISPR/Cas9 system in the Dongjin background. Meta-Expression Analysis was performed with the rice Affymetrix microarray data and tested with qRT-PCR. Subcellular Localization analysis was conducted on tobacco leaf. Pollen germination tests using Ika solution suggest a cytological interpretation of pollen germination.

[Results and Discussion]

Motif and phylogenomic analyses revealed seven NPF-rich and 10 NPF-less subgroups of these proteins, as well as various clathrin-mediated endocytosis-related motifs in their C-terminals. To investigate their roles in pollen germination, we identified five mature pollen-preferred *OsANTH* genes. The subcellular localization of four OsANTH proteins that were preferentially expressed in mature pollen suggested their role in endocytosis in the plasma membrane. Of them, OsANTH3 represented the highest expression in mature pollen. Functional characterization of *OsANTH3* using T-DNA insertional knockout and gene-edited mutants revealed that a mutation in *OsANTH3* decreased seed fertility by reducing the pollen germination percentage in rice. Thus, our study suggests OsANTH3-mediated endocytosis is important for rice pollen germination.

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