

# Functional characterization of the three *Oryza sativa* SPX-MFS proteins in maintaining phosphate homeostasis

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## Abstract

Plant vacuoles serve as the primary intracellular compartments for phosphorus (P) storage and play a central role to maintain P homeostasis. The *Oryza sativa* (rice) genome contains three genes that encode SPX (SYG1/PHO81/XPR1)-MFS (Major Facility Superfamily) proteins (OsSPX-MFS1, 2, 3). OsSPX-MFS1 and OsSPX-MFS3 were shown previously to have vacuolar phosphate (Pi) transporter activities, but the physiological role of the three transporters under varying P conditions and under field grown conditions for a crop plant is not known. To address this knowledge gap, we generated single, double, and triple mutants (7 mutants with at least two lines of each) for the three rice Os *SPX-MFS* genes. All the mutants except *osspx-mfs2* display lower vacuolar Pi concentrations and all Os *SPX-MFS*s overexpression plant lines display higher Pi accumulation, demonstrating that all three OsSPX-MFSs are vacuolar Pi influx transporters. OsSPX-MFS3 plays the dominant role based on the phenotypes of three single mutants in terms of growth, vacuolar and tissue Pi concentrations. OsSPX-MFS2 is the weakest and only functions as vacuole Pi sequestration under *osspx-mfs1/3* background. The vacuolar Pi sequestration was severely impaired in *osspx-mfs1/3* and *osspx-mfs1/2/3*, which led to Pi toxicity and subsequently increased Pi allocation to aerial organs. High Pi in the panicle result in necrotic symptoms on husks and impaired panicle and grain development in *osspx-mfs1/3* and *osspx-mfs1/2/3* mutant lines. The mutation in the weak vacuolar Pi transporter OsSPX-MFS2 resulted more stable yield compared to the wildtype under low P field conditions. The results suggest that alteration of vacuolar Pi sequestration may be a novel effective strategy to improve rice (crop) tolerance to low phosphorus field conditions and maintain yield.

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