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Overexpression of *PSTOL1-like* genes increases maize root surface area and biomass under low and high phosphorus conditions

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Low phosphorus (P) availability in soil is a significant limitation for crop production in tropical regions. The PHOSPHORUS-STARVATION TOLERANCE1 (OsPSTOL1) protein kinase enhances root surface area, P acquisition, and grain yield in rice under P-deficient conditions. Homologs of OsPSTOL1 in sorghum were identified through association mapping in two sorghum panels phenotyped for P uptake, root system morphology, and architecture in hydroponic systems, as well as grain yield and biomass accumulation under low-P conditions in Brazil and Mali. In maize and sorghum, candidate genes were co-localized with quantitative trait loci (QTL) associated with root morphology, dry weight, and grain yield under low P. To validate the function of these genes, the rice *OsPSTOL*1 (as a positive control) and its maize (*ZmPSTOL*3.06, *ZmPSTOL*8.02, and *ZmPSTOL8.05*_1) and sorghum (*Sb07g002840*, *Sb03g031690*, and *Sb03g006765*) homologs were cloned downstream of an ubiquitin promoter in the pMCG1005 vector, with the *Bar* gene serving as a selective marker. Genetic transformation of maize B104 embryos was performed using *Agrobacterium tumefaciens*. Homozygous transgenic events with a single copy of the transgene were selected, and those showing transgene overexpression were evaluated under low and high-P conditions. In a growth chamber, the events *Sb07g002840*, *Sb03g006765*, and *ZmPSTOL3.06* showed greater root length and total fine root surface area compared to the negative control (B104 transformed with an empty vector), under both low and high P-conditions. The Sb03g006765 event exhibited higher root and shoot dry weight under both low and high P. *ZmPSTOL8.02* presented a higher dry weight than the negative control only under low P, while *Sb07g002840* had a higher shoot dry weight under high P. In the greenhouse, the *Sb03g006765*, *ZmPSTOL8.02*, and *ZmPSTOL8.05_1* events showed increased shoot dry weight under low P, while *OsPSTOL1*, *Sb07g002840*, and *Sb03g006765* exhibited higher shoot dry weight under high P. For root dry weight under low P, *Sb03g006765*, *ZmPSTOL8.02*, and *ZmPSTOL8.05_1* were superior to the negative control. Under high P, all events except *ZmPSTOL8.02* outperformed the negative control. Overexpression of the *PSTOL*1 homologs significantly improved vegetative growth and root surface area, demonstrating that these genes function similarly to *OsPSTOL*1 in rice.

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