

PHYLOGENETIC ANALYSIS AND FUNCTIONAL CHARACTERIZATION OF *SbPSTOL1* PROTEINS IN SORGHUM

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Receptor-like kinases (RLKs)/Pelle belong to a monophyletic group of the serine/threonine protein kinase superfamily. These proteins play key roles during cellular development and activate signaling pathways in response to environmental stimuli. *Phosphorus starvation tolerance 1* (*PSTOL1*) genes in rice (*OsPSTOL1*) and sorghum (*SbPSTOL1*) are similar to members of the superfamily RLKs/Pelle and are linked to root morphology and phosphorus (P) acquisition. Here, we report on a phylogenetic analysis, an *in silico* analysis of *cis* regulatory elements and subcellular localization of these proteins. We also studied the expression profiling of *SbPSTOL1* in response to P using sorghum lines contrasting for root morphology traits. Kinase domain alignment of different members of the superfamily RLKs/Pelle was performed with ClustalW and a phylogenetic tree was constructed using the maximum likelihood method (JTT model, with 1000 bootstrap replicates) implemented in the MEGA7 software. Predictions of the protein domains were carried out using Pfam and Smart tools. Analyses of the upstream region, approximately 2000 bp, of each gene (*OsPSTOL1* and *SbPSTOL1*) were performed using the MatInspector software. Transient expression 35S::*SbPSTOL1*::GFP was achieved via particle bombardment of onion epidermal cells. The kinase domain (predicted to be cytoplasmic) is conserved among 6 selected *SbPSTOL1* proteins but the N-terminus differs, featuring a putative extracellular domain containing a signal peptide, a cysteine-rich galacturonan_binding domain (GUB-WAK_bind), a wall-associated receptor kinase domain (WAK_assoc domain) and a transmembrane domain. Phylogenetic and structural analysis support that *OsPSTOL1* in rice and *SbPSTOL1* in sorghum are both members of the LRK10 subfamily and share a common ancestry. *SbPSTOL1* proteins were highly similar to *ZmWAKRLK* in maize, which confers resistance to *Exserohilum turcicum* and represents a new class of immunological receptors in monocots. *In silico* analysis of *cis* regulatory elements suggest that *OsPSTOL1* and *SbPSTOL1* genes can respond to environmental stimuli such as biotic and abiotic stresses. In fact, *SbPSTOL1* was transcriptionally upregulated by low P and showed a tendency towards increased expression in lines that have higher root surface area. The subcellular localization of the *OsPSTOL1* protein is predicted to be cytoplasmic. However, the possible presence of cell-wall association domains as well transmembrane domains in *SbPSTOL1* proteins warrants our current efforts to confirm *SbPSTOL1* as functional wall-associated kinases (WAKs). We hypothesize that *SbPSTOL1* proteins, which are members of the subfamily LRK10, have the capacity to monitoring the extracellular environment, thereby inducing signaling pathways involved in adaptive responses to abiotic and biotic stress.

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