Tissue-specific activation of DOF11 promotes rice resistance to sheath blight disease and increases grain weight via activation of SWEET14

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Sugar will eventually be exported transporter (SWEET) is a family of sugar transporters that plays a critical role during host and pathogen interaction (Bezrutczyk et al., 2018). In rice, SWEET11/Xa13, SWEET13/Xa25 and SWEET14 were identified as targets of Xanthomonas oryzae pv. oryzae effectors (Antony et al., 2010; Hutin et al., 2015; Yang et al., 2006). However, the role of SWEET genes in rice and Rhizoctonia solani, the causative agent of sheath blight disease (ShB), is largely unknown. Our previous transcriptome data showed that SWEET14 expression is sensitive to R. solani infection (De Peng Yuan, 2020). qPCR results verified that R. solani infection dramatically induces SWEET14 expression along with a pathogen-related protein P2B1 (Figure 1a). Furthermore, CRISPR/Cas9-mediated genome editing mutants and overexpression lines were generated, and sequencing of the genomic DNA indicated that the fifth exon of SWEET14 in sweet14-1 and sweet14-2 mutants had a 2-bp deletion and a 1-bp insertion, respectively (Figure 1b). The expression of SWEET14 was slightly higher in sweet14 mutants while significantly higher in the overexpressors, SWEET14 OX (#1, #2) compared to the wild-type plants (Figure 1c). R. solani AG1-IA inoculation demonstrated that sweet14 mutants were more susceptible while SWEET14 OX lines were less susceptible to ShB (Figure 1d). These results suggest that SWEET14 may reduce sugar content in the apoplast to inhibit R. solani growth (Figure 1e). However, SWEET14 OX resulted in a dwarf phenotype, reduced 1,000-grain weight and grain number per panicle, and normal tiller growth, while sweet14 mutants maintained normal growth and yield production (Figure 1f–i).

Further, the transcription factors that directly activate SWEET14 were screened using 2-kb of SWEET14 promoter via yeast one-hybrid assay (Figure 1j). Among the transcription factors isolated, DOF11 was further examined. A previous report indicated that DOF11 modulates sugar transport in rice (Wu et al., 2018). DOF11 genome editing mutant showed 1-bp deletion in the first exon and exhibited higher susceptibility to ShB (Figure 1k, l). Furthermore, DOF11 overexpressors (OXs) (Figure 1m, n) were less susceptible to R. solani than wild-type plants (Figure 1o). DOF11 OX developed similar tiller numbers, but its 1,000-grain weight and grain number per panicle were reduced compared with wild-type (Figure 1p–r). DOF11 overexpression increased rice resistance to ShB, but reduced yield production. Therefore, the VP16, a transcriptional activation domain (Li et al., 2013), was fused to DOF11 in transgenic plants. DOF11-GFP and DOF11-VP16-GFP were localized in the nucleus (Figure 1s). The DOF11-VP16-Myc expression under the control of 2.0 kb DOF11 promoter rescued the dof11 semi-dwarf phenotype (Figure 1t). DOF11-VP16-Myc protein expression was detected using Western blot analysis (Figure 1u). R. solani inoculation showed that dof11/DOF11-VP16-Myc plants (#1, #2) were less susceptible to ShB compared to wild-type (Figure 1v). Further examination showed that dof11 mutants exhibited decreased 1,000-grain weight, tiller number per plant and grain number per panicle while the dof11/DOF11-VP16-Myc plant values increased in all the phenotypes, except tiller number (Figure 1w–y). A chromatin immunoprecipitation (ChIP) assay was performed using DOF11-VP16-Myc transgenic plant calli with an anti-Myc antibody. The results showed that DOF11-VP16-Myc bind to the P1 and P2 regions, but not to the P3-P5 fragments of SWEET14 promoter (Figure 1z).

SWEET14 expression test in wild-type, DOF11 OX1 and dof11/DOF11-VP16-Myc #1 showed that SWEET14 level was higher in the root, leaf and mature booting stage in DOF11 OX1 and dof11/DOF11-VP16-Myc #1 compared with wild-type plants, and higher in the root and at the mature booting stage of DOF11 OX than in dof11/DOF11-VP16-Myc #1. However, the ectopically expressed SWEET14 in mesophyll cells and root hairs of DOF11 OX were not detected in corresponding tissues of DOF11-VP16-Myc #1 (Figure 1ii), suggesting tissue-specific activation of SWEET14 by DOF11-VP16. To analyse whether the increase of yield and resistance in DOF11-VP16 was via activation of SWEET14 transcription, genetic combinations between sweet14 and DOF11-VP16 plants were generated. Investigation of the yield showed that the 1,000-grain weight of DOF11-VP16 was higher compared with wild-type, sweet14 and sweet14/DOF11-VP16 (Figure 1iii). Next, R. solani AG1-IA inoculation results showed that DOF11-VP16 was less susceptible to ShB than wild-type, sweet14 and sweet14/DOF11-VP16 (Figure 1iv).

Taken together, our analyses revealed that SWEET14, a sugar transporter, positively regulates rice resistance to ShB. However, non-specific transport of sugar by overexpression of SWEET14 significantly reduced yield production, suggesting that SWEET14 plays a role in both yield production and defence. DOF11 is identified as a direct transcriptional regulator of SWEET14, with...
DOF11 overexpression increased resistance to ShB but reduced yield production. Interestingly, tissue-specific activation of DOF11 by fusion of VP16 increased both yield production and resistance to ShB. Expression, genetic and pathological analyses suggest that tissue-specific activation of DOF11 simultaneously increases yield production and improves resistance to ShB, possibly partially through activation of SWEET14.

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Authors’ contributions

PK, CYX and YHL and YHX designed the experiments. PK, CYX and HDS performed the experiments. YG and LF manipulated plant materials. PK, YHL and YHX analysed data. YHL and YHX wrote...
the manuscript. All authors read and approved the final manuscript.

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