Weakening of gene function of OsTB1 by genome editing improves rice productivity under phosphorus deficiency

Tillering is an important trait that determines shoot architecture and yield in rice. Many genes are involved in tillering in rice, and among them, rice *TEOSINTE BRANCED1* (*OsTB1*) is a key gene that suppresses tillering. On the other hand, phosphorus, a soil nutrient, is one of the most important environmental factors involved in tillering. Phosphorus deficiency leads to reduced tiller number and is a major constraint on rice production in sub-Saharan Africa. In this study, we generate mutants for *OsTB1* using the CRISPR/Cas9 system in X265, which is a major rice cultivar in Madagascar, and then investigate tillering and productivity under phosphorus deficiency in the resultant mutants.

The CRISPR/Cas9 system has generated two types of mutant lines: an in-frame mutant line with 30-bp deletion (#29418) and a frameshift mutant line with 1-bp insertion (#29430) (Fig. 1). The in-frame mutant line #29418 has 1.2 times more tillers than the background cultivar X265 (WT) at just before heading stage (Fig. 1). On the other hand, the frameshift mutant line #29430 produces 3.4 times more tillers than WT (Fig. 1). This means that OsTB1 weakens its tillering suppression function through the in-frame mutation, while the frameshift mutation loses its tillering suppression function. The expression level of the *OsTB1* gene in #29418 is comparable to that of WT (Fig. 2). The *OsGT1* gene, which is directly regulated by OsTB1 to suppress tillering, is down-regulated in #29430 (Fig. 2). The expression level of *OsGT1* in #29418 is intermediate between those of WT and #29430 (Fig. 2), revealing that the modified OsTB1 expressed in the in-frame mutant line #29418 has a moderate function in the regulation of *OsGT1*. The grain yield of #29418 under phosphorus deficiency is higher than that of WT under low phosphorus application levels: #29418 has approximately 40% higher grain yield than WT under 0 mg/kg phosphorus application (Fig. 3A). The number of panicles, spikelets, and filled grains of #29418 was higher than those of WT, and the 1,000-grain weight was lower than that of WT (Fig. 3B). The gain in filled grain numbers more than compensates for the reduced 1,000-grain weight. On the other hand, the frameshift mutant line #29430 has more filled grains than WT, but it does not improve yields because the gain in filled grain numbers does not compensate for its decreased 1,000-grain weight.

Our study demonstrates that genome editing of OsTB1 can modify tillering in rice and suggests that the breeding of rice cultivars that have a moderately higher number of tillers may effectively improve rice productivity in areas suffering from phosphorus deficiency.

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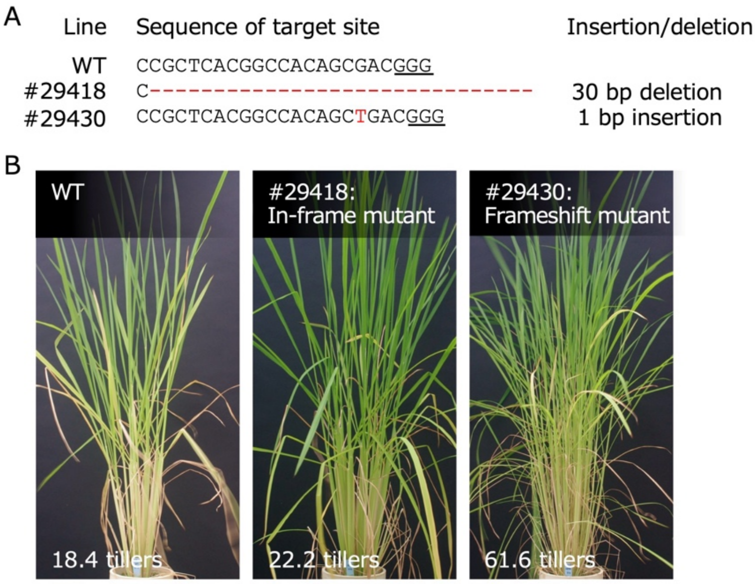

Fig. 1. Generation of mutants for *OsTB1* by genome editing

(A) DNA sequences of mutated *OsTB1* generated by CRISPR/Cas9. Underlines in the target sequences indicate PAM. A red letter indicates an inserted nucleotide. Red dashes indicate deleted nucleotides.
(B) Plants of WT (left), #29418 (in-frame mutant; center), and #29430 (frameshift mutant) at just before heading stage. The mean tiller numbers of each line are indicated at the bottom of pictures.

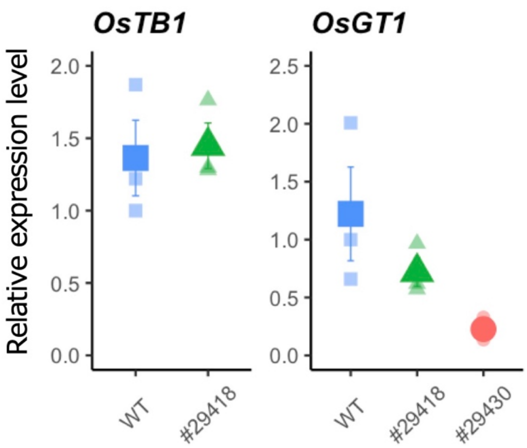

Fig. 2. Expression analysis of *OsTB1* and *OsGT1*

Expression levels in the basal node region of 21-day-old plants are quantified by qRT-PCR. *OsGT1* is a gene that OsTB1 directly binds to its promoter to induce its expression. Each large symbol represents the mean from three biological replications, and each small symbol represents the observed raw value for each replication. #29418, in-frame mutant; #29430, frameshift mutant.

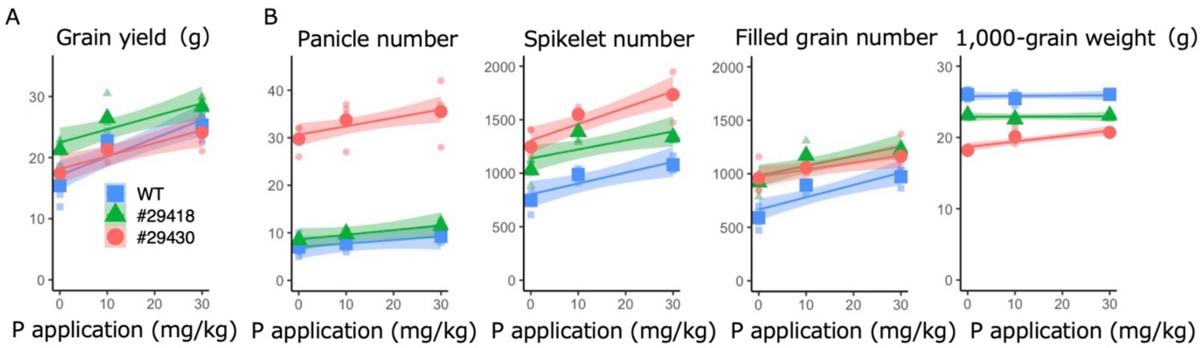

Fig. 3. Grain yield and yield components of mutants for *OsTB1* under different phosphorus (P) applications

#29418 (in-frame mutant), #29430 (frameshift mutant), and WT (X265) were grown in soil supplemented with 0, 10, or 30 mg/kg of P until the mature stage. (A) Grain yield per plant. (B) Yield components of the in-frame mutant line (#29418) and non-mutated X265 (WT). Each large symbol represents the mean from four replications, and each small symbol represents the observed raw value for each replication. Predictions and 95% confidence intervals by ANCOVA are indicated respectively with lines and shading.

Reference: Ishizaki et al. (2023) *Plant Science* 330: 111627.