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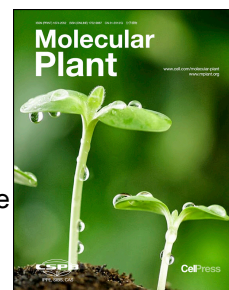
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Strigolactones restrict sugar acquisition in rice tiller buds to inhibit their outgrowth through suppressing the expression and polarized localization of two monosaccharide transporters

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Short summary:

This study uncovers a mechanistic framework wherein strigolactones orchestrate rice tillering by transcriptionally suppressing the monosaccharide transporters *OsMST3* and *OsMST6* via a key transcription factor OsTB1, thereby restricting the direct long-distance flux of glucose and fructose toward tiller buds. Moreover, strigolactones also actively counteract sugars-induced polarized localization of OsMST3&6 on plasma membrane, establishing a pivotal regulatory nexus that integrates hormonal signaling with carbon partitioning to govern rice tillering.

Abstract

Strigolactones (SLs) are fundamental phytohormones that suppress tillering, whereas sugars act as both energy source and signaling molecules to promote tillering. However, the molecular interplay between SLs and sugars remains largely underexplored. Here, through iTRAQ-based quantitative proteomic analysis, we identified two highly homologous monosaccharide transporters OsMST3 and OsMST6 showing increased protein abundance in the SLs signaling mutant *d3^{s2-215}* and biosynthesis mutant *d10^{XJ1705}*. Through feeding with fluorescence- or isotope-labelled sugars, we demonstrated that OsMST3/6 functioned as influx transporters of glucose and fructose, facilitating their translocation from leaf blades to tiller buds to promote bud outgrowth. Interestingly, glucose and fructose induced *OsMST3/6* expression and polarized plasma membrane localization, a process essential for bud outgrowth, whereas SLs suppressed their expression and counteracted sugars-induced polarized localization. OsTB1, a key transcription factor downstream of SLs signaling, directly bound the *OsMST3/6* promoters to inhibit their transcription. Tiller bud outgrowth of the *tb1^{SG0312}* mutant was severely inhibited by a non-metabolizable glucose analog (2-Deoxy-D-glucose) and an inhibitor of sugar transporters (CCCP). Moreover, simultaneous knockout of *OsMST3/6* rescued the high-tillering phenotype of the *d3^{s2-215}*, *d10^{XJ1705}* and *tb1^{SG0312}* mutants. Together, we found a novel mechanism by which SLs control tillering by limiting sugars allocation to buds through suppressing monosaccharide transporters OsMST3/6.

Key words: monosaccharide transporter OsMST3/6, strigolactone, rice tillering, glucose and fructose transport, polarized membrane localization, OsTB1

Introduction

Plant shoot branching/tillering is a critical agronomic trait determining grain yield. It is controlled by a complex interplay of internal hormonal balance, nutrient availability, and external environmental factors (Kun et al., 2023; Patil et al., 2022; Wang et al., 2020; Wu et al., 2020; Xu et al., 2005; Zhao et al., 2001). SLs are a group of carotenoid-derived phytohormones mainly produced in roots of a plant, which inhibit shoot branching/tillering, induce the hyphal branching of symbiotic arbuscular mycorrhizal (AM) fungi, and perform other biological functions (Cook et al., 1966; Jiang et al., 2013; Zhou et al., 2013).

The SLs biosynthesis and signal transduction pathway has been elucidated through the isolation of numerous dwarf and high-tillering mutants and the functional characterization of the corresponding causal genes (Abe et al., 2014; Booker et al., 2005; Hu et al., 2024; Jiang et al., 2013; Kretschmar et al., 2012; Liu et al., 2018; Zhao et al., 2014; Zhou et al., 2013). SLs are derived from β -carotene and their biosynthesis involves multiple enzymes. First, all-trans- β -carotene is converted to 9-cis- β -carotene by a β -carotene isomerase encoded by *Dwarf 27* (*D27*). Then 9-cis- β -carotene is converted to 9-cis- β -apo-10'-carotenal and carlactone through sequential cleavage catalyzed by CAROTENOID CLEAVAGE DIOXYGENASE 7 and 8 encoded by *Dwarf 17* (*D17*) and *Dwarf 10* (*D10*), respectively. Finally, carlactone is modified by the MAX1 cytochrome P450, LATERAL BRANCHING OXIDOREDUCTASE (LBO) and other enzymes to produce various strigolactones (Al-Babili and Bouwmeester, 2015; Alder et al., 2012; Brewer et al., 2016; Liu et al., 2021; Mashiguchi et al., 2021). SLs are perceived by an α/β -hydrolase, DWARF14 (*D14*), which interacts with an F-box protein, DWARF3 (*D3*), in a strigolactone-dependent manner. *D3* forms an SCF^{D3} E3 ubiquitin ligase complex with OSKs (SKP1-like genes in rice) and OsCullin1 to ubiquitinate the transcriptional repressor Dwarf 53 (*D53*), a key negative regulator in the SLs signaling pathway, and target it for proteasomal degradation (Jiang et al., 2013; Zhao et al., 2014; Zhou et al., 2013). *D53* represses the transcriptional activation activity of the transcription factor IPA1, which activates expression of *OsTB1* (Kretschmar et al., 2012). *OsTB1* (TEOSINTE BRANCHED1) is a TCP family transcription factor functioning downstream of SLs to inhibit bud outgrowth (Minakuchi et

al., 2010). BRANCHED1 (BRC1), the *Arabidopsis* ortholog of OsTB1, can upregulate the key ABA biosynthetic gene *NCED3* by activating three HD-ZIP class I transcription factors (HB21, HB30 and HB42) or by directly binding to the *NCED3* promoter, ultimately increasing ABA content in axillary buds (González-Grandío et al., 2017). The cucumber (*Cucumis sativus* L.) CsBRC1 directly represses the auxin efflux carrier *CsPIN3* thus promotes auxin accumulation in axillary buds (Shen et al., 2019). A higher transcript level of *BRC1/OsTB1* is closely correlated with fewer branches/tillers, while a lower *BRC1/OsTB1* transcript level typically leads to more branches/tillers in plants (Choi et al., 2012; Li et al., 2024).

Monosaccharides, including glucose, fructose, and galactose, are simple sugars. Glucose acts as a primary source of energy and a signaling molecule in plant development processes, such as lateral bud elongation and shoot branching (Li et al., 2024; Patil et al., 2022; Rabot et al., 2012; Salam et al., 2021). Glucose plays a signaling role through HEXOKINASE1 (HXK1), which not only catalyzes the conversion of glucose into glucose-6-phosphate but also generates a signal upon glucose binding that regulates shoot branching independently of glucose phosphorylation (Barbier et al., 2021). Sucrose, composed of one glucose and fructose, triggers lateral bud outgrowth when applied (Barbier et al., 2015; Henry et al., 2011; Patil et al., 2022; Rabot et al., 2012).

Sugars can be transported by different types of transporters. Three families of transporters have been identified as key players in sugar transport: monosaccharide transporters (MSTs), sucrose/H⁺ cotransporters (SUTs), and the hexose and sucrose export transporters Sugars Will Eventually be Exported Transporters (SWEETs) (Eom et al., 2015; Zhang and Turgeon, 2018b). Through these three kinds of transporters, sugars can be translocated from source leaves to sink tissues, although other pathways (e.g., plasmodesmata-mediated symplastic pathway) also exist (Wang et al., 2025). MSTs play crucial roles in phloem unloading (Wang et al., 2025; Zhang and Turgeon, 2018b). The transporter activity and biological functions of several MSTs have been characterized (Bavnhøj et al., 2021; Flutsch et al., 2020; Khan et al., 2023; Li et al., 2022; Paulsen et al., 2019; Reyer et al., 2024; Rottmann et al., 2018). OsMST3 has higher transport activity for

D-glucose than D-xylose and 3-O-methyl glucose (3-OMG). OsMST3 is involved in the accumulation of monosaccharides required for cell wall synthesis during root development (Toyofuku et al., 2000). OsMST5 transports monosaccharide substrates in yeast and is associated with pollen development (Ngampanya et al., 2003). OsMST6 has high affinity for xylose, glucose, and galactose (Wang et al., 2008b). The transcription factor OsERF120 could bind to the DRE/CRT element in the *OsMST6* promoter and activate its expression to positively regulate chilling tolerance (Luo et al., 2024). OsMST8 plays an essential role in rice anther development (Mamun et al., 2006). OsMST7 and OsMST8 affect pollen fertility by maintaining sugar transport and confers cold stress tolerance at the reproductive stage (Liu et al., 2019a). Sugar transporter protein 15 in rice (OsSTP15) functions as a glucose efflux transporter. Disruption of *OsSTP15* can enhance crop yield by increasing tiller number due to the accumulation of glucose, sucrose, and Tre6P in shoot base and by stimulating the cytokinin signaling pathway (Li et al., 2024). OsSTP28 acts as a glucose influx transporter that negatively regulates rice tillering. The *osstp28* mutant displayed more tillers owing to high levels of glucose in the apoplast, which releases the inhibition of OSH15 on OsGA2oxs-mediated GA catabolism thus reducing GA content in tiller bud (Zhang et al., 2024).

Recent studies have reported that SLs and sugars antagonistically regulate bud outgrowth across different plant species (Barbier et al., 2023; Bertheloot et al., 2020; Dierck et al., 2016). In rice, the circadian clock-modulated sugars status interacts with the SLs pathway to regulate tillering and panicle development (Wang et al., 2020). Sucrose downregulates *D3* expression thus inhibits the SLs-triggered degradation of D53 by the SCF^{D3} E3 ubiquitin ligase, thereby promoting bud outgrowth (Patil et al., 2022). Similarly, in *Arabidopsis*, sucrose stimulates bud outgrowth in single-node segments by suppressing the expression of *MAX2* and *BRC1* (Barbier et al., 2019; Wang et al., 2015). Consistently, in sorghum (*Sorghum bicolor*), reduced sugar availability caused by defoliation or shade treatments, leads to the upregulation of *SbMAX2* expression and inhibition of bud outgrowth (Kebrom et al., 2010; Kebrom and Mullet, 2015). On the other hand, relatively less are known about how SLs antagonize sugar signaling, except that SLs inhibit T6P biosynthesis by

suppressing expression of *TPS1* (*Trehalose Phosphate Synthase 1*) (Fichtner et al., 2024).

To comprehensively understand the antagonistic relationship between sugars and SLs in bud outgrowth, more studies are needed to explore how SLs feedback on sugar signaling and transport.

In order to identify downstream targets of SLs that regulate tiller bud outgrowth, we conducted a large-scale quantitative proteomic analysis of a new allelic mutant of *D3* (*d3^{s2-215}*) and its wild type (WT), Nipponbare (NIP), using Isobaric Tags for Relative and Absolute Quantification (iTRAQ). The protein abundance of two highly homologous monosaccharide transporters, OsMST3 and OsMST6, was obviously increased in the *d3^{s2-215}* mutant compared with WT, which caused more glucose and fructose transported into tiller buds. OsMST3 and OsMST6 functioned as influx transporters of glucose and fructose, which promoted glucose and fructose transportation from leaf blades to tiller buds to promote tillering. Glucose and fructose induced polarized localization of OsMST3 and OsMST6 from a uniform distribution at the cell membrane to the apical and basal sides of the cell and SLs counteracted their polarized localization induced by glucose and fructose. We also uncovered that SLs could repress *OsMST3* and *OsMST6* expression through the master transcription factor OsTB1 which bound the GGNCCC and GAGGAG motif in their promoters. Furthermore, the transcript and protein levels of OsMST3 and OsMST6, as well as the contents of glucose and fructose, were also increased in tiller buds of the *d10^{XJ1705}* and *tb1^{SG0312}* mutants. Combinational knockout of *OsMST3* and *OsMST6* complemented the high-tillering phenotype of the SLs-deficient mutant *d10^{XJ1705}* and SLs-insensitive mutants *d3^{s2-215}* and *tb1^{SG0312}*. These results suggested that SLs negatively regulated rice tillering by modulating sugars availability through suppressing expression and polarized localization of the monosaccharide transporter OsMST3 and OsMST6.

Results

Increased protein abundance of OsMST3 and OsMST6 in a new allelic mutant of *D3*

A dwarf and high-tillering mutant *s2-215* was isolated from a mutant population of the *japonica* rice variety Nipponbare (NIP) mutagenized by ethyl methane sulfonate (EMS).

This mutant showed dwarfism and excessive tillering at both the seedling and mature stage (Supplemental Figure 1A and 1B). The plant height of *s2-215* was approximately half that of the wild type (WT), while tiller number was four times higher (Supplemental Figure 1C and 1D). These phenotypes closely resembled those previously described for the *d3* mutant (Ishikawa et al., 2005; Zhao et al., 2014). Map-based cloning revealed that a C to T mutation at the 1177th nucleotide in the *D3* coding sequence resulted in a premature stop codon (Supplemental Figure 1E). Introducing the full-length *D3* genomic DNA driven by its native promoter into the *s2-215* mutant could complement its dwarf and high-tillering phenotype (Supplemental Figure 1F-1H), confirming that *s2-215* is indeed a new allelic mutant of *D3*, thus termed as *d3^{s2-215}*.

It is becoming increasingly difficult to identify new components in the SLs signaling pathway through forward genetics, as the isolation of dwarf and high-tillering mutants is nearing saturation. To discover new targets of SLs in regulating rice tillering, a large-scale quantitative proteomic analysis by iTRAQ was performed using callus tissues induced from WT and the *d3^{s2-215}* mutant. By comparison of the global protein profiles of WT and *d3^{s2-215}*, we identified 257 differentially expressed proteins (DEPs). Among these, 146 proteins were more abundant while 111 proteins were less in the *d3^{s2-215}* mutant than in WT, Supplemental Table 1, upregulated ≥ 1.2 -fold or downregulated ≤ 0.80 -fold). A hierarchical clustering heatmap of the DEPs was depicted in Supplemental Figure 2A. Gene Ontology (GO) analysis revealed that the category with carbohydrate transmembrane transporter activity was the most significantly enriched in molecular functions category in the *d3^{s2-215}* mutant. This category included two monosaccharide transporters (OsMST3 and OsMST6, 83% identity), two bidirectional sugar transporters (OsSWEET4 and OsSWEET1b, 43% identity), and one glucose-6-phosphate/phosphate translocator-like protein (Supplemental Figure 2B and Figure 1A). OsMST6 and OsMST3 were the two most significantly upregulated proteins in this category. According to the phylogenetic analysis of the eight OsMST family members in rice (from OsMST1/OsSTP1 to OsMST8/OsSTP8) (Deng et al., 2019), OsMST6 was most closely related with OsMST3, while OsMST8 and OsMST7,

OsMST4 and OsMST1 were more closely related to each other (Supplemental Figure 2C). Compared with other *OsMST* members, the expression of *OsMST3* and *OsMST6* was high in roots and leaf blades during the vegetative stage, whereas in the reproductive stage, it was predominantly detected in roots and anthers. according to the Rice Expression Profile Database (<https://ricexpro.dna.affrc.go.jp>) (Supplemental Figure 2D).

The StrigoQuant-like reporter, in which firefly luciferase (FF) and Renilla luciferase (REN) are expressed in a single transcript, serves as an effective tool for comparing protein abundance between the WT and mutants or different genotypes (Sun et al., 2021). The ratio of FF/REN indicated the relative abundance of the FF: target fusion protein (Figure 1B). To confirm the iTRAQ results, FF: *OsMST3* and FF: *OsMST6* were separately transformed into protoplasts of WT and *d3^{s2-215}*. After incubation overnight, the FF/REN ratios of *OsMST3* and *OsMST6* were determined. The results showed that the abundance of *OsMST3* and *OsMST6* were significantly higher in the *d3^{s2-215}* mutant than in WT, with *OsMST6* showing a more pronounced increase compared with *OsMST3* (Figure 1C and 1D).

***OsMST3* and *OsMST6* promote tiller bud outgrowth during rice tillering**

To investigate the roles of *OsMST6* and *OsMST3* in rice tillering, we first overexpressed *OsMST6* in a *japonica* rice Zhonghua 11 (ZH11), driven by the constitutive *CaMV35S* or *OsTB1* promoter which exhibits high expression specificity in tiller buds (Takeda et al., 2003; Minakuchi et al., 2010). More than 15 independent transgenic lines were obtained, respectively, almost all of which displayed more tillers than WT (Supplemental 3A and 3B). Among them, the *35S: OsMST6-5* and *OsTB1pro: OsMST6-26* lines were selected for detailed analysis (Figure 1F-1K). Transcript levels of *OsMST6* in tiller buds of these lines increased by 6- and 17-fold compared with WT, respectively (Figure 1E). Tiller number was significantly increased, from an average of 7.7 tillers in WT to 10.4 or 14.8 tillers in the *35S: OsMST6-5* and *OsTB1pro: OsMST6-26* line, respectively (Figure 1F and 1I), while their plant height did not show significant change (Figure 1F and 1H). The grain yield per plant

increased by approximately 24% and 31%, respectively, compared with WT (Figure 1G and 1J). The total biomass of the two transgenic lines also significantly increased (Figure 1K). Additionally, there was no discernible difference in the seed setting rates between WT and the *OsMST6*-overexpressing lines (Supplemental Figure 3C). These data reveal that *OsMST6* promotes rice tillering and significantly improves grain yield by increasing the number of productive tillers.

We then created the *osmst3* and *osmst6* single or double mutants using the CRISPR/Cas9 technique in the NIP background. Three independent homozygous *osmst3* mutants (*osmst3*-19, *osmst3*-23, and *osmst3*-25), which harbored 4-bp, 1-bp and 5-bp deletions, respectively, showed no obvious difference in plant height and tiller number compared with WT (Supplemental Figure 4A-4D). Similarly, three independent homozygous *osmst6* mutants (*osmst6*-1, *osmst6*-2 and *osmst6*-9) with 1-bp insertion, 4-bp and 5-bp deletions, respectively, exhibited no significant differences in plant height and tiller number (Supplemental Figure 4E-4H). However, when *OsMST3* and *OsMST6* were simultaneously knocked out, three homologous transgenic lines (*osmst3/6*-12, *osmst3/6*-10 and *osmst3/6*-30) with 1-bp insertion, 2-bp or 1-bp deletion in *OsMST3*, and 3-bp, 6-bp deletions, or 1-bp insertion in *OsMST6* (Supplemental Figure 4I and 4J), respectively, displayed a dwarf and low-tillering phenotype (Figure 1L-1N). The 3-bp or 6-bp deletion in *OsMST6* led to the loss of the lysine residue located within a topological domain flanked by two transmembrane domains, a region critical for forming the substrate transport channel. Tiller number of the *osmst3/6* double mutant was reduced to approximately 9.5, compared with an average of 22.5 in WT, and plant height was about 75% of that of WT (Figure 1L-1N). To exclude the off-target effect of sgRNAs, we designed a second set of sgRNAs to knockout *OsMST3* and *OsMST6* in NIP. Three resulting *osmst3/6* double mutants (B1035), containing 2-bp deletion, 1-bp insertion or 2-bp deletion in *OsMST3*, and 10-bp deletion, 3-bp deletion and 3-bp deletion in *OsMST6* (Supplemental Figure 4K and 4L), displayed a similar dwarf and low-tillering phenotype (Supplemental Figure 4M-4O).

To examine the underlying mechanism of the low-tillering phenotype of the *osmst3/6* double mutants, we anatomically observed the formation of tiller bud and measured its length in WT and the *osmst3 osmst6* double mutant seedlings in relation to the growth of the third leaf blade (Figure 1O). Time course analysis revealed that the reduced tiller number in the *osmst3/6* double mutant was due to decelerated tiller bud outgrowth rather than a decrease in tiller bud formation (Figure 1O and 1P). At the early growth stage, the *osmst3/6* double mutants did not show significant difference in tiller number compared with WT. However, starting from the fifth weeks after germination, the reduction in tiller number became more and more obvious (Figure 1Q). These results suggest that *OsMST3* and *OsMST6* promote tiller bud outgrowth.

The double knockout mutants of *OsMST3* and *OsMST6* were also created in the *d3^{ss2-215}* mutant background. The tiller number of three independent lines (*osmst3/6/d3^{ss2-215}-7*, *osmst3/6/d3^{ss2-215}-8* and *osmst3/6/d3^{ss2-215}-26*) with 2-bp deletion, 1-bp insertion or 1-bp deletion in *OsMST3*, and 6-bp deletion, 6-bp deletion, or 1-bp insertion in *OsMST6*, respectively (Supplemental Figure 4P and 4Q), was extremely reduced, although their plant height remained short (Figure 1R-1T). This result suggests that the high-tillering phenotype of the *d3^{ss2-215}* mutant is dependent on the proper function of *OsMST3* and *OsMST6*.

Expression patterns of the *OsMST3* and *OsMST6* genes and tissue localization of their encoded proteins

To investigate the expression patterns of *OsMST3* and *OsMST6*, RT-qPCR assay was conducted. The transcript levels of *OsMST3* and *OsMST6* were higher in roots, tiller buds, leaf blades than other tissues (Supplemental Figure 5A and 5B). Histochemical staining of the transgenic rice plants expressing the β -glucuronidase (*GUS*) reporter gene driven by the *OsMST3* or *OsMST6* promoter indicated that *OsMST3* and *OsMST6* exhibited strong expression in the major veins of leaf blades and sheaths, tiller buds and roots (Figure 2A). Additionally, GUS activity was detected in young panicles (Supplemental Figure 5C-5H),

which was consistent with the RT-qPCR results. To investigate the cell-type-specific expression profiles of *OsMST3* and *OsMST6*, we analyzed the publicly available single-cell RNA sequencing data of rice (Wang et al., 2025b). UMAP visualization revealed that *OsMST3* and *OsMST6* displayed enriched expression in specific cell clusters predominantly located in photosynthetic and vascular tissues, including the flag leaf, culm, tiller bud and crown root (Supplemental Figure 5I), suggesting their potential involvement in long-distance transport of photoassimilates. To further study localization of the *OsMST3* and *OsMST6* proteins in these tissues, transgenic rice lines expressing *OsMST3-GFP* and *OsMST6-GFP* driven by their native promoters were developed, and immunostaining assays were performed using a GFP-specific antibody. The results revealed that both proteins were mainly localized in the mesophyll cells and phloem parenchyma cells in leaf blade, and distributed throughout the vascular bundle in leaf sheath. Strong signals of *OsMST3-GFP* and *OsMST6-GFP* were observed in tiller bud and the junction region between tiller bud and main stem. In root, the two proteins were mainly located in the root epidermis, exodermis, endodermis as well as pericycle/stele (Figure 2B).

To determine subcellular localization of the *OsMST3* and *OsMST6* protein, constructs expressing *GFP-OsMST3* and *GFP-OsMST6*, driven by the 35S promoter, were transiently transformed into rice protoplasts. The GFP signals of both *OsMST6* and *OsMST3* were found to overlap with the fluorescence signal of FM4-64, a marker for the plasma membrane (Supplemental Figure 5J), suggesting that *OsMST3* and *OsMST6* are localized to the plasma membrane.

***OsMST3* and *OsMST6* function as glucose and fructose influx transporters**

To characterize the sugar transport properties of *OsMST3* and *OsMST6*, *OsMST3* or *OsMST6* was first introduced into the hexose uptake-deficient yeast mutant EBY.VW4000, which only grew on medium containing maltose (Li et al., 2024; Zhang et al., 2024). *SCHXT11* encoding Hexose Transporter 11 in yeast (*Saccharomyces cerevisiae*) (Wieczorke et al., 1999) was transformed into the EBY.VW4000 as a positive control. The

EBY.VW4000 strains expressing either *OsMST3* or *OsMST6* were able to grow well on medium containing different concentrations of glucose or fructose, although less vigorously than the strain expressing *SchXT11* (Figure 2C and Supplemental Figure 6A), while the strains expressing *OsMST3* or *OsMST6* could not grow on medium containing another monosaccharide, ribose (Supplemental Figure 6B). Furthermore, EBY.VW4000 transformants expressing *OsMST3* and *OsMST6* were unable to grow on 1% glucose supplemented with 0.2% 2-Deoxy-D-glucose (2-D-glucose, a non-metabolizable glucose analog that inhibits glycolysis), suggesting that *OsMST3* and *OsMST6* exclusively localize on the plasma membrane rather than the vacuole membrane (Supplemental Figure 6C) (Li et al., 2024; Wang et al., 2008b). However, when 2% maltose was used as carbon source, the inhibitory effect of 2-D-glucose on yeast growth was abolished (Supplemental Figure 6C). To test whether *OsMST3* and *OsMST6* can also transport sucrose, *OsMST3* and *OsMST6* were further transformed into the sucrose uptake-deficient yeast mutant SUSY7/ura3, which can grow specifically on a glucose-containing medium (Li et al., 2024). *OsMST3* and *OsMST6* could not complement the growth of SUSY7/ura3, whereas the positive control vector expressing the sucrose transporter *OsSUT1* grew well on 2% sucrose medium, suggesting that *OsMST3* and *OsMST6* cannot mediate sucrose transport (Supplemental Figure 6D). These results indicated that glucose and fructose, rather than sucrose, are the substrates for *OsMST3* and *OsMST6*.

To further characterize the substrate specificities of *OsMST3* and *OsMST6*, the transport rate of radioactive ^{14}C -glucose was studied in the presence of other sugars supplied at 10 times higher concentrations. The uptake of ^{14}C -glucose in yeast expressing *OsMST3* or *OsMST6* was strongly inhibited by glucose and fructose, and even more dramatically inhibited when both sugars were combined. In contrast, sucrose only slightly decreased ^{14}C -glucose uptake in yeast expressing *OsMST3* and had no effect in yeast expressing *OsMST6* (Figure 2D and 2E). Carbonyl cyanide m-chlorophenylhydrazone (CCCP), a proton uncoupler, disrupts the proton gradient across the plasma membrane. Therefore, measuring transport rate in the presence of CCCP helps determine whether the uptake of

a specific substrate relies on a proton gradient (symport mechanism) or occurs independently (facilitated diffusion) (Li et al., 2020; Li et al., 2015). Glucose uptake was extremely reduced by low concentrations of CCCP in yeasts expressing *OsMST3* or *OsMST6* (Figure 2D and 2E), indicating that sugar uptake via *OsMST3* and *OsMST6* is driven by a proton gradient across the plasma membrane. This finding suggests that *OsMST3* and *OsMST6* are energy-dependent hexose/H⁺ symporter with high affinity for glucose and fructose.

¹⁴C-glucose uptake experiment in yeast revealed that *OsMST3* and *OsMST6* transport glucose from the extracellular space into the yeast cell (Figure 2F). To determine the direction of *OsMST3* and *OsMST6* transport in the plant cells, a hexose uptake assay using rice protoplasts was carried out. Protoplasts of WT, *d3^{ss2-215}*, the *osmst3* and *osmst6* single and double mutants were incubated with 1 mM FITC-glucose or 1 mM CY2-fructose for 30 minutes. Protoplasts of the *d3^{ss2-215}* mutant exhibited a significantly higher fluorescence signal than those isolated from WT, the *osmst3* and *osmst6* single mutant, when comparing the fluorescence intensity in protoplasts of 25-30 µm in diameter. In contrast, protoplasts of the *osmst3/6* double mutant displayed a markedly lower fluorescence signal compared with WT. No significant difference was observed in the fluorescence density among WT, the *osmst3* and *osmst6* single mutant protoplasts (Figure 2G-2I). These findings further suggest that *OsMST3* and *OsMST6* function as a glucose and fructose influx transporter in rice.

Glucose and fructose induce the expression of *OsMST3* and *OsMST6* and promote polarized localization of these transporters on the plasma membrane

We next studied the transcriptional response of *OsMST3* and *OsMST6* to varying concentrations of glucose and fructose. To minimize the influence of the photosynthetic reaction cycle on the experiment, the WT seedlings were first incubated in the water under dark condition for 24 hours, then treated with varying concentrations of glucose or fructose for 12 hours under normal light condition. The contents of glucose and fructose vary

depending on rice variety, developmental stage, and tissue type. In rice leaves, glucose content can reach up to 2% of fresh weight, and fructose content up to 4% of fresh weight (Boriboonkaset et al., 2013). To enable plants to perceive exogenous hexose signals more effectively, 1%-4% glucose and fructose treatments were applied. The shoot bases were collected to assess the expression levels of *OsMST3* and *OsMST6* through RT-qPCR. *OsMST3* was highly induced by 1% glucose and 1% fructose, while the *OsMST6* transcript levels sharply increased in response to 2% glucose and 1% fructose. As sugar concentrations further increased, the expression levels of both genes started to decline (Figure 3A and 3B). To investigate the response of *OsMST3* and *OsMST6* to glucose and fructose treatment at the protein level, transgenic rice lines expressing *OsMST3-GFP* and *OsMST6-GFP* under the control of their native promoters were separately treated with 1.5% glucose or 1% fructose, and samples were collected at different time points. No obvious changes in the protein abundance of *OsMST3* or *OsMST6* were observed within 4 hours. However, both glucose and fructose significantly induced accumulation of the *OsMST3* and *OsMST6* proteins starting at 6 hours post-treatment (Figure 3C and 3D).

To investigate the potential effects of sugars on the subcellular localization of *OsMST3* and *OsMST6*, roots of transgenic rice lines expressing *OsMST3-GFP* and *OsMST6-GFP* driven by their native promoters were treated with different monosaccharides for four hours and then cells in the elongation zone were observed. Under mock condition, *OsMST3-GFP* or *OsMST6-GFP* were evenly distributed along the entire plasma membrane of the cells (Figure 3E and 3F). Interestingly, after treatment with 2% glucose or fructose, *OsMST3-GFP* and *OsMST6-GFP* underwent a polarized localization, leading to a shift of the fluorescence protein from the lateral to the basal and apical sides of the cells (Figure 3E and 3F). The fluorescence ratio at the lateral and basal sides of cells decreased significantly after glucose and fructose treatment (Figure 3G and 3H). The sugar-triggered polarized localization seemed specific to hexose, as 2% ribose (one kind of pentose) had no effect on the localization of *OsMST3-GFP* or *OsMST6-GFP* (Figure 3E and 3F). Additionally, the same apical-basal polarity localization pattern was recapitulated in tiller buds treated with

glucose or fructose as in the root (Supplemental Figure 7). The dynamic changes in subcellular localization of OsMST3 and OsMST6 may represent an adaptation to alterations in intracellular sugar levels during the photoperiod. Polarized localization of OsMST3 and OsMST6 at both the basal and apical sides of the cells may enhance the efficiency of hexose transport to sink tissues.

To investigate the biological role of glucose- and fructose-induced polarized localization of OsMST3 and OsMST6, methyl- β -cyclodextrin (MCD) was used to treat the transgenic rice plant roots to disrupt their polarized localization. MCD is a cholesterol-depleting agent that disrupts lipid rafts by removing membrane cholesterol, thereby inhibiting vesicle trafficking (e.g., endocytosis and exocytosis) (Hamtaux et al., 2011; Menschikowski et al., 2011). MCD effectively inhibited the migration of OsMST3-GFP and OsMST6-GFP from lateral membrane to the basal and apical sides (Figure 3E and 3F). Tiller bud growth in the WT was significantly inhibited with increasing concentrations of MCD. In the $d3^{s2-215}$ mutant, which exhibits higher expression levels of *OsMST3* and *OsMST6* and possesses longer tiller buds, MCD treatment also led to a concentration-dependent and more pronounced suppression of tiller bud elongation. These findings indicate that proper polarized localization of OsMST3 and OsMST6 is essential for normal tiller bud growth (Figure 3I and 3J).

OsMST3 and OsMST6 are responsible for the transport of hexose from the leaf blade to tiller bud

Given the higher abundance of OsMST3 and OsMST6 in the $d3^{s2-215}$ mutant than WT (Figure 1C and 1D), sugar contents in different tissues of these genotypes were measured and compared (Figure 4A). The contents of glucose and fructose in the leaf sheaths and tiller buds were significantly higher in the $d3^{s2-215}$ mutant (Figure 4C and 4D), while their contents in the leaf blades were noticeably lower in the $d3^{s2-215}$ mutant compared with WT (Figure 4B). The sucrose contents in the leaf blades, sheaths and tiller buds were slightly higher in the $d3^{s2-215}$ mutant than in WT (Figure 4B-4D). The ratio of trehalose 6-phosphate

(Tre6P) to sucrose is a critical parameter for plant to modulate its development (Yadav et al., 2014). The Tre6P to sucrose ratio in tiller buds was significantly increased in the $d3^{s2-215}$ mutant compared with WT (Figure 4E), consistent with the increased sugar levels in tiller buds. Furthermore, we compared the sugar contents in different tissues of the *osmst3/6* double mutant with WT. The contents of glucose and fructose were obviously increased in the leaf blades and sheaths whereas dramatically decreased in the tiller buds of the *osmst3/6* double mutant compared with WT (Figure 4F-4H). The levels of sucrose in leaf blades and sheaths showed no significant change between WT and the *osmst3/6* double mutant (Figure 4F and 4G). However, the sucrose levels in tiller bud were reduced to varying degrees compared with those in WT, probably due to the reduced levels of glucose and fructose in the *osmst3/6* double mutant (Figure 4H). *OsNIA1* and *OsCAB2*, two sugar-sensitive reporter genes encoding nitrate reductase 1 and Chlorophyll a/b-binding protein 2, respectively, are positively and negatively regulated by cytosolic glucose content, respectively (Koch, 1996; Wingenter et al., 2010). The expression of *OsNIA1* was increased by 2-fold in the $d3^{s2-215}$ mutant tiller buds and decreased by nearly 10-fold in the *osmst3/6* double mutant tiller buds compared with that in WT tiller buds (Figure 4I). The expression alteration of *OsCAB2* showed an opposite trend to that of *OsNIA1*. Its expression was obviously decreased in the $d3^{s2-215}$ mutant tiller buds but greatly increased in the *osmst3/6* double mutant tiller buds compared with that in WT (Figure 4J). These expression changes further support that the contents of glucose and fructose were higher and the sugar signal was stronger in tiller buds of the $d3^{s2-215}$ mutant but lower in the *osmst3/6* double mutant tiller buds compared with WT. These results suggest that OsMST3 and OsMST6 may transport glucose and fructose from the leaf blade to tiller bud via the leaf sheath.

To confirm the roles of OsMST3 and OsMST6 in transport of glucose from the leaf blade to tiller bud, an *in vivo* glucose transport assay was performed. The radioactive ^{14}C -glucose was applied on the incisions made on the leaf blades of WT, $d3^{s2-215}$, the *osmst3* and *osmst6* single and double mutants to mimic the translocation of photosynthetic products in

leaf blades (Figure 4K). After 24-hour incubation, we quantified the radioactive signals in the leaf sheaths and tiller buds. Compared with WT, there was a noticeable rise in the radioactive signals in the $d3^{s2-215}$ leaf sheaths and tiller buds (Figure 4L). We also visualized the distribution of radioactive signals within the whole plant of WT and the $d3^{s2-215}$ mutant by autoradiography, which confirmed that the radioactive signal in the $d3^{s2-215}$ mutant tiller buds was much stronger than that of WT (Supplemental Figure 8A). Furthermore, we also compared the transport of ^{14}C -glucose in WT, the *osmst3* single, *osmst6* single and *osmst3/6* double mutants. The results showed that less ^{14}C -glucose was detected in leaf sheaths and tiller buds of the *osmst3/6* double mutant compared with WT (Figure 4M and 4N). The radioactive signals in leaf sheaths and tiller buds of the *osmst3* and *osmst6* single mutant were slightly lower than those of WT (Figure 4M and 4N). Since leaf blades were in direct contact with ^{14}C -glucose, we did not measure the radioactive signal in leaf blades.

OsMST3 and OsMST6 facilitates the direct, long-distance transport of glucose from the leaf blade to tiller bud

Since sucrose is the primary transportable form of assimilates in many plant species (Arnold, 1968; Liu et al., 2012; Riesmeier et al., 1994), we also compared the transport of radioactive ^{14}C -sucrose among the $d3^{s2-215}$ mutant, *osmst3/6* double mutant and WT. Although more ^{14}C -sucrose was detected in leaf sheaths and tiller buds of the $d3^{s2-215}$ mutant than WT, there was no significant difference in the amount of ^{14}C -sucrose transported into the leaf sheaths and tiller buds of the *osmst3/6* double mutant compared with WT (Figure 4O and 4P). 5, 6-carboxy fluorescein diacetate (5, 6-CFDA) is a fluorescent tracer of sucrose symplastic translocation in phloem (Zhao et al., 2022). After feeding 5, 6-CFDA on the leaf blades of WT, the $d3^{s2-215}$ mutant and the *osmst3/6* double mutant (Figure 4Q), stronger fluorescence of 5, 6-CFDA was observed in the tiller buds of the $d3^{s2-215}$ mutant than that in WT, whereas there was no obvious difference between WT and the *osmst3 osmst6* double mutant (Figure 4R). These results suggest that MST3 and MST6 mainly transport hexose instead of sucrose.

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488 To confirm that glucose can be directly transported from the leaf blade to tiller bud without
 489 being converted into sucrose, we first fed leaf blades with FITC-glucose, a non-
 490 metabolizable, fluorescently labelled glucose analog in which the FITC label is covalently
 491 bound to the glucose molecule and blocks its recognition by sucrose synthase (Su et al.,
 492 2018), then tested whether fluorescence of FITC-glucose could be detected in tiller buds
 493 (Figure 4Q). Under normal circumstances, sucrose is biosynthesized via formation of a
 494 glycosidic bond between the anomeric carbon (C-1) of α -D-glucose and the anomeric
 495 carbon (C-2) of β -D-fructose through a dehydration condensation reaction. When the C-1
 496 hydroxyl group of glucose is covalently modified with fluorescein isothiocyanate (FITC), the
 497 nucleophilic oxygen required for glycosidic bond formation is blocked, thereby preventing
 498 sucrose synthesis (Supplemental Figure 10). Indeed, the fluorescence of FITC-glucose
 499 was detected in the tiller buds and the signal intensity was stronger in the $d3^{s2-215}$ mutant
 500 but weaker in the *osmst3/6* double mutant compared with WT (Figure 4S), suggesting that
 501 glucose produced in leaf blades can be directly transported into tiller buds by the
 502 monosaccharide transporters OsMST3 and OsMST6. To further clarify that untransformed
 503 glucose/fructose can be directly transported to tiller buds, we performed the feeding assay
 504 with the stable isotope ^{13}C -labeled glucose in leaf blades and tracked the metabolic flux of
 505 ^{13}C in tiller buds via liquid chromatography-mass spectrometry. Compared with WT, tiller
 506 buds of the $d3^{s2-215}$ mutant contained higher amount of both ^{13}C -labeled glucose and ^{13}C -
 507 labeled sucrose. On the other hand, tiller buds of the *osmst3 osmst6* double mutant
 508 contained similar amount of the ^{13}C -labeled sucrose but lower amount of the ^{13}C -labeled
 509 glucose than WT (Figure 4T-4V and Supplemental Figure 9A), supporting that the
 510 monosaccharide transporters OsMST3 and OsMST6 are responsible for transporting
 511 glucose but not sucrose into tiller bud. The ^{13}C -metabolic flux analysis (^{13}C -MFA) confirmed
 512 that monosaccharides such as glucose can be directly transported into tiller buds, although
 513 a portion is first converted to sucrose for long-distance translocation. In sum, feeding
 514 assays with both FITC-glucose and ^{13}C -labeled glucose collectively revealed that similar
 515 to sucrose, the well-recognized transportable form of assimilates, glucose (probably also

fructose) can also be directly transported from the leaf blades (source) over long distance to tiller buds (sink).

OsMST3* and *OsMST6* are directly and negatively regulated by the TCP family transcription factor *OsTB1

To explore why the *OsMST3* and *OsMST6* proteins were more abundant in the *d3^{sg2-215}* mutant (Figure 1A, 1C and 1D), the transcript levels of *OsMST3* and *OsMST6* in callus and tiller buds of WT and the *d3^{sg2-215}* mutant were compared. The transcript levels of *OsMST3* and *OsMST6* were slightly higher in the callus tissue but drastically increased in tiller buds of the *d3^{sg2-215}* mutant compared with those in WT (Figure 5A). Moreover, the transcript levels of other *OsMST* genes were also compared between WT and the *d3^{sg2-215}* mutant. The results showed that only *OsMST3* and *OsMST6* were specifically upregulated in tiller buds of the *d3^{sg2-215}* mutant, whereas *OsMST1*, *OsMST4*, *OsMST5* and *OsMST8* were downregulated to varying degrees in the *d3^{sg2-215}* mutant compared with WT (Supplemental Figure 11A). *In situ* hybridization analysis also showed that the *OsMST3* and *OsMST6* transcripts accumulated to a markedly higher level in the tiller buds of *d3^{sg2-215}* compared with that of WT (Figure 5B), which was consistent with the RT-qPCR results.

Previous studies have reported that expression of the *OsTB1* gene encoding a TCP family transcription factor is down-regulated in the *d3* mutant (Fang et al., 2020; Minakuchi et al., 2010; Mo et al., 2024; Patil et al., 2022). In this study, the expression of *OsTB1* was also dramatically decreased in the new allelic *d3^{sg2-215}* mutant (Figure 5C). Given the opposite expression trends of *OsTB1* and *OsMST3* or *OsMST6* in the *d3^{sg2-215}* mutant, we speculated that *OsTB1* might negatively regulate the expression of *OsMST3* and *OsMST6*. To test this hypothesis, we compared the expression of *OsMST3* and *OsMST6* between WT and the *tb1^{SG0312}* mutant, which carries a frame-shift mutation in coding sequence of *OsTB1* (Takeda et al., 2003). We checked the transcript levels of *OsMST3* and *OsMST6* in both dormant (length < 1 mm) and activated tiller buds (length > 1 mm) of WT and the *tb1^{SG0312}* mutant. The expression levels of *OsDRM1*-*OsDRM4*, marker genes for tiller bud dormancy,

were higher in dormant tiller buds than in activated tiller buds (Luo et al., 2019) (Supplemental Figure 11B), verifying that our sampling of dormant and activated tiller buds was accurate. The transcript levels of *OsMST3* and *OsMST6* were modestly increased in dormant buds whereas more dramatically increased in activated buds of the *tb1^{SG0312}* mutant compared with those in WT tiller buds (Figure 5D). The increased expression of *OsMST3* and *OsMST6* in the activated tiller buds of the *tb1^{SG0312}* mutant was further confirmed by *in situ* hybridization analysis (Figure 5E). In addition, the expression levels of *OsMST3* and *OsMST6* were significantly decreased in the transgenic lines overexpressing *OsTB1* compared with WT (Figure 5F). These results indicate that *OsMST3* and *OsMST6* are likely negatively regulated by *OsTB1*.

To explore whether *OsMST3* and *OsMST6* are the direct targets of *OsTB1*, we first carried out a ChIP-Seq (Chromatin Immunoprecipitation Sequencing) assay of *OsTB1* using the *HA-OsTB1* transgenic rice plants in which the transcript and protein levels of *OsTB1* were obviously increased (Supplemental Figure 12A and 12B). Based on all the binding sites detected in the whole genome, two consensus recognition motifs, Class I (GGNCCC) and Class II (GAGGAG), were predicted as the binding site for *OsTB1* (Supplemental Figure 12C-12D, and 12G). Among them, Class I motif (GGNCCC) is consistent with the maize TB1 binding site identified previously (Dong et al., 2019; Kosugi and Ohashi, 2002), whereas Class II (GAGGAG) is a previously uncharacterized motif. Our ChIP-Seq assay clearly demonstrated that *OsTB1* directly bound to the promoters of *OsMST3* and *OsMST6* (Figure 5G and 5H). Within most of the *OsTB1*-binding peaks in the promoters of *OsMST3* and *OsMST6*, we identified Class I motifs (P1 for *OsMST3*, P1, P2 and P3 for *OsMST6*) and Class II motif, GAGGAG (P4 for *OsMST6*) (Figure 5G-5H, and Supplemental Figure 13A). Electrophoretic mobility shift assay (EMSA) demonstrated *OsTB1* could specifically bind to a 60-bp probe containing the P1 site (from -4894 to -4889 bp) in the *OsMST3* promoter and the P1 (from -187 to -182 bp), P2 (from -2551 to -2546 bp), P3 (from -4737 to -4732 bp), and P4 (from -4030 to -4025 bp) sites in the *OsMST6* promoter. The binding of *OsTB1* to these probes could be effectively blocked by an excessive amount of

unlabeled probe (cold probe) or abolished by specific mutations in the core motif (Figure 5I-5J, and Supplemental Figure 13B). Chromatin immunoprecipitation followed by quantitative PCR (ChIP-qPCR) analysis using the *HA-OsTB1* transgenic rice plants provided additional evidence for the *in vivo* binding of OsTB1 to the *OsMST3* and *OsMST6* promoter regions containing the core sequence GGNCCC and GAGGAG (Figure 5K and 5L). We further investigated whether the promoter activity of *OsMST3* and *OsMST6* is directly regulated by the transcription factor OsTB1. Co-expression with OsTB1 decreased the firefly luciferase (LUC) reporter activity driven by the *OsMST3* and *OsMST6* promoter (*OsMST3pro: LUC* and *OsMST6pro: LUC*) in transient expression assays in rice protoplasts, whereas OsTB1 could not inhibit their LUC activity when P1 site in *OsMST3* promoter or P1, P2, and P3 sites in *OsMST6* promoter were mutated from GGNCCC to GTNCCC (Mo et al., 2024), and P4 site in *OsMST6* promoter was mutated from GAGGAG to AAAAAA (Figure 5M-5N, and Supplemental Figure 13C and 13D). Similarly, the activities of *OsMST3pro: LUC* and *OsMST6pro: LUC* were significantly suppressed in the presence of *OsTB1* when expressed in *N. benthamiana* leaves, while OsTB1 had little effect on their activities when the binding site P1 in *OsMST3* promoter and P1 and P2 sites in *OsMST6* promoter were mutated to GTNCCC (Figure 5O-5R). These results demonstrated that OsTB1 negatively regulates the expressions of *OsMST3* and *OsMST6* by direct binding to their promoters.

The SLs signaling pathway is relatively conserved across different species. To determine whether BRC1, the *Arabidopsis* homolog of OsTB1, can bind to the promoters of the *OsMST3* and *OsMST6* homologs, we first constructed a phylogenetic tree of selected monosaccharide transporters from rice and *Arabidopsis*. The results showed that *OsMST3* and *OsMST6* were closely related to AtSTP1 (Supplemental Figure 12H). It has been reported that AtSTP1 and AtSTP13 exhibited partial functional redundancy (Yamada et al., 2011). Using the published ChIP-seq data for BRC1 (Van Es et al., 2024), we found that there were BRC1-binding peaks in the promoter regions of both *AtSTP1* and *AtSTP13* (Supplemental Figure 12I and 12J). These data suggest that the regulation of

monosaccharide transporters by the TCP family transcription factors might be conserved between rice and *Arabidopsis*.

SLs negatively modulate the expression of OsMST3 and OsMST6 through *D3* and *OsTB1* and counteract their sugar-induced polarized localization

To further confirm the relationship of *OsTB1* and *OsMST3* or *OsMST6*, we treated the WT, *d3^{s2-215}* and *tb1^{SG0312}* mutant seedlings with the synthetic SLs analog GR24^{5DS} for different time intervals. We observed that the expression levels of *OsTB1* in the WT tiller buds first increased significantly from the 4th min after treatment, reached its peak at the 16th min, and then gradually decreased (Figure 6A). Conversely, the transcript level of *OsMST3* was obviously decreased from the 8th min to the 16th min (Figure 6B), and the expression of *OsMST6* gradually decreased from the 8th min, reaching its lowest level at the 20th min (Figure 6C). The dynamic expression patterns of *OsTB1* and *OsMST3* or *OsMST6* under SLs treatment were apparently negatively correlated, and *OsTB1* exhibited an earlier transcriptional response to GR24^{5DS} compared with *OsMST3* and *OsMST6*, supporting its role as an upstream regulator of *OsMST3* and *OsMST6*. Intriguingly, the expression of *OsTB1*, *OsMST3*, and *OsMST6* was unaffected by GR24^{5DS} treatment in the *d3^{s2-215}* and *tb1^{SG0312}* mutants (Figure 6A-6C), suggesting that the regulation of *OsTB1*, *OsMST3* and *OsMST6* by SLs is dependent on SLs signal transduction. These findings further supported that the transcript levels of *OsMST3* and *OsMST6* were negatively regulated by SLs through *D3* and *OsTB1*. Furthermore, the protein abundance of *OsMST3* and *OsMST6* with or without GR24^{5DS} treatment was detected using transgenic rice lines expressing *OsMST3-GFP* and *OsMST6-GFP* driven by their native promoters. Firstly, the protein abundance of *OsMST3* and *OsMST6* in plants overexpressing *OsMST3-GFP* or *OsMST6-GFP* under different genetic backgrounds (WT, *d3^{s2-215}* and *tb1^{SG0312}*) was compared. Under comparable levels of the Hygromycin B phosphotransferase (HPT) protein which confers hygromycin resistance during rice transformation, the protein levels of *OsMST3* and *OsMST6* were higher in the *d3^{s2-215}* and *tb1^{SG0312}* backgrounds than in the WT (Supplemental Figure 15A). Furthermore, the fluorescence intensity of *OsMST3-GFP* and

OsMST6-GFP on the plasma membrane of protoplasts derived from different genetic backgrounds was observed. The results also showed that the fluorescence signals on the plasma membrane were significantly stronger in the *d3^{ss2-215}* and *tb1^{SG0312}* backgrounds than in the WT background (Supplemental Figure 15B and 15C). Subsequently, transgenic plants were treated by GR24^{5DS} for different time intervals. The abundance of OsMST3-GFP in WT exhibited a significant reduction at 120 min after GR24^{5DS} treatment, reaching the lowest point at 240 min. In contrast, the protein amounts of OsMST3-GFP in the *d3^{ss2-215}* and *tb1^{SG0312}* mutants showed no obvious changes after GR24^{5DS} treatment (Figure 6D). OsMST6-GFP showed the same pattern as OsMST3-GFP in response to GR24^{5DS} treatment (Figure 6D). These results suggest that the expression of OsMST3 and OsMST6 is suppressed by SLs in terms of both RNA transcript and protein abundance.

As shown in Figure 3, glucose and fructose could induce polarized localization of OsMST3 and OsMST6 on the plasma membrane, a process essential for normal tiller bud outgrowth. Given that SLs antagonize sugar signaling in the regulation of tiller bud outgrowth, we next investigated whether SLs would affect the glucose- or fructose- induced polarized localization of OsMST3 and OsMST6. Treatment with either glucose or fructose alone led to a clear redistribution of both proteins from a uniform plasma membrane distribution to the apical and basal poles. However, when roots were co-treated with 10 μ M GR24^{5DS}, this sugar-induced polarized localization was severely attenuated (Figure 6E and 6F). The inhibitory effect of GR24^{5DS} was further supported by quantifying the lateral-to-basal fluorescence ratio (Figure 6G). Collectively, SLs not only negatively regulate the expression of OsMST3 and OsMST6, but also counteract sugar-induced polarized localization of these two monosaccharide transporters.

The high-tillering phenotype of *ostb1* can be complemented by simultaneous disruption of *OsMST3* and *OsMST6*

The direct and negative regulation of *OsMST3* and *OsMST6* expression by *OstB1* prompted us to examine sugar levels in the *tb1^{SG0312}* mutant. The levels of glucose,

fructose, and sucrose in the leaf blades were significantly lower in the *tb1*^{SG0312} mutant, whereas their levels in the leaf sheaths and tiller buds were markedly higher compared with those in WT (Figure 7A-7C). Furthermore, we compared the sugar transport activity in the *tb1*^{SG0312} mutant and WT using ¹⁴C-glucose and ¹⁴C-sucrose. More ¹⁴C-glucose and ¹⁴C-sucrose were transported from leaf blades to leaf sheaths and tiller buds in the *tb1*^{SG0312} mutants compared with those in WT (Figure 7D), leading to higher accumulation of radioactive signal in tiller buds of the *tb1*^{SG0312} mutant than those of WT (Supplemental Figure 8B). ¹³C-metabolic flux analysis (¹³C-MFA) showed that the glucose and sucrose isotopologues exhibited higher labeling levels in the tiller buds of *tb1*^{SG0312} compared with WT (Supplemental Figure 9B-9E). The increased transport activity of glucose and sucrose was further validated by stronger fluorescence signal of FITC-glucose and 5,6-CFDA in tiller buds of the *tb1*^{SG0312} mutant compared with those of WT (Figure 7E-7G). To further investigate the roles of glucose and fructose in the elongation of tiller buds, 2-D-glucose (a non-metabolizable glucose analog that inhibits glycolysis), was used together with glucose to feed WT and the *tb1*^{SG0312} mutant. 2-D-glucose extremely inhibited the outgrowth of tiller buds in the *tb1*^{SG0312} mutant in a dose-dependent manner. The outgrowth of tiller buds in WT was also suppressed with increasing concentrations of 2-D-glucose although to a lesser extent (Figure 7H and 7I). In addition, feeding with CCCP, an inhibitor of sugar transport owing to its disruption of the proton gradient across the plasma membrane, dramatically suppressed the outgrowth of the *tb1*^{SG0312} tiller bud compared with WT (Figure 7J and 7K). These results suggest that the *tb1*^{SG0312} mutant showed more tillers due to the higher accumulation of glucose and fructose, which was attributed to stronger sugar transport activity.

To study the genetic relationship between *OsTB1* and *OsMST3* or *OsMST6*, the knockout mutants of *OsMST3* or *OsMST6* were created in the *tb1*^{SG0312} mutant using the CRISPR/Cas9 technique, either separately or simultaneously. The resulting homologous knockout plants were identified by sequencing. There was a 2-bp deletion in the target region of *OsMST3* in the *osmst3/tb1*^{SG0312} double mutant and a 1-bp insertion in the target region of *OsMST6* in the *osmst6/tb1*^{SG0312} double mutant. In the *osmst3/6/tb1*^{SG0312} triple

mutant, there was a 1-bp deletion in *OsMST3* and a 9-bp deletion in *OsMST6* (Supplemental Figure 8C and 8D). The high-tillering phenotype of the *tb1^{SG0312}* mutant could not be complemented by the single *OsMST3* or *OsMST6* mutation but was partially rescued by the simultaneous mutation of *OsMST3* and *OsMST6* (Figure 7L and 7M). Plant height of the *osmst3/6/ostb1* triple mutant was also slightly shorter than that of WT (Figure 7L and 7N). This result suggests that *OsMST3* and *OsMST6* function redundantly downstream of *OsTB1* in regulating rice tillering.

High-tillering phenotype of the SL-deficient *d10^{XJ1705}* mutant can be complemented by simultaneous mutation of *OsMST3* and *OsMST6*

As mentioned above, more sugars were transported from the source leaves to tiller buds due to the upregulation of *OsMST3* and *OsMST6* in the SLs signaling mutants *d3^{s2-215}* and *tb1^{SG0312}* (Figure 1, Figure 4, Figure 5 and Figure 7). To investigate whether the same mechanism works in a SL-deficient mutant *d10^{XJ1705}*, in which the 302th amino acid of D10 was mutated from Ser/S to Leu/L in the NIP background, we detected the protein abundance of *OsMST3* and *OsMST6* in the *d10^{XJ1705}* mutant. The FF/REN ratio for *OsMST3* or *OsMST6* was significantly higher in the *d10^{XJ1705}* mutant than in WT (Supplemental Figure 16A and 16B). Transcript levels of *OsMST3* and *OsMST6* in the callus tissues and tiller buds of *d10^{XJ1705}* were obviously higher than those in WT (Supplemental Figure 16C). The levels of glucose and fructose in the leaf blades of the *d10^{XJ1705}* mutant were noticeably decreased compared with WT (Supplemental Figure 16D), while their levels in the leaf sheaths and tiller buds were significantly increased in the *d10^{XJ1705}* mutant compared with WT (Supplemental Figure 16E and 16F). Although the level of sucrose did not change in the leaf sheath and blade, it increased significantly in the tiller buds of the *d10^{XJ1705}* mutant probably due to the increased levels of glucose and fructose (Supplemental Figure 16D and 16F). The *osmst3/6/d10^{XJ1705}* triple mutant was created using CRISRP/Cas9 technique, with the mutation sites in *OsMST3* and *OsMST6* shown in Supplemental Figure 16J and 16K. Tiller number of the *osmst3/6/d10^{XJ1705}* triple mutant was extremely reduced, even lower than that of WT (Supplemental Figure 16G and

16I), suggesting that the high-tillering phenotype of the *d10^{XJ1705}* mutant can be complemented by simultaneous mutations of *OsMST3* and *OsMST6*. However, the plant height of the *osmst3/6/d10^{XJ1705}* triple mutant was shorter than that of WT (Supplemental Figure 16G and 16H). All these data are consistent with those observed in the *d3^{s2-215}* mutant (Figure 1R-1T). In conclusion, *OsMST3* and *OsMST6* consistently promote tillering in both SLs signaling and SLs biosynthesis mutants, indicating that their functional mechanism is broadly conserved.

Discussion

The chlorophyll content and the rate and efficiency of photosynthesis are key factors affecting the contents of sugars. To exclude the effects of these factors in the *d3^{s2-215}* mutant, the total chlorophyll content and efficiency of photosynthesis were measured. The results showed that there was no significant difference in total chlorophyll and chlorophyll a and b contents between WT and the *d3^{s2-215}* mutant (Supplemental Figure 1I-1K). However, the net photosynthetic rate in the *d3^{s2-215}* mutant was slightly lower than that in WT (Supplemental Figure 1L). There was no obvious difference in conductance, intracellular CO₂ concentration and transpiration rate between WT and the *d3^{s2-215}* mutant (Supplemental Figure 1M-1O). These results suggest that the higher accumulation of glucose and fructose in the leaf sheaths and tiller buds of the *d3^{s2-215}* mutant is not due to the increased photosynthesis rate but due to the stronger monosaccharides transport ability from the leaf blades to tiller buds through the leaf sheaths. Starch, a polymer of glucose, serves as a storage polysaccharide in plants and is closely correlated with rice tillering. Reduced starch content resulted in fewer tillers in the *fl016* mutant due to deficiency in a NAD-dependent cytosolic malate dehydrogenase (Teng et al., 2019). Scanning electron microscopy (SEM) showed that more starch granules accumulate in the leaf sheath of the *d3^{s2-215}* mutant compared with WT (Supplemental Figure 1P-1R), and starch content assay further validated that more starch accumulated in leaf sheath in the *d3^{s2-215}* mutant (Supplemental Figure 1S), which was consistent with higher glucose levels in the *d3^{s2-215}* leaf sheath compared with WT (Figure 4C).

Eight *OsMST* members have been reported in the rice genome. We analyzed the expression patterns of these eight *OsMST* genes in response to varying concentrations of glucose and fructose. *OsMST3* can be sharply induced by 1% glucose and 1% fructose, while *OsMST6* is dramatically induced by 2% glucose and 1% fructose (Figure 3A and 3B). In contrast, other *OsMST* genes were significantly induced by relatively high concentrations of sugars i.e., 3% glucose or 4% fructose, except for *OsMST7*, which showed no response to glucose treatment (Supplemental Figure 17A-17C). These expression patterns are similar to those *ZIP* family genes encoding zinc transporters, among which members induced by low concentrations of zinc encode influx transporters that contribute to zinc uptake under low zinc condition; whereas members induced by high concentrations of zinc encode efflux transporters, which help to remove excessive amount of zinc to avoid metal toxicity (Liu et al., 2019b; Yang et al., 2020). Additionally, the yeast strain EBY.VW4000 transformed with *OsMST3* or *OsMST6* grew better on the medium containing 0.5% to 2% glucose than on the medium containing 3% and 4% glucose. Similarly, these strains grew the best on the medium containing 0.5% fructose compared with medium containing with 1% to 4% fructose (Figure 2C and Supplemental Figure 6A). These results further support the notion that *OsMST3* and *OsMST6* function as the influx monosaccharide transporters, while other *OsMST* members may act as the efflux monosaccharide transporters. Besides *OsMST3* and *OsMST6*, which were shown to synergistically regulate tiller bud outgrowth in this study, we also explored the potential roles of other *OsMSTs* in rice tillering using CRISPR-Cas9 technique. There was no discernible difference in tiller number between WT and the single knockout mutants of *OsMST2* (Supplemental Figure 17D-17G) or *OsMST5* (Supplemental Figure 17H-17K), and the double knockout mutants of *OsMST7* and *OsMST8* (Supplemental Figure 17L-17O), or *OsMST1* and *OsMST4* (Supplemental Figure 17P-17S).

In most plant species, sucrose is the main form of sugars for long-distance transport from the source organs (mature leaves) to sink organs (axillary buds, root, seeds etc.) through

phloem (Arnold, 1968; Doidy et al., 2024; Liu et al., 2012). In most herbaceous plants, sucrose is mainly transported through the apoplastic pathway mediated by the SWEET (Sugars Will Eventually Be Exported Transporters) and SUT (Sucrose Transporter) family transporters (Zhang and Turgeon, 2018b). During this process, SWEETs facilitate sugars export from the plant cells into apoplast while SUTs are responsible for sugars import from apoplast into the plant cells. After reaching the sink tissues through long distance transport in the SE/CC (sieve element/companion cell) system in the phloem, sucrose is unloaded from companion cells into apoplast via SWEETs and subsequently enters sink cells through SUTs (Braun et al., 2014; Ruan, 2014). Alternatively, a portion of sucrose is first hydrolyzed into glucose and fructose by the cell wall invertase (cwINV), which are then imported into the sink cells through MSTs. MSTs are mainly involved in phloem unloading at the sink tissues, transferring photosynthetic products from the apoplast into the sink cell (Zhang and Turgeon, 2018). Sink strength has been defined as the potential capacity of sink tissues to accumulate assimilates (Li et al., 2021; Roitsch, 1999). Crop yield can be improved by enhancing sink strength (Li et al., 2021). Overexpression of the grapevine sucrose transporter genes *VvSUC11*, *VvSUC12*, or *VvSUC27* in tobacco and *Arabidopsis* increased seed yield and abiotic stress resistance (Cai et al., 2017). Overexpression of *VvSWEET4* in grapevine hairy roots significantly increased biomass accumulation and conferred resistance to *P. irregulare* infection by facilitating sugar transport (Meteier et al., 2019). Tonoplast-localized OsSUT2 modulated rice tillering. The *ossut2* mutant showed a growth retardation phenotype with an obvious reduction in tiller number (Eom et al., 2011). The activity of cwINVs also affects the sink strength (Ruan et al., 1997; Ruan et al., 2010). Suppression of *OsCWIN2* (*GIF1*) obviously decreased the rice grain yield (Wang et al., 2008a). The similar phenomenon was observed in the maize *lncw2* mutant (Cheng et al., 1996). *VfCWIN1* in *Vicia faba* was also reported to regulate the seed size (Weber et al., 1996). In this study, *MST3* and *MST6* were highly expressed in the sink tissues such as tiller bud, root and young panicle (Figure 2A and Supplemental Figure 5C-5H). In the high-tillering SL-insensitive *d3^{s2-215}* mutant and SL-deficient mutant *d10^{XJ1705}*, the increased abundance of OsMST3 and OsMST6 (Figure 1 and Supplemental Figure 16) may enhance

the sink strength of tiller bud to promote the export of sugars from the source leaf. Indeed, the abundance of two SWEET transporters, OsSWEET4 and OsSWEET1b, was also increased in the *d3^{s2-215}* mutant (Figure 1A and Supplemental Figure 2E and 2F), probably facilitating the export of sugars from the source leaf cells to the apoplast. Under the combined action of OsSWEET4, OsSWEET1b, OsMST3 and OsMST6, photosynthetic products are continuously transported from the leaves to the tiller buds, providing energy and sugar signals for the elongation of tiller buds. At the genetic level, the double knockout mutants of *OsMST3* and *OsMST6* showed reduced tiller number due to decelerated tiller bud elongation rate, confirming their roles in promoting tiller bud outgrowth (Figure 1L-1P). Although in the classic view sucrose is the primary transportable form of assimilates in plant (Arnold, 1968; Liu et al., 2012; Riesmeier et al., 1994), our physiological and genetic characterization of the monosaccharide transporters OsMST3 and OsMST6 suggests that glucose and fructose can also be directly transported over long distance without being converted into sucrose.

The expression levels and activities of plant sugar transporters are regulated by multiple factors, enabling dynamic photosynthate allocation to support growth and development in response to environmental changes. In *Arabidopsis*, drought stress and abscisic acid (ABA) treatment induced the SnRK2 protein kinase to phosphorylate SWEET11 and SWEET12. This phosphorylation enhanced their oligomerization and sucrose transport activity, thereby promoting sugar translocation from shoots to roots to help the plant adapt to osmotic stress (Chen et al., 2022). In rose, transcript level of *RhSUC2* was induced in buds exposed to light after removing the apical dominance. *RhSUC2* was proposed to play a crucial role in bud outgrowth in decapitated plants under light conditions by modulation of sucrose import into buds (Henry et al., 2011). Upon auxin application, *RhSUC2* expression was suppressed. Conversely, cytokinins promoted bud outgrowth probably by upregulating the expression of *RhSUC2* and *RhSWEET10* (Roman et al., 2016). Comparable to the findings above, we found here that SLs suppress bud outgrowth by downregulating the expressions of *OsMST3* and *OsMST6*, thus limiting sugar allocation to rice tiller buds.

Monosaccharides are the carbon skeleton for the cell wall in plants. The *Arabidopsis* *tb129/esk1* mutant showed a dwarf and collapsed xylem phenotype due to a reduction of xylan O-acetylation. Mutation of the key SLs biosynthesis genes (*MAX3* and *MAX4*) rescued the dwarfism, collapsed xylem, and freezing-tolerant phenotype of *tb129/esk1*. The levels of monosaccharides such as arabinose or xylose in *tb129/esk1* were also restored to the WT level (Ramirez and Pauly, 2019). In this study, we found that the *OsMST3* and *OsMST6* proteins were more abundant in SL-deficient and -insensitive mutants, suggesting that monosaccharide transporters, such as homologs of *OsMST3* and *OsMST6* in *Arabidopsis*, may also be upregulated and help transport monosaccharides to rescue the defective phenotype of *tb129/esk1*.

SLs simulate cambial cell division and suppress vessel formation (Agusti et al., 2011; Zhao et al., 2025). In this study, SL-related mutants exhibited an enhanced capacity for transporting glucose and fructose from leaves to tiller buds. To exclude the possibility that this phenotype might arise from structural alterations in vascular bundles, we examined the morphology and structure of the fifth leaf at the seedling stage from WT and SL-related mutant (including *d3^{ss2-215}*, *d10^{XJ1705}*, and *tb1^{SG0312}*). The results showed that, although the fifth leaves of all mutant lines tended to be narrower than those of WT, all genotypes consistently possessed five main veins (Supplemental Figure 18A-18E and 18J). Cross-sectional analysis further revealed that, apart from a significant reduction in vascular bundle diameter in the SL-related mutants (Supplemental Figure 18F-18I and 18K), the overall vascular bundle structure remained unchanged (Supplemental Figure 18B-18K). The smaller vascular bundle in the SL mutants further support that increased sugar transport is due to increased *MST3/6* expression rather than altered vein structure.

Previous studies have shown that SLs promote the elongation of seminal roots and crown roots while suppress the formation of crown roots and lateral roots in both *Arabidopsis* and rice (Feng et al., 2026; Yuan et al., 2023; Sun et al., 2014). As downstream responsive genes of SLs signaling (Figure 6B-6D), *OsMST3* and *OsMST6* were strongly expressed in

rice roots (Figure 2A and 2B). To investigate their potential roles in root development, we examined the root phenotypes of the *osmst3/6* double mutant. The results showed that the double mutant exhibited significantly longer seminal roots and fewer crown roots compared with WT (Supplemental Figure 19A-19C), a phenotype similar to that induced by SLs treatment. Subsequently, we compared the expression levels of key SLs biosynthesis genes, including *OsMAX1* members (*Os900*, *Os1400*), *D10*, *D17*, and *D27*, between WT and the *osmst3/6* double mutant. These genes were significantly upregulated in both the tiller buds and roots of the *osmst3/6* double mutant compared with WT (Supplemental Figure 19D and 19E). Furthermore, we examined the expression levels of *OsMST3*, *OsMST6*, *OsTB1*, and *Os900* in the shoot base and roots under low-phosphorus conditions. The treatment method was illustrated in Supplemental Figure 20A. The results showed that under low-phosphorus treatment, the expression levels of *OsMST3* and *OsMST6* were significantly downregulated in both the shoot base and roots, whereas the expression levels of *OsTB1* and *Os900* were significantly upregulated (Supplemental Figure 20B-20C). These results suggest that low-phosphorus-induced SLs biosynthesis may promote the expression of *OsTB1* and *Os900* while suppress the expression of *OsMST3* and *OsMST6*.

SL-deficient and -insensitive mutants typically exhibit dwarf and high-tillering phenotype. In contrast, loss of function of *OsMST3* and *OsMST6* impaired the transport of glucose and fructose from source tissues to sink tissues, leading to the dual phenotype of reduced plant height and decreased tiller number in the *osmst3/6* double mutant. These observations indicate that reduced plant height and increased/decreased tiller number are not always coupled but rather represent two independently regulated biological processes. The high-tillering phenotype of the *d3^{s2-215}*, *d10^{XJ1705}*, and *tb1^{SG0312}* mutants can be restored by disrupting *OsMST3* and *OsMST6*; however, it is worth noting that their plant height cannot be complemented. These indicate that SLs may regulate rice tillering and plant height through different downstream genes or that sugars regulate these two developmental processes in a relatively independent manner. A similar phenomenon has been reported in a previous study, in which the reduced branching phenotype of the Arabidopsis *hxx1*

mutant can be complemented by a mutant version of HXK1 lacking catalytic activity, while its plant height cannot be complemented (Barbier et al., 2021).

ChIP-seq analysis of OsTB1 showed that OsTB1 can bind to 7532 genes (Supplemental Table 3). Biological process (BP) enrichment analysis revealed that the top-ranked biological process was DNA-templated transcription, indicating that the target genes of OsTB1 are predominantly involved in transcriptional regulation. Molecular function (MF) enrichment analysis showed that the target genes of OsTB1 were most significantly enriched for DNA-binding transcription factor activity and sequence-specific DNA binding (Supplemental Figure 12E and 12F). These results firmly place OsTB1 at the higher hierarchy of the complex transcription cascade. We also performed RNA-seq analysis to find differentially expressed genes (DEGs) between WT and the *tb1^{SG0312}* mutant tiller bud. We found that 501 genes were upregulated and 1077 genes were downregulated in *tb1^{SG0312}* mutant tiller buds compared with WT (Supplemental Figure 14A). RNA-seq data revealed a globally coordinated enrichment of GO terms related to carbohydrate and sucrose metabolism, photosynthetic dark reactions, thylakoid membrane organization, and cell wall remodeling, alongside oxidoreductase and detoxification activities (Supplemental Figure 14B). These molecular changes are closely associated with the observed phenotypic alterations, including significantly increased tiller numbers and elevated soluble sugar contents in tiller buds (Figure 7C and 7L-7N). Collectively, our results suggested that mutation of *OsTB1* redirects carbon partitioning toward tiller buds, promotes local sugar accumulation, and activates metabolic and membrane-associated processes that facilitate tiller outgrowth. We found a mechanistic link between sugar metabolism and tiller development, highlighting *OsTB1* as a central regulator of carbon allocation and bud outgrowth in rice.

Integrating RNA-seq and ChIP-seq data, we found 385 overlapping target genes of OsTB1 (Supplemental Figure 14C and Supplemental Table 4), including *OsMST3* and *OsMST6*, which showed increased expression levels in the *ostb1* mutant (Supplemental Figure 14D). These results further confirmed OsTB1 functioned upstream of *OsMST3* and *OsMST6* and

negatively regulated their expression level. In addition to *OsMST3* and *OsMST6*, several of these genes, including *OsSPS1*, *OsMYB2*, *OsbHLH044*, *OsSUS4*, *OsCatC*, *OsTPP7*, and *OsCCA1*, have been previously reported to function in tillering regulation pathways (Supplemental Figure 14D).

BRC1, the homolog of *OsTB1* in Arabidopsis, directly activates a group of phylogenetically related genes encoding the Homeodomain-Leucine Zipper (HD-ZIP) transcription factors, *HB21*, *HB40*, and *HB53*. These transcription factors, together with *BRC1*, initiate a cascade that leads to local abscisic acid (ABA) accumulation and response, which is essential for bud dormancy under light-limiting conditions. Under long-day conditions, the *hb21 hb40 hb53-1* triple mutants did not show any noticeable changes in their branching phenotype or bud development compared with the wild-type plants. Additionally, some genes regulated by *BRC1* that were involved in cell division were unaffected in the triple mutant (González-Grandío et al., 2017). These results suggest that only a subset of the transcriptional responses induced by *BRC1* are mediated by *HB21*, *HB40*, and *HB53*. Other downstream genes of *OsTB1/BRC1* may also regulate axillary bud dormancy and outgrowth. In this study, *OsTB1* negatively regulated the expression of *OsMST3* and *OsMST6* by directly binding to their promoters. The *osmst3 osmst6* double mutant displayed reduced tillering and transgenic rice plants overexpressing *OsMST6* showed increased tillering both in Beijing (long-day condition) and Hainan province (short-day condition). All these findings suggest that *OsMST3* and *OsMST6*, as downstream targets of SLs, regulate rice tiller bud outgrowth by controlling glucose and fructose availability.

When roots and tiller buds of the transgenic rice plant expressing *OsMST3-GFP* or *OsMST6-GFP* were treated with glucose and fructose, the subcellular localization of *OsMST3* and *OsMST6* shifted from an even distribution in the plasma membrane to the apical and basal sides of the plant cells. This polarization aligns with the direction of sugar transport from source tissues to sink tissues in plants. We confirmed that the polar localization of *OsMST3* and *OsMST6* was a prerequisite for bud outgrowth or for the release of bud dormancy (Figure 3I and 3J). Intriguingly, SLs counteracted sugar-induced

polarized localization of these two monosaccharide transporters (Figure 6E-6G). However, the underlying mechanism behind this polarized localization phenomenon remains to be elucidated. It is possible that the binding of sugars to OsMST3 and OsMST6 directly induces conformational changes, thereby unmasking cryptic protein–protein interaction interfaces or intracellular sorting motifs. Supporting this notion, structural studies on SWEET transporters have demonstrated that substrate binding induces dynamic conformational shifts (Weigle, A. T., & Shukla, D, 2024), offering a mechanistic paradigm for how sugars can actively regulate the subcellular trafficking and spatial organization of sugar transporters. Another alternative and mechanistically plausible model propose that sugar signaling activates specific protein kinases, which modulate the affinity of OsMST3 and OsMST6 for polarity-determining sorting complexes through phosphorylation of their intracellular loop or C-terminal tail.

In addition to the possibility that SLs affect the polarized localization of OsMST3 and OsMST6 by suppressing their transcription and protein accumulation (Figure 6A-6D), a compelling hypothesis is that sugar signaling modulates cellular sensitivity to SLs, potentially via conformational changes in the SL receptor D14, thereby triggering a downstream signaling cascade and ultimately leading to the polar localization of OsMST3 and OsMST6. Yao et al. discovered the crystal structure of the strigolactone-induced AtD14-D3-ASK1 complex, providing the first structural evidence that SL binding triggers a conformational transition of AtD14 from an open to a closed state. SLs were hydrolyzed into a covalently linked intermediate molecule (CLIM), which initiates a conformational change in AtD14 that facilitates its interaction with D3 (Yao et al., 2024). Furthermore, the activation, fine-tuning and termination mechanisms of D14-mediated signal perception were elucidated. Notably, D3 was shown to adopt two topological conformations, which regulate the initiation and termination of the signaling, respectively. In parallel, D14 phosphorylation was identified as a key post-translational modification regulating its protein stability (Hu et al., 2024). Collectively, these findings provide strong mechanistic support for the proposed hypotheses that sugar signaling modulates cellular sensitivity to SLs through D14 conformational dynamics, which may ultimately contribute to the polar

localization of OsMST3 and OsMST6. In the future, we will conduct further research to elucidate the post-translational modifications underlying sugar-induced polarized localization of OsMST3 and OsMST6 and how SLs antagonize this process in regulating rice tillering.

Based on results in this study and our previous findings that sugars suppressed the expression of *D3* and *OsTB1* in rice (Patil et al., 2022; Mo et al., 2024), we proposed a working model (Figure 7O) to elucidate the functions of *OsMST3* and *OsMST6* in the SLs-regulated rice tillering process. OsMST3 and OsMST6 function as key monosaccharide influx transporters that mediate the phloem-dependent translocation of photosynthetically derived monosaccharides from source tissue (leaf blade) through leaf sheath to sink tissues (tiller bud and root). OsMST3 and OsMST6 exhibit dynamic localization patterns dependent on intracellular monosaccharide levels: OsMST3 and OsMST6 are uniformly distributed on the plasma membrane under normal conditions but become polarly localized to the apical and basal regions of cells when monosaccharide levels are high. Sugars and SLs antagonistically regulate the expression and polarized localization of OsMST3 and OsMST6. Under normal intracellular sugar levels, the SL signaling remains active, which upregulates *OsTB1* expression and consequently enhances the suppression of *OsMST3* and *OsMST6*, thereby restricting sugar acquisition by tiller buds and inhibiting tillering. Conversely, under high sugar conditions, sugars attenuate SLs signaling by suppressing the expressions of *D3* and *OsTB1*, which in turn alleviate the *OsTB1*-mediated repression of *OsMST3* and *OsMST6*. More OsMST3 and OsMST6 transporters are polarly localized on the cell membrane. Consequently, glucose and fructose import is enhanced from the apoplast into cells, thus promoting tillering.

In summary, this study uncovered a molecular mechanism whereby SLs suppressed rice tillering by transcriptionally and functionally regulating the monosaccharide transporters OsMST3 and OsMST6, thereby reprogramming sugar partitioning from source tissues to sink tissues. These findings establish a mechanistic basis for targeted manipulation of

1009 source-sink coordination - specifically, improving photosynthetic efficiency of source
1010 tissues, and increasing sink strength of sink tissues - to simultaneously optimize
1011 carbohydrate homeostasis and grain yield potential.

1012

Journal Pre-proof

Methods

Plant materials and growth conditions

The $d3^{s2-215}$ and $d10^{XJ1705}$ mutants were isolated from a mutant population of the *japonica* rice variety Nipponbare (NIP) mutagenized by ethyl methane sulfonate (EMS). To obtain the $tb1^{SG0312}$ mutant in NIP background, the $tb1^{SG0312}$ mutant originally in the Taichung 65 genetic background (Takeda et al., 2003) was backcrossed with NIP for four generations. The $tb1^{SG0312}$ mutant was selected from the BC₄F₂ progeny.

All rice materials were cultivated in paddy fields located in Beijing city or Hainan province, China, under natural growing conditions. To obtain samples during the seedling stage, rice seeds were germinated in water and grown in Yoshida nutrient solution (NSP1040, Coolaber) in a growth chamber set at 16 hours of light (28°C), followed by 8 hours of darkness (22°C), maintaining a humidity level of 60%.

iTRAQ Labeling

Two-month-old callus of similar size of NIP and $d3^{s2-215}$ were selected to perform iTRAQ. Powder of each sample was put into a new tube and adjusted to 100 µL using 8 M urea mixed with 11 µL 1 M DTT. The protein iTRAQ labeling using an iTRAQ Reagents 8-plex kit (AB Sciex, Foster City, CA, USA) was conducted by Applied Protein Technology Co. Limited (Shanghai, China) according to the manufacturer's instructions. One unit of iTRAQ reagent (defined as the amount of reagent required to label 100 µg of protein) was thawed and reconstituted in 70 µL isopropanol. Two independent biological replicates were performed. Nipponbare replicates were labeled with iTRAQ tag113 and 114, while $d3^{s2-215}$ replicates were labeled with iTRAQ tag117 and 118.

Protein abundance detection

To construct the *StrigoQuant-like-OsMST3* and *StrigoQuant-like-OsMST6* reporter vectors, the CDSs of *OsMST3* and *OsMST6* were inserted into *StrigoQuant-like* vector (Sun et al., 2021), (MH110, Coolaber). The primers used are listed in Supplemental Table 2. For checking the protein abundance of *OsMST3* and *OsMST6*, the purified *StrigoQuant-like OsMST3*, *StrigoQuant-like OsMST6* and *StrigoQuant-like* empty plasmids were transferred to the protoplasts of NIP and $d3^{s2-215}$ or $d10^{XJ1705}$ mutants for transient expression,

respectively. The relative FF/REN of *OsMST3* and *OsMST6* were normalized to that of empty vector.

Vector construction and plant transformation

To generate the *OsMST3-GFP* and *OsMST6-GFP* lines, we amplified a 6208-bp genomic DNA fragment of *OsMST3*, which includes a 2099-bp sequence upstream of the start codon and the full-length genomic DNA of *OsMST3* without stop codon, and a 5253-bp genomic DNA fragment of *OsMST6*, including a 2945-bp sequence upstream of the start codon and the full-length genomic DNA of *OsMST6* without stop codon. These fragments were then fused in frame with GFP in the plant binary vector *pCAMBIA1305.1-GFP* using an In-Fusion HD Cloning Kit (638920, Clontech). For the *promoter-GUS* vectors, we fused the 2544-bp promoter sequence of *OsMST3* and the 2814-bp promoter sequence of *OsMST6* to the *GUS* reporter gene in the plant binary vector *pCAMBIA1305.1*. To knock out *OsMST3*, *OsMST6*, and other *OsMSTs* genes in NIP, *d3*^{s2-215}, *d10*^{XJ1705}, and *tb1*^{SG0312} backgrounds, we cloned each single guide RNA (sgRNA) into an sgRNA intermediate vector before cloning it into the destination vector *pYLCRISPR/Cas9Pubi-H* (Ma et al., 2015). The resulting constructs were introduced into rice callus through *Agrobacterium tumefaciens*-mediated transformation. The primers used are listed in Supplemental Table 2.

RNA extraction and RT-qPCR

Total RNA was isolated from different tissues using the KKFast Plant RNApure kit (ZP405K, Zomanbio). cDNA was synthesized using HiScript IV RT SuperMix for qPCR (+gDNA wiper) (Vazyme Biotech Co., Ltd). RT-qPCR was conducted using Taq Pro Universal SYBR qPCR Master Mix (Vazyme Biotech Co., Ltd). Rice *Ubiquitin* gene (*LOC_Os03g0234200*) was used as an internal control. The data were analyzed using the $2^{-\Delta\Delta CT}$ quantification method. The primers used for RT-qPCR are listed in Supplemental Table 2.

GUS staining

Histochemical analysis of the *MST3 promoter-GUS* and *MST6 promoter-GUS* transgenic rice plants was conducted following the previously described protocol (Jefferson et al., 1987). Various tissues were immersed in the GUS staining buffer under vacuum for 30

minutes, followed by incubation in darkness at 37°C for a duration of 3-12 hours. Subsequently, impurities and chlorophyll were effectively removed through continuous washing with 75% alcohol. Finally, photographs were captured using a stereomicroscope (OLYMPUS SZX16).

Immunostaining assays

To study the spatial distribution of the OsMST3 and OsMST6 protein, the leaf blades, leaf sheaths, tiller buds, and roots from *OsMST3-GFP* and *OsMST6-GFP* transgenic rice plants were embedded in paraffin. The following Immunostaining staining was performed as Zhang et al. (2024b) with minor modifications. In short, sections were prepared using a rotary microtome, deparaffinized, and rehydrated. The sections were then incubated overnight at 4°C with the primary Anti-GFP (598, MBL, 1:500) antibody diluted in blocking buffer. After washing, samples were incubated with the Secondary Antibody, the Goat anti-Rabbit IgG, Highly Cross-Adsorbed, Alexa Fluor™ Plus 555 (A32732, Invitrogen, 1:400) at room temperature in the dark. Fluorescence signals were observed using a confocal laser-scanning microscope (LSM 980, Zeiss).

Functional characterization of OsMST3 and OsMST6 by heterologous expression in yeast

To test the functionality of OsMST3 and OsMST6, the CDS of *OsMST3* and *OsMST6* were cloned into the yeast expression vector *pDR196*, respectively (Li et al., 2024). The vector *pDR196-OsMST3* and *pDR196-OsMST6* were transformed into the hexose uptake-deficient yeast mutant EBY.VW4000 strain (Wieczorke et al., 1999) and sucrose transport-deficient yeast SUSY7/ura3 (Sun et al., 2019) to verify the sugar transport activity of OsMST3 and OsMST6, using OsSUT1 as a positive control (Li et al., 2024) and SchXT11 (Wieczorke et al., 1999) from yeast as an endogenous control. The primers used are listed in Supplemental Table 2.

For the drop assay of yeast growth, a single clone of the EBY.VW4000 or SUSY7/ura3 transformants was cultured in liquid SD-Ura (PM2271-10×0.5L, Coolaber) medium supplemented with 2% (w/v) maltose or 2% (w/v) glucose as the sole carbon source, respectively, at 28 °C until OD₆₀₀ = 0.8~1.0. The cells were collected and diluted 10, 100,

1000, and 10000 folds, and then 7 μ L of each dilution was dropped on the solid SD-Ura medium with 2% (w/v) maltose or 0.5, 1, 2, 3, and 4% glucose or fructose for the EBY.VW4000 strain and with 2% (w/v) glucose or sucrose for the SUSY7/ura3 strain, respectively. In addition, 2-deoxyglucose (2-D-Glu, M5140-1g, Abmole), a glucose analogue and inhibitor of glucose metabolism, was used to determine the transport capacity of OsMST3 and OsMST6. Cells were cultured under dark conditions at 28°C for 3-5 days.

For the uptake experiment, a liquid medium with maltose (filter-sterilized) serving as the only carbon source was prepared. The yeast strains expressing pDR196, OsMST3, and OsMST6, respectively, were shaken in a shaker at 200 rpm and 30°C until the OD₆₀₀ reached 1.0. After being washed with sterile water, the pellet was resuspended in pre-cooled PBS buffer (0.6 M sorbitol, 50 mM NaPi, pH 5.0), and the OD₆₀₀ was adjusted to 2.0. 10 μ L of the resuspended yeast culture was inoculated into 2 ml of liquid medium (containing 2% ¹⁴C-glucose, with a concentration of 100 mM), and grown in a shaker at 200 rpm and 30°C. At 0, 2, 4, 6, 8, 10, 20, 40 and 60 mins, respectively, 1 μ L of the yeast culture was collected and counted with a hemocytometer. Meanwhile, the yeast was washed 5-10 times with pre-cooled PBS buffer. Cells were collected by vacuum filtration onto 0.8 mm glass fiber filters (Whatman) and washed three times with 4 mL of ice-cold sterile water to stop reaction before radioactivity was determined by liquid scintillation counting.

The substrate competitive uptake assay

A single yeast clone was cultured in liquid SD/-Ura medium containing 2% maltose until the optical density (OD₆₀₀) reached 0.8-1.0. The cells were collected at 3000 g for 10 minutes. After rinsing three times with sterile water, the cells were rinsed once with 25 mM sodium phosphate buffer (pH 5.5) and then cells were resuspended in the sodium phosphate buffer to the final OD₆₀₀ of 2.0. For the substrate competitive uptake assay, 100 μ M ¹⁴C-glucose as substrate without competitor was used as one internal control, and competing sugars were added at 30 s before the addition of ¹⁴C-glucose to a final concentration of 1 mM. For the uptake inhibitor assay, a final concentration of 50 μ M proton

uncoupler carbonyl cyanide *m*-chlorophenyl-hydrazon (CCCP, C6700, Solarbio) was added at 30 s before the addition of ^{14}C -glucose. After incubation for 6 minutes on a shaker at 30°C, cells were collected by vacuum filtration onto 0.8 mm glass fiber filters (Whatman) and washed three times with 4 mL of ice-cold sterile water to stop reaction before radioactivity was determined by liquid scintillation counting.

Glucose uptake assay using rice protoplasts

The isolation of rice protoplasts and the subsequent protoplast glucose uptake assay were conducted following previously described methods (Wang et al., 2019). Rice protoplasts isolated from etiolated seedlings of NIP, *d3^{ss2-215}*, and other genotypes were suspended in 1 mL W5 buffer. To assess glucose or fructose uptake, fluorescein isothiocyanate (FITC)-glucose (WH018N07, Weihuabio) or CY2-fructose (Q-CY2-0312, Qiyuebio) was added to the protoplast suspension to a final concentration of 1 mM, respectively. After gently agitating the tube, the protoplasts were incubated for 30 minutes at 28°C in the darkness. Subsequently, excessive FITC-glucose or CY2-fructose was removed by washing the protoplasts five times with W5 solution. The resulting fluorescence was visualized using a confocal laser scanning microscope (LSM980, Zeiss) equipped with an excitation filter range of 520–530 nm and an emission filter range of 490–495 nm.

Radiolabeling experiment

The radiolabeling experiments were performed at the Isotope Laboratory of the Institute of Biophysics, Chinese Academy of Sciences, with specifications of 50 μCi ^{14}C -glucose (9.805 GBq mmol^{-1} in ethanol: water [9:1, vol/vol; MP Biochemicals; NEC042X050UC, Revvity]) and 50 μCi ^{14}C -sucrose (9.805 GBq mmol^{-1} in ethanol: water [9:1, vol/vol; MP Biochemicals; NEC100X050UC, Revvity]). Leaf blades of WT, *d3^{ss2-215}*, and *tb1^{SG0312}* mutant seedlings were scratched. The wounds in leaf blade were placed in water containing 1 μCi ^{14}C -glucose or ^{14}C -sucrose while the roots were immersed in water at room temperature for 24 hours. Then, the leaf blades, leaf sheaths, and tiller buds were collected for subsequent analysis. The collected materials were finely chopped and incubated with 2 mL of scintillation solution (152-24405, Revvity). Radioactivity (counts per minute) was quantified using liquid scintillation counting employing a Beckman LS650

instrument. A minimum of three biological replicates was measured for statistical analysis. For autoradiography of the entire plant, the treated plant specimen was securely fixed onto a phosphorus screen (BAS, 2040; GE HealthCare) and subjected to gentle pressure for one week. Subsequently, the radioisotope signal was detected utilizing an image analyzer (Typhoon 9210, Amersham).

¹³C-metabolic flux analysis (¹³C-MFA)

Following radiolabeling experiment method, ¹³C-metabolic flux analysis of WT, *d3^{s2-215}*, *osmst3 osmst6*, and *tb1^{SG0312}* was conducted through feeding 500 μm ¹³C-Glucose (389374, Sigma-Aldrich) on the scratched leaf blades. The tiller buds were collected for subsequent analysis. The ¹³C-Glucose metabolites were detected by LC-MS/MS (AB 6500+, AB Sciex). The chromatographic conditions were as follows: flow rate, 0.2 ml/min; column, ACQUITY UPLC HSS T3, 100 Å, 1.8 μm, 2.1 mm×150 mm; Precolumn, ACQUITY UPLC HSS T3 VanGuard, 100 Å, 1.8 μm, 2.1 mm×5 mm; column temperature, 40°C; and mobile phase, solvent A: 0.1% formic acid in water; and solvent B: 100% acetonitrile. Gradient elution was performed as follows: 1 min, 5% B, 7 min, 95% B; 8.5 min, 95% B; 8.8 min, 5% B; and 12 min, 5% B. The abundances of the labeled and unlabeled metabolites are indicated as the peak areas of the spectra.

Glucose and fructose treatment

To detect the expression changes of the *OsMSTs* genes following glucose and fructose treatment, NIP seedlings were cultivated in 1/2 MS medium and incubated in a growth chamber for 10 days. Subsequently, the plant roots were thoroughly rinsed to remove any residual medium and transferred to water for a period of 24 hours. The plants were then exposed to glucose and fructose solutions with varying concentrations (0%, 1%, 2%, 3%, and 4% m/v) for 12 hours. Following the treatment, the shoot base of each plant was subjected to freeze-drying using liquid nitrogen before being finely ground into powder for subsequent analysis of the *OsMSTs* gene expression. The primers used for RT-qPCR are listed in Supplemental Table 2.

To examine the subcellular localization of MST3-GFP and MST6-GFP following glucose and fructose treatment, transgenic seedlings expressing *OsMST3-GFP* and *OsMST6-GFP*

were cultured in 1/2 MS medium for 3-4 days under controlled conditions in a growth chamber. Subsequently, plants with roots cleaned were transferred to water and maintained in dark conditions for an additional 24 hours to minimize any potential photoperiod effects on sugar treatment. Following this, the plants were exposed to a 2% (m/v) solution of glucose, fructose, and ribose while being continuously cultured under light conditions for a duration of 4 hours. Plasmolysis was induced by immersing the cutted root in a solution containing 3% KNO₃. The fluorescence emitted by GFP was visualized using an LSM 980 confocal laser scanning microscope (Zeiss), with excitation/emission wavelengths set at 488/507 nm.

To detect the abundance of MST3-GFP and MST6-GFP protein, membrane proteins were extracted from the shoot base of transgenic rice plants expressing *OsMST3-GFP* and *OsMST6-GFP* treated with 1.5% glucose and 1% fructose for 12 hours using a Membrane and Cytosol Protein Extraction Kit (P0033, Beyotime). Protein extracts were separated on an 8% polyacrylamide gel electrophoresis (PAGE) gel and transferred onto a polyvinylidene difluoride membrane (ISEQ00010, Millipore). Anti-GFP (598, MBL, 1:3000), and goat anti-rabbit and mouse IgG-HRP (M21003, Abmart, 1:3000) were used for Western blot assays. A chemiluminescence detector (Tanon, 5200 Multi) was used for detection.

Exogenous methyl- β -cyclodextrin (MCD) treatment

To examine the effect of MCD on sugar-induced changes in the polarity of *OsMST3-GFP* and *OsMST6-GFP*, transgenic seedlings expressing *OsMST3-GFP* and *OsMST6-GFP* were pretreated with 4 mM MCD (332615, Sigma-Aldrich) for 12 hours. Following removal of residual MCD from the plant surface with ddH₂O, the plants were exposed to a 2% (m/v) solution of glucose and fructose while being continuously cultured under light conditions for a duration of 4 hours. The fluorescence emitted by GFP was visualized using a confocal laser scanning microscope (LSM 980, Zeiss), with excitation/emission wavelengths set at 488/507 nm.

To observe the effect of MCD on tiller bud elongation, NIP and *d3^{s2-215}* seedlings were cultured in 1/2 MS medium with 0, 1, 2, 4 and 8 mM MCD for 4 weeks under controlled

conditions in a growth chamber.

Determination of soluble sugar content

Measurement of sugar-related metabolites was performed at the Core Facility Platform of the Institute of Crop Sciences, Chinese Academy of Agricultural Sciences. NIP, the *d3^{ss2-215}* mutant, and other genotypes, were cultivated in fertile soil. After 14 days of growth, tiller buds, leaf blades and sheaths were harvested and finely powdered in liquid nitrogen. At least 50 mg of powder per biological replicate was then transferred to a 1 mL ethanol/water extraction buffer and subjected to ultrasonic disruption for 30 minutes. Following centrifugation, the resulting supernatant was utilized for determining the relevant soluble sugar content. Glucose, fructose, and sucrose levels were measured and quantified through Ultra Performance Liquid Chromatography (UHPLC3600, Watec, Hefei, China). Trehalose 6-phosphate (T6P) levels were determined and quantified using liquid chromatography–tandem mass spectrometry (LC-MS/MS; EXPEC5210; EXPEC Technology; Hangzhou, China).

5, 6-CFDA, FITC-glucose, and CY2-fructose feeding experiments

To examine sucrose and glucose transported into tiller buds of NIP and mutant rice plants, feeding experiments were carried out. The NIP and mutant leaf blades with 1 cm wounds that have been sanded, were placed in aqueous solutions containing 100 µg/µL 5, 6-CFDA (21879-25MG-F, Sigma-Aldrich, Zhao et al., 2022), and 1 mM FITC-glucose (Li et al., 2024) and CY2-fructose. Meanwhile, the roots are submerged in water. After incubation at room temperature and in the darkness for 12 hours, the NIP and mutant tiller buds were examined under a confocal laser scanning microscope (Zeiss LSM980).

***In situ* hybridization**

For *OsMST3*, a 198-bp fragment in the 5'-UTR was amplified and cloned into the *pSPT18* vector. For *OsMST6*, a 220-bp fragment in the 5'-UTR was amplified and cloned into the *pSPT18* vector. The fragment containing the T7 or SP6 promoter was amplified and purified for the synthesis of DIG-labeled sense and anti-sense RNA probes using the DIG Northern Starter Kit (2039672, Roche). Sample fixation and *in situ* hybridization were performed as described previously (Chun et al., 2023). The primers used are listed in Supplemental Table

2.

ChIP-Seq, ChIP-qPCR, and RNA-Seq assay

To perform the ChIP-seq assay, approximately 1.0 g tiller buds of the *ProUBI: HA-OsTB1* overexpression lines were harvested and cross-linked with 1% (v/v) formaldehyde and ground into a fine powder in liquid nitrogen. The chromatin complexes were isolated and sonicated into 0.1 to 0.5 kb fragments and incubated with Anti-HA (ab9110, Abcam, 1:500) antibody. IgG (AC005, ABclonal, 1:500) serum was used as a negative control. The immunocomplexes were collected using Protein A beads (10001D, Thermo Scientific). Finally, the immunoprecipitated DNA was recovered and dissolved in ddH₂O after washing, elution, and reversing the crosslinking.

The above-prepared DNA samples were subjected to sequencing library construction using NEBNext[®] Ultra[™] II DNA Library Prep Kit, and then sequenced with Illumina system. The sequencing data were analyzed using the standard procedure for ChIP-seq available at Galaxy|China (<https://usegalaxy.cn/>). Briefly, the sequencing reads for ChIP and IgG control were mapped to the rice reference genome (https://ftp.ensemblgenomes.ebi.ac.uk/pub/plants/release-60/fastq/oryza_sativa/dna/Oryza_sativa.IRGSP-1.0.dna.toplevel.fa.gz) using Bowtie2 tool to obtain BAM files. The uniquely mapped reads were then used for peak calling by MACS2 tool. Subsequently, the BamCoverage tool was performed to generate coverage bigwig files. For motif identification, the BED files generated by MACS2 were uploaded to and analyzed by the online MEME-ChIP tool (<https://meme-suite.org/meme/tools/meme-chip>).

The remaining precipitated DNA was utilized for ChIP-qPCR assay. The enrichment of specific regions in the *OsMST3* and *OsMST6* promoter were quantified by RT-qPCR. The primers used are listed in Supplemental Table 2.

For RNA-seq assay, total RNA was extracted from tiller buds of 2-week-old wild-type and *tb1*^{SG0312} mutant plants grown under normal conditions, with three biological replicates per group. Sequencing libraries were prepared using the NEBNext[®] Ultra[™] RNA Library Prep Kit (Illumina, San Diego, CA, USA), which included cDNA end repair, adapter ligation and PCR amplification. The sequencing service was provided by Novogene using Illumina

NovaSeq 6000 platform (Novogene, China). Following quality control, clean reads were mapped to the reference genome with HISAT2, retaining only uniquely aligned reads. Transcript abundance was quantified as FPKM using Kallisto. Differential expression analysis was then performed using DESeq2, which employs its own internal normalization method. Significant DEGs were identified using stringent thresholds ($|\log_2FC| > 1$, $P < 0.01$).

EMSA

The GST-OsTB1^{TCP} fusion construct was developed by cloning coding sequence of the N-terminal of OsTB1 into the *pCold-GST* vector. The fusion protein was expressed in the *Escherichia coli* strain BL21 (DE3, CD801-03, TransGen Biotech) and purified by Glutathione Sepharose™ 4 B (17075601, cytiva). The primers used were listed in Supplemental Table 2. The 60-bp probes containing the GGNCCC fragment and 54-bp mutated probes lacking the GGNCCC cis-element in the *OsMST3* and *OsMST6* promoter were labeled at the 5' end with biotin. For P4 site of *OsMST6* promoter, the 60-bp probes containing the GAGGAG fragment and mutated probe containing the AAAAAA fragment were used (Supplemental Table 2). These probes were annealed to form the double-stranded DNA probe before usage. The unlabeled fragments (cold probe) were used as competitors in the competing assays. The concentration of competitors is 50, 100, 200 times of that of the labeled probe, and the GST protein alone was used as a negative control. EMSA (Electrophoretic mobility shift assay) was performed using the LightShift Chemiluminescent EMSA Kit (20148, Thermo Scientific) following the manufacturer's protocol. The labeled probes and their shifted protein complexes were detected in an automatic chemiluminescence image analysis system (Tanon 5200 Multi).

Transcriptional activity assay

The CDS of *OsTB1* was cloned into the *pAN580-GFP* (35S promoter) vector to generate the effector vector (*pAN580-OsTB1-GFP*). The 5 kb promoter fragments of *OsMST3* and *OsMST6* were cloned into the *pGreenII 0800* vector to drive the *LUC* reporter gene. The primers used are listed in Supplemental Table 2. LUC activity was measured using the Dual-Luciferase® Reporter Assay System (E1910, Promega, Wisconsin).

The transient transactivation assay in *N. benthamiana* leaves was performed. The

pCAMBIA1305.1-UPFLAG-OsTB1 overexpression vector was used as the effector, and the *pCAMBIA1305.1-UPFLAG* vector was used as the control. To generate a mutant reporter vector, the Q5 Site-Directed Mutagenesis Kit (NEB, USA, no. E0554S) was used to substitute GGA/TCCC with GTA/TCCC in the *pGreenII 0800-OsMST3/OsMST6 Pro* vector. The primers are listed in Supplemental Table 2. The effector and reporter were then transformed into *A. tumefaciens* EHA105 (*pSoup*). Four different combinations of *A. tumefaciens* were infiltrated into different positions in the same leaf of *N. benthamiana*. The plants were placed in the dark for 12 h followed by 72 h in a growth chamber under normal conditions. The infiltrated leaves were sprayed with 100 mM luciferin and kept in the darkness for 10 min. LUC signals were captured with the NightSHADE LB 985 (Berthold Technologies). Quantitative analysis was performed using IndiGo software (Berthold Technologies).

GR24^{5DS} treatment

The *pCAMBIA1305.1-OsMST3-GFP* and *pCAMBIA1305.1-OsMST6-GFP* plants were cultivated in 1/2 MS medium for one week and then treated with 10 μ M GR24^{5DS} (GR24-EN1-05, New Scene Lab, Xi'an, China) for various time intervals (0, 20, 40, 120, 180, and 240 mins). The extraction and detection methods for membrane proteins refer to the methods mentioned above. For RT-qPCR, the time points for the GR24^{5DS} treatment were 0, 4, 8, 12, 16, 20, and 24 mins. The primers used for RT-qPCR are listed in Supplemental Table 2.

Exogenous 2-deoxyglucose (2-D-Glu) treatment

To assess the response of tiller buds of NIP and the *tb1^{SG0312}* mutant to exogenous application of varying concentrations of 2-D-Glucose (M5140-1g, Abmole), the plants were cultivated in 1/2 MS medium supplemented with different levels (0%, 0.001%, 0.005%, 0.01%, 0.03%, and 0.05% m/v) of 2-D-Glucose for a duration of four weeks within a controlled growth chamber. The disparity in tiller bud length between NIP and the *tb1^{SG0312}* mutant was subsequently observed and compared utilizing an OLYMPUS SZX16 stereomicroscope.

Subcellular localization

Protoplasts isolated from NIP were transformed with the *pAN580* vectors in which the coding sequence (CDS) of *GFP* was translationally fused with the CDS of *OsMST3* or *OsMST6* driven by 35S promoter (*GFP-OsMST3* and *GFP-OsMST6*). The empty *pAN580* vector was used as a control. After overnight incubation, the protoplasts were stained with the membrane dye FM4-64 (SCT127, Sigma-Aldrich). The fluorescence emitted by GFP and FM4-64 was observed using an LSM 980 confocal laser scanning microscope (Zeiss). The excitation/emission wavelengths employed for GFP were 488/507 nm, while those for FM4-64 were 580/610 nm, respectively. The primers utilized are listed in Supplemental Table 2.

Chlorophyll contents analysis

In brief, 0.1 g fresh leaves were cut into small pieces and soaked in an isolation buffer (ethanol: acetone: H₂O = 4.5: 4.5: 1) for 12 h in the dark. The supernatant was analyzed using a spectrophotometer (Varioskan LUX, Thermo, USA) at 645 nm and 663 nm.

Measurement of photosynthetic parameters

Key photosynthetic parameters of flag leaves at the tillering stage were measured using a LI-6400 portable photosynthetic system (LI-COR Biosciences, Lincoln, NE, USA). A minimum of five biological replicates were conducted for each measurement.

Scanning electron microscopy (SEM)

For scanning electron microscopy analysis, transverse section of leaf sheath was fixed in 3.5% glutaraldehyde solution and then dehydrated through a graded ethanol series. The samples were dried by the critical-point drying method and sputter-coated with platinum, and then observed using a variable-pressure scanning electron microscope (S3400N; Hitachi, Tokyo, Japan).

Starch content assay

About 0.1 g of the shoot base tissue was harvested from 7-day-old wild type and the *d3^{s2-215}* mutant seedlings and ground in liquid nitrogen. Then starch was extracted using a starch content detection kit (BC0700, Solarbio Science & Technology Co, China). Starch contents were measured using a spectrophotometer at 620 nm and calculated by the manufacturer's protocol. Each sample had three biological replicates.

Low phosphate treatment

To observe whether the expression of *OsMST3* and *OsMST6* is affected by phosphate availability, a 20 μM KH_2PO_4 solution was supplied as the phosphorus source to the roots of NIP plants grown hydroponically for 14 days. Following an additional 7-day treatment with the same solution, the plant surfaces were rinsed with ddH₂O to eliminate residual solutes. Roots and shoot bases were then harvested and immediately frozen in liquid nitrogen before being finely ground into powder for subsequent analysis of the *OsMST3* and *OsMST6* expression. Plants maintained in hydroponics for 21 days without additional treatment served as a control. The primers used for RT-qPCR are listed in Supplemental Table 2.

Statistical analysis and reproducibility

All graphs were generated by GraphPad Prism version 9 for windows, GraphPad Software, San Diego, California, USA, www.graphpad.com. Student's *t*-test and 1-way ANOVA followed by Turkey's multiple comparison test were used to determine the statistical significance among different materials. * means $P < 0.05$, ** means $P < 0.01$, *** means $P < 0.001$. All experiments were repeated independently at least twice with consistent results.

AUTHOR CONTRIBUTIONS

Conceptualization: X.L.; Formal analysis and supervision: T.W., J.Z., X.L.; Investigation: T.W., T.M., Y.S., M.M, Y.C., A.K.; Writing – original draft: T.W., J.Z.; Writing – Review & Editing: X.L., J.Z.; Funding acquisition: X.L., J.Z.

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DATA AVAILABILITY

The raw sequencing data generated during this study have been deposited in the National Center for Biotechnology Information (NCBI) BioProject database under accession number PRJNA1504566 for ChIP-seq, PRJNA1504872 for RNA-seq and the China National Genomics Data Center (<https://ngdc.cncb.ac.cn>) under the GSA accession number CRA047665 for ChIP-seq, CRA047663 for RNA-seq. The iTRAQ raw data have been submitted into ProteomeXchange database under accession number PXD081966.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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Figure 1. *OsMST3* and *OsMST6* positively regulate rice tillering.

(A) The fold change of carbohydrate transmembrane transporter in the *d3^{s2-215}* compared with WT (NIP) detected in a quantitative proteomic analysis by iTRAQ.

(B) Schematic representation of the construct for protein abundance assay in (C) and (D). Vectors of *OsMST3/OsMST6-luciferase* (FF) and *Renilla* (REN) in one transcript connected via the self-processing 2A peptide were constructed and transfected into the protoplasts of WT and *d3^{s2-215}*. The relative ratio of FF/REN was compared between WT and the *d3^{s2-215}* mutant.

(C) and (D) The relative protein abundance of *OsMST3* (C) and *OsMST6* (D) in WT and *d3^{s2-215}*.

(E) Relative transcript levels of *OsMST6* in WT (ZH11) and transgenic rice plants overexpressing *OsMST6* driven by the *CaMV35S* (35S: *OsMST6-5*) or *OsTB1* promoter (*TB1pro*: *OsMST6-26*).

(F) Gross morphology of WT (ZH11) and the transgenic rice plants overexpressing *OsMST6* at the grain-filling stage. Bar = 15 cm.

(G) Filled grains per plant of WT (ZH11) and the transgenic rice plants overexpressing *OsMST6* driven by the 35S or *OsTB1* promoter. Bars = 4 cm.

(H) and (I) Plant height (H) and tiller number (I) of rice plants shown in (F).

(J) and (K) Grain yield per plant (J) and total biomass per plant (K) of rice plants shown in (F).

(L) Gross morphology of WT (NIP) and the *osmst3/6* double mutant at the grain-filling stage. Bar = 15 cm.

(M) and (N) Plant height (M) and tiller number (N) of WT (NIP) and the *osmst3/6* double mutant shown in (L).

(O) The tiller bud lengths of WT (NIP) and the *osmst3/6* double mutant in relation to the 3rd leaf growth. Bars = 2 mm.

(P) The length of tiller buds of WT (NIP) and the *osmst3/6* double mutant shown in (O).

(Q) Tiller number of WT (NIP) and the *osmst3/6* double mutant at different weeks post germination in the field.

(R) Gross morphology of WT (NIP), $d3^{s2-215}$, and the $osmst3/6/d3^{s2-215}$ triple mutants in the field. Bar = 15 cm.

(S) and (T) Plant height (S) and tiller number (T) of WT (NIP), $d3^{s2-215}$, and the $osmst3/6/d3^{s2-215}$ triple mutants shown in (R).

Data are means $\pm$ SD. Student's t test, *** $P < 0.001$, $n = 6$ in (C); $n = 4$ in (D); $n = 3$ in (E); $n = 10$ in (H), (I), (J), (K), (M), (N), (S) and (T); $n = 6$ in (P) and (Q). The different letters above the histogram indicate significant differences ($P < 0.05$) by 1-way ANOVA followed by Turkey's multiple comparison test in (E), (H), (I), (J), (K), (M), (N), (S) and (T). n.s. means no significance.

Figure 2. Histochemical staining, protein localization, and sugars transport assay of OsMST3 and OsMST6.

(A) Histochemical staining of different tissues of the *OsMST3pro: GUS* and *OsMST6pro: GUS* transgenic rice plants. Bars = 2 mm.

(B) Tissue localization specificity of the OsMST3 and OsMST6 proteins. Immunostaining was performed with an antibody against GFP in different tissues of *OsMST3-GFP* and *OsMST6-GFP* transgenic and WT rice plants. The tissues are leaf blade, leaf sheath, tiller bud, root from left to right of (B). Red colour indicates the signal from the GFP antibody, and blue colour indicates cell walls. Mc, mesophyll cell; Ph, phloem; Xy, xylem; Vb, vascular bundle; Ep, epidermis; Ex, exodermis; En, endodermis; P/St, pericycle/stele. Bars = 50 μ m.

(C) Growth assay of the hexose uptake-deficient yeast mutant strain EBY. VW4000 transformed with the empty vector pDR196 (EV), pDR196-OsMST3 (OsMST3) or pDR196-OsMST6 (OsMST6) on medium containing different concentrations of glucose or fructose. The yeast mutant strain EBY. VW4000 can use maltose as the carbon source but not glucose and fructose. pDR196-SchXT11 was utilized as an endogenous positive control. (D) and (E) Substrate specificity and effects of the proton uncoupler carbonyl cyanide *m*-chlorophenyl-hydrazon (CCCP) on the transport activity of OsMST3 (D) and OsMST6 (E) in EBY. VW4000.

(F) 14 C-glucose transport capacity of OsMST3 and OsMST6 in the yeast mutant strain EBY.VW4000 expressing OsMST3, OsMST6 or EV.

(G) Green fluorescence protoplast images of WT, *d3^{s2-215}*, *osmst3*, *omst6*, and *osmst3/6* double mutant after incubation with fluorescein isothiocyanate (FITC)-glucose or Cyanine (CY2)-fructose. Bars = 5 μ m.

(H) Comparison of the fluorescence intensity of FITC-glucose in protoplasts with equivalent diameters (25-30 μ m) in (F).

(I) Comparison of the fluorescence intensity of Cyanine (CY2)-fructose in protoplasts with equivalent diameters (25-30 μ m) in (F).

Data are means $\pm$ SD. Student's *t* test, ****P* < 0.001. The different letters above the histogram

1816 indicate significant differences ($P < 0.05$) by 1-way ANOVA followed by Turkey's multiple
1817 comparison test in (D), (E), (H), and (I), $n = 3$ in (D), (E), and (F), $n = 5$ in (H) and (I).
1818

Figure 3. Optimal concentrations of glucose and fructose induce the expression of *OsMST3* and *OsMST6* and mediate the polarized localization of MST3-GFP and MST6-GFP fusion protein.

(A) and (B) The transcript levels of *OsMST3* and *OsMST6* in response to different concentrations of glucose (A) and fructose (B) treatment. The WT plants were exposed to glucose and fructose solutions with varying concentrations (0%, 1%, 2%, 3%, and 4% m/v) for duration of 12 hours.

(C) and (D) The protein levels of OsMST3-GFP and OsMST6-GFP in response to glucose and fructose treatment. The transgenic rice plants expressing *OsMST3-GFP* or *OsMST6-GFP* driven by their native promoters were treated with 1.5% glucose or 1% fructose for different time intervals. The total proteins were used for western blotting analysis using Anti-GFP and Anti-Actin antibody. Relative density of individual band had been quantified using Image J software (<http://image.j.nih.gov/ij/download.html>) and then normalized to that of Anti-Actin in each group.

(E) and (F) Subcellular localization of OsMST3-GFP (E) and OsMST6-GFP (F) in response to 2% glucose with and without MCD, 2% fructose with and without MCD, and 2% ribose treatment. The roots of transgenic lines expressing the *OsMST3-GFP* and *OsMST6-GFP* driven by their native promoters were treated with different monosaccharides for 4 h after 12-hour pretreatment with 4 mM MCD and then fluorescence signals were exclusively observed from the lateral surface of the cells. Plasmolysis was induced by immersing the cut roots in a solution containing 3% KNO₃. The red dotted line in the top panel of (E) and (F) represents the locally magnified region of the images, the red dotted line in the bottom panel of (E) and (F) represents the boundary of the cell after plasmolysis. Bars = 50 μ m in the top panel of (E) and (F), bars = 5 μ m in the bottom panel of (E) and (F).

(G) and (H) Comparison of the lateral/basal fluorescence ratio of OsMST3-GFP (G) and OsMST6-GFP (H) after different treatments in (E) and (F). First, define the region of interest (ROI) in ZEN software and extract the corresponding signal intensity. Next, calculate the signal intensity per unit area along both the basal and lateral directions. Finally, integrate these directional metrics to derive the fluorescence ratio of lateral/basal.

1848 (I) Suppression of tiller bud elongation of WT and $d3^{s2-215}$ by treatment with different
1849 concentrations of MCD. Bars = 1 mm.
1850 (J) The length of tiller buds of WT and $d3^{s2-215}$ shown in (I).
1851 Data are means $\pm$ SD. Student's t test, *** $P < 0.001$, $n = 3$ in (A), (B), (G), (H), and (J). The
1852 different letters above the histogram indicate significant differences ($P < 0.05$) by 1-way
1853 ANOVA followed by Turkey's multiple comparison test in (G) and (H).
1854

Figure 4. OsMST3 and OsMST6 promote the transport of glucose and fructose from leaf blades to tiller buds.

(A) This image presents tissue sites for detecting sugar content in (B)-(D) and (F)-(H).

(B)-(D) The contents of glucose, fructose and sucrose in leaf blades (B), leaf sheaths (C), and tiller buds (D) of WT and the $d3^{s2-215}$ mutant.

(E) The Tre6P/sucrose ratio in the tiller buds of WT and $d3^{s2-215}$.

(F)-(H) The contents of glucose, fructose and sucrose in leaf blades (F), leaf sheath (G), and tiller buds (H) of WT and the *osmst3/6* double mutants.

(I) and (J) Expression of two sugar-sensitive reporter genes in tiller buds of WT, $d3^{s2-215}$ and *osmst3/6* double mutant. *OsNIA1* (I) and *OsCAB2* (J) were positively and negatively regulated by cytosolic glucose content, respectively.

(K) This image presents the isotope treatment methodology employed in (L)-(P).

(L) Radioactivity (counts per minute, CPM) in leaf sheaths and tiller buds of WT and $d3^{s2-215}$ after the leaf blades were fed with ^{14}C -glucose.

(M) and (N) Radioactivity (counts per minute, CPM) in leaf sheaths (M) and tiller buds (N) of WT and the *osmst3/6* double mutants after the leaf blades were incubated with ^{14}C -glucose.

(O) and (P) Radioactivity (counts per minute, CPM) in leaf sheaths (O) and tiller buds (P) of WT, $d3^{s2-215}$ and the *osmst3/6* double mutants after the leaf blades were incubated with ^{14}C -sucrose.

Q) This image presents the methodology of feeding rice seedlings with 5,6 CFDA and FITC-glucose employed in (R) and (S).

(R) The fluorescence signals of 5,6-CFDA in tiller buds of WT, $d3^{s2-215}$ and *osmst3/6* double mutant after the leaf blades were fed with 5,6-CFDA. Bars = 100 μm .

(S) The fluorescence signals of FITC-glucose in tiller buds of WT, $d3^{s2-215}$ and *osmst3/6* double mutant after the leaf blades were fed with FITC-glucose. Bars = 100 μm .

(T) LC-MS/MS chromatograms for tiller buds of WT (left trace), $d3^{s2-215}$ (middle trace), and *osmst3 osmst6* mutant (right trace). Red arrows indicate the detection of ^{13}C -glucose.

(U)-(V) ^{13}C -metabolic flux analysis (^{13}C -MFA) after the leaf blades were incubated with ^{13}C -

glucose. The contents of ^{13}C -glucose (U) and ^{13}C -sucrose (V) in the tiller buds of WT, $d3^{s2-215}$, and *osmst3 osmst6* mutant.

Data are means $\pm$ SD. Student's *t* test, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, $n = 3$ in (B)-(E), (I), (J), (L)-(P), (U)-(V), $n = 4$ in (F)-(H). The different letters above the histogram indicate significant differences ($P < 0.05$) by 1-way ANOVA followed by Turkey's multiple comparison test in (I), (J), (M)-(P), (U)-(V).

Figure 5. The expression of *OsMST3* and *OsMST6* is directly and negatively regulated by *OsTB1*.

(A) Relative transcript levels of *OsMST3* and *OsMST6* in callus (calli) and tiller buds of WT and *d3^{s2-215}*.

(B) *In situ* hybridization of *OsMST3* and *OsMST6* in tiller buds of WT and *d3^{s2-215}*. Hybridization with the sense probe was used as a negative control. Bars = 100 μ M.

(C) Relative transcript level of *OsTB1* in tiller buds of WT and *d3^{s2-215}*.

(D) Relative transcript levels of *OsMST3* and *OsMST6* in the dormant (< 1 mm) and active tiller buds (> 1 mm) of WT and *tb1^{SG0312}*.

(E) *In situ* hybridization of *OsMST3* and *OsMST6* in tiller buds of WT and *tb1^{SG0312}*. Bars = 100 μ M. Hybridization with the sense probe was used as a negative control.

(F) Transcript levels of *OsMST3*, *OsMST6* and *OsTB1* in WT and transgenic rice plants overexpressing *HA-OsTB1*.

(G) and (H) Two replicates of the *OsTB1* binding profile in the promoters of *OsMST3* (G) and *OsMST6* (H) as revealed by a ChIP-sequence assay using an anti-HA antibody and the *ProUBI: HA-OsTB1* transgenic rice plants. Sequencing profile of the DNA collected with the IgG serum was shown as the background control. In *OsMST3*, P1 refers to the *OsTB1*-binding motif GGNCCC, whereas P2 refers to a region that does not contain the GGNCCC motif and is located outside of the *OsTB1*-binding peak. In *OsMST6*, P1, P2, P3 refer to the *OsTB1*-binding motif GGNCCC; P4 is located in the *OsTB1*-binding peak containing the GAGGAG motif; P5 refers to a region that does not contain the GGNCCC motif and is located outside of the *OsTB1*-binding peak.

(I) and (J) Gel retardation assay using GST-*OsTB1* and the labeled probes derived from the *OsMST3* (I) and *OsMST6* (J) promoters. The GST tag alone and mutant probe was used as negative controls. Unlabeled probe in 50, 100 and 200 folds excess was used as the competitor (Cold probe).

(K) and (L) ChIP-qPCR assay showed that *OsTB1* binds to the promoters of *OsMST3* (K) and *OsMST6* (L). The shoot base tissues of the *ProUBI: HA-OsTB1* overexpressing seedling were used for ChIP assay using the Anti-HA antibody. ChIP with the IgG serum

was used as a negative control. *OsMST3 P2* and *OsMST6 P5* (which lack an OsTB1-binding site) and the upstream region of *Ubiquitin* were used as negative controls.

(M) Schematic representation of the reporter and effector constructs in the transient transcription activity assay of OsTB1 on the promoters of *OsMST3* and *OsMST6*. OsTB1 driven by 35S promoter was used in (M) and by *UBIpro* in (O)-(R).

(N) Relative luciferase activities driven by the *OsMST3* or *OsMST6* promoter or by the mutated promoters in which the OsTB1-binding sites in *OsMST3* or *OsMST6* promoter were changed from GGA/TCCC to GTA/TCCC when co-transformed with OsTB1 and the empty effector into the rice protoplasts.

(O)-(R) Relative luciferase activities driven by the *OsMST3* (O and P) or *OsMST6* (Q and R) promoter or by the mutated promoters in which cis-elements in *OsMST3* or *OsMST6* promoters were changed from GGA/TCCC to GTA/TCCC when co-transformed with OsTB1 and the empty effector in tobacco leaves.

Data are means $\pm$ SD. Student's *t* test, **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *n* = 3 in (A), (F), (K), (L), (N), (P) and (R). *n* = 4 in (C) and (D). n.s. means no significance.

Figure 6. Strigolactones suppress the transcription, protein accumulation, and polarized localization of OsMST3 and OsMST6 induced by sugars.

(A)-(C) The transcript levels of *OsTB1* (A), *OsMST3* (B) and *OsMST6* (C) in response to GR24^{5DS} treatment. The 7-day-old WT, *d3^{s2-215}* and *tb1^{SG0312}* seedlings were treated with 10 μ M GR24^{5DS} for various time intervals. The plant treatment solution was renewed daily. The shoot base tissues were used for RT-qPCR.

(D) Protein levels of OsMST3-GFP and OsMST6-GFP in response to GR24^{5DS} treatment. The transgenic rice plants expressing the *OsMST3-GFP* or *OsMST6-GFP* driven by its native promoter in the WT, *d3^{s2-215}* and *tb1^{SG0312}* background were treated with 10 μ M GR24^{5DS} for various time intervals. The shoot base tissues were used for Western blotting using the Anti-GFP and Anti-HPT antibody. Relative density of individual band had been quantified using Image J software (<http://image.j.nih.gov/ij/download.html>) and then were normalized to that of Anti-HPT of 0-minute GR24^{5DS} treatment under WT background in each group.

(E) and (F) Subcellular localization of OsMST3-GFP (E) and OsMST6-GFP (F) in response to 2% glucose and 2% fructose with and without 10 μ M GR24^{5DS} treatment. The roots of transgenic rice plants expressing the *OsMST3-GFP* and *OsMST6-GFP* driven by their native promoters in WT background were treated with different monosaccharides with and without 10 μ M GR24^{5DS} for 4 hours. The red dotted line in (E) and (F) represents the locally magnified region of the images. Bars= 50 μ m in the two left panels of (E) and (F), bars= 5 μ m in the right panel of (E) and (F).

(G) Comparison of the lateral/basal fluorescence ratio of OsMST3-GFP and OsMST6-GFP after different treatments in (E) and (F). First, define the region of interest (ROI) in ZEN software and extract the corresponding signal intensity. Next, calculate the signal intensity per unit area along both the basal and lateral directions. Finally, integrate these directional metrics to derive the fluorescence ratio of lateral/basal.

Data are means $\pm$ SD. Student's *t* test, ****P* < 0.001, *n* = 3 in (A)-(C), and (G).

Figure 7. The high-tillering phenotype of the *tb1^{SG0312}* mutant can be rescued by simultaneous disruption of *OsMST3* and *OsMST6*.

(A)-(C) The levels of glucose, fructose and sucrose in leaf blades (A), leaf sheaths (B) and tiller buds (C) of WT and the *tb1^{SG0312}* mutant.

(D) Radioactivity (counts per minute, CPM) in leaf sheaths and tiller buds of WT and the *tb1^{SG0312}* mutant after the leaf blades were fed with ¹⁴C-glucose or ¹⁴C-sucrose.

(E) This image presents the methodology of feeding rice seedlings with FITC-glucose and 5,6 CFDA employed in (F) and (G), respectively. The red dashed lines indicate the positions of the tiller buds.

(F) and (G) The fluorescence signals of FITC-glucose (F) and 5,6-CFDA (G) in the tiller buds of WT and the *tb1^{SG0312}* mutant after the leaf blades were fed with FITC-glucose and 5,6-CFDA. Bar = 100 μ m.

(H) Suppression of tiller bud elongation of WT and the *tb1^{SG0312}* mutant in the presence of different concentration of 2-D-glucose. The WT and *tb1^{SG0312}* mutant seedlings were cultivated in 1/2 MS medium supplemented with different levels (0%, 0.001%, 0.005%, 0.01%, 0.03%, and 0.05% m/v) of 2-D-Glucose for a duration of four weeks. Bars = 1 mm.

(I) The length of tiller buds of WT and the *tb1^{SG0312}* mutant shown in (H)

(J) Suppression of tiller bud elongation of WT and the *tb1^{SG0312}* mutant in the presence of different concentrations of CCCP. The WT and *tb1^{SG0312}* mutant seedlings were cultivated in 1/2 MS medium supplemented with different levels (0, 10, 25, and 50 μ m) of CCCP for a duration of four weeks. Bars = 2 mm.

(K) The length of tiller buds of WT and the *tb1^{SG0312}* mutant shown in (J).

(L) Gross morphology of WT, the *tb1^{SG0312}*, *osmst3/tb1^{SG0312}*, *osmst6/tb1^{SG0312}*, *osmst3/6/tb1^{SG0312}*, *osmst3/6* mutants in grain-filling stage. Bar = 15 cm.

(M) and (N) Tiller number (M) and plant height (N) of WT, the *tb1^{SG0312}*, *osmst3/tb1^{SG0312}*, *osmst6/tb1^{SG0312}*, *osmst3/6/tb1^{SG0312}*, *osmst3/6* mutants.

(O) The working model of *OsMST3* and *OsMST6* in regulating rice tillering.

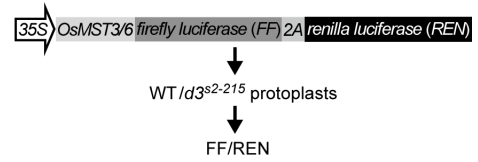
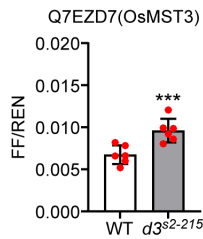
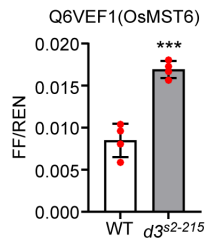
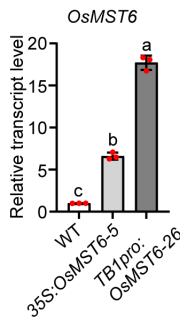
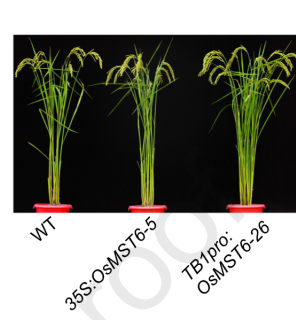
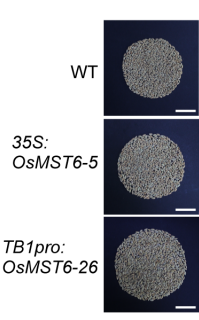
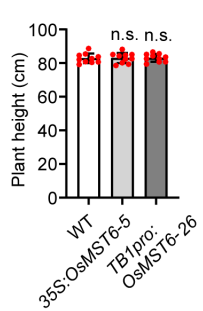
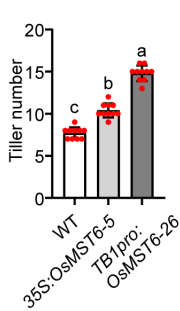
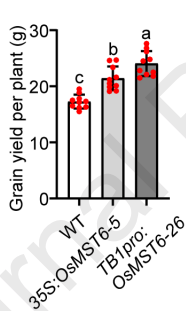
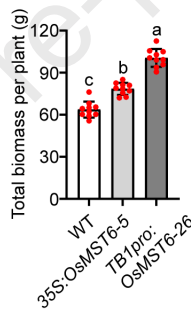
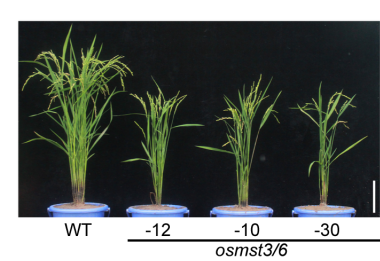
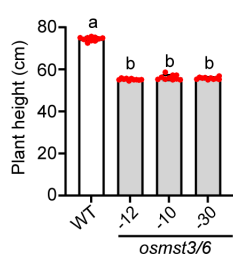
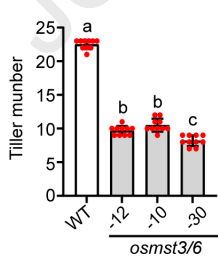
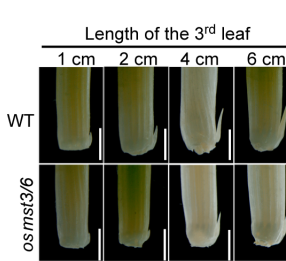
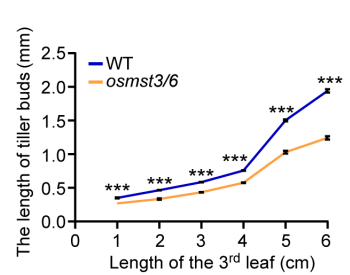
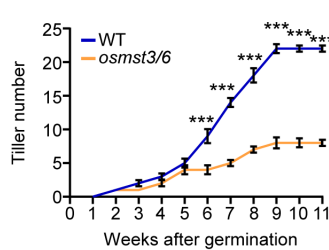
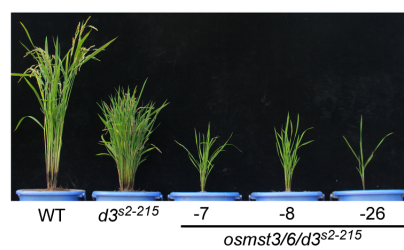
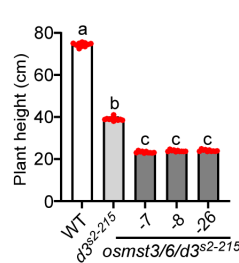
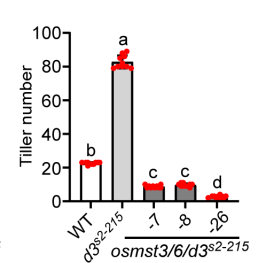
OsMST3 and *OsMST6* function as key monosaccharide influx transporters that mediate the phloem-dependent translocation of photosynthetically derived monosaccharides-from

source tissue (leaf blade) through leaf sheath-to sink tissues (tiller bud and root). *OsMST3* and *OsMST6* exhibit a dynamic localization pattern dependent on intracellular monosaccharide levels: *OsMST3* and *OsMST6* are uniformly distributed on the plasma membrane under normal conditions but become polarly localized to the apical and basal regions of cells when monosaccharide levels are high. Sugars antagonize the function of SLs. Under high sugar conditions, this inhibition of SLs signaling attenuates the expression of *D3* and *OsTB1*, which in turn alleviates the *OsTB1*-mediated repression of *OsMST3* and *OsMST6*. Consequently, glucose and fructose import is enhanced from the apoplast into cells, promoting tillering. Conversely, normal intracellular sugar levels represent more active SLs signaling, which upregulates *OsTB1* expression and consequently strengthens the suppression of *OsMST3* and *OsMST6*, thereby restricting sugar acquisition by tiller buds and inhibiting tillering.

Data are means $\pm$ SD. Student's *t* test, **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *n* = 3 in (A)-(D), *n* = 6 in (I), *n* = 10 in (K), (M) and (N). The different letters above the histogram in (M) and (N) indicate significant differences (*P* < 0.05) by 1-way ANOVA followed by Turkey's multiple comparison test.

AComparison of carbohydrate transmembrane transporters (*d3^{s2-215}*/NIP)

Accession	Fold change	Description
Q6VEF1	1.40	Monosaccharide transporter 6 (MST6)
Q7EZD7	1.32	Monosaccharide transporter 3 (MST3)
Q6K4V2	1.32	Bidirectional sugar transporter SWEET4
Q60EC2	1.23	Bidirectional sugar transporter SWEET1b
Q69VR7	1.22	Glucose-6-phosphate/phosphate translocator-like protein

B**C****D****E****F****G****H****I****J****K****L****M****N****O****P****Q****R****S****T**

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