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OsSPL10, a SBP-Box Gene, Plays a Dual Role in Salt Tolerance and Trichome Formation in Rice (*Oryza sativa* L.)

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ABSTRACT Salinity is one of the major abiotic stress factors limiting rice production. Glabrousness is a trait of agronomic importance in rice (*Oryza sativa* L.). We previously found a single-gene recessive mutant *sst*, which displayed increased salt tolerance and glabrous leaf and glume without trichomes, and identified an SBP-box gene *OsSPL10* as the candidate of the *SST* gene. In this study, *OsSPL10*-knockout and *OsSPL10*-overexpression mutants were created to check the function of the gene. The knockout mutants exhibited enhanced salt tolerance and glabrous leaves and glumes as expected, while the overexpression mutants showed opposite phenotypes, in which both salt sensitivity and trichome density on leaf and glume were increased. These results clearly confirmed that *OsSPL10* is *SST*, and suggested that *OsSPL10* controls the initiation rather than the elongation of trichomes. In addition, expression analysis indicated that *OsSPL10* was preferentially expressed in young panicle and stem, and protein OsSPL10 was localized in nucleus. Taken together, *OsSPL10* negatively controls salt tolerance but positively controls trichome formation in rice.

Rice is sensitive to salt stress, which suppresses rice growth and development, causing severe yield loss. Salinity tolerance is a quantitative trait controlled by multiple genes in rice (Ren *et al.* 2005). Salt tolerance mechanism is complex in rice, involving many pathways. Certain apoplastic proteins are involved in the initial phase of salt stress response (Zhang *et al.* 2009). Some receptor-like kinases (RLKs) mediate salt sensitivity or improve salt tolerance by regulating ethylene homeostasis or H_2O_2 homeostasis (Li *et al.* 2014; Zhou *et al.* 2018). G-protein and small G-protein also play roles in salt-induced cellular senescence and other salt sensitivity (Urano *et al.* 2014; Zang *et al.* 2010). Calcineurin B-like protein S (CBLs), CBL-interacting protein kinases (CIPKs), and calcium-dependent protein kinases (CPKs) function in salt signal transduction (Martínez-Atienza *et al.* 2007;

Campo *et al.* 2014). Some cation transporters of plasma membrane, such as *OsSOS1*, *OsHKT1;5* (*SKC1*), *OsKAT1*, *OsHAK1*, and *OsMGT1*, which are sodium, potassium and magnesium transporter (or channel), respectively, are related to salt tolerance (Martínez-Atienza *et al.* 2007; Ren *et al.* 2005; Obata *et al.* 2007; Chen *et al.* 2015; Chen *et al.* 2017). Many different transcription factors are involved in salinity tolerance or sensitivity, including NAC, OsbZIP23, DST, OsWRKY45-2, DREB1B, OsMYB2, SERF1 and so on (Hu *et al.* 2006; Xiang *et al.* 2008; Huang *et al.* 2009; Tao *et al.* 2011; Datta *et al.* 2012; Yang *et al.* 2012; Schmidt *et al.* 2013). In addition, epigenetics is also involved in salt tolerance in rice (Yuan *et al.* 2015; Srivastava *et al.* 2016; Wang *et al.* 2017).

Glabrous rice varieties have glabrous leaves and glumes without trichomes. In most terrestrial plants, trichomes are specialized structures, which originate from the above-ground epidermal tissues and develop into hair-like projections extending from the epidermal surfaces through growth, differentiation or cell division (Johnson 1975). As trichomes can lead to the generation of dust during harvesting and grain manipulating processes in rice production, glabrous leaves and glumes are a desirable characteristic. Only two glabrous genes have been cloned in rice so far, namely, *OsWOX3B* (*DEP*, *NUDA/GL-1*, *GLR1*) and *OsPLT2* (*HL6*). The former is a *WUSCHEL*-like homeobox gene (Angeles-Shim *et al.* 2012; Zhang *et al.* 2012;
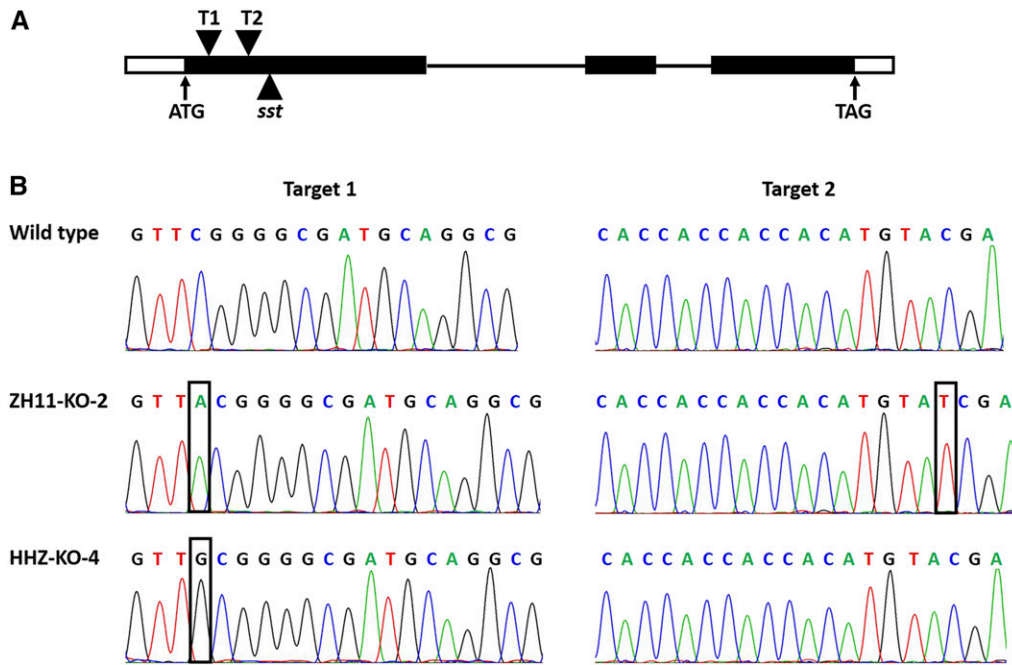
Figure 1 CRISPR/Cas9 editing of *OsSPL10* in ZH11 and HHZ. A The gene framework of *OsSPL10* showing the coding region (filled boxes), UTRs (blank boxes), introns (horizontal lines), positions of *sst* mutation site (filled triangle) and CRISPR/Cas9 target sites (reversed filled triangles). T1, target site 1; T2, target site 2. B The target-site sequences of wild type, ZH11-KO-2 and HHZ-KO-4. The letters in boxes represent inserted nucleotides.

Li *et al.* 2012), while the latter encodes an AP2/ERF transcription factor, which physically interacts with OsWOX3B (Sun *et al.* 2017).

SQUAMOSA Promoter-Binding Protein (SBP) and SBP-Like (SPL) proteins are putative transcription factors, which have a plant-specific SBP domain consisting of 76 amino acids in length (Cardon *et al.* 1997). *SBP* genes (*SBP1* and *SBP2*) were first isolated from *Antirrhinum majus* and found to control early flower development by regulating the MADS-box gene *SQUAMOSA* (Klein *et al.* 1996). Then, *SPL3* involved in floral transition was isolated from *Arabidopsis thaliana* (Cardon *et al.* 1997) and LG1 with SBP domain was found to be required for induction of ligules and auricles during maize leaf organogenesis (Moreno *et al.* 1997). *SPL* gene family is not large, with only 17 members in *Arabidopsis* and 19 in rice (Xie *et al.* 2006). *SPL* genes have been shown to play numerous important roles in plant growth and development, including trichome development and fertility (Unte *et al.* 2003; Xing *et al.* 2010), lateral root development (Yu *et al.* 2015), fruit ripening (Manning *et al.* 2006), plastochron length, flowering pathway and organ size (Wang *et al.* 2008, 2009; He *et al.* 2018), yield (Chuck *et al.* 2014; Si *et al.* 2016; Zhang *et al.* 2017), copper homeostasis (Yamasaki *et al.* 2009; Yan *et al.* 2017), and so on. Some *SPL* genes are related to abiotic stress tolerance. SPL1 and SPL12 confer thermotolerance at reproductive stage in *Arabidopsis* (Chao *et al.* 2017). Down-regulation of *MsSPL8* leads to enhanced salt and drought tolerance in alfalfa (Gou *et al.* 2018).

In rice, it has been found that *OsSPL* genes control a large range of processes underlying plant growth and development (Wang and Zhang 2017). For example, *OsSPL8* (*OsLG1*) controls ligule development and inflorescence architecture (Lee *et al.* 2007; Ishii *et al.* 2013; Zhu *et al.* 2013). *OsSPL13* (*GLW7*) controls grain size (Si *et al.* 2016). *OsSPL14* (*IPA1*, *WFP*) affects tiller number and panicle branching (Jiao *et al.* 2010; Miura *et al.* 2010) and promotes immunity (Wang *et al.* 2018). *OsSPL16* controls grain size, shape and quality (Wang *et al.* 2012) and plays a role in panicle cell death during ER stress (Wang *et al.* 2018). *OsSPL18* controls grain weight and grain number in rice (Yuan *et al.* 2019).

We previously obtained a rice mutant *sst* showing salt tolerance and glabrous leaves and glumes from a restorer line R401. We found that *sst* was controlled by a recessive gene, which was likely to result

from a deletion of one nucleotide in *OsSPL10* (LOC_Os06g44860, Os06g0659100), an SBP-box gene (Wang *et al.* 2013; Lan *et al.* 2015; Song *et al.* 2016). In this study, we confirmed the function of *OsSPL10* as the candidate of *SST* through gene knockout and overexpression, investigated the expression pattern of *OsSPL10*, and analyzed the subcellular localization of OsSPL10, aiming to lay a foundation for deep studies of the molecular mechanism of *OsSPL10* function in salt tolerance and trichome development.

MATERIALS AND METHODS

Plant materials
The following plant materials were used or created in this study: *indica* rice cultivars R401 and Huanghuazhan (HHZ); *japonica* rice cultivars Nipponbare and Zhonghua 11 (ZH11); the salt-tolerant and glabrous-leaf mutant *sst* obtained from R401 by radiation mutagenesis (Wang *et al.* 2013; Lan *et al.* 2015; Song *et al.* 2016); *OsSPL10*-knockout mutant plants from HHZ and ZH11; and *OsSPL10*-overexpression plants from ZH11. All rice plants were grown in plastic trays with paddy soil under a long day condition (approximately 14 h light/day) in the growth chamber (with cool-white light 300 μmol m^2 s^{-1}).

Knockout of OsSPL10
The CRISPR/Cas9 editing system were used to knock out *OsSPL10* in ZH11 and HHZ. Two target sites (5′-GTTCGGGGCGATGCAGGCG-3′ and 5′-CACCACCACCACATGTACGA-3′) upstream to the *sst* mutation site in the first exon of *OsSPL10* were selected and isolated according to the rules of low off-target score and high sgRNA score (http://cbi.hzau.edu.cn/crispr/), and then inserted into the VK005-01 binary vector containing the rice U6 promoter (Viewsolid Biotechnology Company of Beijing). The construct was introduced into ZH11 and HHZ using the stable transformation method (Hiei and Komari 2008). To examine mutations occurred in positive transgenic (T$_0$) plants, a 400-bp genomic DNA fragment harboring the two target sites was amplified from them by PCR using primers 5′-AGCTCCACTTCGTTGGAAGCCA-3′ and 5′-GCGACACGCTGTAG-CACGGT-3′ and then sequenced. Homozygous mutants obtained in
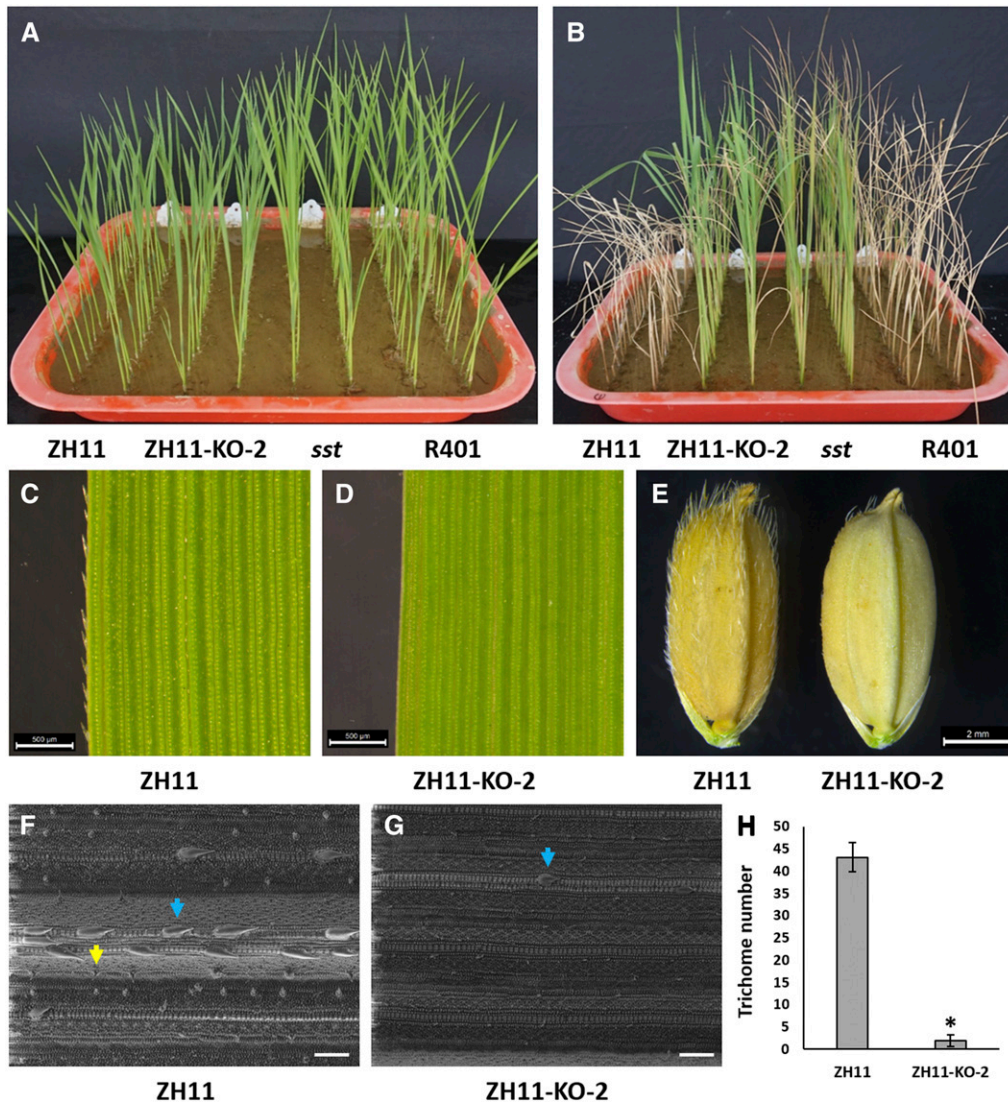
Figure 2 Effects of *OsSPL10* mutation on salt tolerance and trichome development in ZH11. A Ten-day seedlings of ZH11, ZH11-KO-2, *sst* and R401 without salt stress. B Seedlings of ZH11, ZH11-KO-2, *sst* and R401 after salt stress treatment. C–D Mature leaves of ZH11 (C) and ZH11-KO-2 (D) at heading stage. E Seeds of ZH11 and ZH11-KO-2. F–G SEM images of ZH11 (F) and ZH11-KO-2 (G) leaves at heading stage. H Density (number/vision field) of trichomes on leaf in ZH11 and ZH11-KO-2 at heading stage. For each line, the density was calculated based on 6 fields of vision in the SEM images of two penultimate leaves at heading stage. The blue and yellow arrows indicate a macrohair and a microhair, respectively. The asterisks represent significant difference from control ZH11 at $P <$ 0.05. Scale bar = 500 μm (C and D), 2 mm (E) and 100 μm (F and G).

T_1 generation were phenotyped for salt tolerance and trichomes on leaves and glumes.

Overexpression of OsSPL10

Total RNA was extracted from the young panicles (<5 cm) of Nipponbare and converted into cDNA by reverse transcription. RNA extraction was performed using TRIzol reagent (Invitrogen, USA). PrimeScriptTM RT reagent Kit (Takara, Japan) was used to synthesize the first strand of cDNA with OligodT primer. The 1.2-kb coding sequence of *OsSPL10* was amplified from the cDNA by PCR using primers 5′-ATGATGAGCGGTAGGATGAA-3′ and 5′-CTACATGAAGTCGACCTCGA-3′, and then inserted into the pCXUN vector containing the maize *ubiquitin* promoter. The construct was introduced into ZH11 using the stable transformation method (Hiei and Komari 2008). The positive transgenic plants overexpressing *OsSPL10* were phenotyped for salt tolerance and trichomes on leaves and glumes.

Measurement of salt tolerance

Rice seeds were sown on paddy soil in plastic trays (36×28×4.5 cm³) after pregermination and allowed to grow at 26° under a photoperiod of 14 h

light/10 h dark in a growth chamber. Salt stress treatment began from late two-leaf stage. During the treatment, 200 mL of either NaCl solution (150 mM) or fresh water was added into each tray every day. The treatment procedure for the *OsSPL10*-knockout seedlings was: 7 d NaCl → 3 d water → 7 d NaCl → 3 d water, while that for the *OsSPL10*-overexpression seedlings was: 7 d NaCl → 3 d water → 4 d NaCl. The survival rate of seedlings was investigated at the end of the treatment.

Measurement of leaf trichome density

The penultimate leaves of individual plants were collected at tillering stage or heading stage. The adaxial surface of the middle part of each leaf was observed with scanning electron microscopy (SEM). The number of trichomes within a field of vision was counted, and three fields of vision were investigated on each leaf.

Quantitative RT-PCR of OsSPL10

Total RNA was extracted from seedlings as well as flag leaf blades, flag leaf sheaths, mature (second) leaf blades, mature leaf sheaths, stems, pre-emergence inflorescences and young panicles at the booting stage. RNA extraction and cDNA synthesis were conducted
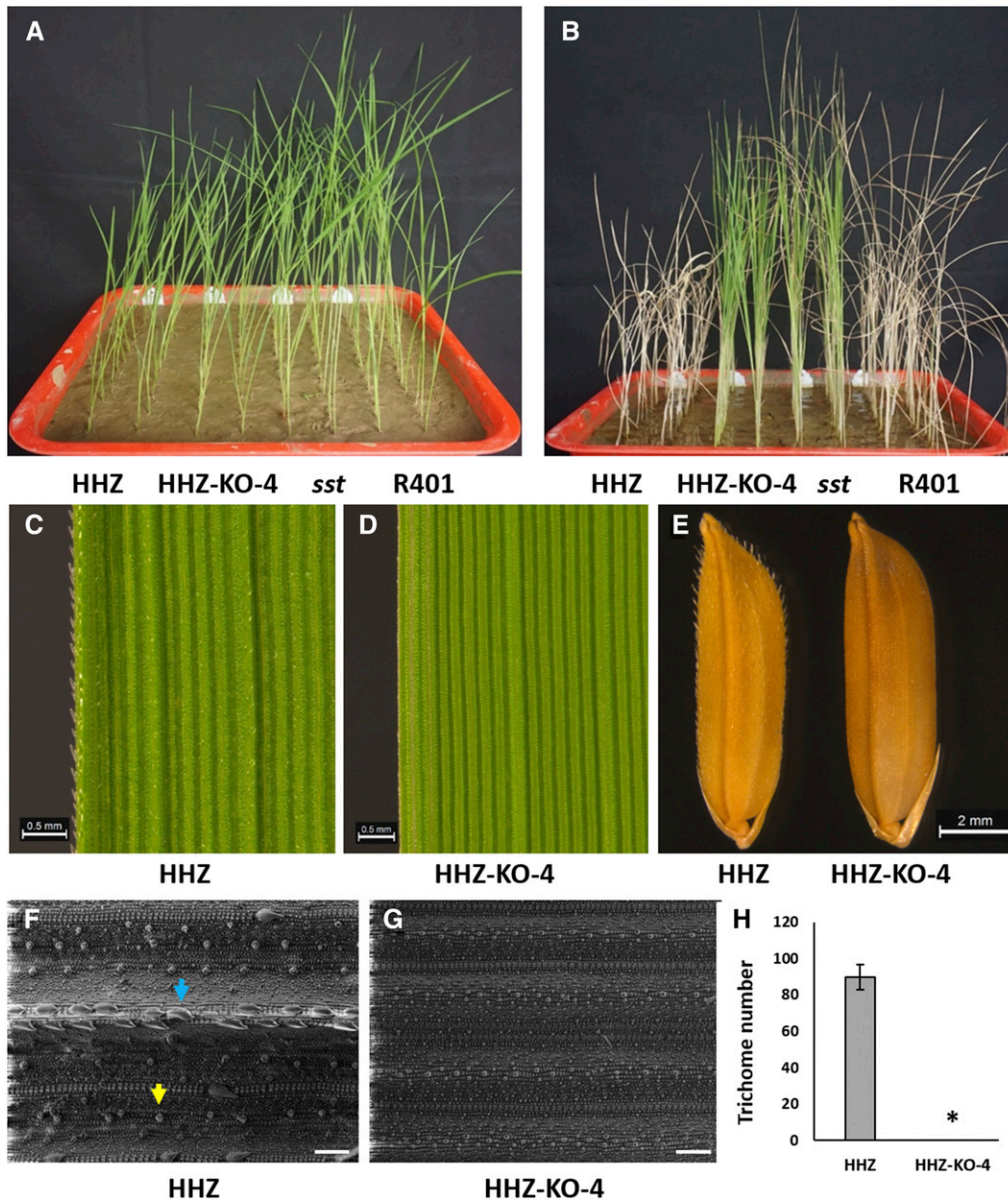
Figure 3 Effects of *OsSPL10* mutation on salt tolerance and trichome development in HHZ. A Twelve-day seedlings of HHZ, HHZ-KO-4, *sst* and R401 without salt stress. B Seedlings of HHZ, HHZ-KO-4, *sst* and R401 after salt stress treatment. C–D Mature leaves of HHZ (C) and HHZ-KO-4 (D) at heading stage. E Seeds of HHZ and HHZ-KO-4. F–G SEM images of HHZ (F) and HHZ-KO-4 (G) leaves at heading stage. H Density (number/vision field) of trichomes on leaf in HHZ and HHZ-KO-4 at heading stage. For each line, the density was calculated based on 6 fields of vision in the SEM images of two penultimate leaves at heading stage. The blue and yellow arrows indicate a macrohair and a microhair, respectively. The asterisks represent significant difference from control HHZ at $P < 0.05$. Scale bar = 500 μm (C and D), 2 mm (E) and 100 μm (F and G).

using the same methods as described above. The qRT-PCR was performed using SYBR Premix Ex TaqTM (Tli RNaseH Plus) (Takara, Japan) on a Prism 7500 96 Real-time PCR System (ABI, USA). The primers for *OsSPL10* were 5′-ACAACGACAACAGC-CACAACAA-3′ and 5′-ACACGAACACATGGTAGGATCGA-3′. The *actin* mRNA level was used as internal reference, for which the primers were 5′-AGTGCGACGTGGATATTAGG-3′ and 5′-TGGCTTAGCATTCTTGGGT-3′. Three independent biological replicates were analyzed by qRT-PCR in triplicate. The changes in gene expression were calculated using the $2^{-\Delta\Delta Ct}$ method.

Subcellular localization of OsSPL10

GFP cDNA was fused to the C-terminus of *OsSPL10* cDNA (without terminator) in the pMDC202 vector through BP and LR recombination (Lambda integrase/excisionase; Elpis-Biotech), resulting in the *35S::OsSPL10-GFP* plasmid. The fusion construct as well as the control (empty pMDC202 vector; *35S::GFP*) were infiltrated into tobacco (*Nicotiana benthamiana*) leaves using a needleless syringe.

For agroinfiltration, agrobacteria were grown overnight in Luria–Bertani containing the appropriate antibiotics. The agrobacteria were collected by centrifugation and then re-suspended in 10 mM $MgCl_2$ containing 100 mM acetosyringone. After incubated for a minimum of 2 h at room temperature, the culture was diluted to an OD600 of 0.2. Tobacco plants were agroinfiltrated with appropriate agrobacterial cultures, and the agroinfiltrated plants were maintained under normal growth conditions for 12 to 72 h. The DAPI (4′, 6-diamidino-2-phenylindole) was used to confirm nucleus. The tobacco cell layers were examined with a confocal laser scanning microscope. (TCS SP8, Leica, Germany).Data availabilityAll data generated or analyzed during this study are included in this published article.

RESULTS

Knockout of OsSPL10 enhances salt tolerance but inhibits trichome development

In the experiment of CRISPR/Cas9 editing of *OsSPL10*, 28 and 20 positive transgenic (T_0) plants were obtained from ZH11 and HHZ,
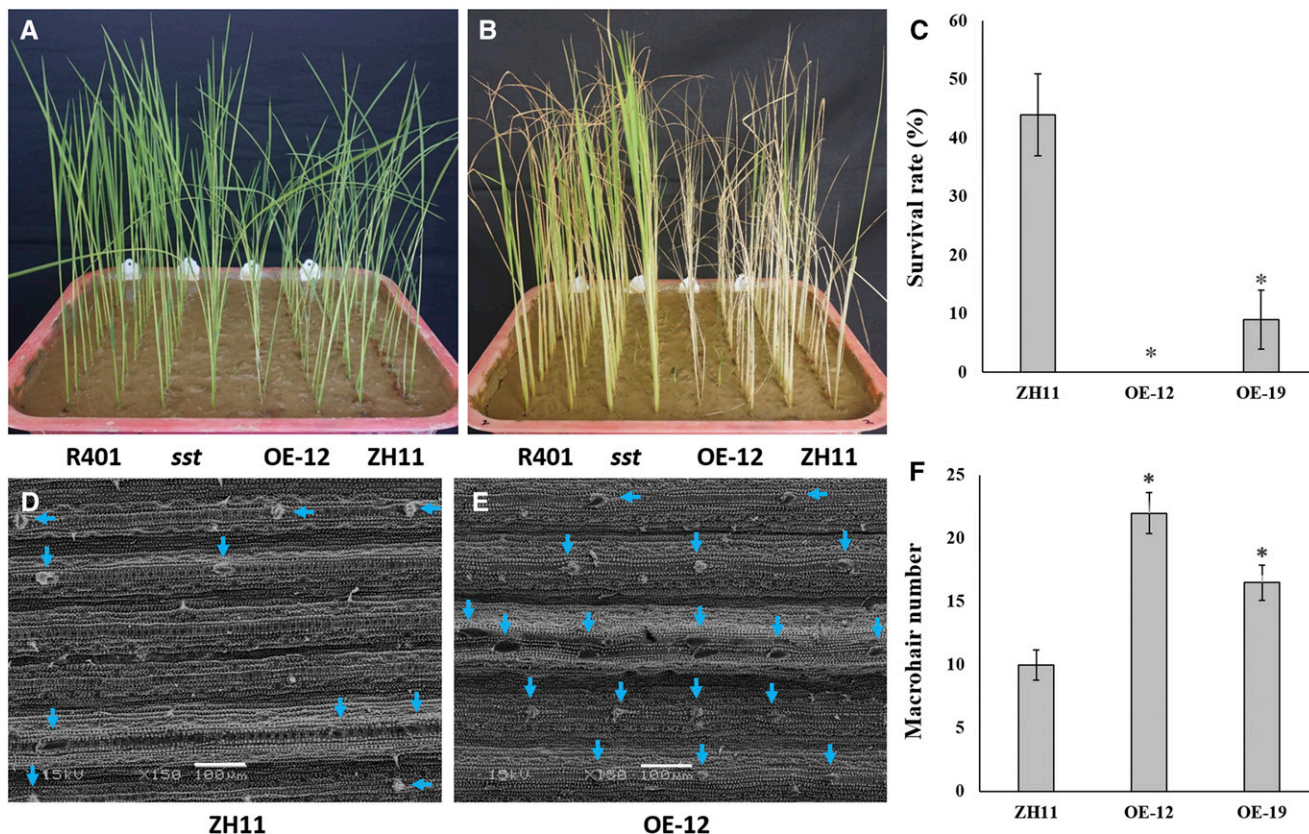
Figure 4 Effects of *OsSPL10* overexpression on salt tolerance and trichome development in ZH11. A Ten-day seedlings of R401, *sst*, ZH11-OE-12 and ZH11 without salt stress. B Seedlings of R401, *sst*, ZH11-OE-12 and ZH11 after salt stress treatment. C Survival rates of ZH11, ZH11-OE-12 and ZH11-OE-19 after salt stress treatment. For each line, the survival rate was calculated based on three replicates (trays) with 22 seedlings in each replicate. D–E SEM images of ZH11 (D) and ZH11-OE-12 (E) leaves at tillering stage. F Density (number/vision field) of macrohairs on leaf in ZH11, ZH11-OE-12 and ZH11-OE-19 at tillering stage. For each line, the density was calculated based on 6 fields of vision in the SEM images of two penultimate leaves at tillering stage. The blue arrows indicate macrohairs. The asterisks represent significant difference from control ZH11 at $P < 0.05$. Scale bar = 100 μm.

respectively. Among the T_0 plants, 25 (89.3%) from ZH11 and 15 (75%) from HHZ had mutations at either or both of the target sites, with 7 from ZH11 and 3 from HHZ being homozygous with the mutant allele. Protein sequence analysis predicted that all of the mutations resulted in a premature stop codon. Therefore, the mutants obtained were all *OsSPL10*-knockout mutants (denoted as ZH11-KO and HHZ-KO, respectively). We chose two mutants, one from ZH11 and HHZ each, named ZH11-KO-2 and HHZ-KO-4, respectively (Figure 1), to investigate the effects of *OsSPL10* mutation on salt tolerance and trichome development.

Both ZH11-KO-2 and HHZ-KO-4 showed significantly higher tolerance to salt stress than their corresponding wild types in the experiment. While all of the ZH11 and HHZ seedlings died (survival rate = 0%) at the end of salt treatment, the ZH11-KO-2 and HHZ-KO-4 seedlings still all kept alive (survival rate = 100%), similar to the case of *sst vs.* R401 (Figure 2A, B and 3A, B). Meanwhile, both ZH11-KO-2 and HHZ-KO-4 displayed glabrous leaves and glumes without or with very few trichomes as expected (Figure 2C-E and 3C-E). SEM observation showed that the trichome density on leaf surface (number of trichomes per vision) at heading stage was ~43 in ZH11 (Figure 2F and H) and ~88 in HHZ (Figure 3F and H), respectively, whereas the density was only ~2 in ZH11-KO-2 (Figure 2G and H) and nearly 0 in HHZ-KO-4 (Figure 3G and H), respectively. These results indicated that loss of *OsSPL10* function can result in higher salt tolerance as well

as glabrous leaves and glumes, confirming that *OsSPL10* is *SST*. In addition, the ZH11-KO-2, HHZ-KO-4 and *sst* seedlings all appeared to be a little taller than those of their corresponding wild types (Figure 2A and 3A), suggesting that loss of *OsSPL10* function has an effect of promoting plant growth.

Overexpression of OsSPL10 reduces salt tolerance but promotes trichome development

A total of 22 positive transgenic (T_0) plants overexpressing *OsSPL10* were acquired, among which plant ZH11-OE-12 showed the highest level of *OsSPL10* expression, followed by plant ZH11-OE-19. We examined the phenotypes of the stably-inherited homozygous progeny lines of ZH11-OE-12 and ZH11-OE-19. Contrary to the *OsSPL10*-knocked-out seedlings, the *OsSPL10*-overexpressed seedlings were a little shorter (Figure 4A) but more sensitive to salt stress than the wild-type seedlings (Figure 4B). At the end of salt treatment there were still ~44% ZH11 seedlings alive, while the ZH11-OE-12 seedlings all died and only ~9% ZH11-OE-19 seedlings survived (Figure 4C). Since *OsSPL10* expression was stronger in ZH11-OE-12 than in ZH11-OE-19, the result suggested that higher *OsSPL10* expression level would lead to higher sensitivity to salt. In addition, SEM observation at tillering stage indicated that the *OsSPL10*-overexpressed plants had higher density of macrohairs on leaf (Figure 4E) than wild type (Figure 4D), and the
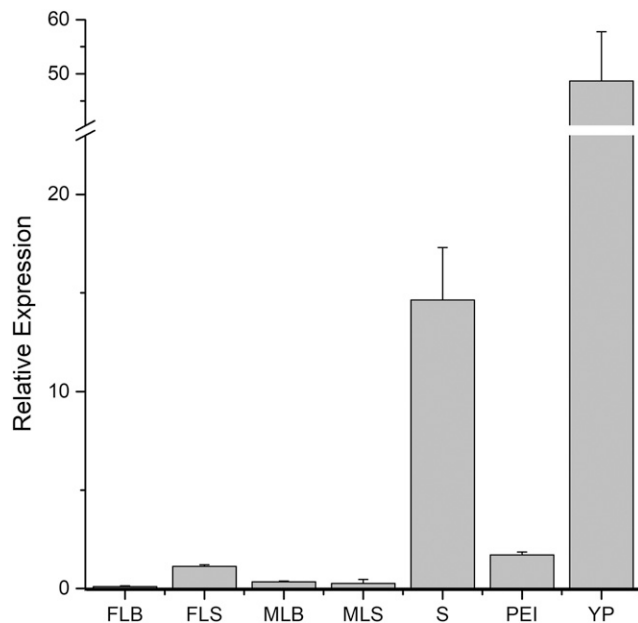
Figure 5 The expression pattern of *OsSPL10* at the booting stage. FLB, flag leaf blade; FLS, flag leaf sheath; MLB, mature leaf blade; MLS, mature leaf sheath; S, stem; PEI, pre-emergence inflorescence; YP, young panicle

density also appeared to be positively proportional to the level of *OsSPL10* expression (Figure 4F). These results indicated that *OsSPL10* overexpression had exactly the opposite effect to that of *OsSPL10* knockout, and the effect increased with the increase of *OsSPL10* expression. This validated the function of *OsSPL10* known from its loss-of-function mutants, further confirming that *OsSPL10* is *SST*.

OsSPL10 is preferentially expressed in young panicle and stem

To examine the potential tissue specificity of *OsSPL10*, we used qRT-PCR to analyze the expression pattern of *OsSPL10* at the booting stage. We found that *OsSPL10* was preferentially expressed in early young panicles (< 5 cm) and stem, while its expression levels in late young panicles (pre-emergence inflorescence, 5-10 cm), leaf blades and leaf sheaths were generally low or very weak (Figure 5). These results suggested that *OsSPL10* is probably involved in the early

development of inflorescence, or in the phase transition from vegetative growth to reproductive development.

OsSPL10 is localized in nucleus

Some *SPL* genes playing important roles in growth and development have been found to function as transcription factors (Wang *et al.* 2009; Jiao *et al.* 2010). Therefore, we predicted that OsSPL10 protein might be also a transcription factor, which should be sorted to nucleus. Transient expression of *35S::OsSPL10-GFP* in the epidermal cells of *Nicotiana benthamiana* (tobacco) leaves clearly showed that the GFP signal of SST-GFP fusion protein was observed only in nucleus (Figure 6A-D). By contrast, the GFP signal due to transformation of *35S::GFP* was observed everywhere in the cell without specificity (Figure 6E-H). These results supported our prediction that SST is localized in the nucleus, suggesting that SST possibly functions as a transcription factor.

DISCUSSION

In this study, we confirmed through gene knockout and gene overexpression that *OsSPL10* is *SST*, which plays a negative role in salt tolerance but a positive role in trichome formation and has a small negative effect on seedling growth as well in rice. In addition, the result of subcellular localization supported the prediction that OsSPL10 probably functions as a transcription factor like other OsSPL proteins.

There are 19 *OsSPL* genes in rice, including one pseudogene. Among them, 11 genes (not including *OsSPL10*) are the targets of miR156 (Xie *et al.* 2006). *OsSPL10* is the first *OsSPL* gene confirmed to control salt tolerance in rice. However, there could be other *OsSPL* genes related to salt tolerance. It has been found that the abundance of miR156 increases in rice plants when subjected to salt stress, and the transgenic rice seedlings overexpressing miR156 show higher salt tolerance (Cui *et al.* 2014). This implies that there might be some *OsSPL* genes targeted by miR156 negatively controlling salt tolerance in rice. If this is true, there will be two different pathways of *OsSPL*-mediated salt tolerance regulation in rice. One is miR156-dependent, the other is miR156-independent (*e.g.*, mediated by *OsSPL10*). But no matter in what pathways, the *OsSPL* genes involved all function as a negative regulator.

To date, two genes controlling trichome development have been reported in rice, namely, *OsWOX3B* (*DEP*, *NUDA/GL-1*, *GLR1*) and *OsPLT2* (*HL6*) (Angeles-Shim *et al.* 2012; Zhang *et al.* 2012; Li *et al.* 2012; Sun *et al.* 2017). *OsWOX3B* belongs to the WOX3 family of plant-specific homeobox transcription factors (Angeles-Shim *et al.* 2012),
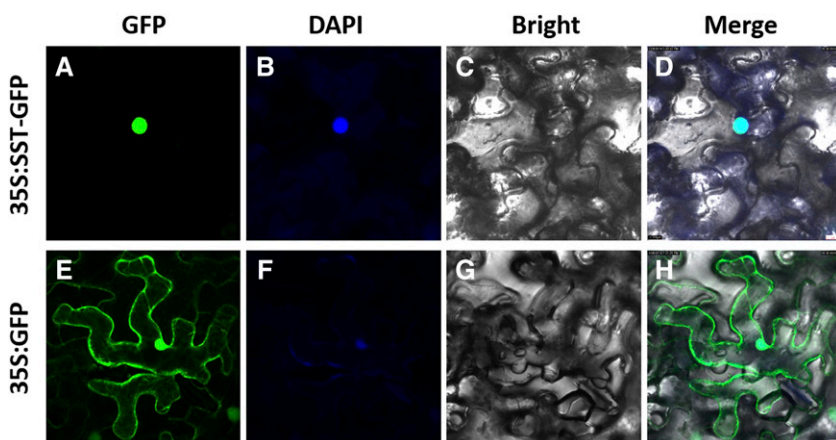

Figure 6 Subcellular localization of OsSPL10-GFP fusion protein. A-D Transient co-expression of fusion protein OsSPL10-GFP and DAPI. E-F Transformation with *35S::GFP* as control. Scale bar = 10 μm, applicable to all photos.

while *OsPLT2* is an AP2/ERF transcription factor. *OsPLT2* regulates trichome elongation, which is dependent on functional OsWOX3B that acts as a key regulator in trichome initiation (Sun *et al.* 2017). In this study, we found that knockout of *OsSPL10* exhibited glabrous leaves and glumes, while overexpression of *OsSPL10* increased the density of trichomes on leaves. In *Arabidopsis*, *SPLs* have also been found to be involved in the development and distribution of trichomes (Unte *et al.* 2003; Yu *et al.* 2010). The effect of *OsSPL10* on trichome development found in this study is more similar to that of *OsWOX3B* than to that of *OsPLT2*. Therefore, we speculate that *OsSPL10* is likely to control trichome initiation. As an SBP-box gene with its protein being localized in nucleus (Figure 6), *OsSPL10* probably function as transcription factor in trichome initiation. Further research is needed to clarify how *OsSPL10* regulates trichome development and what relationship exists among *OsWOX3B*, *OsPLT2*, and *OsSPL10* in rice.

CONCLUSION

OsSPL10 plays a dual role in salt tolerance and trichome formation in rice. It negatively controls salt tolerance but positively controls trichome initiation. The results of this study will help further research and better understanding of the mechanisms of salt tolerance and trichome formation in rice.

LITERATURE CITED

Angeles-Shim, R. B., K. Asano, T. Takashi, J. Shim, T. Kuroha *et al.*, 2012 A WUSCHEL-related homeobox 3B gene, *depilous* (*dep*), confers glabrousness of rice leaves and glumes. Rice (N. Y.) 5: 28. https://doi.org/10.1186/1939-8433-5-28

Campo, S., P. Baldrich, J. Messeguer, E. Lalanne, M. Coca *et al.*, 2014 Overexpression of a calcium-dependent protein kinase confers salt and drought tolerance in rice by preventing membrane lipid peroxidation. Plant Physiol. 165: 688–704. https://doi.org/10.1104/pp.113.230268

Cardon, G. H., S. Höhmann, K. Nettesheim, H. Saedler, and P. Huijser, 1997 Functional analysis of the *Arabidopsis thaliana* SBP-box gene *SPL3*: a novel gene involved in the floral transition. Plant J. 12: 367–377. https://doi.org/10.1046/j.1365-313X.1997.12020367.x

Chao, L. M., Y. Q. Liu, D. Y. Chen, X. Y. Xue, Y. B. Mao *et al.*, 2017 *Arabidopsis* transcription factors SPL1 and SPL12 confer plant thermotolerance at reproductive stage. Mol. Plant 10: 735–748. https://doi.org/10.1016/j.molp.2017.03.010

Chen, G., Q. D. Hu, L. Luo, T. Y. Yang, S. Zhang *et al.*, 2015 Rice potassium transporter OsHAK1 is essential for maintaining potassium-mediated growth and functions in salt tolerance over low and high potassium concentration ranges. Plant Cell Environ. 38: 2747–2765. https://doi.org/10.1111/pce.12585

Chen, Z. C., N. Yamaji, T. Horie, J. Che, J. Li *et al.*, 2017 A magnesium transporter OsMGT1 plays a critical role in salt tolerance in rice. Plant Physiol. 174: 1837–1849. https://doi.org/10.1104/pp.17.00532

Chuck, G. S., P. J. Brownb, R. Meeleyc, and S. Hakea, 2014 Maize *SBP-box* transcription factors *unbranched2* and *unbranched3* affect yield traits by regulating the rate of lateral primordia initiation. Proc. Natl. Acad. Sci. USA 111: 18775–18780. https://doi.org/10.1073/pnas.1407401112

Cui, L. G., J. X. Shan, M. Shi, J. P. Gao, and H. X. Lin, 2014 The *miR156-SPL9-DFR* pathway coordinates the relationship between

development and abiotic stress tolerance in plants. Plant J. 80: 1108–1117. https://doi.org/10.1111/tpj.12712

Datta, K., N. Baisakh, M. Ganguly, S. Krishnan, K. Yamaguchi Shinozaki *et al.*, 2012 Overexpression of *Arabidopsis* and rice stress genes' inducible transcription factor confers drought and salinity tolerance to rice. Plant Biotechnol. J. 10: 579–586. https://doi.org/10.1111/j.1467-7652.2012.00688.x

Gou, J. Q., S. Debnath, L. Sun, A. Flanagan, Y. H. Tang *et al.*, 2018 From model to crop: functional characterization of *SPL8* in *M. truncatula* led to genetic improvement of biomass yield and abiotic stress tolerance in alfalfa. Plant Biotechnol. J. 16: 951–962. https://doi.org/10.1111/pbi.12841

He, J., M. L. Xu, M. R. Willmann, K. McCormick, T. Q. Hu *et al.*, 2018 Threshold-dependent repression of *SPL* gene expression by miR156/miR157 controls vegetative phase change in *Arabidopsis thaliana*. PLoS Genet. 14: e1007337. https://doi.org/10.1371/journal.pgen.1007337

Hiei, Y., and T. Komari, 2008 *Agrobacterium*-mediated transformation of rice using immature embryos or calli induced from mature seed. Nat. Protoc. 3: 824–834. https://doi.org/10.1038/nprot.2008.46

Hu, H. H., M. Q. Dai, J. L. Yao, B. Z. Xiao, X. H. Li *et al.*, 2006 Overexpressing a NAM, ATAF, and CUC (NAC) transcription factor enhances drought resistance and salt tolerance in rice. Proc. Natl. Acad. Sci. USA 103: 12987–12992. https://doi.org/10.1073/pnas.0604882103

Huang, X. Y., D. Y. Chao, J. P. Gao, M. Z. Zhu, M. Shi *et al.*, 2009 A previously unknown zinc finger protein, DST, regulates drought and salt tolerance in rice via stomatal aperture control. Genes Dev. 23: 1805–1817. https://doi.org/10.1101/gad.1812409

Ishii, T., K. Numaguchi, K. Miura, K. Yoshida, P. T. Thanh *et al.*, 2013 *OsLG1* regulates a closed panicle trait in domesticated rice. Nat. Genet. 45: 462–465. https://doi.org/10.1038/ng.2567

Jiao, Y., Y. Wang, D. Xue, J. Wang, M. Yan *et al.*, 2010 Regulation of *OsSPL14* by OsmiR156 defines ideal plant architecture in rice. Nat. Genet. 42: 541–544. https://doi.org/10.1038/ng.591

Johnson, H. B., 1975 Plant pubescence: an ecological perspective. Bot. Rev. 41: 233–258. https://doi.org/10.1007/BF02860838

Klein, J., H. Saedler, and P. Huijser, 1996 A new family of DNA binding proteins includes putative transcriptional regulators of the *Antirrhinum majus* floral meristem identity gene *SQUAMOSA*. Mol. Gen. Genet. 250: 7–16.

Lan, T., S. J. Zhang, T. T. Liu, B. Wang, H. Z. Guan *et al.*, 2015 Fine mapping and candidate identification of *SST*, a gene controlling seedling salt tolerance in rice (*Oryza sativa* L.). Euphytica 205: 269–274. https://doi.org/10.1007/s10681-015-1453-9

Lee, J., J. J. Park, S. L. Kim, J. Yim, and G. An, 2007 Mutations in the rice liguleless gene result in a complete loss of the auricle, ligule, and laminar joint. Plant Mol. Biol. 65: 487–499. https://doi.org/10.1007/s11103-007-9196-1

Li, C. H., G. Wang, J. L. Zhao, L. Q. Zhang, L. F. Ai *et al.*, 2014 The receptor-like kinase SIT1 mediates salt sensitivity by activating MAPK3/6 and regulating ethylene homeostasis in rice. Plant Cell 26: 2538–2553. https://doi.org/10.1105/tpc.114.125187

Li, J. J., Y. D. Yuan, Z. F. Lu, L. S. Yang, R. C. Gao *et al.*, 2012 *Glabrous Rice 1*, encoding a homeodomain protein, regulates trichome development in rice. Rice (N. Y.) 5: 32. https://doi.org/10.1186/1939-8433-5-32

Manning, K., M. Tör, M. Poole, Y. G. Hong, A. J. Thompson *et al.*, 2006 A naturally occurring epigenetic mutation in a gene encoding an SBP-box transcription factor inhibits tomato fruit ripening. Nat. Genet. 38: 948–952. https://doi.org/10.1038/ng1841

Martínez-Atienza, J., X. Jiang, B. Garciadeblas, I. Mendoza, J. K. Zhu *et al.*, 2007 Conservation of the salt overly sensitive pathway in rice. Plant Physiol. 143: 1001–1012. https://doi.org/10.1104/pp.106.092635

Miura, K., M. Ikeda, A. Matsubara, X. J. Song, M. Ito *et al.*, 2010 *OsSPL14* promotes panicle branching and higher grain productivity in rice. Nat. Genet. 42: 545–549. https://doi.org/10.1038/ng.592

Moreno, M. A., L. C. Harper, R. W. Krueger, S. L. Dellaporta, and M. Freeling, 1997 *liguleless1* encodes a nuclear-localized protein

required for induction of ligules and auricles during maize leaf organogenesis. Genes Dev. 11: 616–628. https://doi.org/10.1101/gad.11.5.616

Obata, T., H. K. Kitamoto, A. Nakamura, A. Fukuda, and Y. Tanaka, 2007 Rice shaker potassium channel OsKAT1 confers tolerance to salinity stress on yeast and rice cells. Plant Physiol. 144: 1978–1985. https://doi.org/10.1104/pp.107.101154

Ren, Z. H., J. P. Gao, L. G. Li, X. L. Cai, W. Huang et al., 2005 A rice quantitative trait locus for salt tolerance encodes a sodium transporter. Nat. Genet. 37: 1141–1146. https://doi.org/10.1038/ng1643

Schmidt, R., D. Mieulet, H. M. Hubberten, T. Obata, R. Hoefgen et al., 2013 Salt-responsive ERF1 regulates reactive oxygen species-dependent signaling during the initial response to salt stress in rice. Plant Cell 25: 2115–2131. https://doi.org/10.1105/tpc.113.113068

Si, L. Z., J. Y. Chen, X. H. Huang, H. Gong, J. H. Luo et al., 2016 OsSPL13 controls grain size in cultivated rice. Nat. Genet. 48: 447–456. https://doi.org/10.1038/ng.3518

Song, H. B., B. Wang, R. J. Chen, X. Y. Zheng, S. B. Yu et al., 2016 Genetic analysis and gene mapping of the glabrous leaf and hull mutant glr3 in rice (Oryza sativa L.) (article in Chinese). Yi Chuan 38: 1012–1019.

Srivastava, A. K., C. Zhang, G. Yates, M. Bailey, A. Brown et al., 2016 SUMO is a critical regulator of salt stress responses in rice. Plant Physiol. 170: 2378–2391. https://doi.org/10.1104/pp.15.01530

Sun, W. Q., D. W. Gao, Y. Xiong, X. X. Tang, X. F. Xiao et al., 2017 Hairy leaf 6, an AP2/ERF transcription factor, interacts with OsWOX3B and regulates trichome formation in rice. Mol. Plant 10: 1417–1433. https://doi.org/10.1016/j.molp.2017.09.015

Tao, Z., Y. J. Kou, H. B. Liu, X. H. Li, J. H. Xiao et al., 2011 OsWRKY45 alleles play different roles in abscisic acid signalling and salt stress tolerance but similar roles in drought and cold tolerance in rice. J. Exp. Bot. 62: 4863–4874. https://doi.org/10.1093/jxb/err144

Unte, U. S., A. M. Sorensen, P. Pesaresi, M. Gandikota, D. Leister et al., 2003 SPL8, an SBP-box gene that affects pollen sac development in Arabidopsis. Plant Cell 15: 1009–1019. https://doi.org/10.1105/tpc.010678

Urano, D., A. Colaneri, and A. M. Jones, 2014 Gα modulates salt-induced cellular senescence and cell division in rice and maize. J. Exp. Bot. 65: 6553–6561. https://doi.org/10.1093/jxb/eru372

Wang, B., T. T. Liu, S. J. Zhang, T. Lan, H. Z. Guan et al., 2013 [Genetic analysis and gene mapping for a salt tolerant mutant at seedling stage in rice]. Yi Chuan 35: 1101–1105. https://doi.org/10.3724/SP.J.1005.2013.01101

Wang, J., L. Zhou, H. Shi, M. Chern, H. Yu et al., 2018 A single transcription factor promotes both yield and immunity in rice. Science 361: 1026–1028. https://doi.org/10.1126/science.aat7675

Wang, J. W., B. Czech, and D. Weigel, 2009 miR156-regulated SPL transcription factors define an endogenous flowering pathway in Arabidopsis thaliana. Cell 138: 738–749. https://doi.org/10.1016/j.cell.2009.06.014

Wang, J. W., R. Schwab, B. Czech, E. Mica, and D. Weigel, 2008 Dual effects of miR156-targeted SPL genes and CYP78A5/KLUH on plastochron length and organ size in Arabidopsis thaliana. Plant Cell 20: 1231–1243. https://doi.org/10.1105/tpc.108.058180

Wang, L., and Q. F. Zhang, 2017 Boosting rice yield by fine-tuning SPL gene expression. Trends Plant Sci. 22: 643–646. https://doi.org/10.1016/j.tplants.2017.06.004

Wang, Q., A.-Z. Sun, S.-T. Chen, L.-S. Chen. and F.-Q. Guo 2018 SPL6 represses signalling outputs of ER stress in control of panicle cell death in rice. Nat. Plants 4: 280–288. https://doi.org/10.1038/s41477-018-0131-z

Wang, S. K., K. Wu, Q. B. Yuan, X. Y. Liu, Z. B. Liu et al., 2012 Control of grain size, shape and quality by OsSPL16 in rice. Nat. Genet. 44: 950–954. https://doi.org/10.1038/ng.2327

Wang, Y. M., D. Q. Li, J. B. Gao, X. K. Li, R. Zhang et al., 2017 The 2′-O-methyladenosine nucleoside modification gene OsTRM13 positively regulates salt stress tolerance in rice. J. Exp. Bot. 68: 1479–1491. https://doi.org/10.1093/jxb/erx061

Xiang, Y., N. Tang, H. Du, H. Y. Ye, and L. Z. Xiong, 2008 Characterization of OsbZIP23 as a key player of the basic leucine zipper transcription factor family for conferring abscisic acid sensitivity and salinity and drought tolerance in rice. Plant Physiol. 148: 1938–1952. https://doi.org/10.1104/pp.108.128199

Xie, K. B., C. Q. Wu, and L. Z. Xiong, 2006 Genomic organization, differential expression, and interaction of SQUAMOSA Promoter-Binding-Like transcription factors and microRNA156 in rice. Plant Physiol. 142: 280–293. https://doi.org/10.1104/pp.106.084475

Xing, S. P., M. Salinas, S. Höhmann, R. Berndtgen, and P. Huijser, 2010 miR156-targeted and nontargeted SBP-box transcription factors act in concert to secure male fertility in Arabidopsis. Plant Cell 22: 3935–3950. https://doi.org/10.1105/tpc.110.079343

Yamasaki, H., M. Hayashi, M. Fukazawa, Y. Kobayashi, and T. Shikanaic, 2009 SQUAMOSA promoter binding protein–like7 is a central regulator for copper homeostasis in Arabidopsis. Plant Cell 21: 347–361. https://doi.org/10.1105/tpc.108.060137

Yan, J. P., J. C. Chia, H. J. Sheng, H. Jung, T. O. Zavodna et al., 2017 Arabidopsis pollen fertility requires the transcription factors CITF1 and SPL7 that regulate copper delivery to anthers and jasmonic acid synthesis. Plant Cell 29: 3012–3029. https://doi.org/10.1105/tpc.17.00363

Yang, A., X. Y. Dai, and W. H. Zhang, 2012 A R2R3-type MYB gene, OsMYB2, is involved in salt, cold, and dehydration tolerance in rice. J. Exp. Bot. 63: 2541–2556. https://doi.org/10.1093/jxb/err431

Yu, N., W. J. Cai, S. Wang, C. M. Shan, L. J. Wang et al., 2010 Temporal control of trichome distribution by microRNA156-targeted SPL genes in Arabidopsis thaliana. Plant Cell 22: 2322–2335. https://doi.org/10.1105/tpc.109.072579

Yu, N., Q. W. Niu, K. H. Ng, and N. H. Chua, 2015 The role of miR156/SPLs modules in Arabidopsis lateral root development. Plant J. 83: 673–685. https://doi.org/10.1111/tpj.12919

Yuan, H., P. Qin, L. Hu, S. Zhan, S. Wang et al., 2019 OsSPL18 controls grain weight and grain number in rice. J. Genet. Genomics 46: 41–51. https://doi.org/10.1016/j.jgg.2019.01.003

Yuan, S. R., Z. G. Li, D. Y. Li, N. Yuan, Q. Hu et al., 2015 Constitutive expression of rice MicroRNA528 alters plant development and enhances tolerance to salinity stress and nitrogen starvation in creeping bentgrass. Plant Physiol. 169: 576–593. https://doi.org/10.1104/pp.15.00899

Zang, A. P., X. J. Xu, S. Neill, and W. M. Cai, 2010 Overexpression of OsRAN2 in rice and Arabidopsis renders transgenic plants hypersensitive to salinity and osmotic stress. J. Exp. Bot. 61: 777–789. https://doi.org/10.1093/jxb/erp341

Zhang, B., W. N. Xu, X. Liu, X. G. Mao, A. Li et al., 2017 Functional conservation and divergence among homologs of TaSPL20 and TaSPL21, two SBP-box genes governing yield-related traits in hexaploid wheat. Plant Physiol. 174: 1177–1191. https://doi.org/10.1104/pp.17.00113

Zhang, H. L., K. Wu, Y. F. Wang, Y. Peng, F. Y. Hu et al., 2012 A WUSCHEL-like homeobox gene, OsWOX3B responses to NUDA/GL-1 locus in rice. Rice (N. Y.) 5: 30. https://doi.org/10.1186/1939-8433-5-30

Zhang, L., L. H. Tian, J. F. Zhao, Y. Song, C. J. Zhang et al., 2009 Identification of an apoplastic protein involved in the initial phase of salt stress response in rice root by two-dimensional electrophoresis. Plant Physiol. 149: 916–928. https://doi.org/10.1104/pp.108.131144

Zhou, Y. B., C. Liu, D. Y. Tang, L. Yan, D. Wang et al., 2018 The receptor-like cytoplasmic kinase STRK1 phosphorylates and activates CatC, thereby regulating H_2O_2 homeostasis and improving salt tolerance in rice. Plant Cell 30: 1100–1118. https://doi.org/10.1105/tpc.17.01000

Zhu, Z. F., L. B. Tan, Y. C. Fu, F. X. Liu, H. W. Cai et al., 2013 Genetic control of inflorescence architecture during rice domestication. Nat. Commun. 4: 2200. https://doi.org/10.1038/ncomms3200

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