Cellulose synthase-like protein OsCSLD4 plays an important role in the response of rice to salt stress by mediating abscisic acid biosynthesis to regulate osmotic stress tolerance

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Summary
Cell wall polysaccharide biosynthesis enzymes play important roles in plant growth, development and stress responses. The functions of cell wall polysaccharide synthesis enzymes in plant growth and development have been well studied. In contrast, their roles in plant responses to environmental stress are poorly understood. Previous studies have demonstrated that the rice cell wall cellulose synthase-like D4 protein (OsCSLD4) is involved in cell wall polysaccharide synthesis and is important for rice growth and development. This study demonstrated that the OsCSLD4 function-disrupted mutant nd1 was sensitive to salt stress, but insensitive to abscisic acid (ABA). The expression of some ABA synthesis and response genes was repressed in nd1 under both normal and salt stress conditions. Exogenous ABA can restore nd1-impaired salt stress tolerance. Moreover, overexpression of OsCSLD4 can enhance rice ABA synthesis gene expression, increase ABA content and improve rice salt tolerance, thus implying that OsCSLD4-regulated rice salt stress tolerance is mediated by ABA synthesis. Additionally, nd1 decreased rice tolerance to osmotic stress, but not ion toxic tolerance. The results from the transcriptome analysis showed that more osmotic stress-responsive genes were impaired in osmotic stress, but not ion toxic tolerance. The disruption of OsCSLD4 function decreased grain yield and weight, while overexpression of OsCSLD4 increased grain yield and weight. Taken together, this study demonstrates a novel plant salt stress adaptation mechanism by which crops can coordinate salt stress tolerance and yield.

Keywords: cell wall polysaccharides, salt stress, osmotic stress, ABA, OsCSLD4.

Introduction
Plant cell walls not only determine cell shape and provide structural support, but they also act as the front line of various environmental stresses (Doblin and Pettolino, 2010; Liu et al., 2021a; Tenhaken, 2014; Voeur and Höfte, 2016). Plant cell wall polysaccharides exhibit a striking variety in composition in accordance with environmental conditions, and therefore, there is a close relationship between the variety of plant cell wall polysaccharide compositions and the response of plants to environmental stresses (Corréa-Ferreira et al., 2019; Doblin et al., 2010; Houston et al., 2016; Lenk et al., 2019; Liu et al., 2021b; Yang et al., 2021; Zhang et al., 2021).

Plant cell wall contains three types of polysaccharides, including cellulose, hemicellulose and pectin. Cellulose is composed of β-1,4-glucans and is synthesized by plasma membrane-localized cellulose synthase (CESA) complexes (Doblin et al., 2002; Suzuki et al., 2006). In contrast, hemicelluloses, including several types of glycans such as xyloglucans, xylans, mannans, glucomannans and β-(1,3; 1,4)-glucans (Doblin et al., 2009; Scheller and Ulvskov, 2010). Cellulose synthase-like (CSL) proteins and Golgi-localized β-glycan synthases play a critical role in hemicellulose biosynthesis (Richmond and Somerville, 2000; Yin et al., 2014). According to protein phylogeny, CSL proteins can be grouped into eight subfamilies that include CSLA, CSLB, CSLC, CSLD, CSLE, CSLF, CSLG and CSLH (Kaur et al., 2017; Yin et al., 2014). CSLF subfamily members mediate mannan backbone synthesis (Dhugga et al., 2004; Liepman and Wilkerson, 2005; Zou et al., 2018). CSLC subfamily proteins respond to the biosynthesis of the xyloglucans β-1,4-glucan backbone (Cocuron et al., 2007). CSLF and CSLH subfamilies are considered to be unique in monocots and participate in (1,3;1,4)-β-D-glucan biosynthesis (Burton et al., 2006; Doblin et al., 2009). CSLD share high amino acid similarity with CESA and is involved in the biosynthesis of several types of glycans (Bernal et al., 2007; Galway et al., 2011; Li et al., 2017; Peng et al., 2019). CSLDs can regulate different types of cell wall.
global food security. Plants can remodel cell wall polysaccharide synthesis with tissue and organ specificity, and they play important roles in plant growth and development (Favery et al., 2001; Gu et al., 2016; Hu et al., 2018; Kim et al., 2007; Li et al., 2009; Peng et al., 2019; Wang et al., 2001; Wang et al., 2011a; Yang et al., 2021; Yoshikawa et al., 2013). In Arabidopsis, AtCSDL1 and AtCSDL4 are involved in pollen tube growth (Wang et al., 2011a). AtCSDL2 and AtCSDL3 can affect the morphogenesis and growth of root hair (Favery et al., 2001; Galway et al., 2011; Hu et al., 2018; Wang et al., 2001). AtCSDL5 plays an important role in plant growth by regulating the synthesis of cell wall xylan and homogalacturonan (Bernal et al., 2018; Peng et al., 2019; Tenhaken, 2014; Yang et al., 2021). Salinity stress is one of the most widespread abiotic stresses and has a serious threat to global food security. Plants can remodel cell wall polysaccharide synthesis to cope with salt stress (Fujita et al., 2013; Hu et al., 2018; Peng et al., 2019; Yoshikawa et al., 2013). Therefore, disturbing the activity of cell wall polysaccharide synthesis enzymes seriously interferes with salt stress tolerance (Kesten et al., 2019; Zhang et al., 2016; Zhu et al., 2010). For example, the mutation of cellulose synthase 6 (CESA6) or its companion protein CCA1 (companion of cellulose synthase 1) disturbs cellulose synthesis and decreases Arabidopsis salt tolerance (Endler et al., 2015; Kesten et al., 2019; Zhang et al., 2016). Xyloglucan is one of the most important compositions of hemicelluloses. Xyloglucan endotransglucosylases/hydrolase (XTH) is a member of the xyloglucan-modifying enzyme family that exerts an important effect on cell wall remodelling (Rose et al., 2002). XTH gene DKKXTH1 and CaxXTH3 can modify transgenic Arabidopsis and tomato cell morphogenesis and enhances drought and salt tolerance (Cho et al., 2006; Choi et al., 2011; Han et al., 2017). The Arabidopsis XTH19 is involved in the response of plants to cold stress (Takahashi et al., 2021). The roles of cellulose and hemicellulose synthesis involved in plant stress tolerance are complex. Except for the positive role of cellulose or hemicellulose synthetases in the response of plants to environmental stress, some play a negative role in plant stress tolerance. Disruption of AtCesA5/IRX1 enhances Arabidopsis tolerance to drought and osmotic stress (Chen et al., 2005). Arabidopsis xyloglucan endotransglucosylase-hydrolase30 (XTH30) can modulate xyloglucan side chains. Disruption of XTH30 function partially inhibits xyloglucan-derived oligosaccharide synthesis, sustains crystalline cellulose content and microtubule depolymerization, and enhances plant salt stress tolerance (Yan et al., 2019). These results indicate that cell wall polysaccharide synthesis-related enzymes play a complex and important role in the adaptation of plants to adverse environmental conditions. Although cell wall polysaccharide synthesis enzymes play important roles in the response of plants to salt stress, the detailed mechanism of these enzymes involved in plant stress responses remains obscure (Kesten and Menna, 2017; Novakovici et al., 2018).

OsCSDL4 (rice cellulose synthase-like D4) encodes a CSLD subfamily protein. Previous studies have revealed the critical role of OsCSDL4 in rice growth and development by regulating cell wall polysaccharide synthesis (Ding et al., 2015; Hu et al., 2010; Li et al., 2009; Luan et al., 2011; Wu et al., 2010; Yoshikawa et al., 2013). However, the role of OsCSDL4 in the response of rice to environmental stresses remains unclear. This study revealed that OsCSDL4 was involved in the rice salt stress response by mediating abscisic acid (ABA) content to enhance rice osmotic stress tolerance.

**Results**

OsCSDL4 is highly homologous to AtCSDL5 and its gene expression is induced by salt

The results of the phylogenetic analysis revealed that OsCSDL4 was highly homologous to Arabidopsis AtCSDL5 and maize ZmCSDL4 (Figure 1a,b). AtCSDL5 played a vital role in the Arabidopsis salt stress response (Zhu et al., 2010). The high homology of OsCSDL4 to AtCSDL5 implies that OsCSDL4 may exert a similar function in the rice salt stress response. To elucidate the role of OsCSDL4 in the rice salt stress response, we studied the expression pattern of OsCSDL4 under salt stress by using quantitative RT-PCR (qPCR). The results revealed that the expression of OsCSDL4 was induced significantly by salt stress (Figure 1c). The expression of OsCSDL4 was induced quickly within 1 h and remained high until 8 h. In contrast, the expression of other members of the CSLD subfamily in rice was not noticeably induced by salt treatment (Figure 1c). The result indicates that OsCSDL4 may play an important role in rice salt stress response.

**Disruption of OsCSDL4 impairs rice salt stress tolerance**

To further analyse the role of OsCSDL4 in the rice salt stress response, we used the reported OsCSDL4 function disruption mutant narrow leaf and dwarf1 (nd1) in an indica variety Zhongxian 3037 background (Li et al., 2009) to study the effect of OsCSDL4 on rice salt stress tolerance. nd1 possesses a narrow and rolled leaf and dwarf phenotype (Figure S1a; Hu et al., 2010; Li et al., 2009; Luan et al., 2011; Yoshikawa et al., 2013). When 2-week-old nd1 and WT seedlings were treated with salt (150 mM NaCl) for 7 days with another 7-day recovery (watered without salt), the majority of nd1 leaves were markedly wilted, while only a small number of WT leaves exhibited a wilted phenotype (Figure 2a,b). As presented in Figure 2b, nd1 exhibited a low survival rate of only approximately 37%, while the WT survival rate was as high as approximately 68% after 7-day recovery, thus indicating that OsCSDL4 is important for rice salt stress tolerance.

Salt stress can damage and inhibit the growth of plant roots, therefore, the root growth can represent plant salt stress tolerance (Arsova et al., 2020; Dinneny, 2019; Li et al., 2021; Shao et al., 2021). The results from qPCR assays revealed that OsCSDL4 was expressed in rice roots, stems and leaves (Figure S2; Ding et al., 2015). Different from shoots and crown roots that displayed markedly dwarf and rolled leaf or shortened phenotypes, respectively, in 10-day-old nd1 seedlings, the growth of nd1 primary roots was similar to that of WT (Figure S1; Figure 2c, d). To better analyse and display the effect of OsCSDL4 function on the rice salt stress response, we examined the primary root growth to analyse the role of OsCSDL4 in the rice salt stress response. When germinated WT and nd1 seeds were transferred to 1/2 Murashige & Skoog (MS) medium with 150 mM NaCl, and then grown for 7 days, the growth of nd1 primary roots was observed to be more severely inhibited compared to that of WT (Figure 2c,d). The length of nd1 primary roots was approximately 3.2 cm, and in contrast, the length of WT primary roots was approximately 5.6 cm (Figure 2d). Additionally, to confirm the function of OsCSDL4 in rice salinity stress tolerance, we also analysed the salt tolerance of the nd1 allelic mutant nr17 (in
The results revealed that nd1 also exhibited decreased rice salt stress tolerance (Figure S3). The above results demonstrate that OsCSLD4 is important for salinity tolerance of rice seedlings.

Disruption of OsCSLD4 increases rice seedlings’ osmotic stress sensitivity

Salinity stress can disturb cellular ions and water balance in plant, ultimately resulting in ion toxicity and osmotic stress damage to plant (Amin et al., 2021; Dinneny, 2019; Garcia et al., 2009; Huang et al., 2012; Zhu, 2002). To illustrate the role of OsCSLD4 in the response of rice to salinity stress, we first studied the ion toxicity tolerance of nd1. LiCl is easily absorbed and transported into plants, and this can result in marked ion toxicity at low concentrations. To avoid osmotic stress interference from high concentration salinity, low concentration (18 mM) LiCl was used to analyse the ion toxicity tolerance of nd1 seedlings. The results revealed that the growth of nd1 seedlings was similar to that of WT under LiCl treatment (Figure S4), thus indicating that there was no marked difference in ion toxicity tolerance between nd1 and WT.

Additionally, considering that the effect of OsCSLD4 on cell wall polysaccharide composition and cell wall morphology may disturb ion absorption or transport, we measured Na+ and K+ content in rice roots and leaves before and after salt treatment. The results demonstrated that the Na+ and K+ contents in rice roots and leaves were not significantly different between WT and nd1 before or after salt treatment (Figure S5), thus indicating that the salt-sensitive phenotype of nd1 is not due to ion absorption or transport.

We further analysed the function of OsCSLD4 in the context of osmotic stress tolerance in rice. The results from PEG treatment experiments revealed that the growth of nd1 roots was inhibited more markedly by 10% PEG treatment compared to that of WT (Figure 3a). The primary root length of nd1 seedlings grown under 10% PEG for 10 days was approximately 2.6 cm, and this was only approximately 31% of that grown under normal conditions (approximately 8.8 cm). The primary root length of WT seedlings treated with 10% PEG for 10 days was approximately 4.5 cm, and this was approximately 51% of that in plants grown under normal conditions (Figure 3b), indicating that the disruption of OsCSLD4 impairs rice osmotic stress tolerance.

A previous study demonstrated that the OsCSLD4 homolog AtCSLD5 is involved in the Arabidopsis salt stress response through the ROS scavenging system (Zhu et al., 2010). Here, we sought to determine if OsCSLD4 plays the same role in rice. Firstly, we analysed the oxidative damage in nd1 seedlings under salt treatment. MDA is one of the main products of cellular oxidized components, so MDA content can represent cell oxidative damage degree. The MDA content in rice seedlings

Figure 1 Phylogenetic analysis of OsCSLD4 homologous proteins and OsCSLD4 salt-induced expression patterns. (a) Phylogenetic analysis of OsCSLD4 homologous proteins in Arabidopsis, maize and rice. (b) Alignment of amino acid sequences of AtCSLD5, ZmCSLD4 and OsCSLD4. White or black background represents the identity of amino acid sequences. (c) Expression level of rice OsCSLD subfamily genes under salt treatment (150 mM NaCl). The genes expression level under normal condition (0 h) is standardized to ‘1’. Figure shows the relative expression level of genes under salt treatment to normal condition. The figure presents the average from three independent experiments. Bar represents the standard error (±SE).
revealed that the MDA content in \textit{nd1} seedlings was markedly higher than that in WT after long-term salt treatment (treated with 150 mM NaCl for 3 days) (Figure 3c). The higher MDA content in \textit{nd1} seedlings indicates more serious ROS damage to \textit{nd1} seedlings under salt stress (You and Chan, 2015). To distinguish the ROS damage in \textit{nd1} seedlings under salt treatment, rice seedlings were treated with high salt for a short time period, and the ROS (O$_2^-$) and MDA contents were then analysed immediately. The results revealed that the ROS content was not significantly different in the leaves of \textit{nd1} and WT (Figure 3d,e) under short-term salt treatment (treated with 150 mM NaCl for 1 h and 12 h respectively). Additionally, the results from H$_2$O$_2$ and MV treatment experiments demonstrated that the growth of \textit{nd1} seedlings was similar to that of WT under oxidant treatments (Figure S6). The above results reveal that the ROS content in \textit{nd1} is not due to decreased ROS scavenging ability and is instead due to more serious damage to plant cells caused by the decreased plant salt tolerance, thus indicating that the pathway of OsCSLD4 involved in salt stress response in rice is different from that of AtCSLD5 in Arabidopsis.

\textbf{OsCSLD4 regulates global gene responses to salt stress}

To elucidate the mechanism by which OsCSLD4 is involved in the rice salt response, we studied the global impression of OsCSLD4-dependent gene expression in response to salt stress by analysing the transcriptome of WT and \textit{nd1} that were treated with water (mock) or NaCl (150 mM NaCl). \textit{nd1} possessed 1,318 differentially expressed genes (DEGs, marked as group A) compared to those of WT, and 554 genes were up-regulated and 764 were down-regulated, thus implying that OsCSLD4 plays an important role in rice under normal growth conditions. After treatment with 150 mM NaCl, \textit{nd1} exhibited 888 DEGs (marked as group B) compared to those of WT, and 554 genes were up-regulated and 303 were down-regulated, thus indicating that OsCSLD4 plays an important role in regulating gene expression under salt stress (Figure 4a and Table S2). Statistical gene ontology (GO)-term enrichment analysis revealed that group A converges on cell wall organization or biogenesis, hormone synthesis or response, and transcript regulation. Group B converged in response to oxygen, hormone signal or synthesis and abiotic stress, with the
exceptions of cell wall organization and biogenesis (Figure S7). These results reveal that OsCSLD4 is important for allowing rice to balance its development and salinity tolerance.

Moreover, the WT possessed 1068 differentially expressed genes compared to those in plants treated with water as a control (group C). In contrast, nd1 exhibited 1641 differentially expressed genes (group D). Among these genes, 1200 DEGs exhibited different patterns compared to those in WT, and 331 DEGs between nd1 and WT under salt stress may be responsible for the OsCSLD4-dependent salt stress response (Figure 4a and Table S2). We selected 107 OsCSLD4-dependent DEGs between nd1 and WT for further GO enrichment. The results demonstrated that OsCSLD4-dependent DEGs were primarily involved in cell wall organization or biogenesis (36 genes, about 33.6% of total OsCSLD4-dependent DEGs), processes related to ABA (9 genes, about 8.4%), signal transduction (21 genes, about 19.6%) and stress response (36 genes, about 33.6% of total genes, Figure 4b, Table S3).

We verified the relative expression in response to different treatments of some OsCSLD4-dependent differentially expressed genes using qPCR. For example, the cellulose synthesis genes OsCesA4 (Tanaka et al., 2003; Zhang et al., 2009) and ATP-binding cassette (ABC) transporter G subfamily member 5 OsABCG5 (Matsuda et al., 2016; Shiono et al., 2014), the calcium/calmodulin dependent protein kinase CRINKLY4 (Pu et al., 2012), the ABA-responsive gene RAB16C (El-Esawi and Alayafi, 2019), the ABA-biosynthesis gene 9-cis-epoxycarotenoid dioxygenase 4 OsNCED4 (Hwang and Lee, 2018) and the transcription regulator DREB1C (Dubouzet et al., 2003) all exhibited similar results in both the qPCR and transcriptome experiments (Figure 4c). These results suggest that disruption of OsCSLD4 has an obvious effect on ABA synthesis and signal pathway, and impaired salt stress tolerance may be due to the decreased transcription of stress-responsive genes.
genes by interfering with plant stress signal transduction, particularly ABA signal pathway.

Disruption of OsCSLD4 decreases the ABA sensitivity of rice seedlings

The phytohormone abscisic acid is known to play a key role in the response of plants to osmotic stress by regulating the expression of a large number of osmotic stress-responsive genes in plants (Chen et al., 2016; Geng et al., 2013; Sah and Reddy, 2016; Vishwakarma et al., 2017; Wang et al., 2011b; Zhu, 2002, 2016). We observed that there were a large number of DEGs between WT and nd1 that are involved in ABA synthesis or its response in the transcriptome. To determine if and how OsCSLD4 is involved in the rice salt stress response via the ABA pathway, we first investigated the response of nd1 to exogenous ABA treatment. The results demonstrated that the sensitivity of nd1 roots to ABA treatment was lower than that of WT plants. The length of nd1 primary roots was longer than that of WT under exogenous ABA treatment (Figure 5a,b), thus indicating that the disruption of OsCSLD4 may affect the sensitivity of rice to ABA.

Disruption of OsCSLD4 decreases ABA synthesis under both normal and salt conditions

Plants can regulate ABA responses by activating the action of the ABA signalling pathway or by increasing ABA content (Yoshida...
and Mogami, 2014; Zhu, 2002, 2016). To illustrate the pathway by which the OsCSLD4 mutant alters rice ABA sensitivity, ABA content was measured. The results revealed that the ABA content in nd1 seedlings under normal growing conditions was significantly lower compared to that of WT seedlings. The ABA content in the WT was approximately 169 ng/g FW. In contrast, the ABA content in nd1 was approximately 155 ng/g FW (Figure 5c). The ABA content of nd1 under salt stress was also measured. The results demonstrated that the ABA content in nd1 under salt stress was lower compared to that in WT (Figure 5c). After a 7-day salt treatment, the ABA content in nd1 was approximately 232 ng/g FW. In contrast, the ABA content in the salt-treated WT was approximately 264 ng/g FW. The above results indicated that the disruption of OsCSLD4 decreased rice ABA content.

ABA is critical for OsCSLD4 to modulate rice salt stress tolerance

To further analyse the role of ABA in OsCSLD4-regulated rice salt stress tolerance, we used low concentrations of exogenous ABA to treat nd1 seedlings. The nd1 seedlings that were treated with 50 nM ABA exhibited similar salt tolerance to that of WT. The length of the primary root was not significantly different compared to that of the WT under salt stress (Figure 5d,e), thus implying that ABA plays a crucial role in OsCSLD4-regulated rice salt stress response.

Disruption of OsCSLD4 impairs the expression of ABA synthesis and response genes

We analysed the expression of ABA synthesis genes in nd1. The results of the qPCR assay revealed that the expression of key genes involved in ABA synthesis in nd1 seedlings was significantly lower than that in WT seedlings (Figure 6a). For example, the transcript level of the rice ABA synthesis key gene OsNCED4 was only approximately 20% of that in WT. The expression levels of 9-cis-epoxycarotenoid dioxygenase 1 (OsNCED1), abscisic aldehyde dehydrogenase/reductase-like 2 (OsAAO1), OsAAO2 and rice short-chain dehydrogenase/reductase-like 2 (OsSDR2) in nd1 were approximately half of those in WT (Figure 6a). The above results indicate that the decreased ABA level in nd1 seedlings may be due to the impaired expression of ABA synthesis genes.

Abscisic acid can induce downstream stress-response genes to enhance plant stress tolerance (Yoshida et al., 2014; Zhu, 2002). To analyse the role of ABA in OsCSLD4-mediated regulation of the salt stress response, we tested the expression of ABA-responsive genes in nd1. The results of qPCR assays revealed that the expression levels of ABA-responsive stress tolerance genes, including regulatory genes, such as OsZIP23 and OsDREB1C, and function genes, such as OsRAB16C, OsRAB17, OsLIP9 and OsRD22, were markedly impaired in nd1 (Figure 6b). For example, the expression levels of OsRD22 and OsRAB17 in nd1 were approximately 19% and 10%, respectively, of those in the WT (Figure 6b).

Expression of OsCSLD4 is induced by salt stress through an independent ABA pathway

To analyse the role of ABA in the salt-induced OsCSLD4 expression, we studied the expression pattern of OsCSLD4 under ABA treatment using qPCR assay. The expression of OsCSLD4 under ABA treatment was increased to up to approximately 2.7-fold compared to that under the control conditions after 2 h of ABA treatment (Figure 7a), indicating that the expression of OsCSLD4 was slightly induced by ABA.

Secondly, we analysed the expression of OsCSLD4 in nd1 without ABA or salt treatment. The results from qPCR assays revealed that the OsCSLD4 expression level in nd1 was approximately 70% of that in WT (Figure 7b), and the salt-induced expression of OsCSLD4 was significantly inhibited in nd1, which was only approximately 30% of that in WT (Figure 7b), thus indicating that the disruption of OsCSLD4 impaired its expression under normal conditions or salt stress.

Following, to analyse the role of ABA in salt-induced OsCSLD4 expression, we treated WT and nd1 seedlings with ABA and salt together. The results from qPCR assays demonstrated that exogenous ABA cannot restore the salt-induced expression of OsCSLD4 in nd1. The expression level of OsCSLD4 in nd1 was markedly lower than that in WT treated by salt alone (Figure 7b, c), but was similar to that in WT treated with ABA only (Figure 7a, c), thus indicating that salt-induced OsCSLD4 expression was independent of ABA-induced OsCSLD4 expression.

Overexpression of OsCSLD4 enhances rice salt stress tolerance and grain yield

The above studies demonstrated that the loss function of OsCSLD4 markedly impaired rice salt stress tolerance. To well display the role of OsCSLD4 in rice salt stress response, we further analysed the effect of the gain function of OsCSLD4 on rice salt tolerance. Firstly, we impaired the expression of OsCSLD4 in the japonica variety Kitaake by RNA interference technology (Figure S8b). RNA interference plants (RIs) showed a salt stress-sensitive phenotype (Figure S8a,c), which was similar to that of the OsCSLD4 mutants under Zhongxian 3037 and Zhonghua 11 background, indicating that OsCSLD4 has a similar role in rice salt stress response under different backgrounds.

Secondly, we analysed the expression of OsCSLD4 overexpression lines in KITAKE and Zhongxian 3037. Overexpression lines OE1 and OE2 was approximately 195 ng/g FW and 205 ng/g FW, respectively. Additionally, the expression levels of OsCSLD4 overexpression seedlings exhibited higher salt tolerance after 7-day NaCl for with another 7-day recovery (Figure 8a). The survival rate of OsCSLD4-overexpressing plants was 70%–85%, and in contrast, the survival rate of the wild type (Kit) was only approximately 45% (Figure 8b).

The result also revealed that ABA content in OsCSLD4 overexpression seedlings was higher than that in Kit (wild type) (Figure 8c). The ABA content in Kit was approximately 115 ng/g FW. In contrast, the ABA content in the OsCSLD4 overexpression lines OE1 and OE2 was approximately 195 ng/g FW and 205 ng/g FW respectively (Figure 8c). Additionally, the expression levels of ABA synthesis and responsive genes were markedly up-regulated in OsCSLD4 overexpression plants (Figure 8d). The above results reveal that OsCSLD4 can play a positive role in prompting ABA synthesis to cope with salt stress in rice.

Intriguingly, we observed that overexpression of OsCSLD4 not only enhanced rice salt tolerance but also increased grain size and weight. As presented in Figure S10b,d, the grain width (approximately 3.6 mm) of OsCSLD4-overexpressing plants was obviously larger than that of the wild type (Kit, approximately 3.3 mm). In contrast, the grain width of nd1 (approximately 2.3 mm) was smaller than that of WT (Zhongxian 3037, approximately 2.9 mm) (Figure S10a,c). The 1000-grain weight of nd1 was only approximately 16.8 g, while that of wild type (WT, Zhongxian 3037) was approximately 24.8 g (Figure S10e). The 1000-grain
weights of the OsCSLD4 overexpression lines OE1 and OE2 were approximately 25.6 g and 24.9 g, respectively, while that of Kit was only about 23.3 g (Figure S10f). The above results demonstrate that OsCSLD4 plays a positive role in both salt tolerance and grain yield.

Discussion

Cell wall polysaccharide synthases play critical roles in plant stress tolerance (Hamann, 2012; Liu et al., 2021a; Novaković et al., 2018; Rajashekar and Lafta, 1996; Rao and Dixon, 2017; Wang et al., 2019; Wang et al., 2016). However, the detailed mechanism of polysaccharide synthases in plant stress responses remain obscure. Previous studies have demonstrated that the polysaccharide synthase OsCSLD4 can catalyse the biosynthesis of rice cell wall polysaccharides, and plays an important role in rice growth and development (Hu et al., 2010; Li et al., 2009; Luan et al., 2011; Yoshikawa et al., 2013). The present study further demonstrated the mechanism of OsCSLD4 involved in rice salt tolerance, which will be beneficial for people to

Figure 5 ABA is related to OsCSLD4 function. (a) Phenotype of rice primary roots treated with ABA for 7 days. (b) Primary root length of rice seedlings treated with ABA for 7 days. (c) ABA content in 2-week-old rice seedlings under normal and salt treatment conditions. (d) Phenotype of rice seedlings treated with NaCl and ABA together. Rice seedlings were grown in 1/2 MS medium without or with ABA and 150 mM NaCl for 7 days, respectively, or with both ABA and 150 mM NaCl. (e) Primary root length of rice seedlings treated with NaCl and ABA together. Figures present the average primary root length from three independent experiments. Bars represent the SE (±) from three repeated assays. Asterisks indicate a significant difference. Significance was evaluated using the t-test (*P < 0.05 and **P < 0.01).
understand the roles of polysaccharide synthases in plant salt stress response.

OsCSLD4 plays a positive role in enhancing rice salinity stress tolerance

Studies revealed that cell wall polysaccharide synthesis or modification-related enzymes play critical roles in the response of plants to abiotic stresses (Corrêa-Ferreira et al., 2019; Hamann et al., 2009; Novaković et al., 2018; Tenhaken, 2014; Wang et al., 2019). For example, the mutation of cellulose synthase 6 (CESA6) or its companion protein CC1/2 (companion of cellulose synthase 1/2) disturbs cellulose synthesis and decreases Arabidopsis salt tolerance (Kesten et al., 2019; Zhang et al., 2016). Overexpression of hot pepper endotransglucosylase/hydrolase CaXTH3 enhances the tolerance of transgenic tomato and Arabidopsis to salt stress (Cho et al., 2006; Choi et al., 2011). Overexpression of hot pepper endotransglucosylase/hydrolase CaXTH3 enhances the tolerance of transgenic tomato and Arabidopsis to salt stress (Cho et al., 2006; Choi et al., 2011). The disruption of Arabidopsis XTH genes XTH19 and XHT23 decreased plant salt tolerance (Xu et al., 2020). Except for positive role in enhancing plant salt stress tolerance, some enzymes also play negative roles in plant salt stress tolerance. The overexpression of Arabidopsis β-1,4-galactan synthase GALACTAN SYNTHASE 1 (GALS1) decreased Arabidopsis salt tolerance (Yan et al., 2021). Previous studies have showed that OsCSLD4 plays an important role in the synthesis of rice cell wall polysaccharides (Li et al., 2009, 2010). Here, this study showed that the disruption of OsCSLD4 impaired rice salt and osmotic stress tolerance (Figure 2, 3 and Figure S3), and in contrast, the overexpression of OsCSLD4 enhanced rice salt stress tolerance (Figure 8), thus revealing the positive role of OsCSLD4 in rice salt stress tolerance. Previous study demonstrated that OsCSLD4 homolog AtCSLD5 also positively regulated Arabidopsis salt stress tolerance (Zhu et al., 2010), indicating the function of OsCSLD4 homologous proteins may be conserved in plant salt stress response.

OsCSLD4 enhances rice salt stress tolerance by regulating ABA synthesis to enhance plant osmotic stress tolerance

Studies showed that cell wall polysaccharide synthases have important roles in plant stress response, but the pathways of cell wall polysaccharide synthases involved in salt stress response are still obscure (Liu et al., 2021a; Novaković et al., 2018; van Zelm et al., 2020; Voxeur and Høfte, 2016; Zhao et al., 2021b; Zhu et al., 2010). Zhu et al. (2010) demonstrated that the OsCSLD4 Arabidopsis homolog AtCSLD5 may be involved in the salt stress response by regulating ROS scavenging to enhance Arabidopsis osmotic stress tolerance under high salt stress. Here, ROS (O$_2$•) content in the OsCSLD4-disrupted mutant nd1 was similar to that in wild-type plants during short-term salt treatment (Figure 3d,e). The sensitivity of nd1 to MV and H$_2$O$_2$ was also similar to that of the wild type (Figure S6), thus indicating that the decreased salt tolerance

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Figure 6 Relative expression levels of ABA synthesis and response-related genes in nd1 seedlings. (a) Relative expression levels of ABA synthesis genes in 2-week-old nd1 seedlings. (b) Relative expression levels of ABA-responsive and salt stress-related genes in 2-week-old nd1 seedlings. The expression of OsActin1 was used as an internal control. The figure presents the relative expression levels of genes relative to that in WT. Bars represent SE (±) from three repeated assays.
of nd1 is not due to impaired ROS scavenging ability. The Na⁺ and K⁺ contents in the roots and leaves of nd1 were also similar to those of the wild type before or after salt treatment (Figure S5), thus indicating that OsCSLD4 is not involved in the absorption and transport of ions in rice. In contrast, the sensitivity of nd1 to ABA is impaired (Figure 5a,b), and exogenous ABA can restore nd1 salt tolerance (Figure 5d,e), thus revealing that ABA plays a critical role in OsCSLD4-mediated rice salt stress response.

ABA plays an important role in regulating the response of plants to environmental stress (Sah et al., 2016; Vishwakarma et al., 2017; Zhu, 2002). Plants can induce ABA biosynthesis to stimulate stress-responsive gene expression to adapt to adverse environmental conditions (Sah et al., 2016; Vishwakarma et al., 2017; Wang et al., 2011b; Zhu, 2016). Studies demonstrated that cell wall composition and integrity can affect ABA synthesis and the ABA signalling (Chen et al., 2005; Hernández-Blanco et al., 2007; Liu et al., 2021a; Novaković et al., 2018; Palmeros-Suárez et al., 2017). For example, osmotic stress can induce ABA synthesis in the leaf discs of beans or barley, but this process fails in their protoplasts, thus indicating that the cell wall is essential for osmotic stress-induced ABA synthesis (Lahr and Raschke, 1988; Loveys and Robinson, 1987). Additionally, the cellulose synthase AtCesAB1/IRX1 allelic mutants lew2-1 and lew2-2 both exhibited increased expression levels of ABA synthesis genes and ABA-responsive genes, and accumulated more ABA (Chen et al., 2005; Hernández-Blanco et al., 2007), thus indicating that polysaccharide synthesis enzymes are closely related to ABA biosynthesis and ABA signalling. Here, the disruption of OsCSLD4 function decreased ABA synthesis (Figure 5c), and in contrast, overexpression of OsCSLD4 increased ABA content (Figure 8c). The expression levels of ABA synthesis-related genes were decreased and enhanced in nd1 and OsCSLD4 overexpression plants, respectively, thus indicating that OsCSLD4 plays an important role in mediating ABA content by regulating the expression of ABA synthesis genes. The analysis of gene transcript level showed that amounts of ABA-responsive genes, such as OsRAB16B (Os11g0454200), OsLEB24 (Os01g0702500) and OsRAB16C, can also be obviously induced by salt stress in nd1 (Tables S2 and S3). Additionally, exogenous ABA can restore nd1 salt tolerance (Figure 5d,e). These results indicate that OsCSLD4 may be involved in salinity stress response by mediating ABA content but not the activity of ABA signalling pathway in rice. Moreover, the disruption of OsCSLD4 decreased rice seedlings’ osmotic stress tolerance, which indicated that OsCSLD4 is involved in rice high salt adaptation by modulating rice osmotic stress tolerance.

OsCSLD4 plays a positive role in coordinating rice salinity stress tolerance and grain growth

The cell wall-localized proteins play critical roles in coordinating plant salt stress responses and growth and development (Liu et al., 2021a). Several plasma membrane-localized cell wall...
integrity sensors have been identified that perceive cell wall changes and are involved in plant salt stress responses and growth and development. Wall-associated kinases (WAKs), which can be cross-linked with pectin, are a family of receptor-like Ser/Thr kinases (Decreux and Messiaen, 2005). WAKs can coordinate plant salt stress response and growth. For example, the mutant of barley WAK protein HvWAK1 obviously impaired salt stress tolerance and retarded root growth under salt stress (Kaur and Singh, 2013). The tomato WAK mutant Siwak1 disrupted osmotic homeostasis and reduced plant shoot growth under salt stress (Meco et al., 2020). The rapid alkalinization factors (RALFs) are a type of small signalling peptides. RALFs play important roles in growth and development, and are involved in stress responses (Liu et al., 2021b; Murphy and De Smet, 2014). In Arabidopsis, the constitutive expression of ATRALF8 increased transgenic plant susceptibility to drought stress (Atkinson and Lilley, 2013). The overexpression of ATRALF22 and ATRALF23 can bind to leucine-rich repeat extensins (LRXs) or receptor-like kinases, such as FERONIA (FER), THESEUS1 (THE1), to control plant growth and salt stress responses by modulating the synthesis and signalling of phytohormones, such as ABA and jasmonic acid (Chen et al., 2016; Yu et al., 2012; Zhao et al., 2021a).

Previous studies have revealed that OsCSLD4 is involved in the synthesis of cell wall xylan, cellulose and homogalacturonan and has pleiotropic effects on the growth and development of the leaf, stem and root (Hu et al., 2010; Li et al., 2009; Luan et al., 2013). The constitutive expression of OsCSLD4 in rice increased salt tolerance (Meco et al., 2020). The overexpression of OsCSLD4 enhanced rice salt tolerance by up-regulating ABA synthesis (Figure 8). (a) Phenotype of OsCSLD4 overexpression rice seedlings treated with salt stress in soil. Two-week-old rice seedlings grown in soil were treated with 150 mM NaCl for 7 days with another 7-day recovery. (b) Survival rate of OsCSLD4 overexpression rice seedlings treated with 150 mM NaCl for 7 days with another 7-day recovery. (c) ABA content in 2-week-old OsCSLD4 overexpression rice seedlings. (d) Expression level of ABA synthesis and responsive genes in 2-week-old OsCSLD4 overexpression transgenic rice seedlings. The expression of OsActin1 was used as the internal control. The gene expression levels in transgenic rice seedlings were presented as relative to the levels in the wild type (Kitaake). Kitaake: wild type. OE: independent OsCSLD4 overexpression lines. ‘x’ indicates the numbering of different OsCSLD4 overexpression transgenic lines. The figure presents the average of at least three repeated experiments. Bars indicate the SE ±) from three repeated experiments. Asterisks represent significant difference as evaluated by t-test (*P < 0.05 and **P < 0.01).
et al., 2011; Yoshikawa et al., 2013; Figure 2a and Figures S2a, S4b). The present study showed OsCSLD4 also played a positive role in rice growth. Although the mechanism of its synergistic regulation of rice salt stress response and growth and development needs to be further studied in detail, the results from the transcriptome analysis showed the expressions of WAKs gene OsWAK76 (Os08g0501700, WALL-ASSOCIATED KINASE GENE 76) and RALFs gene RALFL6 (Os01g0358100, Rapid Alkalization Factor 6) in OsCSLD4 mutant nd1 are obviously impaired under both normal and salt stress conditions (Table S3), indicating OsCSLD4 may coordinate rice salt stress response and growth and development by modulating the homeostasis of phytohormones via these cell wall-localized proteins.

Taken together, this study demonstrated that the cellulose synthase-like protein OsCSLD4 plays important roles in rice coping with salinity stress by mediating ABA synthesis to enhance osmotic stress tolerance. Considering the profound influence of OsCSLD4 on cell wall polysaccharide composition and structure, we believe that the cell wall polysaccharide composition or structure altered by OsCSLD4 is critical for rice to regulate the action of cell wall-localized proteins, which further modulate intracellular signal to regulate ABA synthesis to adapt to salt stress and adjust growth and developmental processes (Figure 9).

Materials and methods

Plant materials and growth conditions

The rice indica variety Zhongxian3037 and its mutant nd1 (Li et al., 2009), japonica variety Zhonghua 11 and its mutant nr1 (Wu et al., 2010), japonica variety Kitaake and OsCSLD4 overexpression transgenic plants (OE1, OE2 and OE3, in Kitaake background) were used in this study. Unless otherwise specified, wild type (WT) refers to Zhongxian3037. All rice seedlings were grown at 28 °C under a 14-h light/10-hr dark photoperiod. For rice seedlings grown on 1/2 Murashige & Skoog (MS) medium, rice seeds were first sterilized with 75% alcohol for approximately 2 min, treated with 2.5% sodium hypochlorite for 20–30 min, washed with sterile water and then planted on 1/2 MS medium with or without different exogenous reagents.

Phylogenetic analysis

We used the OsCSLD4 full-length amino acid sequence to identify homologous sequences using the Blastp search program of the National Center for Biotechnology Information (NCBI, www.ncbi.nlm.nih.gov). All of the sequences in the CSLD subfamily from rice (Oryza sativa), Arabidopsis (Arabidopsis thaliana) and maize (Zea mays) were used to construct a phylogenetic tree using MEGA 5.0 with 500 bootstrap repetitions. The accession numbers of the CSLDs used in this study are At2g33100 (AtCSLD1), At5g16910 (AtCSLD2), At3g03050 (AtCSLD3), At4g38190 (AtCSLD4), At1g02730 (AtCSLD5), At1g32180 (AtCSLD6), GRMZM2G436299 (ZmCSLD1), GRMZM5G870176 (ZmCSLD2), GRMZM2G044269 (ZmCSLD3), GRMZM2G015886 (ZmCSLD4), GRMZM2G061764 (ZmCSLD5), Os10g042750 (OsCSLD1), Os06g02180 (OsCSLD2), Os08g25710 (OsCSLD3), Os12g36890 (OsCSLD4) and Os06g22980 (OsCSLD5).

Treatments using NaCl, PEG and LiCl

To analyse the expression level of OsCSLD4 under salt stress, 2-week-old rice seedlings were sprayed with 150 mm NaCl for different time periods. To study the salt stress tolerance of rice seedlings in soil, 2-week-old rice seedlings grown in pots under normal conditions were transferred into trays containing 150 mm NaCl solution for 7 days. Then, the rice seedlings were removed from the NaCl solution and grown under normal conditions. The survival rate of rice seedlings was determined after another 7-day recovery period. To study the effect of OsCSLD4 on the salt tolerance of rice roots, the germinated rice seeds were then planted on 1/2 MS with or without 150 mm NaCl for another 7 days. The seedling phenotype was photographed, and the length of rice seedling primary roots was measured after 7 days of salt treatment.

To study the effect of OsCSLD4 on rice osmotic tolerance, the germinated seeds were planted on 1/2 MS with or without 10% (w/v) PEG (polyethylene glycol 6000) for 10 days. Photos of the seedling phenotype were taken, and the length of the primary roots was measured after 10-day PEG treatment.

To analyse the role of OsCSLD4 in rice iron stress tolerance, germinated rice seeds were grown on 1/2 MS or 1/2 MS supplemented with 18 mm LiCl for another 7 days. The seedling phenotype was photographed, and the length of rice seedling primary roots was measured after a 7-day LiCl treatment.

Transcriptome analysis

Two-week-old 3037 and nd1 plants were cultivated, treated and inoculated as described above. Three biological replicates (each consisting of pooled leaves from ~20 plants from one pot) were collected after 2 d of treatment and then snap-frozen in liquid N2 for total RNA extraction. The mRNA library preparation and sequencing were outsourced (Allwegene Tech., Beijing, China). The 12 libraries were sequenced on an Illumina HiSeq 2500 platform, and paired-end 150-bp reads were generated. Reads containing greater than 10% poly-N and greater than 50% low-quality reads (Q < 20) were removed from the raw data using Trimmomatic v0.33 (Bolger et al., 2014). Concurrently, the Q20 and Q30 values, GC content and sequence duplication levels of the clean data were calculated. All downstream analyses were based on clean and high-quality data. RNA-Seq reads were aligned to the rice reference genome (Oryza sativa Japonica Group, Ensembl_IRGSP_1.0.34) using TopHat 2 (v2.1.0) (Trapnell et al., 2012). Each read was mapped with Cufflinks (v2.1.1), a program that assembled the alignments in the Sequence Alignment/Map format into transfrags (Trapnell et al., 2012). The assembly files were then merged with reference transcriptome annotations for further analysis (Trapnell et al., 2012). The differential expression of each gene was calculated by quantifying the illumina reads based on fragments per kilobase of transcript per million fragments mapped (FPKM; Mortazavi et al., 2008). Gene Ontology (GO, http://www.geneontology.org/) enrichment analysis of the DEGs was performed using GOseq (v 1.22) (Young et al., 2010) using Wallenius’ noncentral hypergeometric distribution that can adjust for gene length bias in DEGs.

ABA treatment

To determine the expression level of OsCSLD4 that was induced by ABA, the leaves of 2-week-old rice seedlings were sprayed with 100 μM ABA for different time periods. To analyse the role of ABA in salt-induced OsCSLD4 expression, 2-week-old rice seedlings were sprayed with 100 μM ABA and 150 mm NaCl for different durations.

To analyse the sensitivity of OsCSLD4 to exogenous ABA, rice seeds were sterilized and germinated, and the germinated rice
seeds were then planted on 1/2 MS medium or 1/2 MS medium with 2 μM ABA for another 7 days. The seedling phenotype was photographed, and the length of primary roots was measured after a 7-day ABA treatment.

To study the effect of ABA on OsCSLD4-regulated salt tolerance, the germinated rice seeds were planted on 1/2 MS medium or 1/2 MS medium with 0.05 μM ABA, 150 mM NaCl alone or with 150 mM NaCl plus 0.05 μM ABA for another 7 days. Photos of the seedling phenotype were taken, and the length of the primary roots was measured after a 7-day treatment.

Measurement of MDA content
To measure the MDA content of rice seedlings, approximately 0.2 g of leaf tissue was homogenized with 1.5 mL of 10% (w/v) trichloroacetic acid. After centrifugation, approximately 1 mL of supernatant was transferred into a clean 15-mL collection tube containing 0.25% (w/v) thiobarbituric acid in 10% (w/v) trichloroacetic acid. The samples were incubated at 100 °C for 30 min, cooled to ambient temperature and centrifuged at a speed of 1 g for 15 min. The absorbance of the supernatant was measured at 450, 532 and 600 nm. The MDA content was calculated using the following equation: 6.45×(OD532-OD600)-0.559×OD450 (Qin et al., 2016).

Measurement of Na⁺ and K⁺ content
To test the Na⁺ and K⁺ content in rice shoots and roots, 2-week-old rice seedlings were grown in 1/2 MS liquid medium with or without 150 mM NaCl for 7 days. The shoots and roots of rice seedlings were collected, rinsed with deionized water and dried at 80 °C in paper bags to a constant weight. The weight of each sample was then measured. Subsequently, these samples were incinerated in a furnace at 575 °C for 9 h, dissolved in 0.1N hydrochloric acid for 4 h and diluted 50 times with 0.1N hydrochloric acid. K⁺ and Na⁺ concentrations were determined using atomic absorption spectrophotometry (SpectrAA-10; Springvale, Australia).

Measurement of ABA content
ABA content was measured according to the ELISA method described by Yang et al. (2001) with some modifications. Approximately 200 mg of frozen 2-week-old rice seedlings were ground to a fine powder with liquid nitrogen. The powder was then transferred into a covered, siliconized borosilicate tube containing 500 μL extraction buffer (90% [v/v] methanol and 200 mg/L sodium diethyldithiocarbamate trihydrate) and mixed thoroughly. The samples were centrifuged at 8000 g for 10 min at 4 °C after incubation at 4 °C for approximately 8 h. The supernatant was transferred to a new pre-cooled tube and
OsCSLD4 regulates salt stress tolerance of rice via ABA

481

evaporated at 4 °C to dry. The residue was dissolved in 500 μL methanolic Tris-HCl buffer (50 mM, pH 7.4), and the ABA content was measured using an ELISA kit according to the manufacturer’s instructions (Enzyme-linked Biotechnology, Shanghai, China; No: mi077235).

O2− staining

To assess the O2− content, 2-week-old rice seedlings were watered with or without 150 mM NaCl for 1 h or 12 h. The rice leaves were then incubated in 25 mmol HEPES buffer (pH 7.6) containing 1% nitrotetrazolium blue chloride (NBT) in the dark at 25 °C for 12 h. Next, these leaves were destained with 80% ethanol (v/v) to remove chlorophyll until clear blue deposits were observed. The detail process was described by Qin et al. (2016).

Quantitative real-time PCR (qPCR)

Total RNA was extracted using TRizol solution (Tiangen, Beijing, China). Next, 2 μg of total RNA was reverse transcribed into cDNA using oligo (dT) as primers and M-MLV as reverse transcriptase (Toyobo, Osaka, Japan). qPCR was performed using 2X SYBR Green mix (Takara, Cat No. 330523) with IQC according to the manufacturer’s instructions (Bio-Rad, iQ5). The expression levels of other genes were normalized using the comparative Ct method (Livak and Schmittgen, 2001).

Genetic transformation in rice

To generate rice plants that limit the expression of OsCSLD4, we used RNA interference (RNAi) technology to decrease its expression in japonica variety Kitaake. One artificial microRNA (amiRNA) sequence (amiRNA-CSLD4, 5′-TAAGAATCTTCTTACTGAACTG-3′) targeting OsCSLD4 transcript was selected among those proposed by the WMD3 program (web MicroRNA designer; http://wm3.weigelworld.org/). This special amiRNA-OsCSLD4 will be in pair to the amiRNA exactly mimic the foldback structure of the endogenous osa-MiR528. The fusion amiRNA-CSLD4 product was further cloned into vector pCAMBIA 5300 containing the Kpn I and BamH I site, placing the DNA sequence of the amiRNA precursor under control of the regulatory region of the maize Ubiquitin promoter. The detailed process was described earlier (Warthmann et al., 2008). The plant expression vectors were transformed into Agrobacterium tumefaciens strain LBA4404. Then, the resultant plasmid was introduced into Kitaake using Agrobacterium mediated transformation. The transformed plants were selected by hygromycin. The efficiency and specificity of the RNAi lines were confirmed using real-time quantitative PCR. The T2 transgenic lines were used in this study.

To create OsCSLD4 overexpression transgenic rice plants, the full-length ORF of OsCSLD4 was cloned into vector pCAMBIA 1307 using Xba I and BamH I sites. The recombinant plasmid was then transferred into Agrobacterium tumefaciens strain LBA4404. OsCSLD4 was introduced into the japonica variety Kitaake using Agrobacterium-mediated transformation. OsCSLD4 overexpression transgenic lines were selected using hygromycin and confirmed by qPCR. The detailed selection process for transgenic plants was described by Qin et al. (2016). Homozygous T3 transgenic lines were used in this study.

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Author contributions

H.Z., Z.L. and Y.W. performed most experiments. M.X. and R.Q. performed the construction of OsCSLD4 overexpression transgenic plants. J.W. measured grain traits. R.H., L.Z. and Z.Z. designed experiments and wrote the manuscript. H.L. and H.Z. edited the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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Hui Zhao et al.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.