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RESEARCH PAPER

Two SLENDER AND CRINKLY LEAF dioxygenases play an essential role in rice shoot development

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Abstract

It is clear that 2-oxoglutarate-dependent dioxygenases have critical functions in salicylic acid (SA) metabolism in plants, yet their role in SA biosynthesis is poorly understood. Here, we report that two dioxygenase-encoding genes, *SLENDER AND CRINKLY LEAF1* (*SLC1*) and *SLC2*, play essential roles in shoot development and SA production in rice. Overexpression of *SLC1* (SLC1-OE) or *SLC2* (SLC2-OE) in rice produced infertile plants with slender and crinkly leaves. Disruption of *SLC1* or *SLC2* led to dwarf plants, while simultaneous down-regulation of *SLC1* and *SLC2* resulted in a severe defect in early leaf development. Enhanced SA levels in SLC1-OE plants and decreased SA levels in *slc1* and *slc2* mutants were observed. Accordingly, these lines all showed altered expression of a set of SA-related genes. We demonstrated that SLC1 interacts with homeobox1 (OSH1), and that either the knotted1-like homeobox (KNOX1) or glutamate, leucine, and lysine (ELK) domain of OSH1 is sufficient for accomplishing this interaction. Collectively, our data reveal the importance of SLC1 and SLC2 in rice shoot development.

Keywords: Homeobox1, OsGA20ox, 2-oxoglutarate-dependent dioxygenase, rice, salicylic acid, shoot development.

Introduction

The 2-oxoglutarate-dependent dioxygenase (2OGD) superfamily is the second largest enzyme family in plants, and its members are involved in various oxygenation/hydroxylation reactions. They require 2-oxoglutarate (2OG) and molecular oxygen as co-substrates, and ferrous iron Fe(II) as a cofactor to catalyse the oxidation of different substrates (Prescott and John, 1996). Previous studies have shown that 2OGDs have oxidative activity on some compounds involved in plant hormone metabolism. For instance, in later reactions of gibberellin (GA) metabolism, activities of several 2OGDs, including Gibberellin 20-Oxidases (GA20oxs), Gibberellin 3-Oxidases (GA3oxs), and Gibberellin 2-Oxidases (GA2oxs), are essential (Hedden and Thomas, 2012; Hedden and Sponsel, 2015). The Green Revolution gene *Semidwarf-1* (*Sd-1*), identified by analysing a loss-of-function mutant of *OsGA20ox2*, has been utilized for breeding and has achieved great success in increasing grain production (Monna *et al.*, 2002; Sasaki *et al.*, 2002; Spielmeyer *et al.*, 2002). Enhancing expression of *Grain Number per Panicle1* (*GNP1*)/*OsGA20ox1* in inflorescence meristems results in higher grain number and grain yield (Wu *et al.*, 2016). The rice *DIOXYGENASE FOR AUXIN OXIDATION* (*DAO*) and its Arabidopsis homologues, *AtDAO1* and *AtDAO2*, encode 2OGDs that convert active indole-3-acetic acid (IAA) into biologically inactive OxIAA, serving as a mechanism to fine tune auxin levels in rice and Arabidopsis (Zhao *et al.*, 2013; Porco *et al.*, 2016; Zhang *et al.*, 2016). 2OGDs also play critical roles in

the inactivation of salicylic acid (SA) and jasmonic acid (JA). In Arabidopsis, SA 5-HYDROXYLASE/DOWNY MILDEW RESISTANT 6 (S5H/DMR6) and SA 3-HYDROXYLASE/DMR6-LIKE OXYGENASE 1 (S3H/DLO1) are 2OGDs that both hydroxylate SA (Zhang *et al.*, 2013, 2017). In addition, the JA oxidases (JAOs) or jasmonate-induced oxygenases (JOXs) belong to the 2OGD superfamily and catalyse specific oxidation of JA to 12OH-JA in Arabidopsis (Caarls *et al.*, 2017; Smirnova *et al.*, 2017). GERMINATION INSENSITIVE TO ABA MUTANT 2 (GIM2), another 2OGD member in Arabidopsis, oxidizes GA_{12} and influences GA-mediated seed germination (Xiong *et al.*, 2018).

Salicylic acid (2-hydroxybenoic acid) is a phenolic plant hormone that plays an essential role in plant growth and development, including seedling establishment, leaf senescence, and disease resistance (Vlot *et al.*, 2009; Rivas-San Vicente and Plasencia, 2011). Functioning as an endogenous signal mediating local and systemic plant defensive responses against pathogens, the function of SA is significant. Application of exogenous SA activates plant *pathogenesis-related* (*PR*) genes and induces systemic acquired resistance (SAR) in plants (Ward *et al.*, 1991). The biosynthesis of SA in plants is not yet fully understood, however two pathways have been proposed: the phenylalanine ammonia lyase (PAL) pathway and the isochorismate synthase (IC) pathway. In the PAL pathway, plants synthesize SA from cinnamate produced by PAL (Lee *et al.*, 1995; Ribnicky *et al.*, 1998). In the IC pathway, SA is synthesized from chorismate through two reactions catalysed by isochorismate synthase (ICS) and isochorismate pyruvate lyase (IPL) (Strawn *et al.*, 2007; Mustafa *et al.*, 2009). To maintain optimal SA levels for growth and development, plants adopt a direct and efficient approach by controlling hormone biosynthesis and catabolism. In the Arabidopsis genome there are two *ICS* genes, *ICS1*, also known as *SA INDUCTION DEFICIENT 2* (*SID2*), and *ICS2* (Wildermuth *et al.*, 2001). In rice (*Oryza sativa*), one *ICS* gene has been identified and its role in SA biosynthesis is characterized (Choi *et al.*, 2015). The most studied SA biosynthetic pathway in rice is the PAL pathway (Sawada *et al.*, 2006). The SA glucosyltransferases, which convert SA into SA *O*-β-glucoside (SAG), have been identified in both Arabidopsis and rice (Dean and Delaney, 2008; Umemura *et al.*, 2009). SA is hydroxylated at the C5 or C3 position of its phenyl ring, leading to the formation of 2,5-Dihydroxybenzoic acid (2,5-DHBA) or 2,3-Dihydroxybenzoic acid (2,3-DHBA), a process catalysed by DMR6 or DLO1, respectively (Zhang *et al.*, 2013, 2017). Usually, disruption of SA homeostasis leads to changes in pathogen resistance and plant development. Mutants that over-accumulate SA, such as *constitutive expresser of PR genes 1* (*cpr1*), *cpr5*, *cpr6-1*, *defense no death 1* (*dnd1*), and the *s5hs3h* double mutant, display a significant reduction in rosette leaf size and have constitutive activation of SAR (Bowling *et al.*, 1994; Bowling *et al.*, 1997; Clarke *et al.*, 1998; Yu *et al.*, 1998; Jirage *et al.*, 2001; Zhang *et al.*, 2017). Although many metabolites in the conjugation and catabolic pathways of SA in plants have been identified, the mechanism for controlling SA homeostasis in rice remains elusive.

In this study, we determined that *SLC1* and *SLC2* are homologous genes in the dioxygenase family and play important roles in the growth and development of rice. Altered expression of *SLC1* and/or *SLC2* produced a significant perturbation in shoot development. Increases in SA levels were achieved by overexpression of *SLC1* in rice, and the involvement of SLC1 and SLC2 in maintaining the balance of SA levels was demonstrated. The interaction between SLC1 and OSH1 suggests a role for SLC1 as part of an important regulatory mechanism in rice development.

Materials and methods

Phylogenetic analysis

The rice GA20ox protein sequences (Han and Zhu, 2011) were obtained from the Rice Genome Annotation Project (https://rice.plantbiology.msu.edu) and aligned using the Clustal W multiple sequence alignment program (Larkin *et al.*, 2007). The phylogenetic tree of the alignment was generated by the MEGA software (Molecular Evolutionary Genetic Analysis, version 6.0) (Tamura *et al.*, 2013), using the neighbour-joining method with bootstrapping based on 1000 replicates.

Plant material and growth conditions

Except for the *slc2* mutants which were generated in the *Oryza sativa* L. *japonica. cv. Nipponbare* (Nip) background, other rice plants were in the *O. sativa* L. *japonica. cv. Hejiang 19* (HJ) background. Rice transgenic lines were generated via *Agrobacterium*-mediated transformation and the transformants were selected by screening for resistance to Hygromycin B (H397, Phytotech) (Nishimura *et al.*, 2006). The *slc1* and *slc2* mutants were generated by BioRun (https://www.biorun.net), using CRISPR (clustered regularly interspaced short palindromic repeats)/Cas9 targeted-genome editing (Feng *et al.*, 2013). All rice plants were grown in pots and placed either in a controlled growth chamber at 24–32 °C with photoperiod 12 h light/12 h dark, or outdoors from April to October in Wuhan, China. Arabidopsis transgenic lines (expressing rice genes) were generated in the Columbia-0 (Col) background using the floral dip method (Clough and Bent, 1998) and the transformants were selected by screening for resistance to Glufosinate-ammonium (45520, Sigma-Aldrich). All selected plants were grown in a growth room at 23 °C with photoperiod 16 h light/8 h dark.

Plasmid construction

The control (empty) vector pCAMBIA1300-YFP was made by inserting the yellow fluorescent protein (YFP) fragment into pCAMBIA1300 (Addgene, https://www.addgene.org), driven by a maize (*Zea mays*) ubiquitin promoter. The plasmids pCAMBIA1300-YFP-SLC1 and pCAMBIA1300-YFP-SLC2 were constructed by cloning the full-length coding sequences (CDS) of *SLC1* and *SLC2* into pCAMBIA1300-YFP, respectively. To generate the transgenic rice lines expressing *SLC1-N* or *SLC1-C*, the fragments *SLC1-N* (+1 bp to +120 bp) and *SLC1-C* (+121 bp to +1152 bp) were inserted into pCAMBIA1300-YFP, respectively.

The plasmids pBA002-SLC1, pBA002-SLC2, and pBA002-OsGA20ox2 were made by cloning the full-length CDS of *SLC1*, *SLC2*, and *OsGA20ox2*, respectively, into the binary vector pBA002 (Kost *et al.*, 1998). To generate the RNAi transgenic lines co-silencing *SLC1* and *SLC2*, pCAMBIA1300-SLC1-SLC2-RNAi was constructed by inserting the dsRNAi cassette containing the 500 bp fragment (+1 bp to +500 bp) of *SLC1* and 420 bp fragment (+211 bp to +630 bp) of *SLC2*, into pCAMBIA1300 in frame. To generate transgenic plants carrying pCAMBIA1300-SLC1pro-GUS, the promoter fragment consisting of 1.9 kb upstream of the start codon of *SLC1* was amplified from the genomic DNA of HJ, fused to the β-glucuronidase (GUS) gene, and then inserted into pCAMBIA1300. For analysing subcellular localization via transient expression assays in rice protoplasts, p35S-YFP-SLC1, p35S-YFP-SLC2, and p35S-CFP-DLT were constructed by following the method previously described (Lu *et al.*, 2013; Zhao *et al.*, 2015; Yin

et al., 2019). For the Bimolecular Fluorescence Complementation (BiFC) assay, CDS fragments of *OSH1*, *OSH6*, *OSH15*, *OSH71*, or *SLC1* were cloned into the vector p35S-MCS-YN or p35S-MCS-YC (Zhao *et al.*, 2015).

For expressing His-tagged recombinant SLC1 in *Escherichia coli* (*E. coli*), the CDS of *SLC1* was cloned into pET28a (Novagen, USA). For expressing GST-tagged recombinant OSH1, the CDS of *OSH1* was inserted into pGEX-4T1 (GE Healthcare, USA). For expressing the MBP-tagged recombinant proteins for enzyme assays, the CDS of *SLC1*, *SLC2* or *OsGA20ox2* were cloned into pET28a (Novagen, USA).

For yeast two-hybrid (Y2H) assays, the CDS of *OSH1*, *OSH6*, *OSH10*, *OSH15*, or *OSH71* were cloned into pGADT7 (AD, Clontech). Truncated-fragments of *OSH1* were cloned into pGADT7. The CDS of *SLC1*, *SLC2*, or *OsGA20ox2* were cloned into pGBKT7 (BD, Clontech). The truncated-fragments of *SLC1* were cloned into pGBKT7. The primer sequences used for plasmid construction are listed in Supplementary Table S1 at *JXB* online.

RNA extraction and gene expression analysis

Total RNA was extracted using the EASYspin Plus Plant RNA Kit (RN38, Aidlab) or TRIzol reagent (Invitrogen, USA) by following the manufacturer's instructions. RNA samples were reverse-transcribed with ReverTra Ace-α-® (Toyobo, Japan). Real time-quantitative PCR (RT–qPCR) was performed using Advanced SYBR Green supermix (Bio-Rad) with the CFX connect real-time PCR detection system 185–5201 (Bio-Rad), and relative gene expression was analysed using the CFX manager software (Bio-Rad). For semi-quantitative RT-PCR, initial denaturation was conducted at 95 °C for 3 min, followed by 30 cycles of denaturation at 94 °C for 20 s; annealing at 56 °C for 30 s and elongation at 72 °C for 90 s. The primer sequences used for analysing gene expression are listed in Supplementary Table S2.

Enzyme assay and measurement of hormone and metabolite levels

The enzyme assay was performed according to a previously described method (Zhao *et al.*, 2013; Xiong *et al.*, 2018). The substrate GA_{12} or GA_{53} (OlChemIm, Czech Rep) was incubated with the cell lysate of *E. coli* expressing MBP-SLC1, MBP-SLC2, or OsGA20ox2, in a total reaction volume of 100 μL. The reaction was incubated at 30 °C for 3 h with gentle agitation. The reaction solution contained 100 mM Tris-HCl (pH 7.0) and cofactor mixture (5 mM 2-oxoglutarate, 5 mM L-ascorbate, and 0.5 mM $FeSO_4$). Acetic acid (10/150, v/v) was added to stop the reaction. The reaction mixture was then lyophilized and eluted with elution buffer containing 510 μL H_2O/ACN (acetonitrile) (10/500, v/v). After elution, the sample was evaporated under nitrogen gas and re-dissolved in elution buffer containing 100 μL H_2O/ACN (90/10, v/v). Samples were analysed as described in previous reports (Chen et al., 2013; Xiong et al., 2018). To measure hormone levels, each sample was prepared from 50 mg (FW) plant tissue. The experiment was carried out according to the method described previously (Wang *et al.*, 2011; Chen *et al.*, 2012). GA, SA, JA, IAA and abscisic acid (ABA) levels were measured using a microTOFq orthogonal-accelerated TOF mass spectrometer (Bruker Daltonics, Germany). To measure the levels of precursors in the SA biosynthetic pathways, 200 mg (FW) of aerial tissue was collected from 57-day-old plants of the SLC1#1 and SLC2#1 Arabidopsis lines. The quantification was performed by Biotree (https://www.biotree.cn).

Yeast two-hybrid assay and transcriptional activation assay

The Y2H assay was performed by following the instructions of the Matchmaker GAL4-based two-hybrid system (Clontech, https://www.clontech.com), using the AH109 yeast strain. The transcriptional activation assay was carried out by following the method described by Zhao *et al.* (2018). Briefly, yeast cells (AH109 strain) were transformed and incubated at 30 °C for 3 days. Each yeast liquid suspension was diluted to an absorbance of 0.5 at OD600, and 2 μL of each dilution was then inoculated onto Trp-, His-, and Ade-negative synthetic dropout medium.

Protein pull-down assay

The pull-down experiments were performed as in previously described reports (Wu *et al.*, 2013; Zhao *et al.*, 2015; Yin *et al.*, 2019). Glutathione sepharose beads (GE Healthcare, USA) were used to purify the GST-OSH1 protein, and the His-SLC1 protein was purified by following the protocol described by Clontech. Purified GST-OSH1 was incubated with His-SLC1 in binding buffer (20 mM Tris–HCl, pH 7.5, 150 mM NaCl, 10% glycerol, 0.1% Triton X-100, 5 mM $MgCl_2$, 1 mM EDTA) overnight at 4 °C. To remove unbound protein, the beads were collected by centrifugation at 700 g for 5 min and then washed five times with binding buffer. After washing, the collected protein (pellet) was re-suspended in 50 μL SDS-PAGE sample buffer. Samples were boiled, briefly spun down, and the supernatants separated on a 12% SDS-PAGE gel with subsequent immunoblotting with anti-His or anti-GST antibodies (1:3000) (ABclonal, China).

Results

Overexpression of SLC1 *or* SLC2 *in rice perturbs shoot development*

To elucidate the function of dioxygenase-encoding genes involved in rice development, we analysed the phylogenetic relationship of putative GA20oxs in rice (Supplementary Fig. S1A), and investigated further the divergent trait of OsGA20ox7 (LOC_Os08g44590). We used the protein sequence of OsGA20ox7 in a BLAST search of the MSU Rice Genome Annotation Project Release 7 (https://rice.plantbiology.msu.edu) and found that the Os09g0570800 (LOC_Os09g39720) locus shares 54.6% amino acid sequence identity with OsGA20ox7, contains a conserved Fe(II) 2OG dioxygenase domain (Supplementary Fig. S1B), and both are classified into the same clade of DOXC37 (Kawai *et al.*, 2014).

We generated transgenic rice lines overexpressing *OsGA20ox7* or *Os09g0570800* in the *Hejiang 19* (HJ) background (Fig. 1). The growth of T_0 plants regenerated from calli overexpressing *OsGA20ox7* was severely perturbed (Fig. 1A–F). After 14 d of regeneration, the leaves of these plants were slender and crinkly (Fig. 1B), therefore we renamed *OsGA20ox7* as *SLENDER AND CRINKLY LEAF1* (*SLC1*). After 30 d of regeneration, shoot growth of *SLC1* overexpressing (SLC1-OE) plants was substantially promoted but root elongation was repressed (Fig. 1C). After 40 d of regeneration, peculiar leaf blades and crinkly leaves were prominent; in severe cases, no leaf was formed in the leaf sheath (Fig. 1D). After 60 d of regeneration (heading stage), the SLC1-OE plants retained slender and taller architecture (Fig. 1E); young panicles were wrapped in the leaf sheath and seeds did not develop in any of these T_0 plants (Fig. 1F). As a result, we performed additional rounds of transformation, and in total, over 100 T_0 SLC1-OE plants were obtained and examined. Unfortunately, all of these plants developed abnormally and no seeds were produced. Similar phenotypes were also produced in the plants overexpressing *Os09g0570800*, including the slender and crinkly leaves (Fig. 1G, H). Hence, we named *Os09g0570800* as *SLENDER AND CRINKLY LEAF2* (*SLC2*). Together, these results suggest an important role for SLC1 and SLC2 in the growth and development of rice.

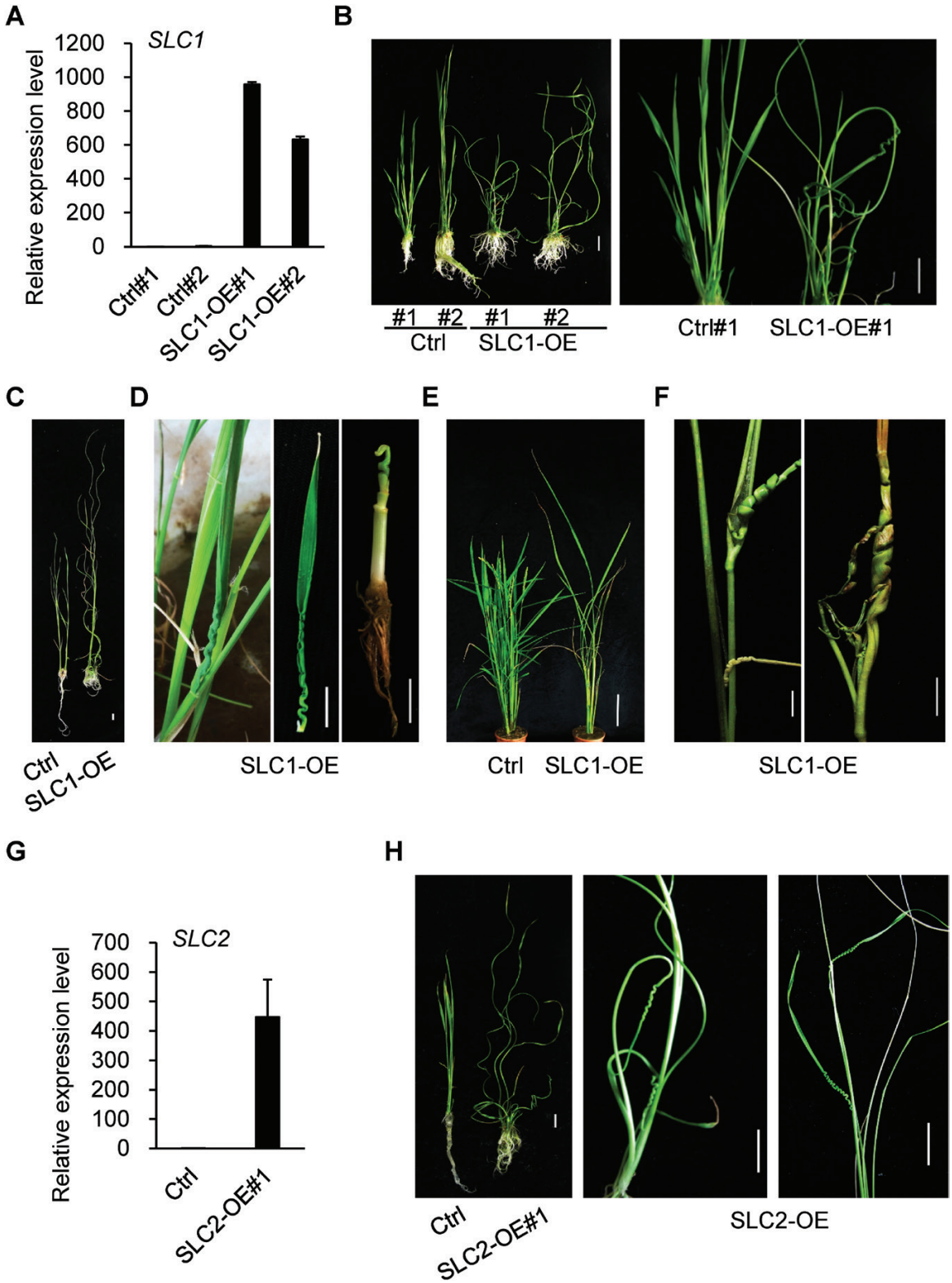

Fig. 1. Overexpression (OE) of *SLENDER AND CRINKLY LEAF1* (*SLC1*) or *SLC2* in rice produces plants with slender and crinkly leaves. (A) Expression level of *SLC1* in SLC1-OE#1 and SLC1-OE#2 T_0 plants was quantified and compared with that of control (Ctrl) plants expressing the empty vector (Ctrl#1 and Ctrl#2). All plants were in the *Hejiang 19* background. The expression of *OsACTIN1* (*Os03g0718100*) was used as the internal control. (B) Slender and crinkly leaves were observed in SLC1-OE T_0 plants but not in Ctrl, after 14 d of regeneration. Scale bars: 1 cm. (C) Taller shoots and shorter roots were observed in SLC1-OE T_0 plants compared to Ctrl, after 30 d of regeneration. Scale bar: 1 cm. (D) Crinkly leaves were observed in SLC1-OE T_0 plants, after 40 d of regeneration. Scale bars: 1 cm. (E) After 60 d of regeneration, slender but taller SLC1-OE T_0 plants were observed. Scale bar: 10 cm. (F) After 60 d of regeneration, twisted leaves and twisted young panicles enclosed in the leaf sheath of SLC1-OE T_0 plants were observed. To visualize the young panicles, the leaf sheath was opened slightly. Scale bars: 1 cm. (G) Expression of *SLC2* was quantified in regenerated plants overexpressing *SLC2* (SLC2-OE#1, T_0). The expression of *OsACTIN1* was used as the internal control. (H) Phenomena such as crinkly, slender and twisted leaves were observed in the SLC2-OE plants, after 14 d of regeneration. The control (Ctrl) plantlet harboring the empty vector displayed a normal growth phenotype. Scale bars: 1 cm.

Expression of SLC1 *or* SLC2 *in Arabidopsis produces dwarf plants*

As rice plants overexpressing *SLC1* or *SLC2* produced no seed, we generated transgenic Arabidopsis plants expressing rice *SLC1* or *SLC2*, and expression of the transgenes was confirmed using semi-quantitative PCR (Fig. 2A). These Arabidopsis lines were morphologically defective, displaying shorter roots (Supplementary Fig. S2A), reduced stature (Fig. 2B, C), and greener leaves (Supplementary Fig. S2B). The chlorophyll content in leaves of 30-day-old plants of SLC1#1 and SLC2#1, was markedly higher than in the wild type control (Col) (Supplementary Fig. S2C). Considering that OsGA20ox2 catalyses the conversion of GA_{53} to GA_{20}, promoting the biosynthesis of endogenous GA_1 (Monna *et al.*, 2002; Sasaki *et al.*, 2002; Spielmeyer *et al.*, 2002), as a comparison we also generated transgenic Arabidopsis plants expressing *OsGA20ox2*. Initially, the growth of 40-day-old plants of OsGA20ox2#1 and OsGA20ox2#2 was morphologically similar to that of Col. At flowering stage (54-day-old), OsGA20ox2 plants were significantly taller than Col (Supplementary Fig. S2D). These results demonstrate that the role of SLC1 or SLC2 in plant development is distinct from that of OsGA20ox2.

slc1 *and* slc2 *rice mutants have reduced stature*

We generated *slc1* rice mutants in the HJ background using CRISPR/Cas9 genome editing. Several mutant lines with a variety of edits were obtained, two of which, *slc1-4* and *slc1-7*, were subjected to subsequent analysis. The homozygous *slc1-4* mutant contained a G base deletion in the first exon of *SLC1*, resulting in a frame-shift and causing a premature stop codon (Supplementary Fig. S3A, B). The *slc1-7* mutant had an A insertion, also leading to a frame-shift in the mRNA sequence of *SLC1* (Supplementary Fig. S3A, B). In addition, we generated three homozygous lines of *slc2* genome-edited rice mutants in the Nip background; *slc2-27*, *slc2-61* and *slc2-62*. All three *slc2* mutants contained a C base deletion in *SLC2*, introducing a stop codon (TAG) at 153 bp (Supplementary Fig. S3C, D). Development of T_2 *slc1* mutants 7 d after germination was significantly different from that of HJ. Height and leaf sheath length of *slc1-4* and *slc1-7* shoots were obviously shorter than those of HJ (Fig. 3A, B). Similarly, height and leaf sheath length of seven-day-old shoots of *slc2-27*, *slc2-61* and *slc2-62* were also reduced (Fig. 3C, D). When compared with HJ, *slc1-4* and *slc1-7* mutants remained shorter at all stages (Supplementary Fig. S3E, F). The length of the main culms in 90-day-old *slc1-4*

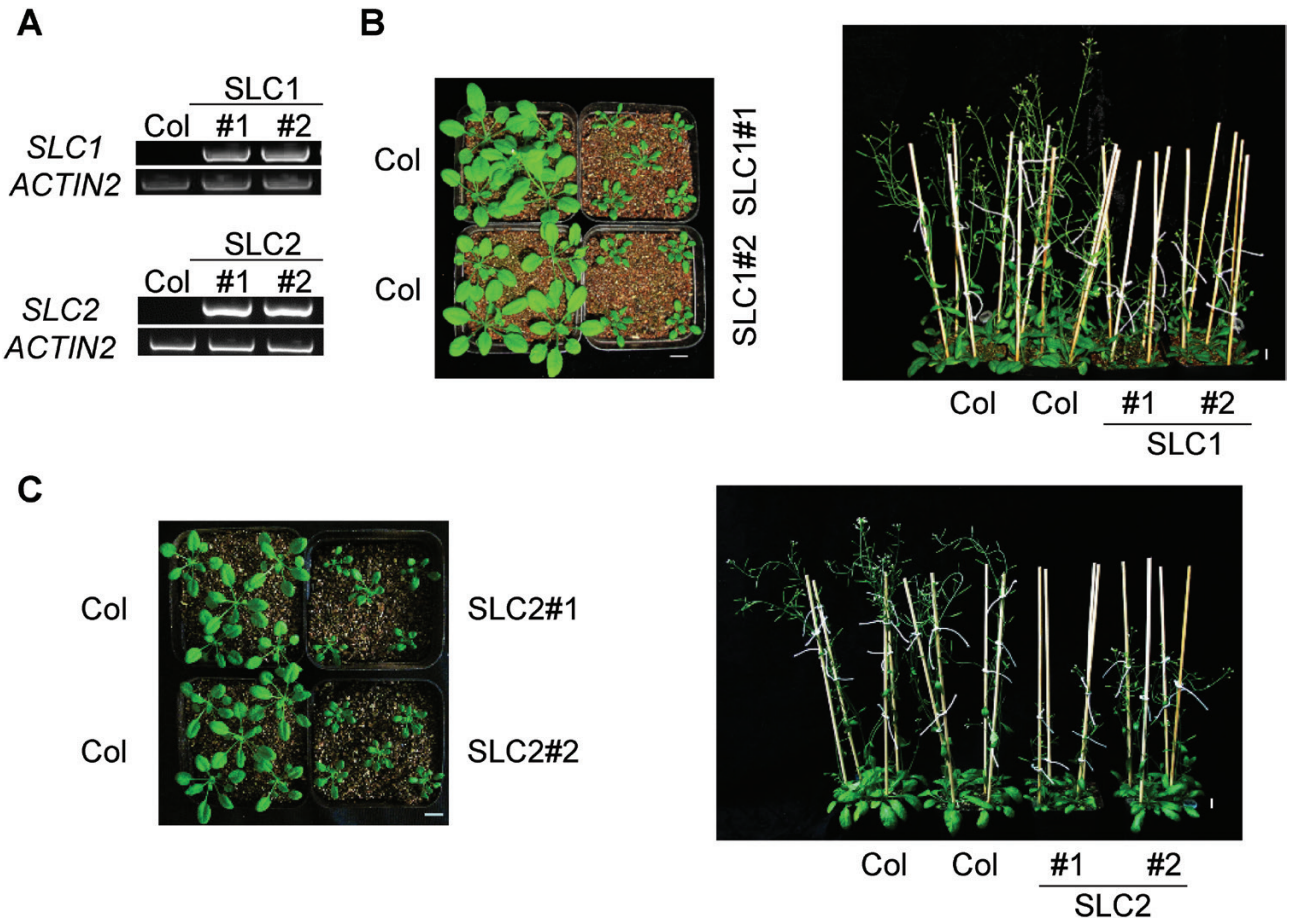

Fig. 2. Ectopic expression of *SLENDER AND CRINKLY LEAF1* (*SLC1*) or *SLC2* in Arabidopsis produces dwarf plants. (A) *SLC1* and *SLC2* expression analysis in SLC1 (#1 and #2) and SLC2 (#1 and #2) lines (Columbia-0, Col, background). Total RNA was extracted from rosette leaves of 30-day-old plants. The expression of *ACTIN2* (*At3g18780*) was used as the internal control. (B) SLC1#1 and SLC1#2 T_2 plants were smaller than Col. Left panel: photo taken at 35 d after germination (DAG). Right panel: photo taken at 53 DAG. Scale bars: 1 cm. (C) SLC2#1 and SLC2#2 T_2 plants were smaller than Col. Left panel: photo taken at 40 DAG. Right panel: photo taken at 54 DAG. Scale bars: 1 cm.

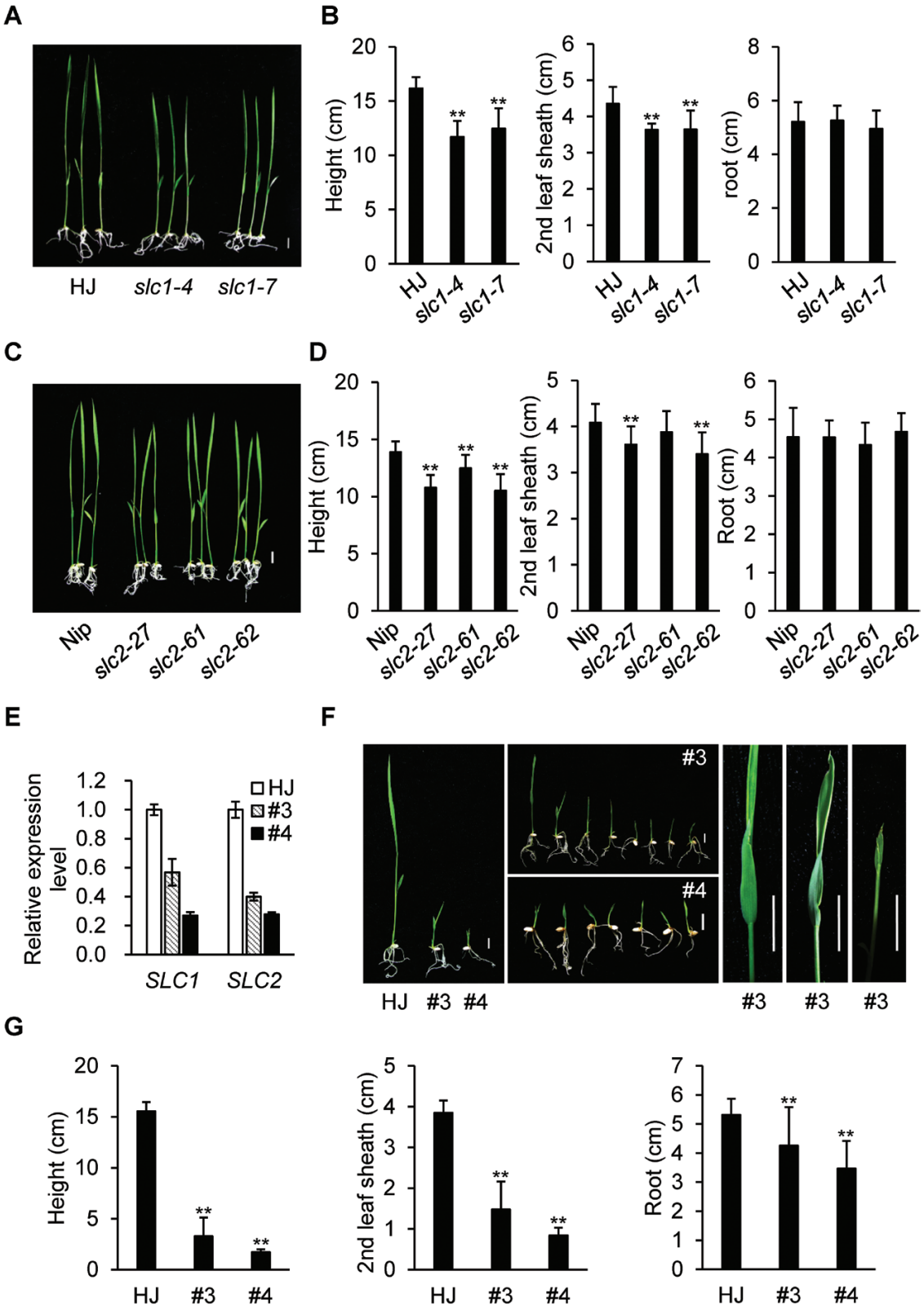

Fig. 3. Phenotype of *slender and crinkly leaf1* (*slc1*) and *slc2* rice mutants. (A) Seven-day-old *slc1* mutants (*slc1-4* and *slc1-7*, T_2) were shorter than *Hejiang 19* (HJ). Scale bar: 1 cm. (B) Plant height, second leaf sheath length, and root length in seven-day-old HJ and *slc1* plants. Data represent the mean ±SD (n=15, $^{**}P$<0.01, Student's t-test). (C) Seven-day-old *slc2* mutants (*slc2-27*, *slc2-61* and *slc2-62*, T_2) were shorter than *Nipponbare* (Nip) plants. Scale bar: 1 cm. (D) Plant height, second leaf sheath length, and root length of seven-day-old Nip and *slc2* plants. Data represent the mean ±SD (n=10, $^{**}P$<0.01, Student's t-test). (E) *SLC1* and *SLC2* expression levels were significantly reduced in SLC1-SLC2-RNAi lines (#3 and #4). *SLC1* or *SLC2* expression level in HJ was taken as 1.0. Data represent the mean ±SD of three independent experiments. (F) Seven-day-old SLC1-SLC2-RNAi plants displayed altered growth. Scale bars: 1 cm. (G) Plant height, second leaf sheath length, and root length of seven-day-old HJ and SLC1-SLC2-RNAi plants. Data represent the mean ±SD (n=8, $^{**}P$<0.01, Student's t-test).

and *slc1-7* mutants was evidently reduced (Supplementary Fig. S3G). Interestingly, the phenotype of older *slc2-27* and *slc2-62* plants was similar to that of Nip (Supplementary Fig. S3H), suggesting that, in terms of plant height, SLC1 and SLC2 might have different functions.

To test if *SLC1* and *SLC2* were functionally redundant, we generated RNAi lines with the pCAMBIA-SLC1-SLC2-RNAi plasmid (Supplementary Fig. S3I). Significant reduction of *SLC1* and *SLC2* expression was confirmed in the SLC1-SLC2-RNAi lines #3 and #4 (Fig. 3E). *OsGA20ox2* expression was also slightly reduced in line #4 (Supplementary Fig. S3J). Growth of seven-day-old plants of SLC1-SLC2-RNAi lines (#3 and #4) was clearly perturbed. For example, the edge of a leaf blade was nicked, and there were substantial reductions in plant height, the length of the second leaf sheath, and root length (Fig. 3F, G).

The spatiotemporal expression of SLC1 *and* SLC2 *in developing culms*

We quantified the spatiotemporal expression patterns of *SLC1* and *SLC2* in different rice tissues. The expression level of *SLC1* in seven-day-old plants was much lower than in mature leaves and developing culms (Fig. 4A), with the highest expression level observed in the second node. A similar pattern of expression of *SLC2* was also detected in developing culms (Fig. 4A). By fusing the *SLC1* promoter to the GUS marker gene (SLC1pro-GUS), we demonstrated that *SLC1* expression was detectable throughout the developmental stages tested (Fig. 4B), with predominant expression in the coleoptile, leaf sheath and blade, and root tip of seven-day-old plants. In mature leaves, GUS activity was restricted to vascular bundles of leaf blades. Strong GUS signal was detected in developing culms, especially in the second node.

To determine the subcellular localization of SLC1 and SLC2, we performed a transient expression assay in rice protoplasts and tobacco leaf epidermal cells (Fig. 4C, Supplementary Fig. S4). Consistent with the expression pattern of known dioxygenases (Xiong *et al.*, 2018), the fusion proteins YFP-SLC1 and YFP-SLC2 were detected in the cytoplasm and in the nucleus, with the latter confirmed using co-localization with a known nuclear protein, DWARF AND LOW-TILLERING (DLT) (Tong *et al.*, 2012).

SLC1 and SLC2 are involved in phytohormone homeostasis

To test our enzyme assay, we first determined the catalytic activity of OsGA20ox2 against its substrates GA_{12} and GA_{53}, and the products GA_{15} and GA_{44} were produced (Supplementary Fig. S5A). We then analysed the catalytic activity of SLC1 and SLC2 by incubating the cell lysate extracted from *E. coli* expressing either MBP-SLC1 or MBP-SLC2 with GA_{12} and GA_{53}. Neither reaction showed catalytic activity (Supplementary Fig. S5A), indicating that neither GA_{12} nor GA_{53} were substrates of either SLC1 or SLC2. The expression of either *SLC1* or *OsGA20ox2* in Arabidopsis created divergent growth phenotypes (Fig. 2B, Supplementary Fig. S2D), indicating that SLC1 might not function as a conventional GA20ox in rice. Therefore, we measured the levels of GA_4, GA_{19} and GA_1 in SLC1-OE, *slc1-4* and *slc2-62* rice plants. GA_4 was not detected, supporting the notion that the GA_4 content in vegetative tissues is normally low (Hirano *et al.*, 2008). The levels of GA_{19} and GA_1 were not significantly affected compared to their appropriate controls (Fig. 5A). These results suggest that SLC1 and SLC2 might not function as conventional GA20-oxidases.

Numerous studies have demonstrated that, rather than acting as a GA20ox to metabolize a GA-intermediate, 2OGDs may catalyse substrates of other hormones (Zhang *et al.*, 2013, 2016, 2017; Zhao *et al.*, 2013; Brewer *et al.*, 2016; Porco *et al.*, 2016; Caarls *et al.*, 2017; Smirnova *et al.*, 2017). To investigate how SLC1 and SLC2 function, we quantified ABA, IAA and SA levels in rice plants of various genetic backgrounds. The level of SA was significantly higher in T_0 SLC1-OE plants (2586 ng g^{-1} FW), when compared to T_0 empty vector control plants (1278 ng g^{-1} FW) (Fig. 5B). In contrast, a reduced level of SA was measured in *slc1-4* (2415 ng g^{-1} FW) compared to HJ (3817 ng g^{-1} FW), and in *slc2-62* plants (3879 ng g^{-1} FW) compared to Nip (4296 ng g^{-1} FW). There was little variation in IAA levels in all tested lines (Supplementary Fig. S5B). ABA levels were reduced in both SLC1-OE and *slc1-4* plants, but more severely in SLC1-OE. The ABA content in *slc2-62* plants was similar to Nip.

We also analysed the levels of several hormones in SLC1#1 and SLC2#1 Arabidopsis plants. In both lines, SA levels were almost 10-fold higher and JA levels were over 2-fold higher, compared to Col (208 ng g^{-1} FW) (Fig. 5C). There was little variation in IAA and ABA levels in all Arabidopsis lines tested (Supplementary Fig. S5C). Collectively, these results implicate a role for SLC1 and SLC2 in SA metabolism.

PR *genes are up-regulated by enhanced expression of* SLC1 *or* SLC2

One of the major roles of SA in plant development is the induction of *PR* gene expression (Stintzi *et al.*, 1993; Hoffmann-Sommergruber, 2000). In Arabidopsis, expression of *PR1*, *PR2* and *PR5* is stimulated by SA (Selitrennikoff, 2001; Zhang *et al.*, 2010); the induction of *PR3* and *PR4* is independent of SA-signaling but dependent on JA-signaling (Thomma *et al.*, 1998); and activation of the SA-signaling pathway suppresses a large set of JA-responsive genes including *PLANT DEFENSIN1.2* (*PDF1.2*) and *VEGETATIVE STORAGE PROTEIN2* (*VSP2*) (van Wees *et al.*, 1999; Van der Does *et al.*, 2013). We analysed the expression of a set of SA- and JA-related genes in SLC1#1 and SLC2#1 Arabidopsis plants compared to Col. Expression of the SA-responsive genes, *PR1*, *PR2* and *PR5*, was enhanced significantly (Fig. 6A). The JA-responsive gene *PR4* was up-regulated, while the expression of *VSP2* was reduced.

In rice, *OsPR1a*, *OsPR1b*, *OsPR2* and *OsPR5* are induced by SA treatment (Jwa *et al.*, 2006), thus we examined the expression of these *OsPR* genes in T_0 SLC1-OE and found their expression was markedly increased compared to control plants (Fig. 6B). In the *slc1-4* mutant, although *OsPR1a* expression was higher

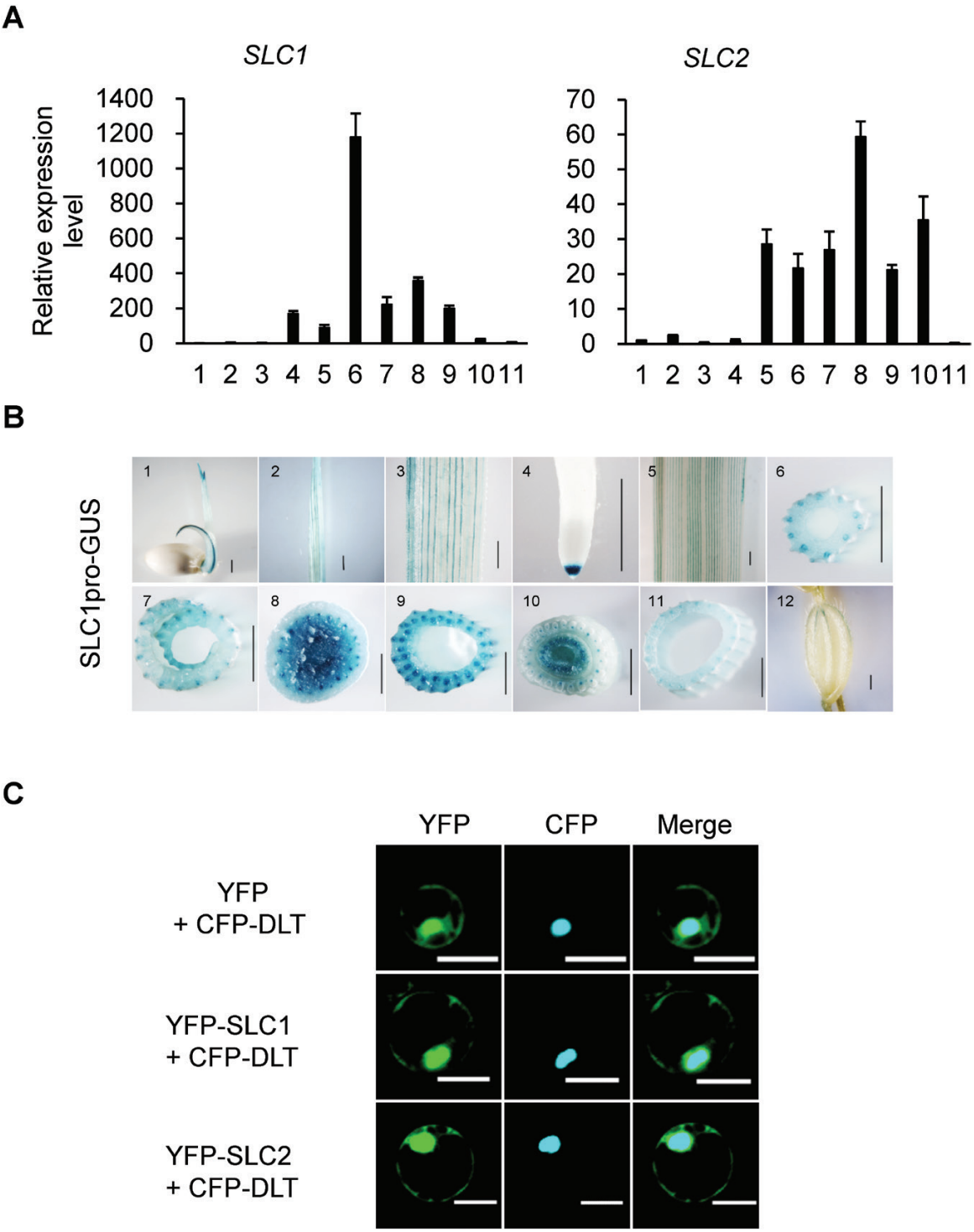

Fig. 4. Characterisation of *SLENDER AND CRINKLY LEAF1* (*SLC1*) and *SLC2* expression in rice. (A) Expression level of *SLC1* (left panel) and *SLC2* (right panel) in different tissues. 1, seeds soaked in water overnight; 2 and 3, shoots and roots of seven-day-old seedlings; 4, leaf blades of 40-day-old plants; 5, first internode; 6, second node; 7, second internode; 8, third node; 9, third internode; 10, spikelet before pollination; 11, spikelet after pollination. The expression of *OsACTIN1* was used as the internal control. (B) Expression pattern of SLC1 visualized through fusion with the β-glucuronidase (GUS) marker gene, SLC1pro-GUS. 1, three-day-old shoot; 2–4, second leaf sheath, third leaf blade and root tip of seven-day-old seedling; 5, mature leaf blade; 6, first node; 7, first internode; 8, second node; 9, second internode; 10, third node; 11, third internode; 12, spikelet. Scale bars: 1 mm. (C) Subcellular localization of SLC1 and SLC2 via transient expression in rice protoplasts. Proteins tagged with yellow fluorescent protein (YFP). Protoplasts isolated from seven-day-old plants of *Hejiang 19* (HJ). YFP: empty vector control; CFP-DLT: marker for nuclear localization. Scale bars: 10 μm.

than that of HJ, the expression of both *OsPR1b* and *OsPR5* was reduced (Supplementary Fig. S6A). In the *slc2-62* mutant, all of these *OsPR* genes were significantly down-regulated. In addition, we observed that in seven-day-old shoots, *SLC1* expression was higher than *SLC2* expression in HJ, while the opposite was true in Nip (Supplementary Fig. S6B).

Two pathways for SA biosynthesis in plants have been proposed (Lee *et al.*, 1995; Ribnicky *et al.*, 1998; Strawn *et al.*,

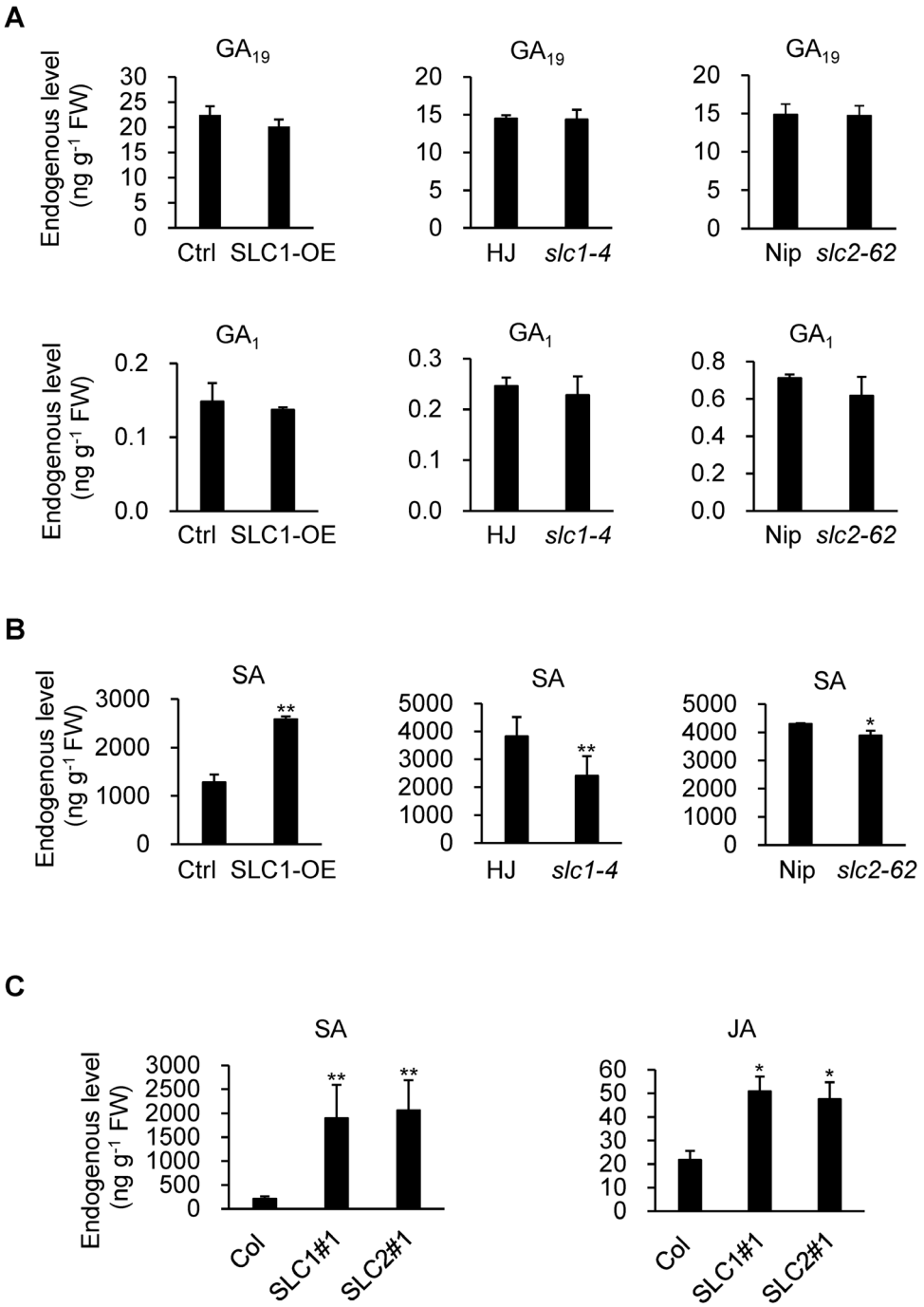

Fig. 5. The relationship between expression of *SLENDER AND CRINKLY LEAF1* (*SLC1*) or *SLC2* and salicylic acid (SA) levels. (A) Level of two gibberellins (GAs), GA_{19} and GA_1, in plants overexpressing *SLC1* (SLC1-OE), and in *slc1-4* and *slc2-62* mutants. Control (Ctrl) plants contain the empty vector. Samples were collected from shoots of SLC1-OE plants after 14 d of regeneration, or from seven-day-old shoots of *slc1-4* and *slc2-62* mutants (T_2 homozygous). (B) Levels of SA in SLC1-OE, *slc1-4* and *slc2-62* plants. For measuring SA levels in SLC1-OE plants, samples were collected from shoots of SLC1-OE or Ctrl after 14 d of regeneration. For measuring SA levels in *slc1-4* and *slc2-62* mutants, samples were collected from seven-day-old shoots of *Hejiang 19* (HJ) and *slc1-4* (T_2 homozygous), or *Nipponbare* (Nip) and *slc2-62* (T_2 homozygous). HJ and Nip control plants were grown from seed. Data represent the mean ±SD of three independent experiments (*P<0.05, **P<0.01; Student's t-test). (C) Increased SA and JA levels in SLC1#1 and SLC2#1 Arabidopsis plants. Samples were collected from aerial tissues of 30-day-old plants. Data represent the mean ±SD of three independent experiments (*P<0.05, **P<0.01; Student's t-test).

2007; Mustafa *et al.*, 2009). To explore the role of SLC1 and SLC2 in SA metabolism, we examined the expression of SA biosynthesis and catabolism genes in SLC1#1 and SLC2#1 Arabidopsis plants. There are four genes encoding PAL and two genes encoding ICS in the Arabidopsis genome (Ohl *et al.*, 1990; Wanner *et al.*, 1995; Wildermuth *et al.*, 2001). Both *DMR6* and *DLO1* encode SA-hydroxylase which fine-tunes SA homeostasis (Zhang *et al.*, 2013; Zhang *et al.*, 2017). In SLC1#1 and SLC2#1, *ICS1*, *DMR6* and *DLO1* were up-regulated, whereas *ICS2* and *PAL4* were down-regulated (Fig. 6C). We then analysed the levels of potential SA precursors in SLC1#1 and SLC2#1. In both lines, the level of

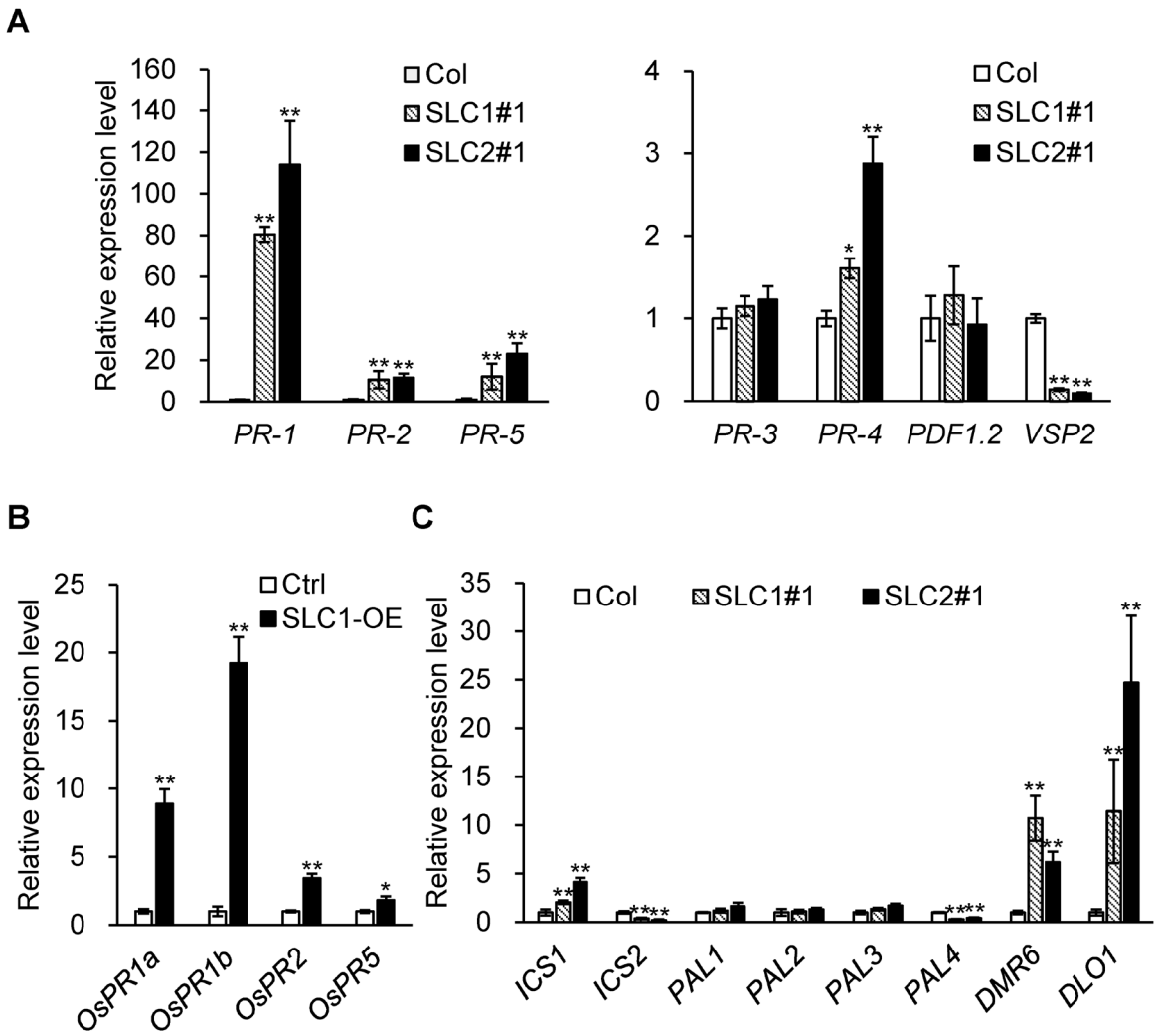

Fig. 6. Up-regulation of *pathogenesis-related* (*PR*) genes in plants overexpressing *SLENDER AND CRINKLY LEAF1* (*SLC1*) or *SLC2*. (A) Expression levels of salicylic acid (SA)- and jasmonic acid (JA)-related genes in SLC1#1 and SLC2#1 Arabidopsis lines. Total RNA was extracted from rosette leaves of 30-day-old plants. The expression of *ACTIN2* was used as the internal control. Data represent the mean ±SD of three independent experiments (*P<0.05, **P<0.01; Student's *t*-test). (B) Expression levels of *OsPR* genes in regenerated plants overexpressing *SLC1* (SLC1-OE, T_0). The expression of *OsACTIN1* was used as the internal control. Data represent the mean ±SD of three independent experiments (*P<0.05, **P<0.01; Student's *t*-test). (C) Expression of SA biosynthesis and catabolism genes in SLC1#1 and SLC2#1. Total RNA was extracted from rosette leaves of 30-day-old plants. Data represent the mean ±SD of three independent experiments (**P<0.01; Student's *t*-test).

L-phenylalanine was higher and the chorismate level remained unchanged compared to Col. Due to their low abundance in Arabidopsis, *trans*-cinnamic acid and *o*-coumaric acid were not detected (Supplementary Fig. S6C).

SLC1 interacts with OSH1 and its N-terminus has transcriptional activation activity

The knotted1-like homeobox (KNOX) transcription factors are crucial for establishment and maintenance of the shoot apical meristem (SAM). Since overexpression of *SLC1* and *SLC2* in rice led to aberrant shoot development, we sought to explore the biochemical relationship of SLC1 or SLC2 with the KNOX proteins. Seven KNOX proteins, OSH1, OSH3, OSH6, OSH10, OSH15, OSH43, and OSH71, function together to establish the SAM in rice (Sentoku *et al.*, 1999). Using a Y2H assay, we screened OSH proteins to determine which, if any, interacted with SLC1. Interactions were observed for SLC1 with OSH1, OSH6, OSH15, and OSH71, but not OSH10 (Fig. 7A, Supplementary Fig. S7A). We verified these interactions in rice protoplasts using BiFC (Fig. 7B), and further confirmed the interaction between SLC1 and OSH1 in a protein pull-down assay (Fig. 7C).

Similarly to SLC1, OsGA20ox2 interacted with OSH1, OSH6, and OSH15, but did not interact with OSH10 or OSH71 (Supplementary Fig. S7B). OSH1 is expressed in a specific region during early embryogenesis and its function may be related to a regulatory process before or independent of organ determination in rice (Sato *et al.*, 1996). The full-length OSH1 protein (361 amino acids) is composed of five conserved domains: KNOX1 and KNOX2 (MEINOX), GSE, ELK, and homeodomain (HD) (Sentoku *et al.*, 1999; Nagasaki *et al.*, 2001). To distinguish the binding specificity of SLC1 and OsGA20ox2 to OSH1, we analysed the interaction of SLC1 with various truncations of OSH1 in Y2H assays (Fig. 7D, E). Interactions were detected between SLC1 and OSH1 (242–361), OSH1 (1–241), OSH1 (1–144), and OSH1 (145–263).

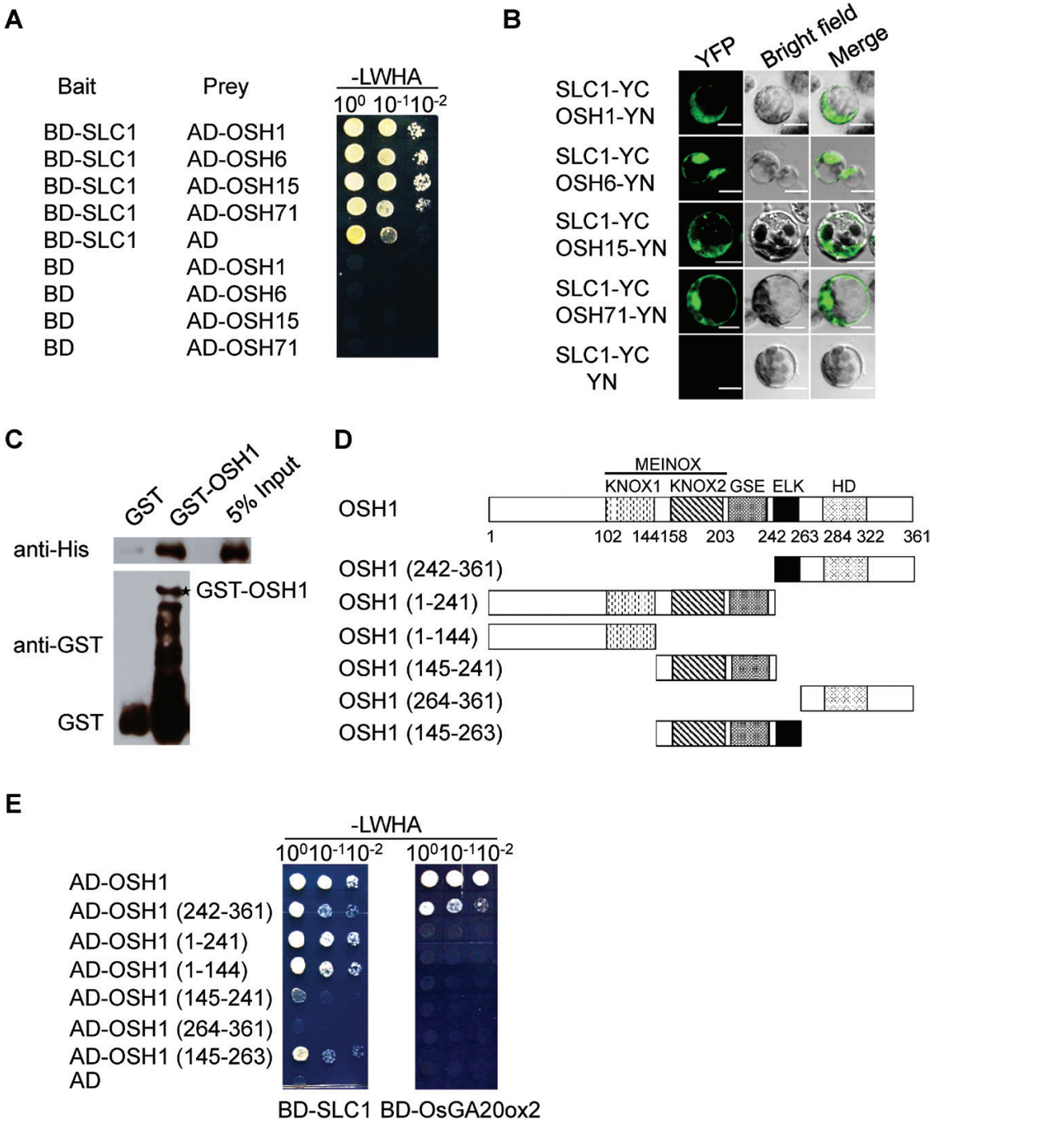

Fig. 7. SLC1 interacts with knotted1-like homeobox (KNOX) proteins. (A) Yeast two-hybrid (Y2H) assay to assess the interaction of SLC1 with homeobox1 (OSH1), OSH6, OSH15, and OSH71. Controls: AD (pGADT7) and BD (pGBKT7); -LWHA, high-stringency selective medium (SD/Leu$^-$/Trp$^-$/His$^-$/Ade$^-$). (B) Bimolecular Fluorescence Complementation (BiFC) assay to assess the interaction of SLC1 (SLC1-YC) with OSH1 (OSH1-YN), OSH6 (OSH6-YN), OSH15 (OSH15-YN), and OSH71 (OSH71-YN). Protoplasts isolated from seven-day-old *Hejiang 19* seedlings. YN, N-terminal YFP fragment; YC, C-terminal YFP fragment. Scale bars: 10 μm. (C) Pull-down assay to confirm the protein interaction between SLC1 and OSH1. Empty vector (GST) and GST-OSH1 immunoblotted with anti-GST antibody. His-SLC1 immunoblotted with anti-His antibody (Input). (D) Diagram of the full-length and truncated proteins of OSH1 (not to scale). (E) Y2H assay to assess the interaction between truncated proteins of OSH1 with SLC1 and OsGA20ox2. Control: AD (pGADT7); -LWHA, high-stringency selective medium (SD/Leu$^-$/Trp$^-$/His$^-$/Ade$^-$).

However, OsGA20ox2 only interacted with OSH1 (242–361), which contained the ELK and HD domains. Hence, either the KNOX1 or ELK domain of OSH1 was sufficient to allow interaction with SLC1. Additionally, we examined the interaction of SLC2 and OSH1 by Y2H, and no interaction was detected (Supplementary Fig. S7C).

To examine the functional specificity of the N- and C-termini of SLC1, we assessed the truncations SLC1-N (1–40) and SLC1-C (41–383) in a Y2H assay. The yeast cells carrying BD-SLC1-N and AD (empty vector) were able to grow on the selection medium (Supplementary Fig. S7D), suggesting that SLC1-N had transcriptional activation activity in

the assay. To confirm this, we performed the transcriptional activation assay by solely expressing BD-SLC1-N in yeast cells. The yeast cells expressing BD-SLC1-N, but not BD-SLC1-C, grew well in the selection medium, indicating strong transcriptional activation activity of SLC1-N (Supplementary Fig. S7E). To further characterize the impact of SLC1-N on the function of SLC1, we generated transgenic rice plants overexpressing SLC1-N (SLC1-N-OE) and SLC1-C (SLC1-C-OE), respectively. The SLC1-N-OE plants showed a similar growth phenotype to HJ, whereas the SLC1-C-OE plants mimicked those shown in the SLC1-OE plants (Supplementary Fig. S7F, G).

Discussion

SLC1 and SLC2 are essential for shoot development

In plants, 2OGDs are involved in a wide range of biological processes, including DNA demethylation, proline hydroxylation, plant hormone biosynthesis, and the biosynthesis of various specialized metabolites. Due to their agricultural significance, the role of 2OGDs in the biosynthesis of GAs has been extensively studied. We previously reported that the dioxygenase GIM2 can oxidize GA_{12} and plays a role in seed germination in Arabidopsis (Xiong *et al.*, 2018). The essential role of 2OGDs in the homeostasis and (in)activation of many plant hormones, including auxin, SA, strigolactones, and JA, has been reported (Zhang *et al.*, 2013, 2016, 2017; Zhao *et al.*, 2013; Brewer *et al.*, 2016; Porco *et al.*, 2016; Caarls *et al.*, 2017; Smirnova *et al.*, 2017). Eight *GA20ox* genes are predicted in the rice genome, named *OsGA20ox1-8* (Han and Zhu, 2011). *OsGA20ox6*, also named *DAO*, encodes a dioxygenase that converts IAA into OxIAA (Zhao *et al.*, 2013), demonstrating that not all predicted OsGA20ox members are involved in GA metabolism. In this study, we renamed OsGA20ox7 as SLC1 and analysed its role, along with SLC2, in rice shoot development. These proteins were classified as 2OGDs and predicted to be OsGA20ox members, and SLC1and SLC2 share 54.6% amino acid identity (Supplementary Fig. S1B). Overexpression of *SLC1* or *SLC2* in rice resulted in slender, rolled and crinkly leaves in regenerated T_0 shoots (Fig. 1B, H). Ectopic expression of *SLC1* or *SLC2* in Arabidopsis produced smaller plants with greener leaves (Fig. 2B, C), which were easily distinguished from Arabidopsis plants expressing *OsGA20ox2* (Supplementary Fig. S2D). In co-silenced SLC1-SLC2-RNAi plants, we observed phenotypes including shorter leaves and roots, and reduced plant height (Fig. 3F, G). Thus, SLC1 and SLC2 may redundantly influence shoot development in rice. The significance of some 2OGDs in rice leaf development has been reported. By affecting secondary cell wall formation in leaf cells, Rolling-leaf14, a 2OG-Fe(II) oxygenase family protein, functions in modulating rice leaf rolling (Fang *et al.*, 2012).

SLC1 and SLC2 are involved in salicylic acid homeostasis

Loss-of-function of the Green Revolution gene *Sd-1* (*OsGA20ox2*) and other GA biosynthesis genes in rice confers a dwarf phenotype (Itoh *et al.*, 2001; Monna *et al.*, 2002; Sasaki *et al.*, 2002; Spielmeyer *et al.*, 2002; Hu *et al.*, 2018). The function of OsGA20ox2 has been extensively analysed since it acts as a typical GA20ox. In this study, we used OsGA20ox2 as a control for analysing the catalytic activity of SLC1 and SLC2. Our data indicated that neither SLC1 nor SLC2 converts GA_{12} or GA_{53} (Supplementary Fig. S5A), suggesting that neither protein possesses GA20-oxidase activity. Furthermore, the levels of GA_{19} and GA_1 were hardly affected in SLC1-OE, *slc1-4* and *slc2-62* plants. Instead, SA levels were dramatically altered (Fig. 5B), demonstrating that the activity of SLC1 and SLC2 correlates with SA homeostasis in rice. The effect of SA on plant growth and development has been documented in numerous reports. For instance, the elevation of SA in the Arabidopsis mutants *cpr1*, *cpr5*, *cpr6-1* and *dnd1*, as well as and the *s5hs3h* double mutant, results in reduced plant stature (Bowling *et al.*, 1994; Bowling *et al.*, 1997; Clarke *et al.*, 1998; Yu *et al.*, 1998; Jirage *et al.*, 2001; Zhang *et al.*, 2017). JA levels were affected in SLC1#1 and SLC2#1 plants (Fig. 5C), indicating the influence of *SLC1* and *SLC2* expression on JA levels in Arabidopsis. SA and JA play interactive roles in plant development and immune signaling networks (Mur *et al.*, 2006; Koornneef *et al.*, 2008). Endogenously accumulating SA antagonizes JA-dependent defense responses, thereby prioritizing SA-dependent defense (Pieterse *et al.*, 2012). In SLC1#1 and SLC2#1 Arabidopsis plants, the SA-responsive genes *PR1*, *PR2* and *PR5* were strongly induced, whereas among four JA-responsive genes (*PR3*, *PR4*, *PDF1.2* and *VSP2*), only *PR4* expression was up-regulated (Fig. 6A). Although JA levels were elevated in SLC1#1 and SLC2#1, *VSP2* expression was drastically suppressed (Fig. 6A), demonstrating a consequence of the antagonism between SA- and JA-signaling. Thus, an improved SA-response occurred in SLC1#1 and SLC2#1.

Increased expression of *OsPR* genes in SLC1-OE rice plants (Fig. 6B) indicated a link between SLC1 and SA homeostasis. In contrast to the low basal level of SA in Arabidopsis and tobacco (less than 100 ng g^{-1} FW), rice has a level of SA two orders of magnitude higher (5000–30 000 ng g^{-1} FW) and appears to be insensitive to exogenous SA treatment (Chen *et al.*, 1997; Yang *et al.*, 2004). We confirmed that SA levels in rice and Arabidopsis are dramatically different (Fig. 5B, C). Thus, the differential phenotypes resulting from expression of *SLC1* or *SLC2* in rice or in Arabidopsis are closely associated with the basal level of SA in each species. Two distinct pathways have been proposed for the biosynthesis of SA in plants: the ICS pathway and the PAL pathway. By analysing the expression of SA biosynthesis and catabolism genes, as well as the abundance of SA precursors (Fig. 6C, Supplementary Fig. S6C), we found that expression of *PAL4* was down-regulated, and the level of L-phenylalanine was elevated in SLC1#1 and SLC2#1 Arabidopsis plants. Although the expression of both *ICS1* and *ICS2* was altered in SLC1#1 and SLC2#1, the level of chorismate was unaffected. In support of the opinion that, in Arabidopsis, SA is synthesized via isochorismate and not through the PAL pathway (Tamaoki, 2008), *trans*-cinnamic acid and *o*-coumaric acid were not detected. Enhanced expression of *DMR6* and *DLO1* in SLC1#1 and SLC2#1 was attributed to the increased SA level in these lines, suggesting the presence of negative feedback regulation. Studies are now required to

elucidate precisely how SLC1 and SLC2 are involved in SA homeostasis, and in which steps of the SA biosynthesis pathway SLC1 and SLC2 implement their functions.

SLC1 interacts with OSH1 and may have diverse functionality

KNOX genes encode HD-containing transcription factors that are expressed in the SAM, but excluded from lateral organ primordia (Tsuda *et al.*, 2014). Loss-of-function mutants of *KNOX* genes, such as *SHOOT MERISTEMLESS* (*STM*) in Arabidopsis and *OSH1* in rice, show defects in SAM formation and/or maintenance (Long *et al.*, 1996; Tsuda *et al.*, 2011). OSH1 is important for specifying cell identity and regionalization for the formation of the shoot and its adjacent tissues in rice (Sato *et al.*, 1996). In our Y2H assay, OSH1 interacted with SLC1 and OsGA20ox2 (Fig. 7A, Supplementary Fig. S7B). However, SLC1 and OsGA20ox2 exhibited distinct binding specificity to OSH1 (Fig. 7E), indicating functional specialization of 2OGDs in rice. The conserved MEINOX and ELK domains in OSH1 are considered to be crucial for protein–protein interaction (Vollbrecht *et al.*, 1991; Bürglin, 1997). Our results indicated that either the KNOX1 or ELK domain of OSH1 is sufficient for the interaction between SLC1 and OSH1 (Fig. 7E). Although our results suggest that SLC1 and SLC2 are functionally redundant, SLC1 may have characteristics that differ from SLC2. For example, SLC1 can interact with OSH1, whereas SLC2 cannot (Fig. 7A, Supplementary Fig. S7C). The N-terminus of SLC1 (SLC1-N) showed transcriptional activation activity in yeast cells. Overexpression of SLC1-N in rice did not affect growth of SLC1-N-OE plants, but abnormal growth, similar to that shown in SLC1-OE lines (Fig. 1), was observed in the plants of SLC1-C-OE (Supplementary Fig. S7G). SLC1 appears to be functionally diverse; it may act as a dioxygenase, and also work together with its associated proteins, such as OSH1, to modulate shoot development in rice.

In this study, we propose that *SLC1* and *SLC2* are homologous genes belonging to the 2OGD family that both play important roles in rice shoot development, and we demonstrate the impact of SLC1 and SLC2 on SA homeostasis. Future studies to determine the enzymatic activity of SLC1 and SLC2, identify their substrates, elucidate the biochemical mechanisms of the interaction between SLC1 and OSH1, and clarify the redundancy and specificity of SLC1 and SLC2, will further our knowledge on the association between SA homeostasis and shoot development in rice.

Supplementary data

Supplementary data are available at *JXB* online.

Fig. S1. The relationships of eight 2-oxoglutarate-dependent dioxygenases in rice.

Fig. S2. Expressing either *SLC1* or *SLC2* in Arabidopsis alters plant development.

Fig. S3. *SLC1* and *SLC2* genome-edited mutants were generated using the CRISPR/Cas9 system.

Fig. S4. Subcellular localization of YFP-SLC1 and YFP-SLC2 in tobacco leaf epidermal cells.

Fig. S5. Assessment of the enzymatic activities of SLC1 and SLC2.

Fig. S6. The relationship of SLC1 and SLC2 with SA.

Fig. S7. SLC1 interacts with KNOX proteins and the N-terminus of SLC1 may have transcriptional activation activity.

Table S1. Primer sequences for plasmid construction in this study.

Table S2. Primer sequences for RT–PCR and qRT–PCR analyses.

Acknowledgements

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