Root angle modifications by the DRO1 homolog improve rice yields in saline paddy fields

Yuka Kitomi, Eiko Hanzawa, Noriyuki Kuya, Haruhiko Inoue, Naho Hara, Sawako Kawai, Noriko Kanno, Masaki Endo, Kazuhiko Sugimoto, Toshimasa Yamazaki, Shingo Sakamoto, Naoki Sentoku, Jianzhong Wu, Hitoshi Kanno, Nobutaka Mitsuda, Kinya Toriyama, Tadashi Sato, Yusaku Uga

The root system architecture (RSA) of crops can affect their production, particularly in abiotic stress conditions, such as with drought, waterlogging, and salinity. Salinity is a growing problem worldwide that negatively impacts on crop productivity, and it is believed that yields could be improved if RSAs that enabled plants to avoid saline conditions were identified. Here, we have demonstrated, through the cloning and characterization of qSOR1 (quantitative trait locus for SOIL SURFACE ROOTING 1), that a shallower root growth angle (RGA) could enhance rice yields in saline paddies. qSOR1 is negatively regulated by auxin, predominantly expressed in root columella cells, and involved in the gravitropic responses of roots. qSOR1 was found to be a homolog of DRO1 (DEEPER ROOTING 1), which is known to control RGA. CRISPR-Cas9 assays revealed that other DRO1 homologs were also involved in RGA. Introggression lines with combinations of gain-of-function and loss-of-function alleles in qSOR1 and DRO1 demonstrated four different RSAs (ultra-shallow, shallow, intermediate, and deep rooting), suggesting that natural alleles of the DRO1 homologs could be utilized to control RSA variations in rice. In saline paddies, near-isogenic lines carrying the qSOR1 loss-of-function allele had soil-surface roots (SOR) that enabled rice to avoid the reducing stresses of saline soils, resulting in increased yields compared to the parental cultivars without SOR. Our findings suggest that DRO1 homologs are valuable targets for RSA breeding and could lead to improved rice production in environments characterized by abiotic stress.

Optimized plant architecture, both above and below ground, is required for plants to adapt to different environments (1, 2). The optimization of plant architecture has also been one of the most effective ways to improve crop productivity. The “Green Revolution,” which began in the 1950s, resulted in new, high-yielding varieties of wheat and rice due to the introduction of dwarfing genes into traditional, tall varieties: Reduced height (Rht) in wheat and semidwarf1 (sd1) in rice (3). In rice, several additional genes associated with its above-ground architecture, such as Tiller Angle Control 1 (TAC1), which controls tiller angle, and Ideal Plant Architecture 1 (IPA1), which regulates tiller number (4–6), have also been identified. Root system architecture (RSA), however, has not experienced the same level of improvement due to the difficult nature of phenotyping the below-ground part of the plants and the limited genetic information available to breeders. Nonetheless, RSA is recognized as an important trait that, if understood, could be improved to allow plants to adapt to a range of soil environments, such as those experiencing deficiencies or excesses of water and/or nutrients (7, 8). Typically, a deep RSA is beneficial for enhancing drought avoidance, whereas a shallow RSA facilitates the acquisition of phosphorus (P) in P-deficient soils. Another unique root system is soil-surface roots (SOR), which may enable upland plants to adapt to waterlogging, by allowing them to obtain oxygen from the air (9). Thus, an improved understanding of the factors controlling RSA could enable the breeding of crop cultivars that are suitable to the different stress conditions caused by global climate change (10).

Many rice genes involved in root development have already been isolated (11). LARGE ROOT ANGLE1 encoding OsPIN2 and DEFECTIVE IN OUTER CELL LAYER SPECIFICATION 1 (DOCS1) belonging to the leucine-rich repeat receptor-like kinase (LRR-RLK) subfamily, control gravitropic responses (12, 13). Rice Morphology Determinant (RMD), encoding an actin-binding protein, controls gravitropic responses to low external phosphate conditions (14). These genes affect the root growth angle (RGA) and determine RSAs in rice. However, only a limited number of previously identified RSA-controlling genes have promise, for the breeding of future climate-resilient rice (15). One of these candidates is DEEPER ROOTING 1 (DRO1), a quantitative trait locus (QTL) that has previously been cloned and shown to affect vertical root distributions in the soil (16). The deeper rooting habit conditioned by the functional allele at DRO1 enhanced grain yields under drought stress, while the

Significance

Genetically improving the root system architectures of plants is an effective strategy for developing climate-resilient crops. In this study, we revealed that a cloned rice quantitative trait locus associated with root growth angle, qSOR1, is a DRO1 homolog involved in root gravitropic responses. The loss-of-function allele qSOR1 resulted in roots that developed on the soil surface and enabled plants to avoid the reducing stress found in saline paddy soils and, consequently, increased yields. We show that the DRO1 homologs could be useful for the controlled breeding of root system architectures that are adapted to the abiotic stress conditions caused by global climate change.
shallow rooting allele, *dro1*, was associated with drought susceptibility. To develop rice cultivars that are robust to environmental stress conditions other than drought, it is necessary to identify and characterize additional QTLs associated with variations in the RSA-related attributes.

Rice cultivars ordinarily develop underground crown roots. We previously identified SOR phenotypes in Gemdjah Beton (GB), an Indonesian lowland rice belonging to the Bulo ecotype (17). SOR may occur due to the selection pressures within Bulu cultivars to adapt to severe anaerobic environments (18). We previously fine-mapped a QTL for *SOIL SURFACE ROOTING 1*, *qSOR1* on rice chromosome 7, using mapping populations derived from a cross between GB and a non-SOR lowland rice cultivar called Sasanishiki (SA) (19). Previously, a gene related to the SOR phenotype, *SOR1*, was identified on rice chromosome 4 using a mutant line (20). *SOR1* has a function like *Arabidopsis WAV3*, which is an E3 ubiquitin ligase that controls root gravitropism by affecting auxin responses (21, 22). The *SOR1* homologs were found in the candidate region of the *qSOR1* locus, which is an E3 ubiquitin ligase that controls root gravitropism by affecting auxin responses (21, 22). The *qSOR1* locus identified in this study maps to a different region (chr 7), and since no *SOR1* homologs were found in the candidate region of the *qSOR1* locus, it was concluded that *qSOR1* may have a different function from that of *SOR1*.

Here, we report that the functional gene, *qSOR1*, is a *DRO1* homolog involved in gravitropic responses that acts through negative regulation via auxin signaling. Furthermore, we demonstrate that the SOR phenotype originates from a loss-of-function of *qSOR1* and contributes to the avoidance of the reducing stress conditions in saline paddies, leading to yield enhancement. Our results suggest that the natural alleles of *DRO1* homologs will be useful for the genetic improvement of the RSA of rice.

Results

Phenotypic Characteristics and Map-Based Cloning of *qSOR1*. To elucidate the effects of *qSOR1* on root morphology, we developed a near-isogenic line (NIL), homozygous for the GB allele of *qSOR1* in a SA background (*qsor1-NIL*) (Fig. 1A). The *qsor1-NIL* readily developed SOR that resembled those of the GB cultivar, whereas the SA cultivar developed relatively fewer SOR (SI Appendix, Fig. SI A–C). The *qsor1-NIL* showed markedly shallower RGA than the SA (Fig. 1 B–D), but there were no differences in the other

---

**Fig. 1.** Phenotypic and molecular characterization of *qSOR1*. (A) Graphical genotypes of Sasanishiki (SA; Left), *qsor1-NIL* (NIL; Center), and Gemdjah Beton (GB; Right). The white and black rectangles indicate the homozygous regions from SA and GB, respectively. Red arrowhead, position of *qSOR1*. (B) Images of rice plants grown in small cups for 20 d after sowing and after the removal of the topsoil from each cup. (C) Images of the basal parts of the rice plants grown in the cups in B. The root growth angle (*θrga*) of each plant was determined by measuring the angle between the horizontal line and the shallowest nodal root. (D) Mean root growth angle of SA, *qsor1-NIL*, and GB. Data are means ± SD; *n* = 40, 38, and 36 plants for SA, *qsor1-NIL*, and GB, respectively. Different letters indicate significant differences (*P* < 0.01, Tukey’s HSD test). (E) Sequence variations between SA and GB in the two putative ORFs detected in the candidate region of the *qSOR1* locus. Red arrowhead, a single 1-bp substitution. Orange rectangles, ORF; gray rectangles, 5’ and 3’ UTRs. (F) *qSOR1* expression in various shoot and root tissues. Samples of the root tips from different depths, leaf blades, leaf sheaths, and shoot bases (1-cm sample from the bottom of the shoot) were taken from plants grown in baskets, 30 d after sowing. Expression of *qSOR1* was normalized to that of rice *Ubiquitin* gene. Data are shown as mean ± SD; *n* = 3 biological repeats. *P* values are based on Student’s *t* tests. (G) Gravitropic curvature in the seminal roots of SA and *qsor1-NIL* plants. *θrac* is the root angle of the curvature after rotation. Asterisks indicate the positions of the root tips at the start of the rotation. Yellow arrows indicate the direction of the gravitational force. (H) Root angle of the curvature of SA and *qsor1-NIL* after rotating 90° from the original vertical axis for 4 h. *P* value is based on Student’s *t* test. (Scale bars: 1 cm.)
root and shoot morphologies (SI Appendix, Fig. S1D). These results indicate that qSOR1 predominantly controls RGA.

To isolate the qSOR1 gene, we identified a candidate region within a 12.51-kb segment using positional cloning (SI Appendix, Fig. S2). Comparing the genomic sequences of the candidate region between GB and SA, a single 1-bp substitution within exon 3 of one of the putative ORFs (Os07g0614400; LOC_Os07g42290.1) was identified. The GB allele at this ORF coded for an unknown protein, and the 1-bp mutation resulted in a premature stop codon (Fig. 1E and SI Appendix, Fig. S3A). Three-dimensional modeling of the qSOR1 candidate protein showed that the premature stop truncated a polypeptide in GB, which inhibited the formation of the helix bundle structure due to a lack of helix–helix interactions (SI Appendix, Fig. S3 B–D). Helaplot analysis for this ORF revealed that the Bulu cultivars with SOR phenotypes commonly possessed this 1-bp substitution, but other accessions that lacked SOR did not (SI Appendix, Table S1). Transgenic plants that carried a 7.57-kb genomic DNA fragment from SA, containing the entire Os07g0614400 ORF in the q sor1-NIL (gSA/NIL) genetic background, showed increased RGAs compared to those transformed with an empty vector (Vec/NIL) (SI Appendix, Figs. S2B and S4 A and B). Therefore, we concluded that the SOR phenotype observed in the GB was caused by the loss-of-function mutation in exon 3 of Os07g0614400.

**Root Gravitropic Responses Mediated by qSOR1 Are Controlled by Auxin Signaling.** qSOR1 was mainly expressed in the tips of deeper roots, the shoot base with crown root primordia, and the floral organs, whereas qSOR1 mRNA was hardly detected in the leaf blade or the sheath (Fig. 1F and SI Appendix, Fig. S5A). Although qSOR1 had its highest expression levels in the floral organs, their sizes in the SA cultivar and in the q sor1-NILs were comparable, indicating that the loss of function of qSOR1 (q sor1) had few effects on floral morphology (SI Appendix, Fig. S5B). The root tip is the main organ involved in gravitational sensing, which determines the direction of root elongation (23), and we employed in situ hybridization to observe the spatial expression of qSOR1 within it. Signals were mainly detected in and around the gravity-sensing columella cells, called statocytes (23), and the expression patterns in SA, q sor1-NIL, and GB were equivalent (SI Appendix, Fig. S6A). The SA roots responded more sharply to rotations from a normal vertical to a horizontal axis than did those of the q sor1-NIL (Fig. 1 G and H). Roots of q SA/NIL also showed sharper gravitropic responses than did those of Vec/NIL (SI Appendix, Fig. S4 C and D). The qSOR1 expression pattern was stable even if the gravitational vector was changed (SI Appendix, Fig. S6B). To investigate whether the qSOR1 was involved in the development of statocytes, we stained amyloplasts in the columella cells of seminal roots. We found that the starch accumulation was indistinguishable between the SA and q sor1-NIL plants (SI Appendix, Fig. S7A). The size and number of the columella cells in the crown roots was also identical between the SA and q sor1-NIL (SI Appendix, Fig. S7 B–F). These findings indicated that the altered gravitropic response in the q sor1-NIL was not due to changes in the spatial expression patterns of qSOR1 or to morphological changes in the root columella cells.

We conducted qRT–PCR to monitor auxin responses in SA and the q sor1-NIL, as the phytohormone auxin plays a key role in root gravitropism. qSOR1 expression in both lines declined within 30 min of the application of the exogenous auxin (SI Appendix, Fig. S8A). We also examined the effects of the protein synthesis inhibitor cycloheximide (CHX) on seedlings with the auxin-dependent reduction of qSOR1 but found that it was not inhibited (SI Appendix, Fig. S8B), suggesting that de novo protein synthesis was not required for qSOR1 reduction by auxin. In the qSOR1 promoter region, we found two auxin response elements (AuxREs) (SI Appendix, Fig. S8C), corresponding to the TGTCTC motif for the ARF protein to bind to, to regulate the transcription of early auxin response genes (24, 25). This indicated that qSOR1 may be an early auxin response gene directly regulated by the auxin signaling pathway.

**Rice DRO1 Homologs Affect RGA.** When qSOR was compared to all protein sequences in rice, it was found to be most closely related to DRO1 (SI Appendix, Fig. S3A). Recently, homologs of rice DRO1 and LAZY, which has sequence similarities with DRO1, have been shown to be involved in the gravitropism of shoots or roots in several plants (26–29). Phylogenetic analysis of the DRO1 gene family in monocots and dicots revealed that many dicots had more similar sequences to qSOR1 (DRO1-like 1, DRL1) than to DRO1 (Fig. 24 and SI Appendix, Table S2). This suggests that the qSOR1 sequence may be more universal than the DRO1 in angiosperms. Phylogenetic analysis also showed that additional DRO1 homologs (DRO1-like 2, DRL2) were found in rice, maize, and sorghum (Fig. 24). To determine whether DRL2 regulates RGA in rice, knockout lines of DRL2 were created using the clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated protein 9 (Cas9) system (SI Appendix, Fig. S9). The knockout lines displayed smaller RGAs than the WT plants, like the qSOR1 and DRO1 knockout lines (Fig. 2 B and C), but with weaker gravitropic responses than the qSOR1 and DRO1 lines (SI Appendix, Fig. S10). DRL2 is located near the same chromosome region as the rice QTLs for RGA (19, 30), suggesting that there may be natural DRL2 variants in rice. Thus, the DRO1 family, consisting of three subgroups, plays a critical role in the genetic variation of RGA in rice.

**Conserved C-Terminal among the DRO1 Homologs Is Indispensable for RGA Control.** A region at the C-terminal of DRO1 homologs, which includes the WxxTD and EAR-like motifs, is well conserved, despite the relatively low homology of the proteins (SI Appendix, Fig. S3A and refs. 28 and 31). Recently, the role of this domain has been revealed in Arabidopsis. Taniguchi et al. designated the domain, including 14 critical amino acid sequences, as the conserved C terminus in the LAZY1 family of proteins (CCL) and demonstrated that the CCL domains in the qSOR1/laZY homologs of Arabidopsis were required for RGA control (28, 29). The interactions between the CCL domains of the laZY1-like (laZY) and the Brevis radix (BRX) domains of the RCC-like domain (RLD) proteins that regulate polar auxin transport are important processes that determine gravitropic responses in Arabidopsis root systems (32). To examine the function of this conserved region in rice, we made a series of transgenic constructs consisting of the 3.4-kb qSOR1 native promoter and truncated qSOR1 cDNA sequences (SI Appendix, Fig. S11A). Deletion lines introducing qSOR1 complimentary DNA (cDNA) without the N-terminal sequences showed similar RGAs to the positive control line, harboring the full-length qSOR1 cDNA from SA (SI Appendix, Fig. S11B). Deletion lines harboring the qSOR1 cDNA without the C-terminal sequences showed shallow RGA phenotypes, like the Vec/NIL. These results suggest that the conserved regions in the C-terminal of the DRO1 homologs are essential for RGA control in both dicots and monocots.

Like DRO1 (16), qSOR1 was involved in gravitropic responses under auxin signaling. However, qSOR1 was mainly expressed around columella cells, while DRO1 was expressed in the whole root meristems, except for the columella cells. To determine the subcellular localization of the qSOR1 proteins, we introduced a construct encoding an EGFP-fusion protein of the full-length qSOR1 from SA and that of a truncated qSOR1 from GB into rice protoplasts. EGFP fluorescence was observed in the plasma membrane for the full-length qSOR1 constructs (SI Appendix, Fig. S12). In contrast, the truncated qSOR1 protein without the conserved C-terminal, which was pivotal for RGA control, was not localized to the plasma membrane. These results indicated that the conserved C-terminal was needed for both protein
function and the subcellular localization of qSOR1. Similar observations were previously found in rice DRO1 (16). These findings suggest that the two genes may have similar functions for RGA control in differently expressed tissues, although further analysis will be needed to clarify the role of each gene with regard to root gravitropic responses.

**Natural Alleles of the DRO1 Homologs Contribute to RSA Variations in Rice.** To clarify the how qSOR1 and DRO1 regulate RGA, we used IR64, lowland rice, and three introgression lines (ILs) carrying DRO1 and qSOR1 alleles in an IR64 background (Fig. 3A). When comparing the DRO1 expression between the qSOR1 and qso1 backgrounds, and the qSOR1 expression between the DRO1 and dro1 backgrounds, respectively, no marked differences were found (SI Appendix, Fig. S13A and B). The amyloplast and columella cell development were also identical among the four lines (SI Appendix, Fig. S7). The RGA assay discovered differences in RGA, resulting in deep to shallow rooting in the order of the lines of [DRO1, qSOR1], [DRO1, qso1], [dro1, qSOR1], and [dro1, qso1], indicating that DRO1 and qSOR1 act additively to determine RGA (SI Appendix, Fig. S13C and D). We also measured the tiller angles of the four lines because LAZY1, which is a gene in the LAZY family, is associated with tiller angle in rice (33). These lines had no significant differences in tiller angle (SI Appendix, Fig. S13E). The expression and phenotypic assays suggested that qSOR1 functions in a distinct pathway from the DRO1 to determine RGA, and that neither gene contributes to tiller angle.

The RGA differences in the four lines observed using the basket assay were confirmed in the upland environment (Fig. 3B–D), although the [dro1, qso1] line showed an extremely small shoot biomass compared to the other three lines (SI Appendix, Fig. S14A). There was little difference between the shoot morphologies of the lines grown in the paddy (SI Appendix, Fig. S14B). The suppressed phenotype in the [dro1, qso1] line grown in the upland environment may be due to the extremely small root zone that resulted in reduced acquisition of water and nutrients from the soil. These results indicated that RGA alterations conditioned by dro1 and qso1 had little impact on plant growth if the plants had access to adequate water and nutrients, as they did in the paddy environment.

*Fig. 2.* Effects of DRO1 homologs on root growth angle. (A) Phylogenetic tree of full-length protein sequences sharing similarities with DRO1 and qSOR1 from both monocots and dicots. The gene names in red and blue are monocots and dicots, respectively. Scale bar shows distance estimated from amino acid substitutions. Correspondences between gene names in the tree and gene IDs are shown in **SI Appendix**, Table S2. (B) Root growth angle ($\theta_{rga}$) of the CRISPR-Cas9 lines. The RGA of the plants grown in stainless-steel mesh baskets for 6 wk after sowing was determined by measuring the angle between the horizontal line and the shallower nodal root. mt, homozygous mutant allele in target gene; WT, homozygous null allele in target gene. Data are shown as mean $\pm$ SD. $P$ values are based on the Student’s $t$ tests. (C) Images of the basal parts of the rice plants grown in the basket described in B. (Scale bars: 1 cm.)
Rice with SOR Can Avoid Reducing Stresses in Saline Paddy Soils. Saltwater incursions into paddy fields result in increased salt concentrations in the soil (Fig. 4A). We hypothesized that the SOR trait allowed rice roots to avoid the salt that settled in the soil. In the paddy fields, the qsor1-NIL had more SOR than the SA, irrespective of the genetic backgrounds of the functional alleles. The Na⁺ concentrations in the xylem exudates were identical for both lines under salinity stress, suggesting that the observed differences in RSA did not affect Na⁺ uptake (SI Appendix, Table S3). The Na⁺ concentrations in the xylem exudates were identical for both lines under salinity stress, suggesting that the observed differences in RSA did not affect Na⁺ uptake (SI Appendix, Table S3). The Na⁺ concentrations in the xylem exudates were identical for both lines under salinity stress, suggesting that the observed differences in RSA did not affect Na⁺ uptake (SI Appendix, Table S3).

Discussion

The SOR phenotype has been reported in cultivated rice and in wild relatives of maize (teosinte) (9, 17). Previously, QTL mapping was conducted to elucidate and utilize the natural variation for SOR in these plants (19, 35, 36), but the gene(s) underlying the SOR QTLs had not been isolated. In this study, we cloned a rice QTL related to SOR using map-based cloning. Phylogenetic analysis found that the qSOR1 gene is a DRO1 homolog in rice (Fig. 2A). We also revealed that, like DRO1, qSOR1 is negatively regulated by auxin signaling and involved in gravitropism (SI Appendix, Fig. S8 and Fig. 1 G and H). Gene expression studies and phenotypic analyses for RGA using qSOR1 and DRO1 introgression lines showed that both genes independently controlled RGA (SI Appendix, Fig. S13 A–D). Compared with the [DRO1, qSOR1] line, the [drol, qSOR1] line had shallow whole root systems, whereas the [DRO1, qSOR1] line had both shallow and deep roots. The differences in the RSA phenotypes of the lines with loss-of-function alleles for DRO1 or qSOR1 may be related to differences in their tissue-specific expression patterns of the two genes. Genes belonging to the LAZY family, including qSOR1 and DRO1, have been identified in both monocots and dicots (28, 37). These genes are classified into three types: those that affect only shoot gravitropism, only root gravitropism, and those that affect the gravitropism of both organs (37). Our results show that qSOR1 and DRO1 are involved only in root gravitropism, but they have different roles in the process, suggesting that multiple DRO1 homologs contribute to the genetic variation of RGA in rice. The fact that DRL2 was shown to control RGA further supports this hypothesis. To apply DRO1
homologs efficiently in molecular breeding for RSA, it is necessary to clarify in detail, the different functions of each gene.

Yield trials using SA and qSOR1-NIL over 4 y demonstrated that SOR contributed to improved yields in saline paddies, although the presence or absence of SOR did not affect the yield performance between SA and qSOR1-NIL in the control paddies. We initially anticipated that SOR would enable the rice root system to avoid soil with high-salts concentrations in saline paddies. However, SOR did not affect salt absorption (SI Appendix, Fig. S1E). Soil reduction, however, was observed in our experimental paddy fields that were treated with saline water for more than 20 y. The accumulation of excess Na⁺ in the soil generally results in undesirable soil structures, like increased soil bulk density. These soil structures have adverse effects on other soil physical properties, such as aeration and drainage performance (38), resulting in increased soil reduction. Such soil physical properties in saline paddies cause rice root development to be stunted (39). We also know, from previous studies, that saline soils create toxic reduced environments, similar to those created by Fe, Al, and organic acids (40). Consequently, it is presumed that SOR help plants to avoid reducing conditions, rather than directly avoiding the salinity stress, however, further investigations on the physiological benefits of SOR in saline paddies are needed.

SOR is one of the most reliable adaptations of upland crops to avoid waterlogging, since it enables the roots to obtain oxygen from the air (9, 41). To improve the tolerance to waterlogging, SOR and adventitious root formation under hypoxic environments have been studied in maize and soybean (42, 43). In rice, SOR is specifically found in the Bulu ecotype, which is grown in severe anaerobic conditions (18). Even in rice, which is a marsh plant, excessively reduced soils decrease yields, as the roots are damaged by mineral toxicities (44, 45). Therefore, the Bulu ecotype may have used SOR to avoid such anaerobic conditions. Our haplotype analysis showed that only Bulu cultivars from Indonesia with SOR carried the loss-of-function allele at qSOR1 (SI Appendix, Table S1). Natural populations of Arabidopsis grown in high-latitude regions (i.e., north of Sweden) are known to carry a specific allele of CYTOKININ OXIDASE 2 (CKX2) that produces a shallower RGA; this allele may confer a selective advantage allowing plants to adapt to soil hypoxia caused by snow and thaw (46). For a similar reason, the loss-of-function allele of qSOR1 may confer a selective advantage in Bulu cultivars grown in severe anaerobic conditions.

Since salinity is a major abiotic stress that is expected to impact negatively on an estimated 50% of all arable soils worldwide by 2050 (47), our work suggests that global food production could be improved by selecting for RSA that can avoid the damage caused by saline conditions (10). Saltwater intrusions and waterlogging in coastal regions due to sea level rise and cyclones, which will be exacerbated by climate change, threaten crop productivity worldwide (48). In the case of paddy rice, which is a staple food in those swampy regions, qSOR1 could prove to be a valuable breeding target to improve yields in saline paddy fields. The allele is globally rare, but locally common; the only rice cultivars known to carry this allele are Bulu ecotypes from Indonesia. In addition to saline paddy fields, SOR may be effective at avoiding damage caused by other stresses that result from reduced paddy conditions, such as excess iron. Moreover, as shallow rooting is known to be advantageous for P uptake in P-deficient soils (7), SOR may be beneficial for rice in P-deficient paddies. To clarify the relationship between SOR and nutrient uptake, further investigations are required. Our results also suggest that the qSOR1 homologs may help other upland crops such as maize and soybean to avoid waterlogging, as they were found in many other terrestrial plants.
**qSOR1** and **DRO1** function independently to determine RGA and have few adverse effects on shoot morphologies, making them useful for RSA breeding. **TAC1** homologs, which share conserved motifs with the **DRO1** homologs (but not the C-terminal EAR-like motif), determine shoot growth angle in several species (4, 49, 50). **TAC1** has been utilized in rice breeding to make shoot architecture more efficient and to increase photosynthetic efficiency in dense planting regimes (4). **DRO1** homologs could become powerful driving forces of the “Second Green Revolution” by improving root growth angles to enable plants to avoid a variety of environmental stresses, including drought, waterlogging, and saltwater intrusions.

**Materials and Methods**

**Plant Materials.** **GB** is a traditional *japonica* lowland rice cultivar (ecotype Bulu) that originated in Indonesia and grows crown roots on the soil surface. SA is a modern *japonica* lowland rice cultivar released in Japan that does not grow soil-surface roots. To characterize **qSOR1**, we developed a near-isogenic line (NIL) from which recombination had occurred within the region. **TAC1** homologs could become powerful driving forces of the “Second Green Revolution” by improving root growth angles to enable plants to avoid a variety of environmental stresses, including drought, waterlogging, and saltwater intrusions.

**High-Resolution Mapping of **qSOR1**.** We previously mapped **qSOR1** between the simple sequence repeat (SSR) markers RM21941 and RM21976 (19) (mozygous for the GB (donor parent) allele. We developed 4,806 BC3F2 plants progeny derived from a cross between SA (recurrent parent) and one we selected recombinant homozygous lines from an advanced-backcross experiment are shown in the SI Appendix, Table S4. PCR conditions were: 30 s at 95 °C followed by 40 cycles of 15 s at 95 °C and 1 min at 60 °C. Expression of the target genes was normalized to a ubiquitin gene. To examine the effects of the exogenous auxin treatments, seedlings were soaked in water containing 10 μM 2,4-dichlorophenoxyacetic acid for designated lengths of time. For the inhibition of protein synthesis, seedlings were soaked in water containing 100 μM CHX (Wako) or DMSO (control) for 1 h as a pretreatment, and then 2,4-dichlorophenoxyacetic acid was diluted with growth media to 10 μM (DMSO for control) and incubated for 3 h.

**In Situ Hybridization.** To investigate the spatial expression patterns of **qSOR1**, we collected root tips (3–4 mm in length) from 2-d-old SA, qsor1-NIL, and GB plants grown on 0.4% agarose (Sigma). To investigate **qSOR1** expression in planta, we performed in situ hybridization after rotation. We collected the root tips (3–4 mm in length) after rotations of 90° from the original vertical axis for 1.5 h, using 2-d-old seedlings grown on 0.4% agarose. Tissue fixation, hybridization, and immunological detection of the hybridized probes were performed as described previously (56), with minor modifications. For the probes, amplified **qSOR1** fragments were subcloned into pBluescript II KS+ (Stratagene). Digoxigenin-labeled antisense and sense probes were transcribed using a MAXIscript T7 In Vitro Transcription Kit (Ambion).

**Evaluation of Root Gravitropic Curvature.** We measured root gravitropic curvature of the seedlings in the SA, qsor1-NIL, and transgenic plants after rotating the roots from the normal vertical axis to the horizontal axis for 4 h, as described previously (16). Seedlings were grown for 36–48 h on 0.4% agarose, and then the root tissues were fixed with formalin–acetic acid–alcohol, 8-μm-thick paraffin sections of each line were made and stained with hematoxylin. The longitudinal length and width of the root system were measured using ImageJ (rsweb.nih.gov/ij/).

**Measurement of Columella Cell Size.** To observe the amyloplasts in the columella cells, we collected root tips (3–4 mm in length) from 1-d-old seedlings of SA, qsor1-NIL, [**DRO1**], **qSOR1**, [**DRO1**], qsor1, and [**DRO1**], qsor1 plants grown on 0.4% agarose in the dark at 30 °C. The root tips were treated with 1:1 KI solution for 1 min. The stained roots were then spread on a microscope slide and mounted either with chloral hydrate solution (8 g of chloral hydrate, 2 ml of water, 1 ml of glycerol). The root tips were observed under a light microscope (AX70, Olympus). To measure the cell size, we collected the crown root tips (3–4 mm in length) from 1-mo-old plants of the six lines grown in stainless-steel mesh baskets. After tissue fixation with formalin–acetic acid– alcohol, 8-μm-thick paraffin sections of each line were made and stained with hematoxylin. The longitudinal length and width of the root system were measured using ImageJ. The length of the root cells was divided by the total length of the root system of the **DRO1** and **qSOR1** functions.

**Vector Construction and Rice Transformation.** For the complementation tests (gSA), BACs of SA (SA008070) and GB (GB36304) were each selected from the corresponding BAC libraries that were constructed as described previously (52). A 7.57-kb genomic fragment of SA containing the **qSOR1** region was excised from the BAC clone SA008070 by Apal and XbaI, and then cloned into the pP2P2H-lac binary vector (53). For genome editing, the CRISPR/Cas9 cleavage sites of **qSOR1**, **DRO1**, and DRL2, were designed using CRISPRDirect (https://crispr.dlbcs.org), and the vectors were constructed using a previously published method (54). We then cloned the gRNA expression cassettes into the pZDgRNA binary vector by AscI and PacI. The primers used in this experiment are shown in the SI Appendix, Table S4. All generated constructs were introduced into the *Agrobacterium tumefaciens* strain EHA105 by electroporation. *Agrobacterium*-mediated transformation of the rice was then performed as described previously (55). Control plants were generated by introducing the empty binary vectors. Single-copy selection was conducted using the hygromycin phosphotransferase gene.

**RNA Isolation and Expression Analysis by qRT-PCR.** Total RNA was isolated from various tissues, using the RNaseq Plant Mini Kit (Qiagen), according to the manufacturer’s instructions. First-strand cDNA was synthesized using SuperScript II reverse transcriptase (Invitrogen). qRT-PCR using TaqMan probes was performed using specific primers and probes that are listed in the SI Appendix, Table S4. PCR conditions were: 30 s at 95 °C followed by 40 cycles of 15 s at 95 °C and 1 min at 60 °C. Expression of the target genes was normalized to a ubiquitin gene. To examine the effects of the exogenous auxin treatments, seedlings were soaked in water containing 10 μM 2,4-dichlorophenoxyacetic acid for designated lengths of time. For the inhibition of protein synthesis, seedlings were soaked in water containing 100 μM CHX (Wako) or DMSO (control) for 1 h as a pretreatment, and then 2,4-dichlorophenoxyacetic acid was diluted with growth media to 10 μM (DMSO for control) and incubated for 3 h.

**Sequence Alignment and Phylogenetic Tree Construction.** BLAST searches were performed using the amino acid sequences of **DRO1** or **qSOR1** as queries, and the protein sequences used to construct the phylogenetic trees can be found in the UniProtKB (https://www.uniprot.org/) database. The tree was constructed using a maximum likelihood algorithm with MEGA X (57). Full-length amino acid sequences were aligned by ClustalW to build the tree.

**Subcellular Localization Analysis.** The ORF sequences of **qSOR1** and **qSOR1** were amplified by PCR with cDNA as the template, using specific primers (SI Appendix, Table S4). The fragments were subcloned into p ENTER (Invitrogen) with the in-fusion directional cloning kit (Takara). The verified plasmids

---

Kitomi et al.
were mixed with the destination vector pSAT6-DEST-EGFP-N1 (SB) by Gateway LR Clonase (Invitrogen). Then, 2 × 335prom·qsOR1a(α)-EGFP, 2 × 335prom·qsOR1b(β)-EGFP and the plasmid membrane marker UBQ10prom·2×CHERRY-1×PHOSBP (59) were transfected to the protoplasts of young rice seedlings, using a previously described method, with a slight modification (60, 61). Briefly, rice stems of 1-wk-old seedlings were cut into 1-cm segments with a razor blade and then digested with cellulase solution including 10 mM β-mercaptoethanol. The protoplast suspension was prepared as described previously (61), and then 40 μL of protoplast suspension (ca. 1,000 cells) was transfected with 1 μg of DNA for these constructs into a 96-well plate. The EGFP and mCherry fluorescence were observed with a TCS SP5 confocal laser scanning microscope (Leica), according to the manufacturer’s protocol, after incubation overnight.

Quantification of Vertical Root Distribution. We investigated the vertical root distributions of IR64 and its three introgression lines using the trench method, as described previously (16). Plants were grown in an upland field under well-watered conditions at NARO (36°03′N, 140°10′E) in Tsukuba, Japan, in the summer of 2016. One hundred days after sowing, the soil was dug up near the hills of the plants and their root zones were observed. To quantify the root distributions of the rice plants, we took soil monoliths (30 × 30 × 5 cm) from the root area of the 97-d-old plants, using a metal monolith sampler and following the method as described previously (16). Samples were taken from the same field in which we used the trench method before conducting soil excavations. Each soil monolith was divided into 12 blocks, as illustrated in Fig. 3C. The roots in each block were washed, oven dried at 80 °C for 3 d, and then weighed to obtain the root dry weight.

Evaluation of Yield Performance under Salinity Stress Conditions. We investigated the grain yields of SA and the qSOR1-NIL in the experimental paddy fields irrigated with fresh (control) and saline water from 2015 to 2018. Field experiments were conducted at the Experimental Farm Station (38°47′N, 141°06′E), Graduate School of Life Sciences, Tohoku University, in Kashiwadai, Osaki, Miyagi prefecture, Japan, as described previously (62). Forty-nine fields, with at least three replications, in late May. The space between the hills of the plants was 15 cm), using a stainless-steel tube (5 cm diameter × 60 cm), to maintain a constant water level in the greenhouse. The oxidation-reduction potential (ORP) at a 5-cm depth in the soil was measured with ORP Electrodes (9300-10D, HORIBA).

Statistical Methods. All statistical analyses were performed with JMP v. 11.2.1 software (SAS Institute).

Data Availability. The data supporting the findings of the study and associated protocols are available in this article and its SI Appendix. Plant materials used in this study are available upon request to the corresponding author. The DNA Data Bank of Japan (DDBJ) accessions for qSOR1 from SA and GB are LC494454 and LC494455, respectively.

ACKNOWLEDGMENTS. We thank Y. Jaillais (Université de Lyon) for kindly providing the UBQ10prom·2×CHERRY-1×PHOSBP vector. We thank H. Kamamori, H. Fujiwasa, R. Motoyama, and Y. Nagamura from NARO and A. Nakamura from AIST for their technical support with the genomic and molecular analyses; Y. Itai, M. Takimoto, S. Tatsumi, J. Nakatsui, N. Maruyama, Y. Fukuda, S. Takayasu, E. Odajima, and S. Teramoto for their technical assistance with the plant phenotyping at NARO; the staff of the technical support section of NARO for their technical support with the genomic and molecular analyses; K. Ichijyo for technical assistance in the saline paddy field trials in Tohoku University; and S. McCouch (Cornell University) for critical reading of the manuscript. This research was supported by JSPS KAKENHI Grants 15K18630, 18K14447, 19H02936, EST Grant JP18C17210, Japan; and the Salt Damage Environment Research Foundation, Tohoku University.

1. S. Roychoudhry, S. Kepinski, Shoot and root branch growth angle control—the wonderfulness of lateralization. Curr. Opin. Plant Biol. 23, 124–131 (2015).
2. G. T. Freschet, E. Kichenin, D. A. Wardle, Explaining within-community variation in plant biomass allocation: A balance between organ biomass and morphology above vs below ground? J. Veg. Sci. 26, 431–440 (2015).
3. R. Heiden, The green revolution. Trends Genet. 19, 5–9 (2003).
4. B. Yu et al., TaC1, a major quantitative trait locus controlling tiller angle in rice. Plant J. 52, 891–898 (2007).
5. Y. Jiao et al., Regulation of OsSPL14 by OsMIR156 defines ideal plant architecture in rice. Nat. Genet. 42, 541–544 (2010).
6. K. Miura et al., OsSPL14 promotes panicle branching and higher grain productivity in rice. Nat. Genet. 42, 545–549 (2010).
7. J. P. Lynch, K. M. Brown, Topsoil foraging—An architectural adaptation of plants to low phosphorus availability. Plant Cell 23, 225–237 (2001).
8. Y. Uga, Y. Kitomi, S. Ishikawa, M. Yano, Genetic improvement for root growth angle under low phosphorus availability. Breed. Sci. 65, 111–119 (2015).
9. Y. Mano, F. Omori, Breeding for flooding tolerant maize using “teosinte” as a germplasm resource. Plant Root 1, 17–21 (2007).
10. E. D. Rogers, P. N. Benfey, Regulation of plant root system architecture: Implications for crop advancement. Curr. Opin. Biotechnol. 32, 93–98 (2015).
11. Y. Kitomi, J. Inoh, Y. Uga, “Genetic mechanisms involved in the formation of root system architecture” in Rice Genomics, Genetics and Breeding, T. Sasaki, M. Ashikari, Eds. (Springer Nature, Singapore, 2018), pp. 243–274.
12. L. Wang et al., LARGE Root ANGLE1, encoding OsPIN2, is involved in root system architecture in rice. J. Exp. Bot. 69, 385–397 (2018).
13. M. BETTENBROCK et al., Root cone angle is enlarged in docs1 LRR-RLK mutants in rice. Rice (N. Y.) 10, 50 (2017).
14. G. Huang et al., Rice actin binding protein RMD controls root angle in response to external phosphate. Nat. Commun. 9, 2346 (2018).
15. M. V. MICKELBART, P. M. HASEGAWA, J. BAILEY-SERRES, Genetic mechanisms of abiotic stress tolerance that translate to crop yield stability. Nat. Rev. Genet. 16, 237–251 (2015).
16. Y. Uga et al., Control of root system architecture by DEEPER ROOTING 1 increases rice yield under drought conditions. Nat. Genet. 45, 1097–1102 (2013).
17. Y. Ueno, T. Sato, Aerial root formation in rice etotype Bulu. Jpn. J. Trop. Agric. 33, 1773–1779 (1989).
18. H. R. LAFITTE, M. C. CHAMPoux, G. MCLEAREN, J. C. O’TOOLE, Rice root morphological traits are related to isozyme group and adaptation. Field Crops Res. 71, 57–70 (2001).
19. Y. Uga et al., Identification of qSOR1, a major rice QTL involved in soil-root surface rooting in paddy fields. Theor. Appl. Genet. 124, 75–86 (2012).
20. E. HANZAWA et al., The loss-of-function mutant gene for root-soil surface rooting in rice (Oryza sativa L.). Rice (N. Y.) 16, 30 (2013).
21. T. Sakai et al., The wavy growth 3 E3 ligase family controls the gravitropic response in Arabidopsis roots. Plant J. 70, 303–314 (2013).
22. H. Chen et al., E3 ubiquitin ligase SOR1 regulates ethylene response in rice root by modulating stability of Aux/IAA protein. Proc. Natl. Acad. Sci. U.S.A. 113, 4513–4518 (2018).
23. F. D. Sack, Plant gravity sensing. Int. Rev. Cytol. 127, 193–252 (1991).
24. T. Ulmasov, G. Hagen, T. J. Guilfoyle, ARF1, a transcription factor that binds to auxin response elements. Science 276, 1865–1868 (1997).
25. G. Hagen, T. Guilfoyle, Auxin-responsive gene expression: Genes, promoters and regulatory factors. Plant Mol. Biol. 49, 373–385 (2002).
26. T. Yoshihara, E. P. Spalding, M. lino, ATLAZY1 is a signaling component required for gravitropism of the Arabidopsis thaliana inflorescence. Plant J. 74, 267–279 (2013).
27. L. Ge, R. Chen, Negative gravitropism in plant roots. Nat. Plants 2, 16155 (2016).
28. J. M. Guseman, K. Webb, C. Srinivasan, C. Dardick, DROI influences root system architecture in Arabidopsis and Prunus species. Plant J. 89, 1093–1105 (2017).
29. M. Taniguchi et al., The Arabidopsis LAZY1 family plays a key role in gravity signaling within statocytes and in branch angle control of roots and shoots. Plant Cell 29, 1984–1999 (2017).
30. Y. Kitomi et al., QTLs underlying natural variation of root growth angle among rice cultivars with the same functional allele of DEEPER ROOTING 1. Rice (N. Y.) 8, 16 (2015).
31. A. Ashraf et al., Evolution of Deeper Rooting T-like homeologs in wheat entails the C-terminus mutations as well as gain and loss of auxin response elements. PloS One 14, e0214145 (2019).
32. M. Furutani et al., Polar recruitment of RLD by LAZY1-like protein during gravity signaling in root branch angle control. Nat. Commun. 11, 76 (2020).
33. P. Li et al., LAZY1 controls rice shoot gravitropism through regulating polar auxin transport. Cell Res. 17, 402–410 (2007).
34. F. Sun et al., Salt modulates gravity signaling pathway to regulate growth direction of primary roots in Arabidopsis. Plant Physiol. 146, 178–188 (2008).
35. Y. Mano, F. Omori, M. Muraki, T. Takamizo, QTL mapping of adventitious root formation under flooding conditions in tropical maize (Zea mays L) seedlings. Breed. Sci. 55, 343–347 (2005).
36. Y. Mano, F. Omori, C. H. Loaisiga, R. M. Bird, QTL mapping of above-ground architecture in Arabidopsis and Prunus species. Plant J. 74, 397–402 (2018).
37. S. Sakamoto, K. Matsui, Y. Oshima, N. Mitsuda, Efficient transient gene expression system using buckwheat hypocotyl protoplasts for large-scale experiments. Plant J. 385, 128–134 (2020).
38. M. Qadir, S. Schubert, Degradation processes and nutrient constraints in sodic soils. Land Degrad. Dev. 50, 175–182 (2016).