Root hairs facilitate rice root penetration into compacted layers

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SUMMARY

Compacted soil layers adversely affect rooting depth and access to deeper nutrient and water resources, thereby impacting climate resilience of crop production and global food security. Root hairs (RHs) play well-known roles in facilitating water and nutrients acquisition. Here, we report that RHs also contribute to root penetration into compacted layers. We demonstrate that longer RHs, induced by elevated auxin response during a root compaction response, improve the ability of rice roots to penetrate harder layers. This compaction induced auxin response in the RH zone is dependent on the root apex-expressed auxin synthesis gene *OsYUCCA8* (*OsYUC8*), which is induced by compaction stress. This auxin source for RH elongation relies on the auxin influx carrier OsAUX1 mobilizing this signal from the root apex to the RH zone. Mutants disrupting *OsYUC8* and *OsAUX1* genes exhibit shorter RHs and weaker penetration ability into harder layers compared to wild-type (WT). RH specific mutants (*rhl1-1* and *csld1*) phenocopy these auxin-signaling mutants, as they also exhibit an attenuated root penetration ability. We conclude that compaction stress upregulates OsYUC8-mediated auxin biosynthesis in the root apex, which is subsequently mobilized to the RH zone by OsAUX1, where auxin promotes RH elongation, improving anchorage of root tips to their surrounding soil environment and aiding their penetration ability into harder layers.

INTRODUCTION

A large proportion of arable land exhibits vulnerability to soil compaction stress, which represents a major challenge to agriculture and food security [1, 2]. Compacted subsoil layers adversely affect root downward growth [3], thereby impeding access to water and mobile nutrients at greater depths. The mechanical impedance of compacted layers constitutes a significant challenge, imposing physical and physiological limitations on plant growth, including diminished stomatal aperture, leaf size, plant height and yield [4, 5]. The reduction in biomass observed in crops grown in compacted soil is attributable in part to the impact of impaired root penetration and resource capture [6, 7]. In order to tackle these challenges, it is imperative to cultivate crops that possess an augmented capacity to penetrate compacted soils [1]. This is of particular significance given the projected global population of 10 billion by 2050 [8]. Thus, developing crops that can better penetrate compacted soils promise to aid food security efforts in the coming decades.

Identifying root adaptive mechanisms that aid penetration of compacted soil is vital [9]. When roots come across a compacted soil layer, they are confronted with two potential outcomes: either penetrate the layer or deviate from their initial growth trajectory [10]. In situations where the root lacks adequate lateral support or anchorage strength, the root tips bend above the compacted layer, impeding their access to deeper resources [11]. This provision of anchorage strength is purportedly facilitated by RHs, which are elongated, tubular outgrowths originating from root epidermal cells [12, 13]. RHs have long been attributed to enhancing the interactions between roots and soil, leading to increased water absorption and nutrient uptake [14, 15]. Despite the common association of RHs with anchorage during seedling establishment [9, 13], their functional roles during root penetration of compacted soil remain less explored.

In this study, we explore how RHs serve as a crucial component aiding rice root penetration of compacted layers. We report when encountering compacted layers, how

root tip expression of the auxin synthesis gene *OsYUC8* is upregulated, increasing auxin levels that are mobilized by the auxin transporter OsAUX1 to the RH zone. The attenuation of auxin responses in the RH zone of mutants lacking *OsYUC8* and *OsAUX1* reduces their ability to penetrate compacted layers. Moreover, two RH mutants also diminished root penetration capacity akin to mutants of *OsYUC8* and *OsAUX1*. Collectively, the augmentation of auxin response in roots induced by compaction stress stimulated RH elongation, aiding anchorage required for enhanced root penetration ability in compacted layers.

RESULTS

Rice root growth trajectory is altered when encountering a compacted layer

Compacted soils typically feature elevated mechanical strength due to increased bulk density, resulting in decreased gas diffusion [16, 17]. This reduction impedes ethylene diffusion out from root tissues, triggering root growth inhibition [16]. The impact of high soil strength on root growth also imposes water stress (matric potential) [18, 19], rendering it challenging to discern whether the plant is responding to low water availability, high soil strength, or both [7, 16]. To address this concern, we have devised an agar-based synthetic screening system to specifically study barrier strength, which has been extensively employed in the examination of mechanical strength in *Arabidopsis* [11, 20, 21]. This comprises a standardized system using agar composed of a top layer of 1% agar and a bottom layer of 3% agar. By integrating a high-density agar gel layer to simulate the hardpan (high strength layer) present in compacted soils (Figure 1A), we have generated a partial barrier that emulates the mechanical impedance encountered by roots.

To evaluate the capacity of root penetration through a strong layer, we monitored root growth angle and length after mechanical impedance. Following a growth period of five days, we observed that the growth of WT (cv. Nipponbare) rice roots was impeded at higher levels of compaction (3% agar, as depicted in Figure 1B), consistent with previous studies conducted in soil [22, 23]. We here chose 3% agar to study mechanical impedance responses as 3% agar was sufficient to reduce the root growth up to 25% as compared to 1% agar. Notably, in our split system (1%/3% agar, as illustrated in Figure 1B), roots exhibit curvature after encountering the boundary, indicating that an increase in mechanical impedance (stress) alters root growth angle.

Auxin acts downstream of ethylene in modulating root elongation through compacted soil [24] and also controlling root angle [25]. To investigate whether auxin plays a role in the root's response to mechanical stress, we utilized the DR5-VENUS reporter to reveal changes in auxin response during this adaptive growth process [25]. Confocal imaging revealed an elevated auxin response in the root apex and epidermal cells in the elongation zone of WT (cv. Dongjin) roots upon encountering the barrier (Figures 1C-1E). Our observations suggest mechanical impedance triggers a local auxin response in root tip tissues, but it is unclear how this elevated auxin response is mediated.

OsYUC8-mediated auxin biosynthesis regulates root penetration through barriers

YUC family members control auxin biosynthesis via the conversion of indole-3-pyruvic acid (IPA) to indole-3-acetic acid (IAA) in plants [26]. Among the 14 members in rice,

OsYUC8 was found to play a major role in regulating primary root growth [27]. Consistently, only *OsYUC8* was induced by mechanical impedance compared to other root-expressed *OsYUC* genes including *OsYUC3*, OsYUC5, and *OsYUC11*) (Figure 2A). In order to localize the expression of *OsYUC8*, a *GUS* reporter gene was transcriptionally fused to a 3.0 kb fragment upstream of the *OsYUC8* start codon. Histochemical analysis revealed that the localized *GUS* transcripts were predominately accumulated at the root apex of transgenic plants (Figure 2B). This *OsYUC8* reporter revealed a significant elevation in GUS activity when roots encountered a stronger layer (Figures 2C and 2D), suggesting that its expression was induced in response to mechanical impedance. Given this pattern of expression of *OsYUC8* in root apex, it is plausible that the induction of higher auxin response by root tissues encountering mechanical impedance depends on OsYUC8-mediated auxin biosynthesis.

To experimentally verify the OsYUC8-mediated model, we crossed the rice DR5-VENUS auxin reporter into the *osyuc8-2* background, a T-DNA insertion knockout mutant [27]. The fluorescence intensity of the DR5-VENUS reporter in epidermal cells of differentiated zone of WT (cv. Hwayoung) was observed to be reduced in *osyuc8-2* roots, particularly after encountering the harder agar layer (Figure 3A and 3B). Consistently, mechanical impedance induced expression of *OsIAA20*, an auxin-responsive gene [25], yet it remains unchanged in *osyuc8-2* (Figure 3C). Hence, the upregulation of auxin response observed in root tip tissues following mechanical impedance is dependent on *OsYUC8*.

To test the physiological function of OsYUC8-mediated auxin biosynthesis in root response to strong layers, we tested whether *osyuc8-2* roots exhibited an altered response after encountering a stronger agar barrier. This revealed that *osyuc8-2* plants grown in a split system exhibited significantly greater bending angles than their WT background (Figures 3D-3G). To test if other OsYUC family members are required to mediate auxin biosynthesis during the root response to mechanical impedance, we employed a specific auxin biosynthesis inhibitor called yucasin, which specially inhibits the function of YUC proteins [28]. Significantly, roots of both WT and *osyuc8-2* mutants treated with yucasin displayed a comparable phenotype to untreated *osyuc8-2* controls (Figure 3D-3G). Hence, OsYUC8-mediated auxin biosynthesis is specifically required to facilitate root penetration into harder layers.

Next, we investigated whether the root penetration deficiency of *osyuc8-2* lines could be ascribed to a compromised gravitropic response. However, *osyuc8-2* and WT roots exhibited comparable gravitropic responses, regardless of yucasin treatment (Figure 4A-4C). Hence, another root characteristic regulated by OsYUC8 appears to promote rice root ability to penetrate harder layers. RH elongation is known to be regulated by auxin [29] and is linked with improved soil penetration during seedling establishment [13]. Examination of RH length revealed that they are much longer in WT roots grown in the split (1%/3%) system compared to lower-compaction (1%/1%) system (Figures 4D-4L). However, induction of RH length by the split system was completely suppressed in the roots of *osyuc8-2*, and *osyuc8-2* and WT treated with yucasin (Figures 4D-4L). These findings suggest OsYUC8-mediated auxin promotion of RH elongation may aid rice root penetration when encountering harder layers.

The *OsAUX1* mutation disrupts rice root penetration into harder layers

Auxin-mediated promotion of rice RH elongation is dependent on OsAUX1 to facilitate auxin transport from the root apex to the RH zone [29]. To examine the impact of the

osaux1 mutation on the induction of the auxin response when encountering a harder layer, we compared DR5-VENUS expression in WT versus mutant backgrounds. In contrast to WT, *osaux1-3* displayed no change in the DR5-VENUS auxin response reporter when mutant root tips encountered the boundary of the harder layer (Figures 5A-5E). These findings reveal that the elevated auxin responses triggered by the mechanical impedance were disrupted in the root epidermal cells of the *osaux1-3* mutant. Consequently, *osaux1-3* provides a useful genetic tool to explore the functional importance of the elevated auxin response in the root elongation zone during penetration of harder layers.

As anticipated, WT treated with yucasin and *osaux1-3* mutant roots exhibit quicker elongation compared to WT under uniform impedance conditions (i.e. 1%:1% or 3%:3%; Figures 5F-5J), which is consistent with previous findings in compacted soil (22). However, *osaux1-3* mutant roots exhibited a reduced ability to penetrate harder agar layers (1%/3%) and instead featured a horizontal growth pattern along the boundary of the high strength agar layer (Figures 5F-5I and 5K). This behavior was in contrast to the successful penetration by WT (cv. Dongjin) roots into the high strength agar layers (Figures 5F-5I and 5K). Moreover, *osaux1-3* exhibits less response to external yucasin treatment (Figures 5F-5K). Mutational studies indicate that the increased auxin responses in the elongation zone, which is reliant on OsAUX1-mediated shootward auxin transport, also play a crucial role in facilitating root penetration into harder layers.

How can OsAUX1 facilitate root penetration into harder layers? OsAUX1-mediated shootward auxin transport is required for root gravitropism and RH elongation in response to environmental stimuli [29]. Consistently, *osaux1-3* exhibits a reduced gravitropic response, as evidenced by a bending angle of approximately 30 degrees, in contrast to the approximately 90 degrees exhibited by the WT (Figure S1A and S1B). The *osaux1-3* mutant also displays shorter RHs (but with a normal number of RHs) in the split system, compared to the WT (Figure S1C-S1H), indicating that the elongation of RHs in response to encountering a harder layer is dependent on OsAUX1. Whilst it is plausible that the defective penetration ability of *osaux1-3* into harder layers was attributable to the mutant's reduced gravitropic response, it appears more likely that the impaired length of *osaux1* RHs disrupts root penetration into compacted layers (through decreased anchorage).

Our results imply auxin plays a crucial role modulating root penetration by promoting RH elongation. To further verify this, we examined the effects of auxin treatment on the root penetration ability of auxin mutants, *osyuc8* and *osaux1* when challenged with our compacted layer bioassay. We observed that the defective penetration phenotypes observed in these mutants were effectively restored after exogenous treatment with the synthetic auxin 1-NAA (Figure S2A-S2F). Furthermore, the penetration ability of WT (cv. DJ) was further increased following auxin treatment (Figure 2D-2F). Consistently, longer RHs were observed in WT, and defects in RH length of auxin mutants were completely restored upon auxin treatment (Figure 2G-2P). Taken together, it appears plausible that auxin promotes root penetration by promoting RH elongation.

RH mutants exhibit reduced root penetration ability

To determine whether RH elongation (rather than gravitropism) was important for enabling roots to penetrate harder layers, we next characterized rice mutants that specifically disrupt RH elongation. Mutant lines were characterized for *RHL1*, which encodes a novel basic helix-loop-helix (bHLH) transcription factor expressed exclusively in RH cells [30].

The RH length of *rhl1-1* is significantly reduced in comparison to WT (cv. Kas) [30]. Similarly, *cellulose synthase-like D1* (*CSLD1*) gene is expressed solely in RH cells [31]. Compared with WT (cv. DJ), RHs of *csld1* are shorter, but there is no defect in RH density [31]. To validate their RH specific expression patterns, promoter fragments of *RHL1* and *CSLD1* were fused to the VENUS-N7 nuclei reporter, confirming that they were exclusively detected in RH cell nuclei (Figure S4A-S4D). Consequently, *rhl1-1* and *csld1* mutants, which exhibit RH specific defects were deployed to evaluate the contribution of RHs in helping roots penetrate harder layers.

Closer analysis demonstrated that RHs were shorter in RH mutants compared to WT counterparts. Nevertheless, when grown in a uniform hardness split agar system, the root length of *rhl1-1* and *csld1* mutants was comparable to their respective WT backgrounds (Figure S4E), indicating root elongation ability of RH mutants is unimpaired under these constant impedance conditions. Furthermore, RH mutants and WT plants showed no discernible differences in their root gravitropic responses (Figure S4F-S4G), further supporting their specific role in RH development. To assess the impact of RHs on the ability of roots to penetrate stronger layers, we grew RH mutants in the split system. We observed only a minor deviation in growth trajectory of WT roots, in contrast, RH mutants exhibited enhanced bending (Figures 6A and 6B). Furthermore, it is observed that RH mutants do not display any response to external auxin treatment (Figures S5), suggesting that RH genes function downstream of auxin in the regulation of RH-mediated root penetration. This finding suggests that RH mutants encountered difficulties penetrating harder layers, which is reminiscent of the phenotype observed in *osyuc8-2* (Figures 3D-3G).

RHs are reported to aid seedling establishment through providing anchorage for emerging roots to penetrate the soil surface [13]. Quantification of the maximum reaction force (anchorage) provided by RHs is frequently based on the force required to extract a root. In uniform systems, the force needed to pull out a WT root was significantly greater than that required for RH mutants, across different densities of agar layers (Figure S6). Our findings support that increased RH lengths enhance the anchorage of growing root tips to penetrate harder layers.

To investigate whether rice RH mutants exhibit comparable penetration defects in both split agar- and soil- based systems, we imaged RH mutants growing through compacted layers of varying bulk densities (upper: 1.0 g cm^{-3} bulk density [BD]; lower 1.4 g cm^{-3} BD; Video S1). Using micro-computed tomography (CT), we observed that RH mutants displayed a sharp ‘S-shape’ in their root growth direction compared to WT lines when encountering a compacted soil layer (Figures 6C-6G; Videos S2-S5). This altered root growth behaviour resembled what was observed in the artificial agar-based system (Figure 6A). Additionally, RHs were shorter in mutants compared to WT (Figures 6H-6L). Our findings reveal that RH elongation is necessary for effective root penetration into compacted layers. We conclude that compacted layers induce elevated auxin biosynthesis and transport to the RH zone. This, in turn, leads to the formation of longer RHs, which provide enhanced anchorage for root penetration.

DISCUSSION

Compacted soil, particularly in the form of a hardpan, is prevalent in lowland rice fields and hinders the penetration of roots to deeper soil layers [32, 33]. As a result, nutrient

uptake is reduced, and rain fed crops become more susceptible to drought stress [33]. In this work, we demonstrate impedance induced promotion of RH length plays a crucial role in anchoring growing roots and aiding their penetration of compacted layers. In addition, we report the molecular mechanisms underlying the elongation response of RHs to compacted layers facilitated by auxin (Figure 6M). The presence of strong agar layers triggers the induction of *OsYUC8*, a key gene involved in auxin biosynthesis, leading to an elevated auxin concentration at the root apex. Subsequently, this auxin is transported via OsAUX1 from the root apex to the differentiation zone, promoting the elongation of RHs. The elongation of RHs results in an increased surface area of root-soil contact, generating the required anchorage force to support root penetration into compacted layers. Our previous research has also unveiled the pivotal role of AUX1 in facilitating RH elongation under low phosphate conditions [29]. This process is crucial for transporting auxin back to the differentiation zone where RH elongation takes place. This finding underscores the possibility that longer RHs in compacted soil may have contributed to enhanced phosphate uptake [34]. These results collectively indicate that AUX1-mediated RH elongation is a conserved mechanism across different plant species and under a diverse array of environmental conditions.

Previous studies have demonstrated that reduced ethylene diffusion in compacted soil leads to the accumulation of auxin and abscisic acid (ABA) in root tip tissues, thereby inhibiting root elongation within compacted soils [16, 24]. Mutants of the related hormone-signaling genes exhibit better elongation ability within compacted soil [16, 24]. In line with these findings, our research also indicates that mutants of auxin transport genes do not exhibit a response to uniform compaction conditions (Figures 5F-5J). However, mutants with disrupted auxin biosynthesis and transport pathways display reduced penetration ability into compacted layers, accompanied by significant changes in growth trajectory (Figures 3D-3G; Figures 5F-5I and 5K). These observations suggest that successful root penetration is reliant on rice roots' ability to avoid bending when encountering a hard layer. Hence, differences in the ability of rice roots to penetrate compacted layers are not solely determined by their elongation ability in strong soil, but also by their capacity to overcome mechanical impedance when faced with a rapid increase in soil resistance.

Our study reports that increased root auxin responses in strong agar layers are dependent on the induction of OsYUC8-mediated auxin biosynthesis at the root apex and subsequent OsAUX1-mediated shootward auxin transport, triggering auxin responsive markers like *OsIAA20* (Figure 3C). Disrupting OsYUC8 blocked upregulation of auxin responsive markers, revealing the importance of auxin biosynthesis (rather than auxin catabolism and response). The mechanistic basis of *OsYUC8* upregulation after encountering mechanical impedance remains unclear (Figure 2). Mechanical stimulation induces higher expression of the mechano-inducible calcium channel PIEZO1 (PZO1) in columella and lateral root cap cells in *Arabidopsis* [21]. Furthermore, *pzo1* seedlings exhibited reduced calcium transients and failed to penetrate hard agar, indicating the involvement of PZO1 in the root's short-term response to mechanical detection of compacted soil layers [21, 35]. This calcium-signaling pathway may act upstream of auxin (and OsYUC8) in the root barrier-touching response.

The primary advantage of RHs facilitating root penetration into high-strength layers lies in their ability to increase the number of roots that can successfully penetrate these layers, rather than solely improving the rate of elongation once within the compacted layer. This trait is particularly valuable in agricultural fields, as enhancing root penetration has a

significant impact on enlarging the overall root system size and increasing access to soil resources, especially those situated in deeper layers. Additionally, enhanced root penetration ability is imperative for establishment of plants under conditions prone to topsoil drying. Our results provide new insights into a key root trait for breeders to select to enable crops to be more resilient to soil stresses, by exploiting variation in RH length.

STAR★Mothods

Key Resource Table

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Bacterial strains		
E. coli (TOP10)	Widely used	N/A
Agrobacterium tumefaciens (strain EHA105)	Widely used	N/A
Critical commercial assays		
RNeasy Plant Mini	QIAGEN	Cat# 74904
HiScript II Q RT SuperMix for qPCR (+gDNA wiper)	Vazyme	Cat# R223-01
KOD One^{TM} PCR Master Mix	TOYOBO	Cat# KMM-101
Agar (Phytagel)	Sigma	Cat# P8169
ClonExpress II One Step Cloning Kit	Vazyme	Cat# C112-02
Experimental models: Organisms/strains		
Rice: Nipponbare (wild type)	Widely used	N/A
Rice: DongJin (wild type)	Widely used	N/A
Rice: Hwayoung (wild type)	Widely used	N/A
Rice: Kasalath (wild type)	Widely used	N/A
Rice: *osyuc8-2*/HWY	Qin et al.[27]	N/A
Rice: osaux1-3/DJ	Giri et al.[29]	N/A
Rice: DR5-VENUS	Huang et al.[25]	N/A
Rice: *oscsld1*/DJ	Kim et al.[31]	N/A
Rice: *osrhl1-1*/Kas	Ding et al.[30]	N/A
Rice: *ProRHL1::VENUS-N7*	This study	N/A
Rice: *ProCSLD1::VENUS-N7*	This study	N/A
Rice: DR5-VENUS/*osyuc8-2*	This study	N/A
Rice: DR5-VENUS/*osaux1-3*	Giri et al.[29]	N/A
Rice: *ProOsYUC8::GUS*	This study	N/A
Oligonucleotides		
OsYUC3 qRT-PCR Forward: GTGAGAACGGGCTCTACTCGGTCG	This paper	N/A
OsYUC3 qRT-PCR Reverse: GCTTATGCATGACCGATGAACACG	This paper	N/A
OsYUC5 qRT-PCR Forward: GAGAAATACGGCCTCCGACG	This paper	N/A
OsYUC5 qRT-PCR Reverse: CGACCCCATCCTCTGTGAAG	This paper	N/A
OsYUC8 qRT-PCR Forward: CCAACATCTCCTCGGTGTAG	This paper	N/A
OsYUC8 qRT-PCR Reverse: GCATCAGACAAGCAACATCC	This paper	N/A
OsYUC11 qRT-PCR Forward: ATGCCCAAGAAGGACTTCCC	This paper	N/A
OsYUC11 qRT-PCR Reverse: GAAGGCCTTGACGTCATTAGCA	This paper	N/A
OsIAA20 qRT-PCR Forward: CGGGATTATTTTGTTCACGTTTC	This paper	N/A

OsIAA20 qRT-PCR Reverse: CGAGATTTCATTCGTCATGCTTA	This paper	N/A
TUB qRT-PCR Forward: GCTGACCACACCTAGCTTTGG	This paper	N/A
TUB qRT-PCR Reverse: AGGGAACCTTAGGCAGCATGT	This paper	N/A
Software		
ImageJ	Widely used	https://imagej.nih.gov/ij/
Excel 2023	Widely used	N/A

326
327

Resource Available

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Guoqiang Huang (huang19880901@sjtu.edu.cn).

Materials availability

DNA constructs and transgenic rice seeds generated in this study are available from the Lead Contact, Guoqiang Huang, upon request.

Data and code availability

All data reported in this paper will be shared by the lead contact upon request.

This paper does not report original code.

Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

Experimental model and subject details

Plant materials and growth conditions

The genetic backgrounds of rice cultivars are Dong Jin (DJ) (*Oryza sativa*, *Japonica*), Kasalath (Kas) (*Oryza sativa*, *Indica*) and Hwayoung (HWY) (*Oryza sativa*, *Japonica*). *Osaux1-3* and *csld1* are two T-DNA insertion mutants under DJ background. *Osyuc8-2* is a T-DNA insertion mutant in HWY background. *rhl1-1* harbors a point mutation disrupting the splicing site and causing a shift in the reading frame. All rice plants were cultured in Shanghai (31°3′ N, 121°44′ E) and Sanya (8°33′ N, 109°16′ E), China, in the summer and winter seasons, respectively. The seedlings were cultured in a light incubator with 18h-lightness/6h-darkness at 28 °C.

Root penetration treatment

The rice seeds were dipped into the water under dark for 4 days at 28 °C. The germinated seeds were transferred and inserted into the medium containing 1% (w/v)/1%, 1%/3% and 3%/3% low-melting agars (pH = 5.8) melted by ddH_2O. The germinated seeds were cultured at 12-h lightness/12-h darkness at 28 °C. After 5-day growth, the excess agar around the root system was carefully chopped with maintaining its original growth state, then the closely surrounding agar was melted and then removed by 50 °C heat treatment for 5 min prior to imaging. Finally, the seedlings were used to be imaged and analyzed. The root length and growth angle were calculated via ImageJ (https://imagej.nih.gov/ij/).

Soil materials preparation and CT scanning

Soil was collected from Yingtan, Jiangxi Province, CHN (28°15′ N, 116°5′ E), passed through a 2 mm sieve. Soil basic properties: pH 4.9, soil organic carbon 10.23 g kg^{-1}, total nitrogen 0.90 g kg^{-1}, available phosphorus 34.15 mg kg^{-1}, available potassium 235.11 mg kg^{-1}. Equally germinated seedlings of WT and mutants were grown in columns (60 mm diameter x 130 mm height) filled with the soil packed to a bulk density of either 1.0 g cm^{-3} (noncompacted) or 1.4 g cm^{-3} (compacted). These plants were grown in a controlled growth chamber maintained at 28 °C, 16-hour photoperiod with 70% relative humidity. The root systems of 5-day-old seedlings were imaged non-destructively using an Xradia 520 Versa (Carl Zeiss, Germany) based at INSTRUMENTAL ANALYSIS CENTER, Shanghai

Jiao Tong University. Scans were acquired by collecting 1200 projection images at 140 kV X-ray energy. Scan resolution was 40 microns. Image reconstruction was performed using Datos|REC software (GE Inspection Technologies, Wunstorf, Germany) and roots were visualized and measured using the polyline tool in VGStudioMax (Volume Graphics GmbH, Germany).

Chemical treatment

Rice seeds (WT, *osyuc8-2*, and *osaux1-3*, and other mutants) were germinated for 4 days in water at 28 °C. The germinated seeds were transferred and inserted into the medium with/without 20 μM yucasin (5–(4–chlorophenyl)-4H-1,2,4–triazole-3–thiol (WAKO, 352–12001)) or 10 nM NAA. After 5-day growth, the roots were imaged and analyzed as above description.

RNA extraction, RT-PCR, qRT-PCR and vector construct

The samples were harvested from 0 to 5 mm distal from the primary root tips of the 5-day-old seedlings when root encountering the mechanical impedance. Total RNA was extracted using TRIzol reagent (Invitrogen) with accordance to the instruction of manufacturer. 1 μg RNA was used to synthesize the first strand cDNA using the Rever Tra Ace-a-First strand cDNA synthesis kit (Vazyme). The quantitative RT-PCR (qRT-PCR) analysis was conducted as previously described [25]. The rice *TUB* gene was used as internal control.

All PCR amplifications were done with KOD ONE DNA polymerases (TOYOBO) with the recommended annealing temperature and extension time. Sequences were analyzed with SnapGene. PCR products were recovered with QIAquick® Spin miniprep kit, and DNA midipreps were with the Qiagen TIP-100 kit. The function *proYUC8::GUS* reporter construct was constructed via the ClonExpress II One Step Cloning Kit (Vazyme). For this vector, more than 15 independent transgenic lines were obtained for each vector. One representative transgenic plant was used for further analysis.

GUS staining

The roots of 5-day-old *ProOsYUC8::GUS* transgenic plants were immersed into GUS solution (50 mM Na_3PO_4 (pH 7.0), 50 mM NaH_2PO_4, 10 mg/mL X-Gluc and 0.02% (v/v) TritonX-100, 10 mM Na_2EDTA, 0.5 mM $K_3[Fe(CN)_6]$, 0.5 mM $K_4[Fe(CN)_6]$) under dark at 37 °C for 12 h. After that, the samples were washed with 70% ethanol for 36 h. The GUS staining images were taken via Leica light microscope (M205A) with a CCD camera.

RH assays

The rice seeds were germinated in the water under dark for 4 days at 28 °C. Uniformly germinating seeds were inserted into the agar system. After 5-day growth, the excess agar around the root system was carefully punctured without disrupting its original growth state, then the closely surrounding agar was melted and removed by 50 °C heat treatment for further imaging. RH was recorded on elongated zone of the primary root using a Leica transmission microscope (dark field). RH length was measured as the average of 10 fully elongated root hairs of one seedling via ImageJ (https://imagej.nih.gov/ij/).

Laser scanning microscope assays

Auxin response DR5-VENUS reporter seeds were germinated in dark for 4 days and then transferred into different densities of agar to grow for another 5 days under 28 °C. The roots were extracted from the agars and used to be observed via Leica Laser Scan

Microscope (SP5) using an excitation wavelength of 488 nm and emission wavelength of 500-550 nm. Confocal images were analyzed via ImageJ (https://imagej.nih.gov/ij/).

Accession numbers

OsYUC3 (*Os01g0732700*); *OsYUC5* (*Os12g0512000*); *OsYUC8* (*Os03g0162000*); *OsYUC11* (*Os12g0189500*); *OsRHL1* (*Os06g0184000*); *OsCSLD1* (*Os10g0578200*); *OsAUX1* (*Os01g0856500*); *OsEIL1* (*Os03g0324200*); *OsEIN2* (*Os07g0155600*).

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Author Contributions

X.K., S.Y.., Y.X., X.S., L.N., X.W., J.R., and G.H. performed experiments; X.K., S.Y., B.P., H.Z., and G.H. performed data analysis; G.H., M.B., and H.Z. designed the experiments; and G.H., B.P., H.Z., and M.B. wrote the paper.

Declaration of interests

The authors declare no competing interests.

Inclusion and diversity

We support inclusive, diverse, and equitable conduct of research.

Supplemental information

Document S1. Figures S1-S6

Video S1.

3D images of split soil system used in this system. Upper soil: 1.0 g cm^{-3} BD (non-compacted soil), Lower soil: 1.4 g cm^{-3} BD (compacted soil), related to Figure S6.

Video S2

3D images of WT (Kas) used in this system. 5-day-old seeding of WT was imaged by CT, related to Figure 6.

Video S3

3D images of *rhl1-1* used in this system. 5-day-old seeding of *rhl1-1* was imaged by CT, related to Figure 6.

Video S4

3D images of WT (DJ) used in this system. 5-day-old seeding of WT was imaged by CT, related to Figure 6.

Video S5

3D images of *csld1* used in this system. 5-day-old seeding of *csld1* was imaged by CT, related to Figure 6.

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576