Rice auxin influx carrier OsAUX1 facilitates root hair elongation in response to low external phosphate

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Root traits such as root angle and hair length influence resource acquisition particularly for immobile nutrients like phosphorus (P). Here, we attempted to modify root angle in rice by disrupting the OsAUX1 auxin influx transporter gene in an effort to improve rice P acquisition efficiency. We show by X-ray microCT imaging that root angle is altered in the osaux1 mutant, causing preferential foraging in the top soil where P normally accumulates, yet surprisingly, P acquisition efficiency does not improve. Through closer investigation, we reveal that OsAUX1 also promotes root hair elongation in response to P limitation. Reporter studies reveal that auxin response increases in the root hair zone in low P environments. We demonstrate that OsAUX1 functions to mobilize auxin from the root apex to the differentiation zone where this signal promotes hair elongation when roots encounter low external P. We conclude that auxin and OsAUX1 play key roles in promoting root foraging for P in rice.
ood security represents a pressing global issue. Crop production has to double by 2050 to keep pace with predictions of global population increasing to 9 billion. This target is even more challenging given the impact of climate change on water availability and the drive to reduce fertilizer inputs to make agriculture environmentally sustainable. In both cases, developing crops with improved water and nutrient uptake efficiency by manipulating root architecture, which critically influences nutrient and water uptake efficiency would provide part of the solution. For example, root angle impacts phosphate acquisition efficiency (PAE) as this nutrient preferentially accumulates in the top soil1,2.

Very few genes that regulate root architecture traits such as root angle have been identified in crop plants to date3. In contrast, major progress has been made characterizing genes and molecular mechanisms controlling root angle in the model plant Arabidopsis thaliana4. AUX1 was one of the first genes identified in Arabidopsis to control root angle5,6 and later shown to encode an auxin influx carrier7,8. AUX1 regulates root angle by transporting auxin from gravity-sensing columella cells at the root tip via the lateral root cap to elongating epidermal cells that undergo differential growth to trigger root bending9,10. Such detailed functional information in model organisms opens possibilities to perform translational studies to manipulate equivalent root traits in crops controlled by orthologous genes.

In this study, we describe how a translational approach was initially adopted to improve PAE in rice by genetically manipulating the orthologous AUX1 sequence. Reverse genetic studies in rice combined with non-invasive X-ray (microCT) imaging in soil confirmed that root angle was significantly altered in osaux1 compared to wild-type plants. Nevertheless, physiological experiments performed on osaux1 (versus wild-type) failed to demonstrate improvement in PAE, suggesting that OsAUX1 controls other traits important to P acquisition. Further studies revealed OsAUX1 was also required for rice root hair elongation, an important adaptive response designed to forage for immobile nutrients such as P in the soil11. Auxin quantification and reporter lines revealed that under low P conditions, auxin levels are elevated in the root hair zone. We conclude that in response to low external P supply, OsAUX1 is required to transport elevated auxin from the root apex to the differentiation zone to promote root hair elongation and hence facilitate rice P acquisition. In parallel papers, we demonstrate that this auxin-dependent root hair response to low external P is highly conserved in the dicotyledonous model Arabidopsis thaliana12 and which relies on AUX1 to promote hair elongation via intracellular auxin and calcium signaling13.

Results

Rice root angle is altered by disrupting the OsAUX1 gene. The AUX1 gene family in rice is encoded by five closely related OsAUX1/LAX genes (Supplementary Figure 1a). Bioinformatic analysis revealed that the two rice sequences (Os01g63770 and Os05g37470) were closely related to AUX1. In order to identify which rice sequence(s) represents an orthologous gene, we tested the ability of each of their cDNA sequences to complement the Arabidopsis aux1 agritropic phenotype. This genetic assay revealed that only one of the OsAUX1 sequences (Os01g63770) was able to successfully rescue the aux1 mutant’s root agritropic defect (Supplementary Figure 1b, c). Our observations are consistent with previous complementation experiments using Arabidopsis AUX/LAX sequences, which revealed that gene family members had undergone a process of sub-functionalization14.

To test the in planta function of OsAUX1 in rice directly, we characterized two independent T-DNA insertion lines (3A-51110 and 3A-01770) disrupting the Os01g63770 genomic sequence in the Dongjin background (see “Methods”). The T-DNA insertion lines were termed osaux1-1:1 and osaux1-1:3 (in agreement with Zhao et al.15). Southern hybridization confirmed that single T-DNA insertion events had disrupted the OsAUX1 gene in osaux1-1:1 and osaux1-1:3, respectively. PCR amplification of genomic fragments adjacent to each T-DNA followed by sequencing confirmed that T-DNA insertions in osaux1-1:1 and osaux1-1:3 had disrupted the gene coding sequence in exon 3 and exon 6, respectively (Fig. 1a). Reverse transcription quantitative-PCR (RT-qPCR) analysis also revealed that both T-DNA alleles exhibited significantly reduced OsAUX1 transcript abundance (>80%; Supplementary Figure 2). Hence, osaux1-1:1 and osaux1-1:3 appear to represent null alleles.

Phenotypic analysis of young seedlings (homozygous for the T-DNA inserts) germinated on vertical agar plates revealed a reduced root angle phenotype in both osaux1-1:1 and osaux1-1:3 alleles compared to the positive gravitropic behavior of the wildtype control roots (Fig. 1b). The gravitropic defect became apparent in both primary and crown roots of osaux1 seedlings 4–8 days after germination (Fig. 1b). Mutant seedling primary and crown roots exhibited altered root angles compared to wildtype roots that grew closer to the vertical (Supplementary Figure 3). Similarly, seedling primary roots of both osaux1 alleles failed to reorient after a 90° gravity stimulus in contrast to wildtype roots (Supplementary Figure 4). Hence, the OsAUX1 gene appears to control primary and crown root gravitropic responses and angle in rice.
Phosphorus acquisition efficiency is not improved in osaux1. Root angle represents an important determinant for PAE. Many crops with roots whose angles deviate more from the vertical exhibit greater P foraging ability since this nutrient preferentially accumulates in the upper soil volume\textsuperscript{11}. We initially investigated whether OsAUX1 controls root angle in rice plants grown in soil. The architecture of wild type versus osaux1 lines was compared using X-ray microCT and rhizotron-based root phenotyping approaches\textsuperscript{16}. When using microCT, rice lines were grown in soil for a total of 4 weeks, non-invasively scanning samples each week. This non-destructive imaging approach helped reveal the temporal evolution of wild-type and mutant rice root architecture. Clear differences in root distribution within the soil volume were apparent at week 2 (Fig. 2) with osaux1 lines preferentially colonizing the upper soil space compared to wild type. Large rhizotrons (1.5 M depth by 0.5 M width) enabled imaging of 2D root architecture in older rice plants, and independently validated differences observed using microCT in root angle and colonization of the upper soil profile by osaux1 mutant roots (Supplementary Figure 5). Hence, rice plants lacking OsAUX1 exhibit a major change in the vertical distribution of roots.

Given the striking difference in osaux1-1 root angle compared to wild type when grown in soil (Fig. 2 and Supplementary Figure 5), we next tested whether the mutant also had improved PAE. We performed a series of experiments designed to assess whether the osaux1 mutant’s root angle phenotype conferred a selective advantage for P foraging. When plants were provided with limited, sufficient and high levels of this immobile nutrient in the soil, no significant difference was evident in P accumulation in shoot tissues of osaux1 compared to the wild-type control (Supplementary Figure 6). Rather surprisingly, split nutrient treatments (where sufficient or high P were provided in the top 50% soil volume) revealed that osaux1 accumulated less P in shoot tissue compared to the wild type (Supplementary Figure 6). We conclude, based on the latter observations, that OsAUX1 must also control other root traits of importance for soil P acquisition.

OsAUX1 promotes root hair growth in low phosphate conditions. Root hairs play an important role in accessing immobile nutrients like P from the soil. We therefore examined whether mutating OsAUX1 disrupted root hair development, in addition to root angle. We initially observed that both osaux1 mutant alleles retained the ability to form root hairs (Fig. 3a and Supplementary Figure 7). However, closer examination revealed that mutant root hairs were shorter than wild type (Fig. 3b and Supplementary Figure 7). The reduced root hair length in osaux1 phenocopies the previously reported root hair elongation defect in Arabidopsis aux1 mutant alleles\textsuperscript{17,18} and reveals that this growth response represents a highly conserved AUX1-dependent process.

External phosphate availability has been reported to control root hair length in several plant species\textsuperscript{11}. We also observed that external P concentration had a major effect on wild-type rice root hair length (Fig. 3a, b and Supplementary Figure 7), which increased more than threefold to >500 μM under the most limiting nutrient conditions. In contrast, the osaux1-1;1 and osaux1-1;3 alleles either exhibited a highly attenuated root hair response or this was completely abolished, respectively (Fig. 3b and Supplementary Figure 7). The marked reduction in root hair length of the osaux1 alleles phenocopies the typically reported root hair elongation defect in Arabidopsis aux1 mutant alleles\textsuperscript{17,18} and reveals that this growth response represents a highly conserved AUX1-dependent process.

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Root auxin response is elevated by low phosphate and OsAUX1. The observed functional link between OsAUX1 and root hair elongation response to P deficiency suggests roots employ auxin as a signal during this important adaptive response. To directly test whether auxin levels are elevated in rice roots under P limiting conditions, we grew wild-type plants

![Figure 2](image_url) MicroCT imaging reveals OsAUX1 controls root angle in soil. Comparison of root angles from X-ray CT images of soil grown wild-type (WT), osaux1-1;1 and osaux1-1;3 roots at 1-, 2-, 3-, and 4-week-old stages (denoted W1–4). Scale bar represents 2 cm.

![Figure 3](image_url) OsAUX1 promotes root hair growth at low external P levels. a 9-day-old WT, osaux1-1;1 and osaux1-1;3 seedlings were grown for 6 days in hydroponics at three different P concentrations. Scale bar 1 mm. b Quantitation of RH length in WT, osaux1-1;1 and osaux1-1;3 mutants reveal low P. Each bar represents the average length of 30 fully elongated RH on >10 nodal roots. *, **, and *** indicate significant difference p value <0.05, 0.001, and 0.0001, respectively. Error bars mean ± SE, n = three biological replicates and p values were calculated by Student’s t test.
Low P increases root hair zone auxin response via AUX1. a, b Two photon laser scanning microscopy images of auxin response reporter DR5:VENUS (green) fluorescence in transgenic rice seedlings grown at either low (a) or high P levels (b). Inset shows close-up of the distal elongation zone. c–f Maximum projection confocal images of Z-stacks of DR5:VENUS fluorescence in the roots of wild type (c, d) or osaux1-1;3 (e, f) seedlings grown in either low (c, e) or high P (d, f). g AUX1pro::GUS lines reveal OsAUX1 root apical expression. Scale bar represents 100 μm.

Our study has uncovered a novel role for OsAUX1 in facilitating root hair elongation to better forage for this immobile resource in soil.

Discussion
Our study has uncovered a novel role for OsAUX1 in facilitating root adaptation to low external P by promoting hair elongation, thereby helping increase the volume of soil being explored by the roots.
observed two decades ago that auxin and AUX1 promote root hair elongation response in many plant species. Availability triggers a root hair elongation response in many species. Arabidopsis seedlings grown in high P medium for 7 days and then transferred to high P (i) for a further 6 days. (ii) and (iii) show DRS::VENUS fluorescence of split P experiment roots, where 7-day-old high P roots were split into two halves: one half was grown in high P and the other in low P medium (iii) for a further 6 days. (iv) Maximum projection confocal image of 13-day-old low P grown rice root. b Raw integrated fluorescence intensity quantification of DRS::VENUS roots (from Fig. 5a and Supplementary Table 1). Each bar represents the average raw integral density of fluorescence intensity of DRS::VENUS under high P, low P to high P, high P to low P, and low P conditions. Fluorescence intensity of at least 19 roots under low P and high P grown DRS::VENUS seedlings and 10 roots of split P conditions were used for fluorescence intensity measurement in three independent replicates. Scale bar represents 50 μm. Student’s t test was performed to calculate p values.

**Methods**

**Plant material and growth conditions.** Arabidopsis thaliana seeds (Col-0) were surface sterilized and grown in a growth room under 16 h light (50–200 μmol m⁻² s⁻¹; 23 °C) and 8 h dark cycle (18 °C). Rice (Oryza sativa L japonica) AUX1 T-DNA insertion lines osaux1-1 and osaux1-3 (Dongjin background and Dongjin wild-type seeds were provided by Pr G An, Kyung Hee University, Korea). Rice plants were grown in 13 cm pots (volume 804 cc) filled with a 1:1 (w:w) ratio of John Innes No1 (John Innes, Norwich, UK): Levington M3 (JFC Monro, Devon, UK) soil mix, at 28 °C in 12 h light and 12 h dark cycle and regularly irrigated with plant media (0.642, 7000, 8000, 5.6E-10).

**AUX1 complementation experiments.** cDNA sequences for OsAux/Lax genes were PCR amplified from rice root or leaf cDNA libraries, other than OsLAX1 which was obtained from the rice BAC clone AK111849. Each cDNA was initially cloned into pGEM-T Easy and then the binary vector pMogorfLaux14, which contains the 2 kb promoter region, start codon and the 3’UTR of the Arabidopsis AUX1 gene. Constructs were then transformed into the Arabidopsis mutant aux1-22 using the floral-dip method. Prizers used for cDNAs amplification are listed in Supplementary Table 1. Root growth and gravitropism analyses were performed on vertical agar plates and quantified as described earlier.
Characterization of osaux1 root architecture. Two independent T-DNA insertion mutant lines of Osaux1 were identified using OryGeneDB software\textsuperscript{[25]}. In line 3A, 511.10, 220 DNA inserts were disrupted within intron 6 of the T-DNA was inserted in exon 6 (and termed osaux1-1 and osaux1-1, respectively). T- DNA insertions were confirmed using the site-finder approach\textsuperscript{[28]}. Root growth and gravitropism analyses were performed on vertical agar plates and quantitated as described earlier\textsuperscript{[7]}. Root architecture analysis of root grown plants was performed using each of the mutant lines in low phosphate grown agarose. In the latter case, the germinated seeds were planted in plastic columns containing sandy loam (Newport) soil. For phosphate amendment crushed TSP (44% P\textsubscript{2}O\textsubscript{5} in 50 mL of deionised water) was mixed thoroughly with the soil. The amended soil was sieved to <2 mm and then packed in polystyrene columns (5.5 cm diameter, 10 cm height, and 0.23 cm thick) to a 1.2 g cm\textsuperscript{-3} density. Columns were microCT scanned at weekly intervals for 4 weeks using a GE NanoTom CT model. Typical settings were 130 kV, 240 µA, 1080 projections, 73 min total scan time, sample-source distance of 22.7 cm, 27.3 µm voxel size with a 0.1 mm copper filter. The relatively long scan time (73 min) was used to obtain the best quality X-ray CT images for the sample size. Each sample received an approximate X-ray dose of 5.9 Gy over the four scans (1.5 Gy each scan). For each sample, grey-level transformation was performed to calculate the density. After 7 days of growth seedlings were transferred to glass tubes available from the corresponding author on request.

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