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

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Abstract

Root traits such as root angle and hair length influence resource acquisition particularly for immobile nutrients like phosphorus (P). To improve rice P acquisition efficiency (PAE) we attempted to modify root angle in rice by disrupting the *OsAUX1* auxin influx transporter gene. X-ray microCT imaging reveals *osaux1* root angle is altered, causing mutant lines to preferentially forage in the topsoil where P normally accumulates, yet surprisingly, did not improve PAE. Closer investigation revealed *OsAUX1* also promotes root hair elongation in response to P limitation. Reporter studies revealed that the auxin response increased in root hair zones grown in a low P environment. We demonstrate that *OsAUX1* functions to mobilise 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.

(147 words)

Food 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 topsoil[1,2].

Very few genes that regulate root architecture traits such as root angle have been identified in crop plants to date[3]. In contrast, major progress has been made characterizing genes and molecular mechanisms controlling root angle in the model plant *Arabidopsis thaliana*[4]. AUX1 was one of the first genes identified in *Arabidopsis* to control root angle[5,6] and later shown to encode an auxin influx carrier[7,8]. 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 bending[9,10]. 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 wildtype plants. Nevertheless, physiological experiments performed on *osaux1* (versus wildtype) 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 soil[11]. 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 *OxAUX1* 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 thaliana*[12] and which relies on AUX1 to promote hair elongation via intracellular auxin and calcium signalling[13].

Results

Rice root angle is altered by disrupting the *OsAUX1* gene

The *AUX1* gene family in rice is encoded by 5 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* agravitropic phenotype. This genetic assay revealed that only one of the *OsAUX1* sequences (*Os01g63770*) was able to successfully rescue the *aux1* mutant's root agravitropic 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-functionalization[14].

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 materials and methods). The T-DNA insertion lines were termed *osaux1-1* and *osaux1-3* (in agreement with Zhao et al, 2015[15]). Southern hybridisation confirmed that single T-DNA insertion events had disrupted the *OsAUX1* gene in *osaux1-1* and *osaux1-3*, respectively. PCR amplification of genomic fragments adjacent to each T-DNA followed by sequencing confirmed that T-DNA insertions in *osaux1-1* and *osaux1-3* had disrupted the gene coding sequence in intron 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* and *osaux1-3* 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* and *osaux1-3* alleles compared to the positive gravitropic behaviour 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 90^0 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[11]. We initially investigated whether *OsAUX1* controls root angle in rice plants grown in soil. The architecture of wildtype versus *osaux1* lines was compared using X-ray microCT and rhizotron-based root phenotyping approaches[16]. When using microCT, rice lines were grown in soil for a total of 4 weeks, non-invasively scanning samples every week. This non-destructive imaging approach helped reveal the temporal evolution of wildtype 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 wildtype. Large rhizotrons (1.5M depth by 0.5M 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 wildtype when grown in soil (Fig. 2 & 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 wildtype 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 wildtype (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[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[11]. We also observed that external P concentration had a major effect on wild type rice root hair length (Fig. 3 a,b and Supplementary Figure 7), which increased more than 3 fold to >500µm under the most limiting nutrient conditions. In contrast, the *osaux1-1 and osaux1-3* 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 (particularly under P limiting conditions) will negatively impact their ability to forage for P in soil. Root hairs account for up to 90% of P uptake[19], and the benefits of increased root length in the top soil profile is more than cancelled by the loss of surface area induced by shorter root hairs considering that 91% of the total root surface area is contributed by hairs[20].

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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 hydroponically under low external P supply, then surgically excised root tips and root hair zones and measured levels of the major form of auxin, indole-3-acetic acid (IAA) using GC-MS/MS (see materials and methods). Hormone quantification revealed IAA levels were indeed elevated in wild type root tip and root hair zone under low external (compared to high) P conditions (Supplementary Figure 8).

To visualize if low external P conditions triggered an auxin response, rice reporter lines encoding the auxin responsive reporter *DR5:VENUSX3* were created (see materials). We monitored changes in rice root auxin response to external P levels employing two forms of laser scanning microscopy (see materials). Multi-photon microscopy was used to image

deep inside rice root tissues, revealing that the *DR5:VENUSX3* reporter signal was elevated in root cap and epidermal cells when grown under low external P (versus high P) conditions (Fig. 4a,b). In parallel, confocal microscopy was employed to image root surface tissues under both external P conditions. A maximal surface projection image was taken to capture the entire cylindrical root surface (Fig. 4c-f). This revealed low *DR5:VENUSX3* auxin response expression in root surface tissues grown in high external P (Fig. 4d), but under low external P conditions reporter activity was strongly upregulated in all root epidermal cells between the apex and hair zone (Fig. 4c).

Lateral root cap and epidermal tissues have been shown in *Arabidopsis* roots to represent the AUX1-mediated conduit for auxin to be transported 'shootward' from the root apex to root hair zones[9]. Transgenic rice roots encoding an *OsAUX1* promoter GUS reporter (*OsAUX1:GUS*) revealed that the rice orthologue was expressed in lateral root cap and epidermal tissues (Fig. 4g). To test whether the *osaux1-3* mutation reduced auxin dependent root hair elongation by disrupting 'shootward' auxin transport, we monitored *DR5:VENUSX3* reporter expression in the mutant background (Fig. 4e,f). This revealed *DR5:VENUSX3* auxin response expression remained low in root surface tissues grown in either high or low external P. In the latter case, the *DR5:VENUSX3* reporter was clearly elevated in *osaux1-3* epidermal cells close to the root apex, but (unlike wildtype) was not expressed in more distal cells within the elongation and differentiation zones (Fig. 4e,f). This behavior concurs with model simulations of auxin transport in root tissues which reveal that influx carrier activity is necessary for this hormone signal to move efficiently from cell to cell[9,10]. We conclude auxin response is elevated in root epidermal cells due to this signal being upregulated at the root apex by low external P, then mobilized to the root hair zone in an OsAUX1 dependent manner.

Auxin and root hair growth are induced by local phosphate availability

Given that P is relatively immobile in soil, roots are likely to employ mechanisms to fine tune their hair length in response to this nutrient's heterogeneous distribution. This would necessitate a *local* (rather than *systemic*) signaling solution by roots to monitor external P availability and then trigger adaptive responses like hair elongation. To investigate whether root hair length is regulated by either a local or systemic signaling system, rice plants were grown employing a split root experimental set-up, where roots from a single plant were grown in 2 separate hydroponic chambers to control external P availability. As reported above (Fig. 3), control split roots grown under just low or just high P exhibited long and short root hairs, respectively (Supplementary Figure 9). Interestingly, when roots from individual

rice plants were grown simultaneously in high and low external P conditions, they exhibited short and long root hair lengths, respectively (Supplementary Figure 9). Hence, root hair elongation in rice appears to be controlled by local (rather than systemic) P availability. However, when we performed a split plate experiment in soil where seminal roots from the same rice plant were exposed (at the same time) to replete P and low P conditions, the latter roots exhibited an attenuated hair elongation response compared to control roots (Supplementary Figure 10). This suggests that, whilst root hair length is strongly influenced by local P availability, a systemic signal(s) may also communicate the P status of shoot tissues.

We next examined whether auxin response plays a role in local and/or systemic signaling mechanisms to P availability using our split root hydroponic system. As reported above, *DR5:VENUSX3* rice split roots grown under just low or just high external P conditions exhibited high and low reporter signals, respectively (Fig. 5a,b and Supplementary Figure 11 & 12). Similarly, when roots from individual rice *DR5:VENUSX3* plants were grown simultaneously in high and low external P conditions, they also exhibited low and high auxin response reporter expression, respectively (Fig. 5a,b and Supplementary Figure 11 & 12). Hence, root auxin response appears to be inversely related to local P availability, where low levels of this key nutrient triggers an increase in root epidermal auxin response, which promotes 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 plant root. Plant physiologists have long known that low P availability triggers a root hair elongation response in many species[11]. *Arabidopsis* developmental biologists have also observed two decades ago that auxin and AUX1 promote root hair elongation[17,18]. Our current study in rice provides the experimental evidence that integrates these observations and stimulated subsequent efforts in the model plant *Arabidopsis thaliana*[12,13] to develop a mechanistic framework for this adaptive response pathway.

The conservation of the AUX1-regulated root hair adaptive response between model dicot and monocot species provides confidence that we have uncovered a highly conserved auxin regulatory mechanism controlling plant responses to external P availability. A central role for auxin has been further substantiated by the observation that *Arabidopsis* mutants

either disrupting auxin response (e.g. *arf19*), synthesis (e.g. *taa1*) or degradation (e.g. *dao1*) also modify the P deficiency induced root hair elongation response[12]. In addition, hormone quantification, pharmacological treatment and reporter studies in rice and *Arabidopsis* have revealed that P deficit elevates IAA levels and response (Supplementary Figure 8)[12,13], triggering enhanced auxin responsive gene expression in key root tissues that include epidermal root hair cells. Targeting AUX1 to just lateral root cap and epidermal root tissues rescued the *aux1* P deficiency root hair defect, demonstrating the functional importance of the shootward auxin transport pathway from the root apex via the lateral root cap to elongation and differentiation zones[12]. Auxin-inducible transcripts that exhibit elevated expression in the elongation and differentiation zones during P deficit conditions include the transcriptional factor genes *ARF19* and (its targets) *RSL2* and *RSL4*. Given the recent demonstration that the abundance of RSL4 exhibits a linear relationship with root hair length[21], *RSL4* mRNA up-regulation by auxin (in response to P deficit) would promote hair elongation. Collectively, our experimental results can be placed into a mechanistic framework initiated by auxin up-regulation at the root apex in response to low external P

Materials and Methods

Plant material and growth conditions

Arabidopsis thaliana seeds (Col-0) were surface-sterilised and grown in a growth room under 16h light (150-200 µmols $m^{-2}s^{-1}$; 23°C) and 8h dark cycle (18°C). Rice (*Oryza sativa* L. japonica) *AUX1* T-DNA insertion lines (Dongjin background) and Dongjin wildtype seeds were provided by Pr G An, Kyung Hee University, Korea[24]. Rice plants were grown in 13 cm pots (volume 804 cc) filled with a 1:1 (w:w) ratio of John Innes No1 (John Inness, Norwich UK) : Levington M3 (JFC Monro, Devon, UK) soil mix, at 28°C in 12h light and 12h dark cycle and regularly irrigated with plant media[25].

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 *pMOGORFLAUX1*[14] 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[26]. Primers 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[27].

Characterization of *osaux1* root architecture

Two independent T-DNA insertion mutant lines of *Osaux1* were identified using OryGeneDB software27.2

Root hair assays

Dehusked rice seeds were surface sterilised with 2% bleach and 0.1% Triton for 15 min followed by 5 washes with sterile water. Seeds were then germinated on moist Whatman paper for three days in dark. Uniformly germinated seedlings were then transferred on 1/4th strength MS plates (pH 5.6 with 1% agar) containing 1 µM, 31 µM or 312 µM P. Low P media were complemented with equimolar concentration of KCl. Seedlings were grown vertically in 12 inch square plates in growth chamber maintained at 28°C with 12h of light and 12h of darkness. After nine days of growth seedlings were transferred to glass tubes filled with same media without agar (hydroponic system). Liquid media was changed every day and root hair growth was recorded on nodal roots of 15-days-old seedlings using a Zeiss stereo zoom microscope (optical zoom 2.5X, digital zoom 1.2X). Experiments were repeated three times. RH length was measured as the average of 30-60 fully elongated root hairs from one seedling. Data from > 10 seedlings was used to calculate final RH length.

Split root experiments

Rice seeds *(DR5:VENUS3X)* were dehusked and were cut into halves to retain only embryo portions (onwards referred as seeds). Seeds were then surface sterilised with 50% bleach for 10 min followed by 10 washes with sterile water. After washing, seeds were dried on sterile Whatman paper for 10 min. Seeds were germinated for 3 days on vertical ½ MS (Murashige and Skoog) plates (supplemented with 0.5% phytagel) in a growth chamber maintained at 28°C (250-300 µM photons/m^2/sec). Uniformly germinated seedlings were then transferred to hydroponic solutions of modified Yoshida medium[24] containing 1 µM (low) P in phytotron growth chamber (16 h day (30°C)/8 h night (30°C) photoperiod, 250-300 µM photons/m2/sec photon density and ~70% relative humidity). After seven days of growth in low P (1µM), 10 low Pi starved seedlings were split into two glass tubes filled with low (1µM) and high (312µM) P Yoshida medium. The liquid medium was changed every day and fluorescence images and Z-stacks were recorded on nodal roots of 13-days-old seedlings using Leica SP5 confocal microcope. All recorded images and Z-stacks were processed in Fiji to generate maximal surface projection images and to measure raw integrated densities of fluorescence. The .lif file format was opened in Fiji and all z slices were summed and duplicated. The duplicated image was used for thresholding to visualize the maximum fluorescence pixels. After thresholding, each fluorescence pixel was selected using the ROI manager tool and a ROI number added to that image. Finally, raw integral densities were calculated using the measurement tool.

Auxin and P measurements in rice plants

Root tip (~1.5 mm) and differentiation zone (next 2 mm region) from 15-days-old rice seedling grown under low and high P were excised under a dissecting stereo microscope and frozen immediately in liquid nitrogen. 12-15 roots were used per sample with four biological replicates. Five-hundred picograms of $^{13}C_6$-IAA internal standard was added to each sample before purification. Auxin quantification was performed using GC-MS/MS as

described earlier[32] with minor modifications. P levels in shoot tissues were measured using ICP-MS.

Generation of rice reporter lines

The *DR5$_{rev}$::VENUS* fragment was composed of a generic synthetic promoter with nine repeats of the auxin response element (AuxRE) motif (TGTCTC) linked to minimal 35S CaMV promoter[33,34], driving the expression of 3 copies of the YFP VENUS sequence with the nuclear localization signal N7 from maize[35]. The construct was inserted into the pMLBART[36] vector to form the *DR5rev::3xVENUS* construct. The vector was transformed into rice japonica cultivar 9522 calli using *Agrobacterium tumefaciens* strain EHA105[37]. To create the *OsAUX1$_{pro}$*:*GUS* construct, 1.8 kbp of the *OsAUX1* promoter sequence was PCR amplified and cloned into Gateway binary vector pGWB3 which contains the GUS gene (Supplementary Figure 2). This vector was then transformed into *Agrobacterium.* Rice transformation was carried out as described earlier[38].

Two photon Laser Scanning Microscopy (TLSM)

Plant seeds were sterilized in ethanol 70% for 1 minute, and then in 40% sodium hypochlorite for 30 minutes under agitation. Seeds were transferred to ½ strength MS plates (supplemented with half strength vitamins; 0.8% agar; pH 5.8). Plates were kept at an angle of 15% from the vertical in a growth chamber maintained at 25°C, 60% humidity, and under a 12 hour photoperiod for 3 days. Root tips were counter stained with Propidium iodide (PI; 10µg/ml) for 10 minutes and were then briefly washed with distilled water thrice. Root tips were mounted in low melting agarose (0.5%) and were scanned typically using a Two photon Laser Scanning Microscope. The GFP and PI emissions were collected in separate channels with excitation at 836 nm (Chameleon Ultra II) and 1096 nm (Chameleon Compact OPO), respectively,with a gain set at 600 nm using 2PMT NDD and 2 PMT BiG detectors. All images were processed using Zeiss ZEN software. For images stack, the auto brightness correction was applied. In some cases, roots were scanned using Leica SP5 confocal microcope with 1.5 µm step size for Z-stacks. Maximum projections were generated using Leica SP5 software,

RT-qPCR and reporter imaging

qRT-PCR was performed in three biological and four technical replicates per sample. Total RNA (2 ug) was used for cDNA synthesis using transcriptor first-strand cDNA synthesis kit (Roche). Gene expression assay was performed as described earlier[14]. For GUS assays, samples were kept immersed in ice-cold 90% acetone with gentle shaking for 1h followed by three washes with sodium phosphate buffer pH 7 for 1 h. Tissues were incubated in GUS staining solution for 3h at 37^0C[14] and images were taken on a Leica microscope using DIC optics.

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END NOTES

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Author Contributions

J.G, R.B., G.H., B.K.P., H.P, S.Z., J.Y., A.D., C.B., K.L., A.P., T.R., A.L., S.M. and C.J.S. performed experiments and contributed experimental data; J.G., R.B., B.K.P., K.L., T.R., P.W., L.D., M.H., C.P., W.L., B.P., C.T.H., J.L., M.W., D.Z., T.P., S.M., E.G., R.S. and M.J.B. designed experiments; and J.G., R.B., G.H., B.K.P., R.S. and M.J.B. wrote the manuscript.

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Figure Legends

Fig. 1 *OsAUX1* controls rice root angle. (a) schematic representation of T-DNA insertion sites in Os*AUX1* gene. **(b)** time course images of root angle in WT, os*aux1-1;1 and osaux1-1;3* T-DNA mutants. Images were taken after three days after seed germination (3DAG) to eight days post germination (8DAG). White bars represent 0.5cm.

Fig 2. MicroCT imaging reveals *OsAUX1* controls root angle in soil. Comparison of root angles from X-ray CT images of soil grown wildtype (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.

Fig 3. *OsAUX1* promotes root hair growth at low external P levels. **(a)** 9 day old WT, os*aux1-1;1 and osaux1-1;3* seedlings were grown for 6 days in hydroponics at three different P concentrations. Scale bar 1mm. **(b)** Quantitation of RH length in WT, os*aux1-1;1 and osaux1-1;3* mutants reveal low P . Each bar represents the average length of 30-60 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 replicate and *p*-values were calculated by Student's *t*-test.

Fig 4. 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 wildtype **(c,d)** or *osaux1-1;3* **(e,f)** seedlings grown in either low **(c,e)** or high P (d,f). g. $AUX1_{pro}$*:GUS* lines reveal *OsAUX1* root apical expression. Scale bar represents 100 µm

Fig 5. Low P root auxin response is independent of plant P status. **(a)** Maximum projection confocal images of Z stacks of *DR5::VENUS* fluorescence in the seedlings grown initially in high P medium for 7 days and then transferred to high P (i) for a further 6 days. (ii) and (iii) show *DR5::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 (ii) 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 *DR5::VENUS* roots (from Fig 5A and Supplemetary Figure 11). Each bar represents the average raw integral density of fluorescence intensity of DR5::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 *DR5::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

Supplementary figure legends

Supplementary Figure 1. Identification and characterization of rice *OsAux1*. **(a)** Phylogenomic tree of the *AUX/LAX* gene family in *Arabidopsis* and rice. **(b)** Functional complementation of the *Arabidopsis aux1-22* mutant line by Arabidopsis *AtAUX1* and rice *OsAUX1* cDNA sequences driven by the AtAUX1 promoter. The seedlings were allowed to grow for three days and then the plates were turned 90° for 24 h. **(c)** Quantification of the direction of root growth of the denoted lines

Supplementary Figure 2. T-DNA mutant lines exhibit reduced OsAUX1 levels. RT- qPCR profiling of Os*AUX1* transcripts in WT, *osaux1;1;1* and *osaux1;1;3* lines revealed a significant reduction in Os*AUX1* mRNA abundance in both mutant alleles. Error bars mean ± SE, n = three biological replicate and four technical replicates of each lines.

Supplementary Figure 3. Root angle measurement of WT and aux1 seedlings The graphical representation of root angles of WT and *aux-1* alleles. The root angles were calculated using horizontal line coming from the root emergence point as 0 degree at the initiation site for WT, *aux1-1;1* and *aux1-1;3* after 6-day's growth on plates. All crown and primary roots were included for angle measurement. Error bars represents means ± SD, n = 11, two asterisks mean significant differences (p < 0.01 from Student's *t*-test).

Supplementary Figure 4. *OsAUX1* mutants exhibbit defective root gravitropic responses. **(a)** Representative images of WT, *aux1-1;1* and *aux1-1;3* after 8-h gravity stimulation. Scale bar, 1 cm. **(b)** The quantified data for the curvature degree. Error bars mean ± SE, n = three independent biological repeats with at least 40 roots analyzed in each assay.

Supplementary Figure 5 Mature *osaux1-1;1* rice plants exhibit reduced root angle. Rice plants were grown in large soil-filled rhizotrons (1.2 x 0.3 x 0.015 m) and representative photographs (four replicates) of the rhizotrons containing 15d (a,b) and 40 d (c,d) old rice plants were taken. The images show that rice *osaux1-1;1* mutants (left a and c) exhibit reduced root angle compared to wildtype (lowe left b and extereme right d).

Supplementary Figure 6. Assessing the impact of *OsAUX1* on P foraging in soil. (a) Experiments used P at three levels (low/no added P, sufficient P and high P) distributed uniformly throughout the soil column (upper panel), in the top layer of soil (bottom left) or in the bottom layers of soil (bottom right). (b) Total plant P status in WT and *osaux1;1;1* mutant grown under the different split soil P conditions. Error bars represent standard error (n = 5).

Supplementary Figure 7. Low P root hair growth response is *OsAUX1* dependent
Representative images of WT and *aux1-1;1* root hairs under low and high P conditions
Quantitation of WT and *aux-1-1;1* root hair length under high and low P conditions. Each bar represents at least 10 replicates and each root was analyzed for at least 30 to 50 root hairs on 15 day old seedlings grown for 6 days in hydroponics with three different P levels. p value was calculated from Student's *t*-test

Supplementary Figure 8. IAA quantification in rice root tips and hair zones.

Root tip (~1.5 mm) and root hair zone (next 2 mm region) from 15-days-old rice seedlings either grown under low P (3 µM) and high P (312 µM) conditions for 6 days, then were excised under a dissecting stereo microscope, frozen, then analysed using LC-MS/MS. Error bars represents mean ± SE, n = four biological replicates with at least 12-15 roots for each sample.

Supplementary Figure 9. Root hair growth is regulated by local P availability
Root hair length under high, low P and split P conditions shown by representative images (top line) and quantitation (lower line). Seedlings (4 days old) were transferred to low and high P Yoshida nutrient media. After 6 days treatment half of the roots were either placed in high or low P levels for another four days. At least 10 roots were used for each treatment. Scale bar represents 200 µm. Error bars mean ± SE, n = two independent biological repeats with 10 roots analyzed in each assay.

Supplementary Figure 10. Local P levels control root hair length. Root hair measurement under high, low and mixed P nutrient regimes revealed the importance of local P levels on the regulation of root hair length. Different letters indicate significant differences ranked by Fisher's Least Significant Difference (LSD) test ($p < 0.05$).

Supplementary Figure 11. Low P induced root auxin is independent of plant P status. Maximum projection confocal images of Z stacks of *DR5::VENUS* fluorescence in seedlings initially grown at low P for 7 days and then transferred to low P medium (i) for a further 6 days. (ii) and (iii) show *DR5::VENUS* fluorescence for split P roots where 7 day old low P roots were divided into two halves: one half was grown in low (ii) and the other in high P media (iii) for a further 6 days**.**