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RESEARCH PAPER

Saturated humidity accelerates lateral root development in rice (*Oryza sativa* L.) seedlings by increasing phloem-based auxin transport

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Abstract

Auxin transport plays a significant role modifying plant growth and development in response to environmental signals such as light and gravity. However, the effect of humidity on auxin transport is rarely documented. It is shown here that the transport of labelled indole-3-acetic acid (IAA) from the shoot to the root is accelerated in rice (*Oryza sativa* L. ssp. *indica* cv. IR8) seedlings grown under saturated humidity (SH-seedlings) compared with plants grown under normal humidity (NH-seedlings). The development of lateral roots in SH-seedlings was greatly enhanced compared with NH-seedlings. Removal of the shoot from SH-seedlings reduced the density of lateral roots, and the application of IAA to the cut stem restored the lateral root density, while the decapitation of NH-seedlings did not alter lateral root development. Phloem-based auxin transport appeared responsible for enhanced lateral root formation in SH-seedlings since (i) the rate of IAA transport from the shoot to the root tip was greater than 3.5 cm h^{-1} and (ii) naphthylphthalamic acid (NPA)-induced reduction of polar auxin transport in the shoot did not influence the number of lateral roots in SH-seedlings. It is proposed that high humidity conditions accelerate the phloem-based transport of IAA from the leaf to the root, resulting in an increase in the number of lateral roots.

Key words: *AUX1*-like gene, auxin transport, humidity, lateral root development, *Oryza sativa* L. ssp. *indica* cv. IR8, phloem transport, rice seedlings.

Introduction

Auxins play a pivotal part in plant growth and development, including cell enlargement and division, lateral branching of shoots and roots, vascular differentiation, gravitropism, and early embryonic development (Davies, 1995; Hobbie, 1998). The auxin indole-3-acetic acid (IAA) is unique amongst plant hormones in being directionally transported (Lomax *et al*., 1995). In addition to directional transport, auxin can also move through phloem (Morris and Kadir, 1972). These auxin pathways are not constant processes but change thoroughout the plant life cycle or in response to environmental stimuli such as light and gravity (Lomax *et al*., 1995).

In shoot tissues, polar auxin transport occurs in a basipetal direction from the shoot apex to the base. In contrast, auxin transport is more complex in roots, where IAA is transported acropetally through the central cylinder and basipetally via the outer layers of root cells (Tsurumi and Ohwaki, 1978; Estelle, 1996; Marchant *et al*., 1999; Muday, 2001; Blilou *et al*., 2005; Swarup *et al*., 2005). Recent knowledge about auxin transport has been primarily obtained through a series of studies on auxin influx and efflux carriers using *Arabidopsis* mutants. Auxin is taken up by plant cells via a carrier protein termed AUX1

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Abbreviations: NH, normal humidity; NPA, naphthylphthalamic acid; SH, saturated humidity.

(Bennett *et al*., 1996; Swarup *et al*., 2004; Yang *et al*., 2006) and is mobilized out of cells via auxin efflux carriers encoded by a family of *PIN* genes (Gälweiler *et al*., 1998; Benková *et al*., 2003; Petrásek *et al*., 2006) or *PGP*s (Geisler *et al*., 2005). Mutations of *AUX1* and *AGR1*/*EIR1*/*PIN2*/*WAV6* (Chen *et al*., 1998; Luschnig *et al*., 1998; Müller *et al*., 1998; Utsuno *et al*., 1998; Marchant *et al*., 1999) disrupt gravity response in roots and/or lateral root formation, demonstrating that auxin transport plays a critical role in root growth and development.

Auxin synthesized in young apical parts and leaves is transported to the roots through the phloem or a polar transport system (Reed *et al*., 1998; Marchant *et al*., 2002; Ljung *et al*., 2005). However, the respective role of the two auxin pathways for root development is still unclear.

In the present study, it is shown that the saturated humidity surrounding the shoot of rice seedlings influences lateral root development by impacting phloem-based auxin transport from the shoot to the root. This represents a novel mechanism for how an environmental stimulus could modify root architecture by influencing auxin transport.

Materials and methods

Plant materials and growth condition

Seeds of rice cultivar IR8 (*Oryza sativa* L. ssp. *indica*) were surface-sterilized in a 0.2% (v/v) Benomyl (Dupont Company, Tokyo, Japan) solution for 24 h, rinsed, and soaked in water overnight in the dark at 30 °C. Germinated seeds were transferred to a floating net and grown hydroponically in a 400 ml glass cup under continuous white light at an irradiance of approximately 100 μmol m^{-2} s^{-1} for 4 d at 25 °C in a growth room, where the relative humidity was kept at 60%. For saturated humidity, glass cups were placed in a closed glass chamber of 3.0 l with or without a continuous flow (1.8 l min^{-1}) of water-saturated fresh air (Fig. 1; see Supplementary Fig. S1 at *JXB* online). Relative humidity was measured with a digital humidity meter (HN-K, Chino Corp)

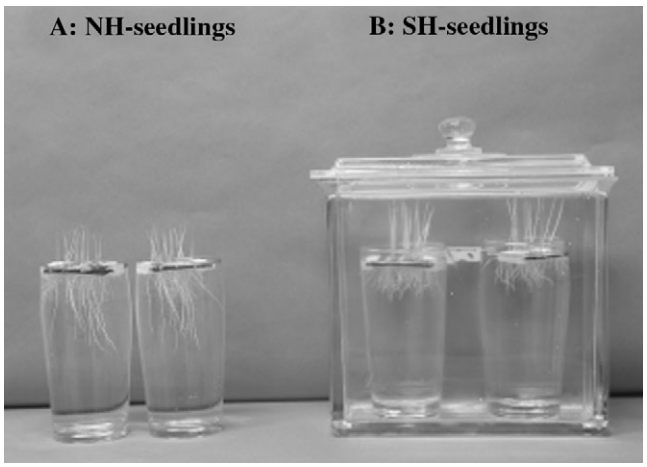

Fig. 1. Growth conditions for NH-seedlings propagated at 60% relative humidity (A) versus SH-seedlings grown at 100% relative humidity in a closed glass chamber (B). Rice seedlings were grown hydroponically in a 400 ml glass cup under continuous white light at an irradiance of approximately 100 μmol m^{-2} s^{-1} for 4 d at 25 °C in a room, where the relative humidity was maintained at 60%.

Transport of labelled IAA from the shoot to the root

Auxin transport from the shoot to the root was performed mainly as described by Chhun *et al.* (2004) with a slight modification. The shoots of 4-d-old seedlings were decapitated and the cut stem of 0.5 cm in length was capped with a small plastic tip containing 3 μl of 10 mM MES buffer (pH 5.7) supplemented with 1 μM 5-[^{3}H]IAA (specific activity 740 MBq μmol^{-1}, American Radiolabelled Chemicals, Inc., St Louis, MO, USA). The [^{3}H]IAA-treated seedlings were incubated for various transport periods under 60% or 100% relative humidity. Radioactivity was measured either on the whole root or after dividing the root into three or four segments. The length of root segments was approximately 1 cm while the length of the root tip segment was slightly longer than 1 cm depending on the length of the whole root. Ten whole roots or root segments were combined, and were placed into 5 ml of scintillation fluid overnight. Radioactivity was counted with a scintillation counter (model LS6500, Beckman Instruments, Fullerton, CA, USA).

Basipetal transport of labelled IAA in root segments

Basipetal auxin transport in roots was measured as described by Chhun *et al*. (2005) with a slight modification. An apical root segment of 1.6 cm in length was obtained from a 4-d-old seedling and placed vertically on a small filter paper containing 1 μM [^{3}H]IAA in a 1.5 ml Eppendorf plastic tube after decapitating the apical 1 mm in order to keep the contact of the apical end of the root segment with the donor filter paper. After 1 h incubation at room temperature, a 3 mm segment was cut from the basal end of the root segment. Five 3 mm root segments were combined and the radioactivity counted.

Application of auxin and NPA to shoot

Two-day-old SH-seedlings were decapitated by removing the apex to retain 0.3 cm of shoot tissue. The decapitated shoot was capped with a small plastic tube containing 5 μl of 0 μM and 10 μM IAA. IAA-treated seedlings were grown under 100% relative humidity for an additional 2 d and the number of lateral roots was measured.

For *N*-(1-naphthyl)phthalamic acid (NPA, Tokyo Kasei Co., Tokyo, Japan) treatment, NPA was applied to the basal part of the shoot as a ring after mixing with lanolin.

Cloning of four AUX1*-like genes of rice (cv. IR8)*

By searching the DNA Data Bank of Japan database (DDBJ), four *AUX1*-like genes were found (GenBank accession numbers AK100090, AK103524, AK111849, and AK102295) in rice (ssp. *japonica*). Specific primers for the *AUX1*-like genes were designed on the basis of DNA sequences in the DDBJ database (see Supplementary Table S2 at *JXB* online). Messenger RNA (mRNA) was extracted from roots of 7-d-old seedlings of rice cv. IR8 according to the manufacturer's instruction (QuickPrep Micro mRNA Purification Kit, Amersham Biosciences Ltd, UK). Reverse transcription was then performed with reverse transcriptase (PowerScript RT, BD Biosciences, Palo Alto, CA, USA) in a total volume of 20 μl at 42 °C for 90 min. PCR amplification was performed with 1 μg of cDNA essentially based on the manufacturer manual (Ex Taq, TaKaRa, Shiga, Japan). Four cDNA fragments were obtained, termed *OsRAU1* (related to AUX1), *OsRAU2*, *OsRAU3*, and *OsRAU4*, which were cloned into the pCR2.1 TOPO vector using the TOPO TA cloning Kit (Invitrogen, Carlsbad, CA, USA) as described in the instruction manual. Positive identification was performed by the dye-primer cycle sequencing method using

a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) and a DNA sequence analyser (ABI PRISM-Avant Genetic Analyzer, Applied Biosystems). Sequence comparisons with databases were performed at the US NCBIs GenInfo Network BLAST server.

Gene expression analysis

Total RNA was extracted from various rice organs including the shoot, embryo, upper and lower seminal roots, lateral roots, and crown roots using the RNeasy Plant Mini Kit (Qiagen, Hilden, Germany). Reverse transcription was then performed in a total volume of 20 µl using 5 µg of DNase-treated total RNA as template at 42 °C for 90 min. PCR amplification was performed with 1 µg of cDNA essentially based on the manufacturer's manual (Ex Taq, TaKaRa, Shiga, Japan). For gene expression analysis, specific primers for *OsRAU1*, *OsRAU2*, *OsRAU3*, and *OsRAU4* genes were designed (see Supplementary Table S2 at *JXB* online) to generate 300 bp sized products while the rice actin gene was used as a positive control. RT-PCR conditions were carefully manipulated to measure transcripts at the exponential phase of amplification. The PCR products in the exponential range of amplification were separated on an 1.2% agarose gel and stained with ethidium bromide.

Results

Rice seedling root morphology is modified in response to saturated humidity

Rice seedlings were grown hydroponically under 60% relative humidity in the light at 25 °C for 4 d (termed NH-seedlings). Seedlings were also grown under saturated humidity in a closed glass chamber (termed SH-seedlings) (Fig. 1). As shown in Table 1 and Fig. 2, it was observed that the root morphologies of SH-seedlings were distinct from those of NH-seedlings. The number of lateral roots per plant and the density of lateral roots were greatly increased in SH-seedlings. Furthermore, the length of the lateral roots was longer in SH-seedlings than in NH-seedlings and dense crown roots appeared on the basal part of shoots in SH-seedlings (Fig. 2), whereas the length of seminal roots of SH-seedlings was shorter compared with NH-seedlings. The direction of SH-root tip growth deviated from the gravity vector, suggesting that the response to gravity was altered in the SH-roots, whereas NH-roots grew downward in response to gravity.

Since the SH-seedlings were grown in a closed chamber for 4 d, it was possible that the seedlings could be influenced by some gaseous factors accumulated in the chamber. To rule out this possibility, the air inside the chamber was refreshed with a continuous flow of water-saturated air (see Supplementary Fig. S1 and Table S1 at *JXB* online). It was observed that this did not alter SH-root architecture, suggesting that saturated humidity represents the cause of the root morphology of SH-seedlings including enhanced lateral root development and root tip curving.

Table 1. *Characteristics of NH- and SH-seedlings grown under normal and saturated humidity, respectively*

Rice seedlings were grown hydroponically in a 400 ml glass cup under 60% relative humidity (NH-seedlings) or saturated humidity (SH-seedlings) in continuous white light at 25 °C for 4 d (Fig. 1). Lateral roots emerged at least 0.5 mm from the root surface were counted. Data represent averages (±SE) of 30 seedlings from three independent experiments. *, ** indicate significant differences from NH-seedlings at the 5% and 1% levels, respectively, as judged by the Student's *t* test.

Characters	NH-seedlings	SH-seedlings
Lateral roots (plant^{-1})	10.00±1.20	18.60±1.50**
Lateral roots (cm^{-1})[a]	2.86±0.30	5.09±0.20**
Lateral root length (cm)[b]	0.26±0.02	0.60±0.04**
Root length (cm)	4.05±0.10	3.67±0.20*
Root tip angle (degree)[c]	5.70±0.90	31.15±5.20**
Plant height (cm)	1.42±0.08	2.91±0.10**

[a] Density of the lateral roots was calculated by dividing the number of lateral roots by the length of the seminal root.
[b] The average of the five longest lateral roots was determined for each plant and then the average for 10 plants was obtained.
[c] The direction of gravity is shown as 0 degrees.

Excision of SH-seedlings shoot tissue blocks humidity-induced changes in root architectures

Lateral root development in *Arabidopsis* is controlled by auxin derived from the shoot (Reed *et al*., 1998; Bhalerao *et al*., 2002). To test this possibility in this experimental system, the effect of removing the shoot apex on the lateral root development in rice seedlings was examined. The shoot of 2-d-old seedlings was removed and root morphology was observed 2 d after decapitation (Fig. 3A). The removal of the shoot apex of SH-seedlings reduced the density of lateral roots compared with intact seedlings and, unexpectedly, the roots grew downward as if the gravity response was restored (Fig. 3B). In contrast, the removal of the shoot apex of NH-seedlings did not alter lateral root density and root gravity response as compared with intact seedlings (Fig. 3).

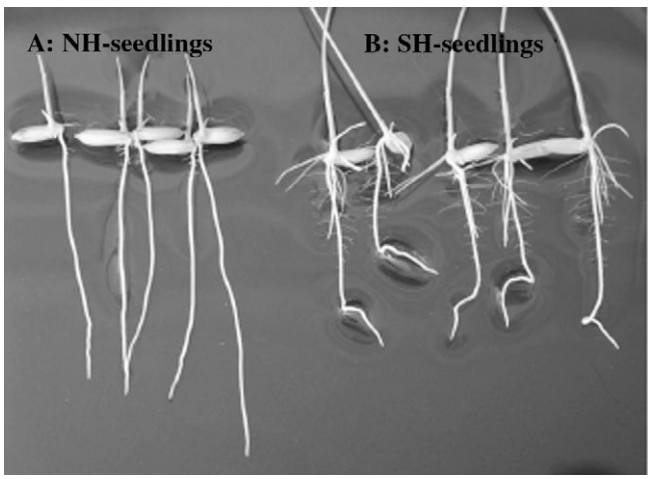

Fig. 2. Four-day-old seedlings grown under 60% relative humidity (A, NH-seedlings) and 100% relative humidity (B, SH-seedlings). SH-seedlings display dense and longer lateral roots, enhanced shoot height and root curling compared with NH-seedlings.

Application of auxin to the decapitated SH-seedlings restores lateral root density

The effect of removing the shoot apex on lateral root development could be due to a reduction in auxin supply from the shoot. To test this idea IAA was applied to the decapitated shoot of 2-d-old SH-seedlings and lateral root density was measured 2 d after IAA application. As predicted by our model, application of 10 μM IAA increased the density of lateral roots in the SH-seedlings to a level equivalent to intact seedlings (Fig. 4). These results raise the possibility that saturated humidity increases auxin transport from the shoot to the root, and that decapitation of the shoot nullifies the auxin supply from this source.

The transport of labelled IAA from shoot to root is enhanced in SH-seedlings

To test the idea that saturated humidity alters auxin transport, the transport of labelled IAA from shoot to root tissues was examined in 4-d-old seedlings. As shown in Fig. 5, a greater amount of [^{3}H]IAA was transported from the shoot to the root tissues in SH-seedlings compared with NH-seedlings. The difference was significant at 1 h and became greater after 2 h incubation, implying that saturated humidity accelerated the auxin transport from the shoot to the root.

To analyse the distribution of labelled IAA in roots, radioactivity was measured in each 1 cm root segment.

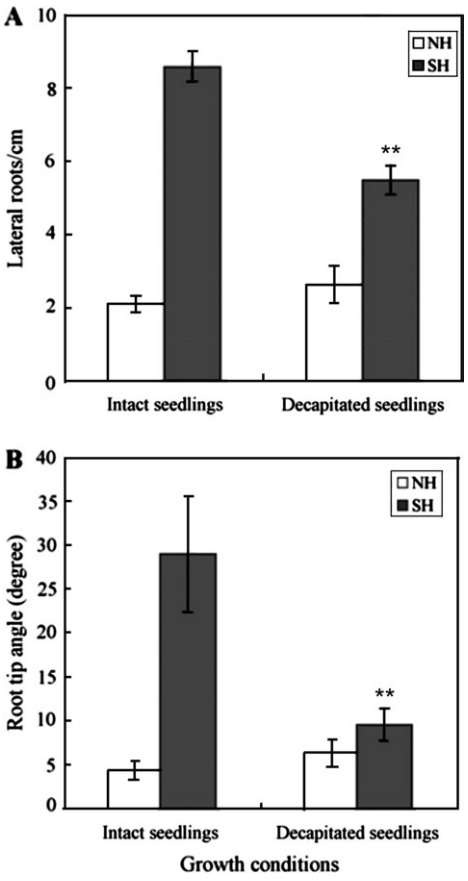

Fig. 3. The effect of removing the shoot apex in NH- and SH-seedlings on lateral root density (A) and root gravity response (B). The shoots of 2-d-old NH- and SH-seedlings were removed and the root morphology was observed 2 d after decapitation. Shoot decapitation was done two or three times a day by excising the shoot apex with a razor blade to avoid new growth. The direction of gravity is shown as 0 degree. Data are averaged (±SE) for 30 seedlings. Two asterisks represent significant differences from intact seedlings at P <0.01 as judged by the Student t test.

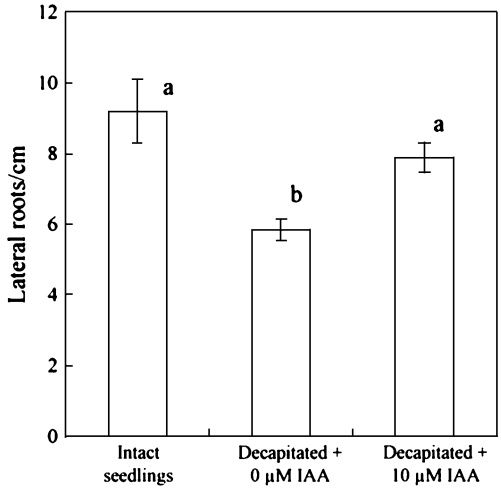

Fig. 4. Effect of IAA on lateral root density when IAA was applied to the decapitated stem of SH-seedlings. The shoot apex of 2-d-old SH-seedlings was removed before mature lateral root formation. The decapitated stem was then capped with a small tube containing 5 μl of 0 μM or 10 μM IAA. Lateral root density was measured following 2 d incubation. Data are averaged (±SE) for 30 seedlings. b is significantly different from a at P <0.01 as judged by the Student t test.

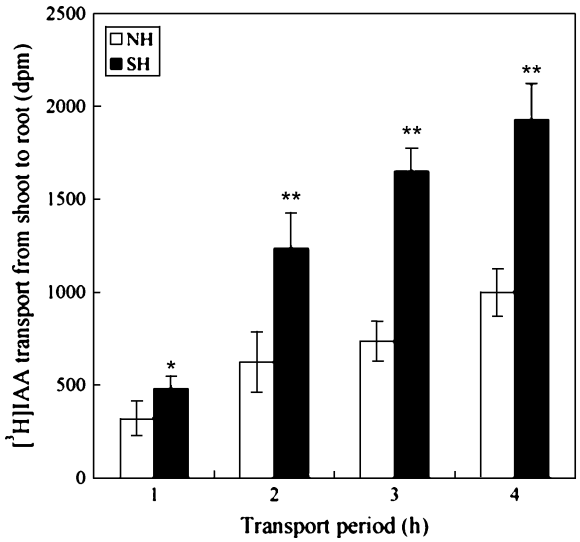

Fig. 5. Time-course of [^{3}H]IAA movement from shoot to root in NH- and SH-seedlings. [^{3}H]IAA was applied to decapitated shoots of 4-d-old NH- and SH-seedlings under NH- and SH-condition, respectively, and the radioactivity of whole root was measured after various transport periods. Data are averaged (±SE) from at least 30 roots. One asterisk and two asterisks represent significant differences from NH-seedlings at P <0.05 and P <0.01, respectively, as judged by the Student t test.

After various transport periods, roots were divided into four segments for NH-seedlings and into three segments for SH-seedlings. As shown in Fig. 6, labelled auxin reached the apical root segment in both seedling treatments after 1 h incubation. Since the distance from the application site of the shoot to the apical root segment in NH-seedlings was about 3.5 cm, the velocity of labelled IAA from the shoot to the root tip is estimated to be 3.5 cm h^{-1} or greater. Such rapid transport of IAA is characteristic of phloem (rather than polar) transport (Goldsmith *et al*., 1974).

In NH-roots (Fig. 6), the radioactivity per segment decreased from segment 1 to segment 3 and this pattern did not change for 4 h. In contrast, in SH-seedlings, the radioactivity of segment 2 increased greatly after 2 h incubation and reached the same level as segment 1 at 3 h. Segment 2 of SH-roots represents the position where lateral roots emerge under SH-conditions (Fig. 2). In NH-seedlings, lateral roots were formed only in segment 1, whereas in SH-seedlings they emerged in a wider region including segments 1 and 2.

NPA does not block the humidity-induced increase in lateral root number and density in SH-seedlings

It could be predicted that if auxin transport from the shoot to the root were mediated by a polar transport system, the auxin transport inhibitor NPA would block auxin movement. To address this issue, the transport of labelled auxin from the shoot to the root was examined after treating the basal part of shoots in 4-d-old SH-seedlings with NPA lanolin. As shown in Fig. 7A, NPA slightly but significantly reduced [^{3}H]IAA movement from the shoot to the root by 15%, implying that only a small proportion of auxin transport from shoot to root is mediated by a polar transport system.

To evaluate the role of polar auxin transport in shoot tissues in root morphology, the effect of NPA was examined on lateral root formation. The basal part of the shoot of 2-d-old seedlings was treated with NPA lanolin in a ring

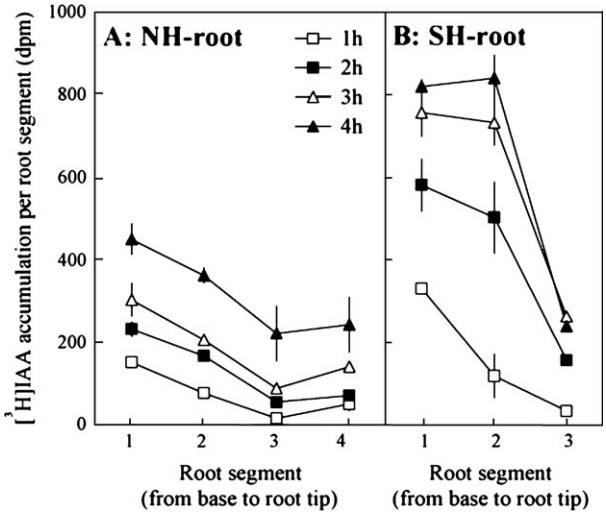

Fig. 6. Distribution of radioactivity in roots when [^{3}H]IAA was applied to the shoots of NH- (A) and SH- (B) seedlings. [^{3}H]IAA was applied to decapitated shoots of 4-d-old NH- and SH-seedlings under NH- and SH-condition, respectively. After various transport periods, the root was divided into four (A) or three (B) segments and the radioactivity of each segment was measured. The length of each root segment was 1 cm except at the root tip. The length of the tip segment was slightly longer than 1 cm depending on the length of whole root. Data represent averages (±SE) of 30 roots from three independent experiments.

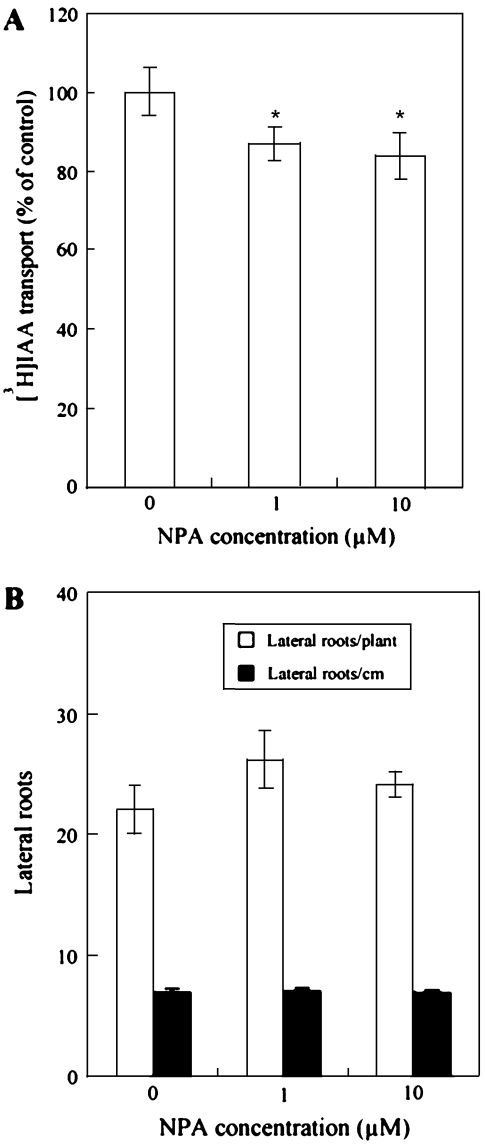

Fig. 7. Effect of an auxin transport inhibitor, NPA, on [^{3}H]IAA transport from shoot to root (A) and lateral root number and density (B). The NPA solution was mixed with lanolin and applied to the basal part of the shoot as a ring. (A) [^{3}H]IAA was applied to the decapitated shoot of 4-d-old SH-seedlings, which were treated with NPA, and the radioactivity of the whole root was measured after a 4 h transport period. (B) Two-day-old SH-seedlings, treated with NPA, were grown for a further 2 d and the number of lateral roots was measured. To confirm the effect of NPA, the inhibitor was re-applied every day to the basal part of the shoot. Data represent averages (±SE) of 40 seedlings from four independent experiments. The asterisk represents significant differences from 0 μM-NPA treated seedlings at P <0.05 as judged by the Student t test.

and the number of lateral roots was counted 2 d later. To ensure the effectiveness of NPA treatment, the NPA lanolin ring was applied every day to the basal part of the shoot. Surprisingly, the number or density of lateral roots per plant was not reduced in NPA-treated seedlings (Fig. 7B). These results suggest that the phloem-based transport of auxin is responsible for humidity-induced lateral root development. However, it is still possible that some signal from the shoot, other than auxin, is acting in this system.

Basipetal transport of IAA in roots is reduced in SH-seedlings compared with NH-seedlings

SH-seedlings exhibit altered gravitropic growth (Fig. 2). Since the basipetal transport of auxin in the roots (from the root tip backward to the base) is the important factor for gravitropism (Rashotte *et al*., 2000; Swarup *et al*., 2005), the basipetal transport of labelled IAA was measured in the roots of 4-d-old SH- and NH-seedlings (Fig. 8). The basipetal [^{3}H]IAA transport in the roots was suppressed in SH-seedlings compared with NH-seedlings, consistent with the reduced gravitropic response of SH-seedling root tips.

Sequence and expression analysis of four AUX1*-like genes in rice (ssp.* indica *cv. IR8)*

AUX1 gene expression in *Arabidopsis* leaves has been proposed to regulate the phloem-based IAA transport by facilitating hormone loading into the leaf vascular system (Marchant *et al*., 2002). In order to examine the possible involvement of *AUX1*-like genes in the phloem-based IAA transport from the shoot to the root in rice, cloning and expression analysis of homologous gene were carried out. By searching the DDBJ database, four *AUX1*-like genes were found in rice (ssp. *japonica* cv. Nipponbare); AK100090 on chromosome 1, AK103524 on chromosome 3, AK111849 on chromosome 5, and AK102295 on chromosome 10. These genes shared a high degree of identity at the amino acid level with *AtAUX1*; 83.7, 77.5, 82.5, and 76.6%, respectively. Primer sets were designed to clone the whole open reading frame of each gene in rice (ssp. *indica* cv. IR8) by RT-PCR. The deduced amino acid sequences of the four obtained cDNAs were aligned with AtAUX1 (see Supplementary Fig. S2 at *JXB* online). The difference in sequence between IR8 and Nipponbare was only three or four bases per gene and one or two amino acids per protein as for the *AUX1*-like genes. Amino acids, which were substituted in the 13 *aux1* missense alleles (Swarup *et al*., 2004), are conserved in four homologous genes in rice (see Supplementary Fig. S2 at *JXB* online). Based on their sequence identity, these cDNAs were termed *OsRAU1* (GenBank accession number AB275160), *OsRAU2* (AB275161), *OsRAU3* (AB275162), and *OsRAU4* (AB275163).

To examine the role of *AUX1*-like genes of rice, the expression of *OsRAU1*, *OsRAU2*, *OsRAU3*, and *OsRAU4* genes was monitored in various organs of rice seedlings by performing RT-PCR to estimate their transcript levels. As shown in Fig. 9, the *OsRAU1* transcript was observed in all organs tested and particularly high levels of the *OsRAU1* transcript accumulated in the upper root and lateral root samples. The *OsRAU2* transcript was observed in shoots, the upper root, lower root, and lateral root samples. Transcripts were present at low to moderate levels in upper and lower roots, lateral roots, and crown root samples for *OsRAU3*, and in the shoot and upper roots for *OsRAU4*.

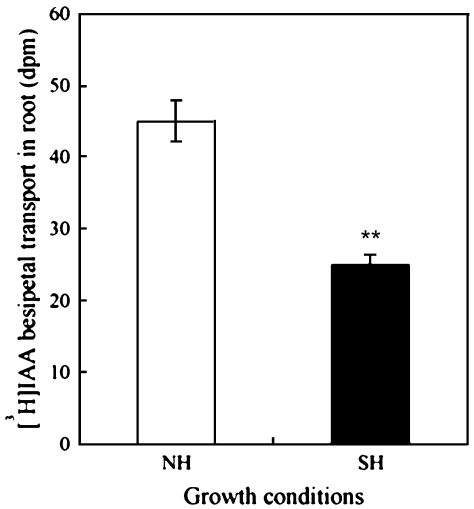

Fig. 8. The basipetal transport of [^{3}H]IAA in NH- and SH-roots. [^{3}H]IAA was applied to the apical end of 1.5 cm root segments for 1 h and the radioactivity accumulating at the basal 3 mm end of the segment was measured. Data represent averages (±SE) of 40 seedlings from four independent experiments. Two asterisks represent significant differences from NH-seedlings at P <0.05 as judged by the Student t test.

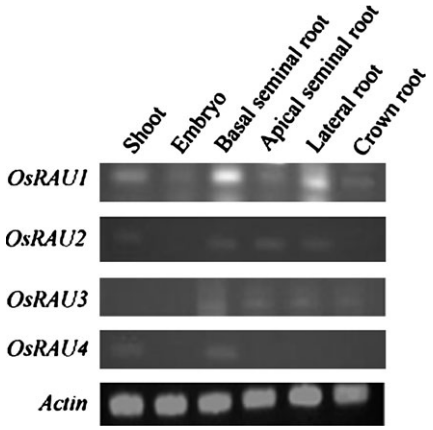

Fig. 9. Expression of *AUX1*-like genes, *OsRAU1*, *OsRAU2*, *OsRAU3*, and *OsRAU4*, in various organs of rice (cv. IR8). Semi-quantitative RT-PCR analysis was performed and the *actin* gene was used as control. One μg of cDNA was used for PCR amplification. Total RNA was extracted from various organs. The embryo was obtained from seeds before benomyl treatment. Shoot and basal and apical seminal root samples were prepared from 3-d-old seedlings. The apical root was taken from the apical 1 cm root tip and the basal root includes the remaining part of the root. Lateral and crown roots were prepared from 5-d-old seedlings.

The expression of the *AUX1*-like genes in 4-d-old SH-seedlings was then compared with that in NH-seedlings. Interestingly, SH-seedlings exhibited a greater abundance of the *OsRAU1* transcript in shoot tissues compared with NH-seedlings (Fig. 10). In contrast to *OsRAU1*, the expression levels of *OsRAU2*, *OsRAU3*, and *OsRAU4* in SH-seedlings were the same as those in NH-seedlings.

Discussion

It is reported here that the shoot and root morphologies of rice seedlings grown under saturated humidity are distinct from those under normal humidity (Fig. 2). The shoot height of SH-seedlings also increased compared with NH-seedlings (Table 1), consistent with a previous observation that shoot elongation of floating rice was enhanced under SH-conditions (Azuma *et al*., 1991). Although ethylene and gibberellins promoted internodal elongation of rice seedlings, Azuma *et al*. (1991) found that a moist environment was required for ethylene-induced internodal elongation in floating rice while the stimulatory effect of gibberellins was independent of moisture conditions. Ethylene's requirement for high humidity conditions suggests that this signal is involved in promoting the shoot height of SH-seedlings. In the present study, it has been shown that the SH-seedlings exhibit altered root morphology, including dense root branching and root agravitropism (Fig. 2; Table 1), despite the roots having been kept in water, implying that the highly humid air surrounding the shoot influences root development. Ethylene is not likely to cause the humidity-induced changes in root morphology because the SH-induced increase in lateral root density was also observed, even when the air surrounding the shoot was continuously refreshed with water-saturated air (see Supplementary Table S1 at *JXB* online).

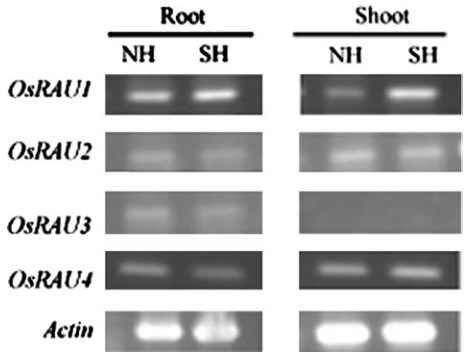

Fig. 10. Comparison of transcription levels of *AUX1*-like genes, *OsRAU1, OsRAU2, OsRAU3*, and *OsRAU4*, in shoot and root tissues of 4-d-old NH- and SH-seedlings. Semi-quantitative RT-PCR analysis was performed and the *actin* gene was used as control. One μg of cDNA was used for PCR amplification.

It is proposed that the SH-induced increase in lateral root density is due to an enhanced translocation of auxin from the shoot to the root tissue as discussed below. First, removal of the shoot apex of SH-seedlings greatly reduced lateral root density in the seminal root (Fig. 3A), indicating that the humidity-induced changes in root morphology are dependent on the presence of shoot tissues. In contrast, removal of shoot tissues did not alter lateral root density in NH-seedlings. Secondly, application of IAA to the decapitated shoot of SH-seedlings restored the density of lateral roots to the intact SH-seedling level. These results indicate that a supply of auxin from shoot to root tissues is required for the humidity-induced increase in lateral root density. It is interesting to note that the requirement of the shoot for lateral root development was only observed in SH-roots (and not NH-roots). This difference in the role of the shoot raises the possibility that the auxin supply from shoot to root tissues is enhanced in SH-seedlings. This idea was supported by the finding that the transport of labelled IAA from the shoot to the root was greater in SH-seedlings compared with NH-seedlings (Fig. 5). Furthermore, the elevated level of transport of labelled IAA from the shoot to the root tissues in SH-seedlings resulted in an enhanced accumulation of radioactivity in the basal part of the roots (Fig. 6), just where the lateral roots emerged under SH-conditions (Fig. 2). These results are consistent with the idea that SH-induced lateral root formation is ascribed to the accelerated transport of auxin from the shoot to the root.

Auxin transport from shoot to root tissues could occur either through phloem or through polar transport routes. Polar transport of auxin occurs at a velocity of about 10 mm h^{-1} in the roots of *Phaseolus vulgaris*, *Pisum sativum*, and *Arabidopsis thaliana* (Iversen *et al*., 1971; Rowntree and Morris, 1979; Rashotte *et al*., 2003), whereas the rate of phloem-mediated auxin transport is over 5 cm h^{-1} in the roots of *Populus tremula* and *Vicia faba* (Eliasson, 1972; Tsurumi and Wada, 1980). Measuring the rate of auxin transport is one of the useful ways to discriminate between these transport routes (Goldsmith *et al*., 1974). When labelled IAA was applied to the shoot, in these experiments, radioactivity was detectable in the root apex within the first hour of transport in either NH- or SH-seedlings (Fig. 6). The longer the transport period, the greater the level of radioactivity that accumulated in the root apex. Based on the distance of translocation from the decapitated shoot to the root tip and the detection of radioactivity in the root apex within 1 h (Fig. 6), the velocity of auxin translocation from the shoot to the root tip was estimated as 3.5 cm h^{-1} or greater, consistent with the idea that auxin transport from the shoot to the root was mediated by the phloem.

Treatment with NPA, an inhibitor of polar auxin transport, represents another method to distinguish the

transport pathway (Lomax *et al*., 1995). Application of NPA to the shoot base reduced the transport of labelled IAA from the shoot to the root by 15% compared with its control (Fig. 7A), indicating that polar transport represents only a minor route for the translocation of auxin from the shoot to the root. Consistent with this conclusion, similar treatment with NPA in 2-d-old seedlings did not impair the humidity-induced increase in lateral root number and density (Fig. 7B). In contrast to NPA treatment of shoot tissues, NPA application to the root has been shown to reduce lateral root numbers (Reed *et al*., 1998). It remains possible that a proportion of the phloem auxin in the shoot may move into the polar transport system in roots. Indeed, the migration of auxin from the phloem to polar systems has been observed in aerial tissues (Cambridge and Morris, 1996).

It is interesting to note that the transcript abundance of an *AUX1*-like gene, *OsRAU1*, was elevated in the shoot of SH-seedlings compared with NH-seedlings, whereas, in roots, the *OsRAU1* level was unchanged (Fig. 10). The amino acid sequence of OsRAU1 protein is 83.7% identical to AtAUX1 which has been proposed to play a role in phloem-based IAA transport pathway (Parry et al., 2001*b*; Swarup *et al*., 2001; Marchant *et al*., 2002). On the other hand, AtAUX1 does not seem to be involved in the polar auxin transport system in shoots (Parry *et al*., 2001*a*). Further experiments are required to prove the involvement of OsRAU1 in the enhanced phloem translocation of labelled auxin.

Labelled IAA applied to shoot tissues accumulated in the basal part of roots in SH-seedlings (Fig. 6), consistent with the previous observation reporting the accumulation of labelled auxin in lateral roots and their primordia (Rowntree and Morris, 1979; Tsurumi and Wada, 1980; Kerk and Feldman, 1995). The accumulation of labelled IAA in primordia may be due to the expression of the AUX1 protein in primordia (Marchant *et al*., 2002). Although basipetal IAA transport in root tips was reduced in SH-seedlings (Fig. 8), auxin accumulation in the basal part of roots in Fig. 6B is not likely to be due to transport inhibition, because this would result in a reduction in lateral root numbers (Reed *et al*., 1998).

In SH-seedlings, the direction of the root tip deviated from the gravity vector (Fig. 2). This pattern of root growth was not observed in NH-seedlings. It was found that the basipetal transport of labelled IAA was greatly reduced in SH-roots compared with NH-roots (Fig. 8), suggesting that reduced basipetal IAA transport was the cause of the gravitropic defect. Although the removal of the shoot apex of SH-seedlings reduced root curvature and restored normal downward root growth as for NH-roots (Fig. 3B), shoot auxin is not likely to be the cause of root curving because the application of auxin to the cut stem did not induce such curving in roots (data not shown).

In contrast to SH-seedlings, the removal of the shoot apex of NH-seedlings did not alter lateral root density compared with intact seedlings (Fig. 3). The role of shoot auxin on lateral root formation is dependent on plant age (Bhalerao *et al*., 2002). In young NH-seedlings, seed-derived auxin (Hall, 1980) may be sufficient for lateral root development. The removal of rice seedlings from their seed severely reduced lateral root number and led to their death 10 d after germination (data not shown). This result is consistent with a previous report that radioactivity from [^{14}C]IAA applied to the seed of *Vicia faba* seedlings accumulated in lateral root primordia along with vascular bundles (Tsurumi and Wada, 1980).

In the present study, the shoot environment is the cause of SH-induced root morphology because the roots of both SH- and NH-seedlings had been kept in water. The seminal root of SH-seedlings is greatly branched and the root tip is curved (Fig. 2). Decapitation of the shoot in SH-seedlings restored the gravitropic response of the seminal root tip as well as normalizing root branching (Fig. 3), implying that the shoot surrounded by moist air is the source of signals responsible for the observed changes in root morphogenesis. The phloem-based transport of auxin from shoot to root tissues is likely to promote root branching. Auxin functions as a mediator in phototropism and gravitropism by moving from sensor tissues to responding tissues in the shoot and the roots (Swarup *et al*., 2005; Whippo and Hangarter, 2006). In the present experiments, the shoot represents the sensing organ for the humid environment and the root functions as the responding organ. The transmitted signal is also likely to be auxin, which is delivered via the phloem from shoot to root tissues. This represents a novel example of how an environmental signal can cause morphological changes by altering auxin transport from shoot to root tissues in rice seedlings.

Supplementary data

Supplementary data in the form of two figures and two tables can be found at *JXB* online.

Fig. S1. Schematic illustrations of growth condition for NH-seedlings grown under 60% relative humidity and SH-seedlings grown under 100% relative humidity.

Fig. S2. Predicted amino acid sequence of *AUX1*-like genes in rice (ssp. *indica* cv. IR8).

Table S1. Effect of refreshing the shoot environment on root morphology of SH-seedlings.

Table S2. List of primers for isolation and expression of *AUX1*-like genes.

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