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Isolation of a novel lateral-rootless mutant in rice (*Oryza sativa* L.) with reduced sensitivity to auxin

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Abstract

Lateral roots play an important role in the acquisition of nutrients and anchorage of the whole plant. To better understand the mechanisms underlying lateral root development, we isolated a new lateral-rootless mutant *lrt2* in screening for 2,4-dichlorophenoxyacetic acid (2,4-D) resistance in M_2 lines of rice (*Oryza sativa* L. cv. Nipponbare) generated by tissue culture. *lrt2* failed to form lateral roots and exhibited altered root response to gravity. Analysis for auxin resistance showed that *lrt2* was less sensitive to various auxins including 2,4-D, indole-3-acetic acid (IAA), indole-3-butyric acid (IBA) and 1-naphthaleneacetic acid (NAA) compared with wild type, but was similarly sensitive to auxin transport inhibitor *N*-1-naphthylphthalamic acid (NPA). This suggests that the reduced sensitivity to auxin in *lrt2* might be caused by a disruption in auxin response rather than in auxin transport. Genetic analysis indicated that the lateral-rootless phenotype of *lrt2* is due to a recessive mutation. To map the *lrt2* gene, we tested molecular markers by bulk segregant analysis. The *lrt2* gene was localized to a 10.8 cM interval on the short arm of chromosome 2, flanked by two sequence-tagged site (STS) markers Lrt2P1 and Lrt2P2.

Keywords: Auxin resistance; Gravitropism; Lateral root formation; *lrt2*; Rice; STS markers

1. Introduction

Lateral root formation is the primary way that plants increase their root mass to absorb water and nutrients as well as anchor the whole plant. By analyzing two rice mutants RM109 and RH2, which lack lateral roots and root hairs respectively, Ma et al. [1] demonstrated that silicon is actively taken up through the lateral roots but not through the root hairs. Likewise, Bailey et al. [2] concluded that lateral roots rather than root hairs play a pivotal role in the anchorage of *Arabidopsis* plants. Thus, investigating the factors that determine lateral root development is of agronomic importance. To date, however, the precise mechanisms involved in the lateral root formation in crops have not been elucidated.

Auxins are known to regulate diverse developmental processes including stem elongation, apical dominance, gravitropism and lateral root formation [3]. Roots are a particularly attractive system to study auxin action because of their morphological simplicity and well-characterized auxin responses [4]. In *Arabidopsis*, a number of auxin-resistant mutants have been isolated, such as *aux1* [5], *axr1* [6], *axr2* [7], *axr3* [8], *axr4* [4], *axr5* [9], *axr6* [10], *slr-1* [11], *tir1* and *tir3* [12,13]. Molecular evidence revealed that these genes are classified into auxin transporter-related proteins, Aux/IAA family, ARF family or ubiquitin-related proteins. Furthermore, almost all of these mutants are defective in lateral root formation and root gravitropism, confirming the importance of auxin in these processes [4].

Previously, Hao and Ichii [14] isolated a dominant lateral-rootless mutant *Lrt1* (lateral-rootless) of rice (*Oryza sativa* L. ssp. *japonica* cv. Oochikara) in screening for 2,4-dichlorophenoxyacetic acid (2,4-D) resistance. *Lrt1* also exhibited resistance to other synthetic and natural auxins including 1-naphthaleneacetic acid (NAA), indole-3-acetic acid (IAA) and indole-3-butyric acid (IBA) [15]. In addition to auxin resistance, *Lrt1* is characterized by its defects in root gravitropic response and reduced root hairs, suggesting that auxin is required for normal root growth.

This paper describes the isolation and characterization of a new recessive mutant defective in lateral root formation in rice, which we have designated *lrt2* (lateral-rootless 2). The *lrt2* mutant also shows high resistance to auxins and altered root

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response to gravity as characterized in the *Lrt1* mutant [14]. The gene responsible for the absence of lateral roots was mapped by using both sequence-tagged site (STS) and cleaved amplified polymorphic sequence (CAPS) markers.

2. Materials and methods

2.1. Plant materials and growth conditions

The rice lateral-rootless mutant *lrt2* was used in this study. *lrt2* was originally isolated from *japonica* cultivar Nipponbare M_2 lines in a mutant screen for 2,4-D resistance. Homozygous seeds of M_4 generation were used in the experiments.

To observe the adult plant phenotype, *lrt2* and Nipponbare were planted in 1/5000 a Wagner's pots (Kiya Seisakusho, Ltd., Tokyo, Japan) containing soils (one plant per pot), and grown during the period of June–September of 2004 under natural condition.

2.2. Screening for mutants

Two thousand M_2 lines derived from selfing of M_1 plants, which were generated by tissue culture of Nipponbare for *Tos17*-tagged mutation [16] were used for screening. Twenty seeds per line were surface sterilized in a 0.2% (v/v) Benomyl (Dupont Company, Tokyo, Japan) solution for 24 h, rinsed, and then soaked in water for 24 h in the dark at 30 °C. Germinated seeds were sown on floating nets and grown at 25 °C in deionized water supplemented with 0.5 μM 2,4-D in a plastic container for 5 days, and then screened for seedlings with longer roots compared with the control of wild type Nipponbare. Putative 2,4-D resistant mutants were transferred to 1/2 concentration of Kimura B nutrient solution for 7 days to allow recovery and then transplanted to pots in a glasshouse to produce M_3 seeds. The nutrient solution contained 0.18 mM $(NH_4)_2SO_4$, 0.27 mM $MgSO_4 \cdot 7H_2O$, 0.09 mM KNO_3, 0.18 mM $Ca(NO_3)_2 \cdot 4H_2O$, 0.09 mM KH_2PO_4, 0.04 mM K_2SO_4 and 0.02 mM NaEDTA-$Fe \cdot 3H_2O$. M_3 progeny of the selected M_2 plants was retested for 2,4-D resistance and investigated for root morphology. Among them, one line *lrt2* was selected for consistent 2,4-D resistance and a lack of lateral roots and used in this experiment.

2.3. Genetic analysis

To investigate inheritance of the lateral-rootless phenotype, *lrt2* was crossed to wild type Nipponbare. Lateral roots of 10 F_1 seedlings cultured in water for 7 days were investigated. After investigation, the F_1 seedlings were transferred into 1/2 concentration of Kimura B nutrient solution, then transplanted to soil in 3 L-pots and grown to maturity. The segregation in F_2 generation was investigated at the seedling stage with respect to lateral root formation.

2.4. Root staining

To observe lateral root primordia formation, the whole seminal root was stained with methylene blue as described by Johnson et al. [17]. Briefly, the excised roots were fixed in an absolute ethanol: glacial acetic acid (3:1, v/v) solution at 4 °C for at least 24 h. Then they were rinsed for 5 min with distilled water and cleared by immersion in sodium hypochlorite solution diluted with distilled water (50%, v/v) under vacuum for 10 min and for an additional 10 min at atmospheric pressure. After fixing and clearing, roots were stained with methylene blue (0.01% in distilled water) to visualize the nucleolar material in the meristems.

2.5. Assay for auxin and NPA resistance

To score auxin resistance, seedlings were grown in black glass cups containing 200 ml of deionized water supplemented with or without various concentrations of 2,4-D, IAA, IBA, NAA or auxin transport inhibitor *N*-1-naphthylphthalamic acid (NPA). IAA solutions were renewed every day in order to avoid the oxidation of IAA. The seedlings were allowed to grow at 25 °C in an incubator with continuous white fluorescent light at irradiance of approximately 67 μmol m^{-2} s^{-1} for 7 days. Data were presented as percentage of root length in water culture supplemented with auxin relative to root length on auxin-free medium.

2.6. Assay for root gravitropism

Seedlings were grown vertically in plastic plates containing 0.7% agar under continuous white light at 25 °C for 3 days and then rotated by 90°. The gravitropic bending of root tips was measured every 2 h after reorientation.

2.7. Mapping of the lrt2 gene

lrt2 was crossed to an *indica* variety Kasalath to generate an F_2 mapping population. A total of 123 F_2 individuals from this population were used for mapping. Genotypes of these plants were determined by F_3 progeny test. Isolation of rice genomic DNA was performed according to the following modified CTAB method. Briefly, approximately 100 mg (fresh mass) of crashed leaf tissue was added to 700 μl of DNA extraction buffer (125 mM Tris–Cl pH 8.0, 20 mM EDTA, 150 mM sorbitol, 30 mM *N*-lauroylsarcosine sodium, 800 mM NaCl, 20 mM CTAB) in a 2.0 ml tube. After incubation at 65 °C for 40 min, the lysate was mixed with 700 μl of chloroform: isoamyl alcohol (24:1). After centrifugation at 10,000 rpm for 10 min, the upper aqueous phase (∼500 μl) was transferred into a 1.5 ml Eppendorf tube. The DNA was precipitated by adding 500 μl of cold isopropanol and collected by centrifugation at 14,000 rpm for 10 min. The pellets of DNA were washed with 1.0 ml of 70% ethanol, then dried, and dissolved in 1/10 TE (pH 8.0). The concentration of the available DNA using this protocol is about 200 ng/μl.

To identify the molecular markers linked to the *lrt2* gene, we first used 80 PCR-based markers developed by RGP (http://rgp.dna.affrc.go.jp/publicdata/caps/index.html) that are dispersed on 12 chromosomes. Bulked segregant analysis employed the DNA mixture contributed equally by 10

homozygous lateral-rootless plants derived from the F_2 mapping population.

PCR was performed in a 10 μl volume containing 15 ng/μl of genomic DNA, 2 μM of each primer, 0.2 mM of dNTPs, 2.5 mM of Mg^{2+}, 1× PCR buffer and 0.3 unit of AmpliTaq (ABI Inc.). A PTC-100TM (MJ Research Inc.) was used for amplification with the following PCR program: 95 °C for 10 min, followed by 35 cycles of 94 °C for 45 s, 52 °C for 45 s, 72 °C for 1 min, and final extension at 72 °C for 10 min. PCR products were resolved on 1.5% agarose gels in TAE buffer.

2.8. Development of sequence tagged site (STS) markers

To develop PCR-based markers, we compared the Nipponbare contig sequences from RGP (http://rgp.dna.affrc.go.jp/) with the *indica* variety 93-11 sequence (http://rise.genomics.org.cn/rice/index2.jsp). The insertion/deletion differences of larger than 20 bp were selected for marker development. Primers were designed by the software Primer Premier 5.0 (PREMIER Biosoft International).

2.9. Linkage analysis

Linkage analysis was performed by MapMaker 3.0 [18] using the Kosambi function [19].

3. Results

3.1. Isolation of the lrt2 mutant

Of 2000 M_2 lines screened for resistance to 0.5 μM 2,4-D, one line included resistant seedlings. Root growth of other lines and wild type Nipponbare was almost completely inhibited. Resistant seedlings showed no lateral roots and later genetic analysis revealed that this mutant defines a new locus involved in lateral root development, named *lrt2* (lateral rootless). Genomic Southern analysis showed that the mutation in the *lrt2* mutant is independent of insertion of retrotransponson *Tos17* and is likely attributable to other factors that occurred during tissue culture (data not shown).

3.2. Genetic analysis

F_1 hybrids between *lrt2* and wild type showed the normal lateral root development, and in the F_2, the segregation of normal and lateral-rootless seedlings fitted well to a ratio of 3:1 (Table 1). Thus, the *lrt2* mutation is recessive and segregates in a manner consistent with a single Mendelian gene.

Table 1
Genetic analysis of the rice lateral-rootless mutant *lrt2*

Line	Lateral root		Total	χ^2 (3:1)	*P*
	+	−			
Nipponbare (WT)	10	0	10		
lrt2	0	10	10		
(*lrt2* × WT) F_1	10	0	10		
(*lrt2* × WT) F_2	102	31	133	0.203	0.65

+: with lateral roots; −: without lateral roots. Seedlings were grown at 25 °C in water for 7 days.

Table 2
Morphological characteristics of Nipponbare (WT) and *lrt2* mutant at the seedling stage

Character	Nipponbare[a]	*lrt2*
Shoot length (cm)	5.2 ± 0.6	4.8 ± 0.7[ns]
Seminal root length (cm)	7.1 ± 0.7	7.9 ± 0.5[**]
Crown root length (cm)	4.6 ± 0.6	4.3 ± 0.8[ns]
Number of crown roots	3.1 ± 0.6	2.9 ± 0.7[ns]
Number of lateral roots[b]	103.0 ± 8.6	0 ± 0[**]

Data of 7-day-old seedlings grown in distilled water (25 °C, 24 h light) were shown. ns: no difference. Student *t*-test was performed.
[a] Values represent mean ± S.D. ($n = 10$).
[b] Lateral root number was counted on the seminal root.
[**] Significant difference at 1% level.

3.3. Morphological analysis

When seedlings were grown in water for 7 days, *lrt2* seedlings had a longer seminal root compared with wild type ($P < 0.01$), whereas no difference was found in shoot length, crown root number or crown root length (Table 2). The most striking phenotype of *lrt2* is its lack of lateral roots. The primary roots of 7-day-old wild type seedlings produced many lateral roots (Fig. 1A and Table 2). Furthermore, no lateral root primordia were observed in *lrt2*, suggesting that the lateral root formation is impaired at the initiation stage in the mutant (Fig. 1B).

The aerial part of *lrt2* plants was investigated. At maturity, *lrt2* showed the reduced plant height and shorter panicles compared with wild type. The total number of tillers observed on *lrt2* plants was nearly 1.5-fold the number seen on wild type (Fig. 1C and Table 3). These growth habits of *lrt2* plants indicated a reduction in apical dominance.

The *lrt2* plants exhibited greatly reduced seed fertility relative to wild type (Table 3). However, *lrt2* plants showed normal seed fertility when pollinated with wild type pollen (data not shown), indicating that female fertility in *lrt2* was normal. The low seed fertility of *lrt2* was probably due to the low pollen fertility.

Table 3
Agronomic characteristics of Nipponbare (wild type) and *lrt2*

Character	Nipponbare[a]	*lrt2*
Heading date	16 August	19 August
Plant length (cm)	81.8 ± 2.3	64.7 ± 1.4 (79)[b]
Panicle length (cm)	20.1 ± 1.2	14.2 ± 0.6 (71)
Tiller number	43.0 ± 4.0	62.3 ± 4.9 (145)
No. of spikelets per panicle	110.8 ± 11.4	59.3 ± 4.2 (54)
Seed fertility (%)	87.1 ± 2.2	46.5 ± 5.5 (53)
Thousand grain weight (g)	25.5 ± 0.3	20.6 ± 0.2 (81)

Data of pot-cultivated plants were shown.
[a] Values represent mean ± S.D. ($n = 6$) except for heading date.
[b] Numbers in parentheses indicate the percentage to that of the wild type.

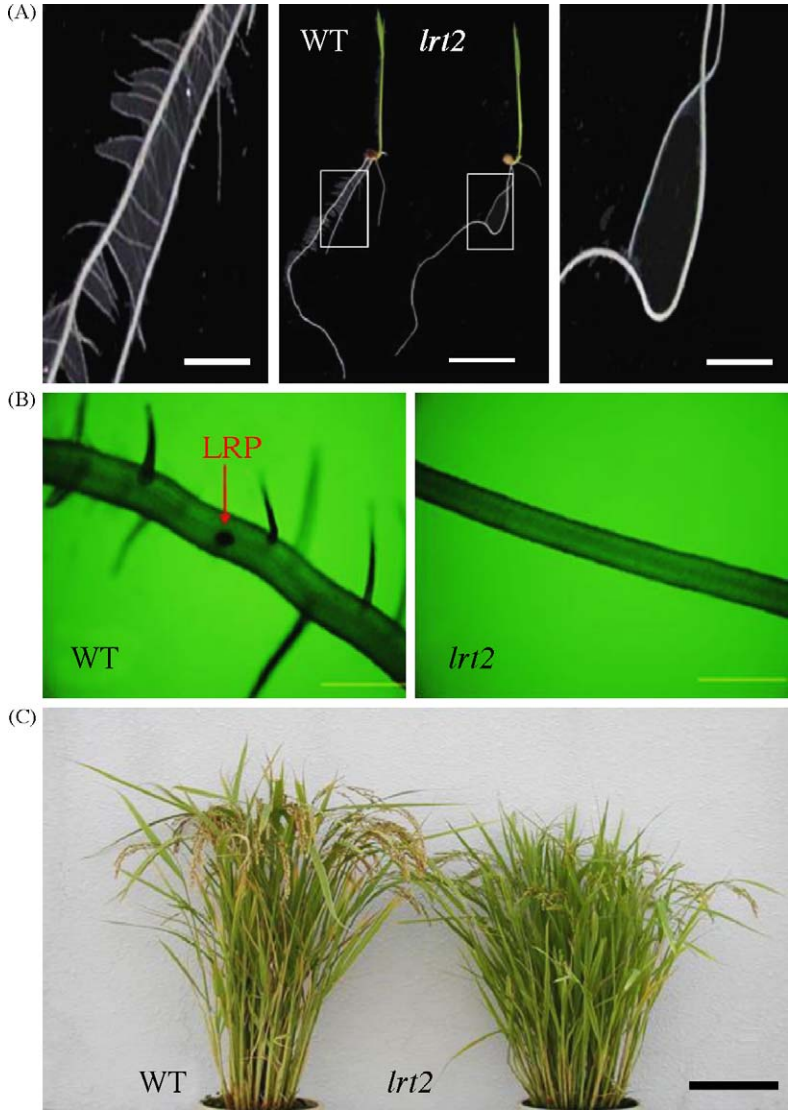

Fig. 1. Morphology of wild type Nipponbare (WT) and *lrt2* mutant. (A) seedlings of 7-day-old wild type Nipponbare (WT) and *lrt2* mutant grown in deionized water at 25 °C with a continuous white light. Left (WT) and right (*lrt2*) are magnified sections of the middle figure, showing the primary root region where lateral roots developed. Bars = 2.0 cm in middle, 0.7 cm in left and right. (B) Methylene blue staining of seminal roots of 7-day-old WT and *lrt2* seedlings. LRP, lateral root primordia. Bar = 1 mm. (C) Adult plant of WT and *lrt2* grown in soils. Bar = 23cm.

3.4. Analysis for root gravitropism

Results of root reorientation experiment using 3-day-old seedlings are shown in Fig. 2. Roots of *lrt2* seedlings exhibited a slower response to a gravity stimulus than the roots of wild type seedlings. Within 4 h of gravistimulation, wild type roots achieved an angle of curvature of more than 80°. In contrast, the roots of *lrt2* seedlings had curved only about 10° toward the gravity stimulus after 10 h. The slower root gravitropic response in *lrt2* did not appear to be associated with root growth rate, because *lrt2* roots elongated slightly rapidly than wild type roots during the reorientation experiment (Fig. 3).

3.5. Analysis for auxin and NPA response

lrt2 was originally selected as a 2,4-D resistant mutant. To evaluate the level of resistance to other auxins, the dose-response of root elongation was scored for 2,4-D, NAA, IAA and IBA. Seedlings of *lrt2* and wild type Nipponbare were grown in various concentrations of the four kinds of auxins for 7 days. The results in Fig. 4A, C and D showed that growth of the root of *lrt2* seedlings was less inhibited than wild type roots over a range of 2,4-D, IAA and IBA concentrations (0.01–10 μM). At 0.01 μM of NAA, *lrt2* roots unexpectedly exhibited the same sensitivity to auxin as wild type roots ($P = 0.317$). At higher concentrations (0.1–10 μM), however, *lrt2* roots showed a greater level of resistance to NAA than wild type roots (Fig. 4B). In order to compare the resistance to various auxins quantitatively, the auxin concentrations that inhibited root elongation by 50% (IC_{50}) were calculated. As shown in Table 4, the mutant showed a high level resistance to 2,4-D, but the level of resistance to other auxins were intermediate (NAA and IAA) and low (IBA). Fig. 5 showed the effect of exogenous application of various auxins on lateral root number. Low doses

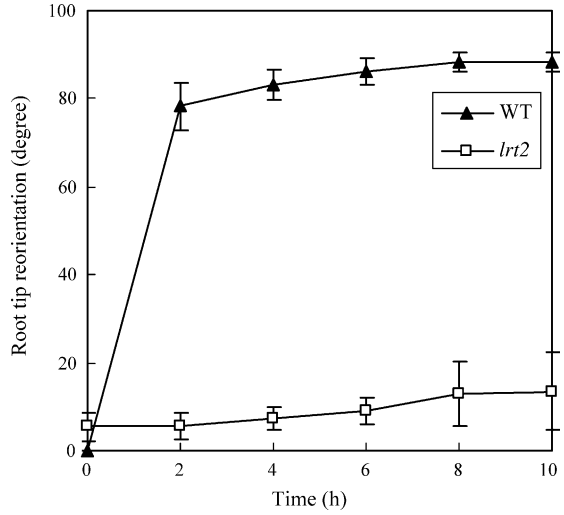

Fig. 2. Root reorientation in response to gravity. Seedlings of wild type Nipponbare and *lrt2* mutant were grown vertically in 0.7% agar plates for 3 days and then turned 90° to a horizontal position. The orientation of the root tips was measured at time indicated. 90° represents complete reorientation downward. Data are the average for 10 seedlings (±S.D.).

of 2,4-D, NAA, IAA and IBA (0.001 μM) induced more lateral root formation in wild type Nipponbare. At higher concentrations (0.01–10 μM), auxins exhibited the inhibitory effect on lateral root formation in wild type (Fig. 5). Nevertheless, all tested auxins failed to rescue the defect in lateral root formation of *lrt2* seedlings (Fig. 5). In addition, the response of *lrt2* roots to auxin transport inhibitor NPA was similar to wild type (Fig. 6). Taken together, these results suggest that *lrt2* has defects in auxin response rather than in auxin transport.

3.6. Molecular mapping of Lrt2 gene

In 123 F_2 plants of the cross between the *lrt2* mutant and Kasalath, 37 were homozygous mutants without lateral roots, 86 were normal lateral root formation, 61 of which were heterozygous and the remaining 25 were homozygotes. This

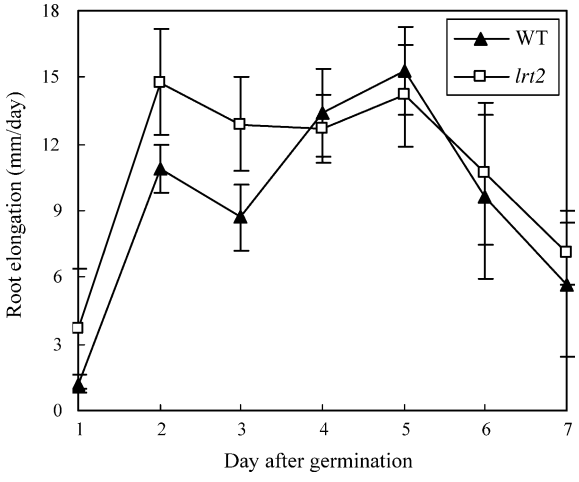

Fig. 3. Time course of root growth rate. Roots of wild type Nipponbare (WT) and *lrt2* seedlings grown in auxin-free medium at 25 °C were measured daily. Values are the average ± S.D. of 10 seedlings.

Table 4
Auxin concentrations showing for 50% inhibition (IC_{50}) in elongation of *lrt2* and wild type Nipponbare (WT) roots

Line	Auxin concentration (μM)			
	2,4-D	NAA	IAA	IBA
WT	0.06	0.05	0.1	0.35
lrt2	4.34 (72)	0.5 (10)	1.0 (10)	0.87 (2.5)

The numbers in parentheses represents the multiple compared with wild type.

segregation ratio fitted to a 1:2:1 ratio ($\chi^2 = 2.35$, $P = 0.309$), confirming that the lateral-rootless phenotype in *lrt2* is controlled by a single recessive gene as mentioned above.

Bulked segregant analysis showed that a CAPS marker C1357 on chromosome 2 is linked to the *lrt2* gene. Further mapping using STS markers developed around C1357 (Table 5) indicated that the *lrt2* gene was located in a 10.8 cM interval, flanked by two STS markers Lrt2P1 and Lrt2P2 (Fig. 7).

4. Discussion

Since auxin plays a crucial role in lateral root development, screening for auxin resistance of roots has been an important method for isolating auxin-mediated lateral root mutants. In this study, *lrt2*, a new lateral-rootless mutant has been isolated by a seminal root elongation assay of seedlings grown in a 0.5 μM 2,4-D solution. The *lrt2* mutant is controlled by a single recessive gene (Table 1) and displays a defect in root gravity response (Fig. 2).

By studying tobacco cultured cells, Delbarre et al. [20] revealed that different auxins enter the cells in distinct ways. The natural auxin IAA and the synthetic auxin 2,4-D were taken up by the cells through auxin transport, while the lipophilic auxin NAA was independent of auxin transport to enter the cell via diffusion. The *Arabidopsis* auxin transport mutant *aux1* showed resistance to IAA and 2,4-D but not to NAA, and had the defects in lateral root formation and root gravitropism. These mutant phenotypes in *aux1* roots could be rescued by exogenous application of 0.01–0.1 μM of NAA, but could not by IAA or 2,4-D [21,22]. Unlike *aux1*, *lrt2* was resistant not only to the transport-dependent auxin such as IAA and 2,4-D, but also to the cell membrane-diffusible auxin NAA (Fig. 4). In addition, *lrt2* showed the same sensitivity to the auxin transport inhibitor NPA as the wild type (Fig. 6) and the defect in lateral root formation could not be rescued by the exogenous NAA and other auxins (Fig. 5). These differences between *aux1* and *lrt2* infer that the *lrt2* mutant is unlikely to have a disruption in auxin transport.

The Cholodny–Went theory postulated that the gravitropic curvature arises with differential elongation of upper and lower sides of roots due to an asymmetric distribution of auxin across the root [23]. Roots of *lrt2* seedlings responded much more slowly to a gravity stimulus than roots of wild type seedlings (Fig. 2). Although auxin appears to be transported normally in *lrt2* roots, the roots of *lrt2* seedlings were unable to generate the differential growth upon perception of a gravity stimulus probably because of reduced auxin sensitivity. Several auxin-

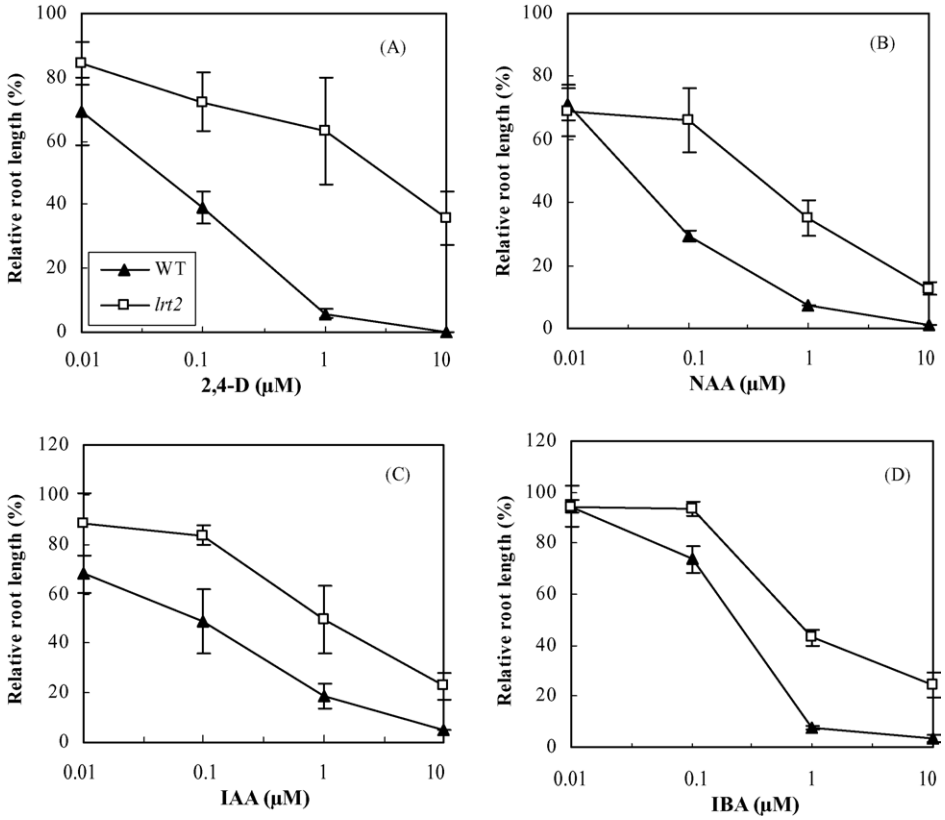

Fig. 4. Dose-response curves for wild type Nipponbare (WT) and *lrt2* mutant seedlings on auxins. Inhibition of root growth by 2,4-D (A), NAA (B), IAA (C) and IBA (D) was expressed relative to growth on auxin-free medium. Each value represents the average for 10 seedlings (±S.D.).

resistant mutants of *Arabidopsis* such as *slr-1* [11] and *axr4* [4] displayed defects in root gravitropic response as well, providing the evidence that auxin functions in regulating root gravitropism.

Root elongation in the absence of exogenous auxins was greater in *lrt2* than in wild type seedlings (Fig. 3), consistent with the observations in other auxin-resistant mutant seedlings of *Arabidopsis* like *axr1* [6] and *axr4* [4]. This strong correlation between reduced auxin response and increased root elongation seems to be explained by the function of auxin. However, we cannot exclude the possibility that other factors affect root growth in these auxin-resistant mutants because *axr1* mutations are also known to function in other signaling pathways besides the auxin signaling pathway [24]. It is possible that these additional pathways also affect root growth.

To date, only two lateral-rootless mutants have been characterized in *Arabidopsis*, although several mutants with altered lateral root morphology were reported [25]. One is the recessive mutant *alf4* that encoded a nuclear-localized protein required for lateral root formation. This mutant showed the normal root response to gravity and was independent of auxin pathway [26,27]. The other was the dominant *slr-1* mutant, which was caused by a mutation in IAA14, a member of the Aux/IAA protein family, and showed a decreased response to auxin [11]. The present rice *lrt2* mutant is rather similar to *slr-1* than *alf4* with respect to lateral root formation and auxin response. However, the gain-of-function and the enhanced apical dominance observed in *slr-1* [11] are opposite to the loss-of-function and decreased apical dominance found in *lrt2*, respectively. Thus, *lrt2* seems to

Table 5
List of the PCR-based markers used in this study

Marker name	Marker type	Primer sequence (5′ → 3′)	Restriction enzyme for CAPS marker	Expected PCR product size[a] (bp)	
				lrt2	Kasalath
Lrt2P1	STS	*F(GCACATCGGTAACGGTAGAGG); R(CGGTGGATAAAGACAAAGAGG)*		206	238
Lrt2P2	STS	*F(ATGGCAGTTTTGTTTGTGGC); R(TCCGATTCTGAGGCTTGG)*		242	281
C1357	CAPS	*F(CCTCACAAGTCACAACCAGT); R(ACCAAAGTCACGTTCAGATG)*	*Hae*III	277	121, 156

Lrt2P1 and Lrt2P2 markers were developed according to rice database. C1357 was derived from RGP.
[a] For C1357, the size represents the PCR products after digestion with *Hae*III.

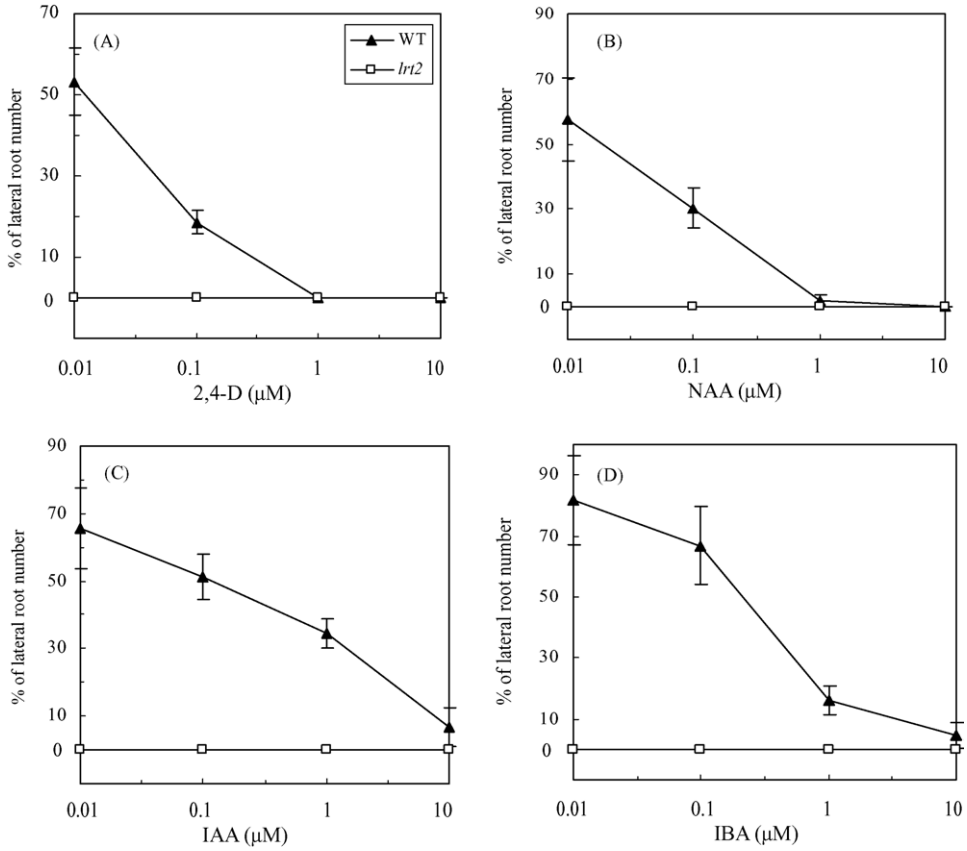

Fig. 5. Effect of auxins on lateral root formation in *lrt2* and wild type Nipponbare. Lateral root number on the seminal root of 7-day-old seedlings was calculated. Induction or inhibition of lateral root formation by 2,4-D (A), NAA (B), IAA (C) and IBA (D) was expressed relative to the values on auxin-free medium. Each value represents the average for 10 seedlings (±S.D.).

be a novel gene required for auxin-mediated lateral root formation that has not been reported in *Arabidopsis* yet.

According to the mapping result, the recessive lateral-rootless *lrt2* gene was localized to the short arm of rice chromosome 2 (Fig. 7). Another lateral-rootless mutant *Lrt1* was isolated by a similar screening and it exhibited a higher resistance to auxins including 2,4-D, IAA, IBA and NAA [15]. The mutant *Lrt1* was controlled by a semidominant gene and showed altered root response to gravity [15] as characterized in *lrt2*. The lateral-rootless in *Lrt1* and *lrt2*, however, are caused by the mutations in the different genes because we have mapped the *Lrt1* gene to a chromosome other than chromosome 2 (Wang et al., unpublished data), and that the direction of dominance is opposite between *Lrt1* and *lrt2*. These observations suggest that many auxin-related genes may be involved in lateral root development in rice. Thus, it is interesting to investigate whether or not the *lrt1* and *Lrt2* loci share the same pathway for controlling the lateral root formation. Analysis of the *Lrt1lrt2* double mutant is likely able to address the possibility of interaction between the two genes.

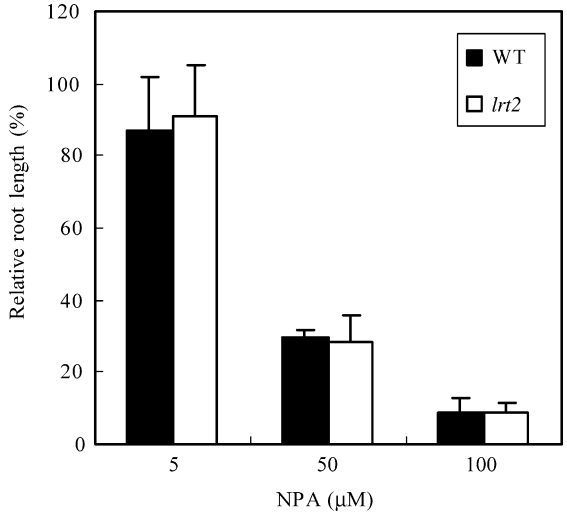

Fig. 6. Effect of auxin transport inhibitor NPA on root elongation in *lrt2* and wild type Nipponbare. Seedlings were grown at 25 °C for 7 days in deionized water containing a range of concentrations of NPA. Values are the average ± S.D. of 10 seedlings.

In rice, no information has been so far available on the molecular mechanisms underlying lateral root development. The isolation and characterization of the *Lrt2* gene will enable us to investigate the cellular function of the *Lrt2* gene product and to better understand the mechanisms for auxin-mediated regulation of lateral root formation. By searching the TIGR Rice Genome Annotation Database (www.tigr.org), we have found several candidate genes in the *lrt2* locus that are homologous to known auxin signaling genes. Currently, we are

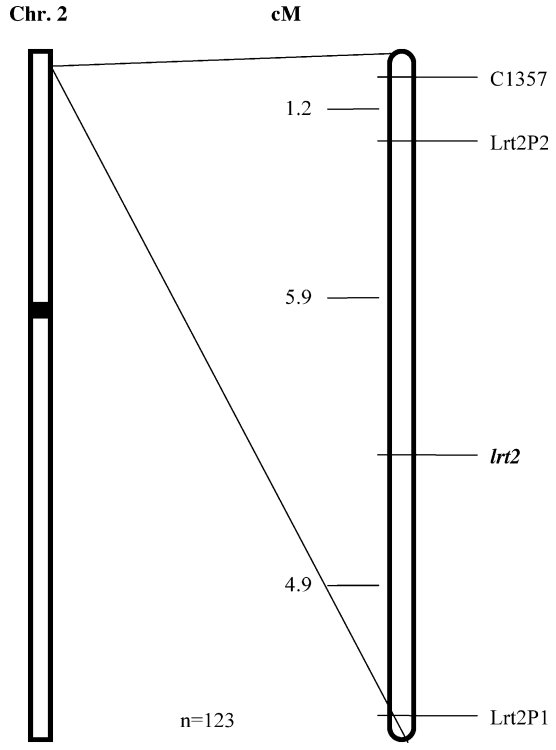

Fig. 7. The estimated location of the *Lrt2* locus on the rice chromosome 2. Linkage analysis was conducted in 123 F_2 plants derived from the cross between *lrt2* and Kasalath. Left is the overview of rice chromosome 2 and filled box indicates the centromere position. Right is a linkage map constructed in this study.

conducting a high-resolution mapping of *lrt2* toward its cloning and functional analysis.

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