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RESEARCH PAPER

Dro1, a major QTL involved in deep rooting of rice under upland field conditions

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Abstract

Developing a deep root system is an important strategy for avoiding drought stress in rice. Using the 'basket' method, the ratio of deep rooting (RDR; the proportion of total roots that elongated through the basket bottom) was calculated to evaluate deep rooting. A new major quantitative trait locus (QTL) controlling RDR was detected on chromosome 9 by using 117 recombinant inbred lines (RILs) derived from a cross between the lowland cultivar IR64, with shallow rooting, and the upland cultivar Kinandang Patong (KP), with deep rooting. This QTL explained 66.6% of the total phenotypic variance in RDR in the RILs. A BC_2F_3 line homozygous for the KP allele of the QTL had an RDR of 40.4%, compared with 2.6% for the homozygous IR64 allele. Fine mapping of this QTL was undertaken using eight BC_2F_3 recombinant lines. The RDR QTL *Dro1* (*Deeper rooting 1*) was mapped between the markers RM24393 and RM7424, which delimit a 608.4 kb interval in the reference cultivar Nipponbare. To clarify the influence of *Dro1* in an upland field, the root distribution in different soil layers was quantified by means of core sampling. A line homozygous for the KP allele of *Dro1* (Dro1-KP) and IR64 did not differ in root dry weight in the shallow soil layers (0–25 cm), but root dry weight of Dro1-KP in deep soil layers (25–50 cm) was significantly greater than that of IR64, suggesting that *Dro1* plays a crucial role in increased deep rooting under upland field conditions.

Key words: Drought avoidance, *Oryza sativa* L., quantitative trait locus, root distribution, root growth angle, upland rice.

Introduction

Drought is the most serious abiotic stress that limits crop production under rainfed conditions. In particular, rice (*Oryza sativa* L.), which is generally grown under flooded conditions, is susceptible to drought stress owing to its shallow root distribution and limited capacity to extract water from deep soil layers (Kondo *et al.*, 2000, 2003). The global warming that has occurred in recent years has caused serious drought damage in rice-growing areas that rely on rainwater and that lack access to irrigation. Therefore, the enhancement of drought resistance in rice is becoming an important strategy to stabilize rice production in areas with rainfed agriculture.

Plant roots play an important role in the absorption and translocation of water and nutrients. A deep root system is thought to enable plants to avoid drought stress by absorbing water from deep soil layers (Yoshida and Hasegawa, 1982). Typical upland rice cultivars have deeper rooting than lowland cultivars (O'Toole and Bland, 1987). The deep root system in upland rice may contribute greatly to its drought resistance through enhanced water uptake (Price *et al.*, 1999). Therefore, introducing the deep rooting characteristic of upland rice into lowland rice cultivars may be one of ways to improve their drought resistance.

Deep rooting is a complex trait that combines the effects of the root growth angle and root length in seminal and nodal roots of cereal crops (Araki *et al.*, 2002). Neither growth angle nor length of roots alone determines the vertical root distribution (Abe and Morita, 1994). Thus,

Abbreviations: CIM, composite interval mapping; CTAB, cetyltrimethylammonium bromide; DAS, days after sowing; InDel, insertion–deletion; LOD, logarithm of odds score; NIL, near-isogenic line; QTL, quantitative trait locus; RDR, ratio of deep rooting; RDW, root dry weight; RIL, recombinant inbred line; SSR, simple sequence repeat; STS, sequence-tagged site.

a combination of a near-vertical growth axis and increased root length along that axis is important for deep root development. Although varietal differences in root length have been reported in cultivated rice in many previous studies (reviewed by O'Toole and Bland, 1987), there have been few studies of the genetic variation in root growth angle (Kato *et al.*, 2006; Uga *et al.*, 2009). To support studies of rooting depth, Oyanagi *et al.* (1993) developed the 'basket' method (in which root angle is defined by the position where roots leave a basket surrounding the plants) for quantifying the root growth angle in wheat. They showed that a near-vertical root growth angle was positively correlated with deep root development in wheat. Their method is an easy and efficient way to quantify root growth angle in cereal crops.

Using the basket method, Kato *et al.* (2006) and Uga *et al.* (2009) investigated the frequency of high root growth angles (50–90 ° with respect to the horizontal) in cultivated rice. Using 59 cultivated rice accessions, Uga *et al.* (2009) demonstrated that cultivated rice had wide genetic variation in root growth angle in upland fields. Using seven lowland and five upland rice cultivars, Kato *et al.* (2006) observed that the frequency of high root growth angles was associated with deep root development in an upland field. They postulated that the root growth angle provided a useful rough estimate of the variation in the vertical root distribution of rice accessions. However, how the root growth angle contributed to deep rooting has not yet been clarified, because previous studies used rice accessions with different genetic backgrounds. In studies of the relationship between root growth angle and deep rooting in diverse rice accessions, differences in their root length affected deep rooting simultaneously with differences in root growth angle. To identify the relative roles of the two parameters, it is necessary to evaluate the influence of root growth angle on deep rooting using homogeneous genetic material such as near-isogenic lines (NILs) in which only the root growth angle differs.

In rice, many analyses of quantitative trait loci (QTLs) for root morphological traits such as maximum length, thickness, volume, and distribution have been performed with different mapping populations (reviewed by Price *et al.*, 2002*a*). To date, 675 QTLs related to root traits have been detected (summarized by Courtois *et al.*, 2009). Among them, 103 QTLs for maximum root length have been identified on the 12 chromosomes (Redona and Mackill, 1996; Yadav *et al.*, 1997; Price *et al.*, 1999, 2002*b*; Hemamalini *et al.*, 2000; Zhang *et al.*, 2001; Kamoshita *et al.* 2002*a*, *b*; Courtois *et al.*, 2003; Xu *et al.*, 2004; Horii *et al.*, 2006; MacMillan *et al.*, 2006; Yue *et al.*, 2006). Recently, Obara *et al.* (2010) mapped *qRL6.1*, a major QTL for root length, on chromosome 6 in rice seedlings grown under hydroponic conditions, and delimited its candidate genomic region within a 337 kb region of the Nipponbare genome. Although vast amounts of genetic information have been accumulated on rice root length, there have been no reports of QTLs associated with root growth angle in rice. Thus, there is little knowledge of the genetics of root growth angle in rice.

Here, QTL analysis was performed to identify the genetic factors that determine deep rooting in rice, and particularly those that determine the root growth angle, using recombinant inbred lines (RILs) from a cross between the lowland cultivar IR64, with shallow rooting, and the upland cultivar Kinandang Patong, with deeper rooting, in hydroponic culture. Next, advanced backcross progeny were used to validate and delimit the candidate genomic region of a major QTL for deep rooting that was detected by means of the QTL analysis. To clarify whether this QTL was involved in deep rooting under field conditions, core samples were used to investigate the variation in the root mass distribution in different soil layers under field conditions among the parental lines and advanced backcross progeny homozygous for both parental alleles at the target QTL.

Materials and methods

Plant materials

For the QTL analysis of deep rooting, 117 F_6 RILs were developed using the single-seed-descent method from F_2 plants produced by crossing IR64 with Kinandang Patong in a previous study (Uga *et al.*, 2008). IR64 is a modern lowland cultivar (*indica*) developed by the International Rice Research Institute in the Philippines, and is widely grown in South and Southeast Asia. Kinandang Patong is a traditional upland cultivar (tropical *japonica*) that originated in the Philippines.

For the fine mapping of *Dro1*, eight BC_2F_3 lines in which recombination occurred within the region that included *Dro1* were used. Two lines homozygous for the entire *Dro1* region, one homozygous for the IR64 allele (IR64-homo) and the other for the Kinandang Patong allele (KP-homo), were also used as genotype references for the linkage analysis. The eight BC_2F_3 lines were developed as follows: an F_1 plant from an IR64×Kinandang Patong cross was obtained, and it was backcrossed with IR64. The resulting BC_1F_1 plants (n=96) were genotyped by means of whole-genome analysis using 95 DNA markers selected from the work of Uga *et al.* (2008). In the rest of this description, specific lines of interest have been named by combining the generation name (e.g. BC_1F_1) with a number that represents a line within that generation (e.g. 16) or its progeny lines (e.g. 16-1). One plant (BC_1F_1-16), which was heterozygous for the region containing *Dro1*, was backcrossed with IR64. One plant from the progeny (BC_2F_1-16-12), in which the *Dro1* region was heterozygous but almost all other regions were homozygous for IR64, was then identified. Plants from line BC_2F_1-16-12 (n=160) were screened using two markers flanking the target region (ID07_07 and E61552). Eight BC_2F_2 recombinants that contained both markers were obtained, and selfing of these plants produced homozygous recombinant BC_2F_3 plants (i.e. BC_2F_3-16-12-1 to BC_2F_3-16-12-8; Table 1). These 10 lines (eight BC_2F_3 lines and two lines homozygous for the entire *Dro1* region) were the same lines previously used for fine mapping of *Sta1*, a QTL that determines the stele transversal area (Uga *et al.*, 2010), because QTL analysis of the ratio of deep rooting (RDR) showed that the putative location of *Dro1* was close to *Sta1*. The BC_2F_4 progeny of the recombinant and reference lines were phenotyped to determine the *Dro1* genotypes of these lines.

To evaluate the influence of *Dro1* on root distribution in an upland field, two BC_2F_4 lines, Dro1-IR64 (which had the IR64 alleles of *Dro1* and *Sta1*) and Dro1-KP (which had the Kinandang Patong allele of *Dro1* and the IR64 allele of *Sta1*) were used. These lines were newly developed by means of marker-assisted selection. This approach was adopted because KP-homo, which was used for fine mapping of *Dro1*, was homozygous for the Kinandang Patong

Table 1. Genotypes of five DNA markers on chromosome 9 in the BC_2F_3-16-12 lines and the ratio of deep rooting (RDR) in the BC_2F_4 progeny

Lines	Genotype of the marker on chromosome 9 in the BC_2F_3 lines[a]					RDR (%) in the BC_2F_4 lines		Genotype of *Dro1*[b]
	ID07_07	ID07_12	ID07_14	ID07_17	E61552	Mean ±SD	*P*	
IR64	A	A	A	A	A	1.6±1.2	0.9975	IR64
Kinandang Patong (KP)	**B**	**B**	**B**	**B**	**B**	72.6±6.5	<0.00001*	KP
IR64-homo	A	A	A	A	A	2.6±1.9	–	IR64
KP-homo	**B**	**B**	**B**	**B**	**B**	40.4±7.2	<0.00001*	KP
BC_2F_3-16-12-1	**B**	A	A	A	A	1.7±2.6	0.9992	IR64
BC_2F_3-16-12-2	**B**	**B**	A	A	A	0.6±1.6	0.7827	IR64
BC_2F_3-16-12-3	**B**	**B**	**B**	A	A	47.7±4.3	<0.00001*	KP
BC_2F_3-16-12-4	**B**	**B**	**B**	**B**	A	46.7±6.2	<0.00001*	KP
BC_2F_3-16-12-5	A	**B**	**B**	**B**	**B**	33.8±6.7	<0.00001*	KP
BC_2F_3-16-12-6	A	A	**B**	**B**	**B**	43.8±8.3	<0.00001*	KP
BC_2F_3-16-12-7	A	A	A	**B**	**B**	53.9±6.7	<0.00001*	KP
BC_2F_3-16-12-8	A	A	A	A	**B**	2.9±2.7	1.0000	IR64

[a] Genotypes of the DNA markers are represented by A (normal) for IR64 homozygous and B (bold) for Kinandang Patong homozygous.
[b] Genotypes of *Dro1* were estimated from the results of Dunnett's test at the 0.1% significance level.
P, probability of no significant difference between IR64-homo and the recombinant BC_2F_4 line in Dunnett's test; *, significant at the 0.1% level.

allele in a chromosome region that included both *Dro1* and *Sta1*. The Dro1-KP line could therefore be used to evaluate the effects of *Dro1* without having to account for the effect of *Sta1* on the root distribution.

Measurement of the RDR

Deep rooting was evaluated from the position where the root penetrated the mesh of hemispherical baskets that held the rice plants (Oyanagi *et al.*, 1993). Kato *et al.* (2006) evaluated the variation in deep rooting of rice from the frequency of high root growth angles (50–90 ° with respect to the horizontal). According to their criteria, the RDR was defined as the number of roots that penetrated the lower part of the mesh (i.e. the part defined by an angle of 50 ° from the horizontal, centred on the stem of the rice plant) divided by the total number of roots that penetrated the whole mesh (Uga *et al.*, 2009). Although it would not be appropriate to assume that this angle is valid for all plant species or rice cultivars, the results of previous research (Kato *et al.*, 2006; Uga *et al.*, 2009) suggested that it is a suitable criterion for use in the present study because of its ability to discriminate between shallow- and deep-rooting rice cultivars. For the QTL analysis of RDR, open stainless-steel mesh baskets with a top diameter of 7.5 cm and a depth of 5.0 cm (PROUD, Ushiku, Japan) were used. Here and in the subsequent experiments, the mesh size (2 mm) was sufficiently large that the mesh did not interfere with root emergence from the baskets. To delimit the top and bottom parts of the basket, a stainless-steel ring was welded around the basket 3 cm from the open top (i.e. to represent the position of an angle of 50 ° from the top), as in the study by Kato *et al.* (2006). For fine mapping of *Dro1*, a plastic mesh basket with a 15 cm wide top, an 8.5 cm wide bottom, and a height of 6 cm (Yazaki Kako, Shizuoka, Japan) was used. The bottom of this basket was defined as all parts of the basket below an angle of not 50 ° but 53 ° from the horizontal because of a practical reason based upon the structure of the baskets. The smaller stainless-steel baskets was used for the QTL analysis because that analysis required the cultivation of many lines when greenhouse space was limited. The larger plastic baskets were used to provide a growing environment as close as possible to the conditions used in a previous study (Uga *et al.*, 2009).

For the QTL analysis, the baskets were filled with soil but without fertilizer, and groups of 40 baskets were put together in a large container filled with tap water (pH 6.0) in a greenhouse (average air temperature, 30 °C; average relative humidity, 50%; natural lighting). Seeds were pre-germinated at 30 °C for 2 d in an incubator, then each seed was sown at the center of a basket; 7 days after sowing (DAS), the water was replaced with half-strength Kimura B hydroponic solution [182.5 μM $(NH_4)_2SO_4$, 45.5 μM K_2SO_4, 273.5 μM $MgSO_4$, 91.5 μM KNO_3, 182.5 μM $Ca(NO_3)_2$, 91.0 μM KH_2PO_4, 8.9 μM $FeCl_3$, pH 6.0]. The solution was replaced with normal-strength Kimura B solution (pH 6.0) 14 DAS. The hydroponic solution was renewed every other day. The RDR was determined 36 DAS, and the means of four plants in each line were calculated.

For the fine mapping, the baskets were filled with soil that had been mixed evenly with inorganic fertilizer (rates of 26 kg of N, 36 kg of P, and 28 kg of K ha^{-1}) and they were installed in 3.5 l pots (Yazaki Kako) filled with the same soil and fertilizer. Seeds were pre-germinated at 30 °C for 2 d in an incubator, then 24 seeds from each line were sown in separate baskets in a greenhouse. The RDR was determined 39 DAS, and the means of the 24 plants in each line were calculated.

Effect of Dro1 *on root distribution in the field*

To investigate the effect of *Dro1* on root distribution in an upland field, the root mass of IR64, Kinandang Patong, Dro1-IR64, and Dro1-KP was measured by means of core sampling (Kondo *et al.*, 2003). Rice plants were grown under rainfed conditions in an upland field at the National Institute of Agrobiological Sciences (36°1'N, 140°6'E) in Tsukuba, Japan, in the summer of 2008. The soil at the experimental site is a volcanic ash soil of the Kanto loam type (Humic Andosol). The topsoil (0–30 cm) is a dark humic silty loam (pH 6.2). The subsoil (below 30 cm) is a red-brown silty clay loam (pH 5.8). A hardpan existed at a depth of 30 cm. Inorganic fertilizer was applied at the time of sowing at rates of 26 kg of N, 36 kg of P, and 28 kg of K ha^{-1}. Top-dressing (N and K, both at 10 kg ha^{-1}) was performed 40 DAS. Weeds were controlled by hand combined with herbicide application. Soil water potential at a depth of 30 cm was monitored with a tensiometer (UIZ-SMT; Uizin Co., Ltd, Tokyo, Japan). Precipitation data were obtained from the MeteoCrop DB coupled crop–meteorological model database (http://meteocrop.dc.affrc.go.jp/).

Four plots per line were arranged in a randomized block design. Two plots were used for measurements of root mass and the other

two plots for measurement of shoot traits. Each plot included 28 hills. Three seeds were sown in each hill (40 cm between plants within a row and 60 cm between rows), and plants were thinned to one per hill after seedling establishment. Four plants were randomly chosen, excluding the border rows in each plot to avoid edge effects, for the root samples. Two soil cores (50 cm deep by 8 cm in diameter) were obtained near each plant 95 DAS, both just beside the hill and 15 cm away from the hill between the rows of plants, using a powered soil sampler (GES-30W, Fujiwara Scientific Co., Ltd, Tokyo, Japan) (Supplementary Fig. S1 available at *JXB* online). Core samplers with smaller diameters (4.5–5 cm) were used in previous studies (Kondo *et al.*, 2000; Kato *et al.*, 2006; Hirayama *et al.*, 2007), but the sampling errors were large. In this study, a sampler with a larger diameter was chosen in order to decrease the sampling error.

The soil cores were divided into two segments (shallow soil, 0–25 cm, and deep soil, 25–50 cm), and the segments were washed carefully to separate the roots from the soil. Four root samples per plant were obtained from the shallow (S) and deep (D) soil: beside the hill (samples S0 and D0), and 15 cm from the hill (samples S15 and D15). the root dry weight (RDW) was measured after oven-drying the samples at 80 °C for 3 d. In general, eight samples (four plants×two plots) were used to calculate the mean RDW for each line. At harvest time (145 DAS), 10 plants in each plot, excluding the border rows to avoid edge effects, were randomly harvested to measure the above-ground parts of the plants (culm length, panicle length, panicle number, shoot dry weight, and panicle weight). Twenty samples (10 plants×2 plots) were used to calculate means in each line.

DNA marker analysis

The genotypes of the RILs were determined by using 77 simple sequence repeats (SSRs), 24 sequence-tagged sites (STSs), and 30 insertion–deletion (InDel) markers selected from Uga *et al.* (2008). Total DNA was extracted from leaves by the cetyltrimethylammonium bromide (CTAB) method (Murray and Thompson, 1980). PCR amplifications were performed in a 5 µl reaction mixture containing 1 µl (20 ng) of DNA, 0.5 µl of 10× PCR buffer, 0.5 µl of 2 mM dNTPs, 0.02 µl (5 U) of *Ex Taq* DNA polymerase (Takara Bio Inc., Otsu, Japan), 0.12 µl of 20 pM mixed solutions of forward and reverse primers, and 2.86 µl of H_2O. PCR was carried out in initial denaturation for 1 min at 95 °C and then 35 cycles of 15 s at 93 °C followed by 30 s at 55 °C for the SSR markers and 60 °C for the STS and InDel markers, with a final extension for 2 min at 72 °C. PCR products were electrophoresed in a 3% agarose gel at 150 V for 90 min.

Statistical and QTL analyses

The broad-sense heritability (h_B^2) was calculated from the estimates of genetic (σ^2_G) and residual (σ^2_E) variances derived from the expected mean squares of the analysis of variance to understand the genetic effects of RDR:

$$h_B^2 = \sigma_G^2/(\sigma_G^2 + \sigma_E^2)$$

Linkage maps were constructed from the genotype data in MAPMAKER/EXP 3.0 software (Lander *et al.*, 1987). The genetic distance was estimated by using the software's Kosambi map function (Kosambi, 1944). Putative QTLs were detected using the composite interval mapping (CIM) function of QTL Cartographer 2.5 (Wang *et al.*, 2005). The CIM threshold was based on the results of 1000 permutations at a 5% significance level (Churchill and Doerge, 1994). The additive effect and the phenotypic variance explained by each QTL (R^2) were estimated at the maximum LOD score.

To compare the mean RDR in the eight recombinant BC_2F_4 lines, the Dunnett's test provided by JMP version 7.0 (SAS Institute, Cary, NC, USA) was used. All BC_2F_3-16-12 lines were compared with IR64-homo as the reference. The genotypes of each line were estimated from the results of Dunnett's test at a 0.1% significance level.

To compare the mean values of the RDW and the above-ground traits in the IR64, Kinandang Patong, Dro1-IR64, and Dro1-KP lines, the Tukey's multiple-comparison test provided by JMP version 7.0 was used.

Results

Detection of a QTL for deeper rooting in the RILs

The comparison of the two parental lines using the small baskets (Fig. 1) showed shallow rooting by IR64 (RDR=12.2%) and deep rooting by Kinandang Patong (RDR=92.5%). The RDRs of the RILs were distributed between the values of the two parental lines, ranging from 10.0% to 81.9%, and showed a bimodal distribution, indicating that at least one major QTL was associated with this trait in this population. The RDR of the RILs had a relatively high h_B^2 (77.7%; Fig. 1).

The RIL linkage map, composed of 131 markers, covered almost the whole rice genome (Supplementary Fig. S2 at *JXB* online). The total map distance was 1343.7 cM, and the average distance between markers was 11.3 cM. The QTL analysis based on an LOD threshold of 4.3 detected only one QTL for RDR, near InDel marker ID07_17 on chromosome 9 (Fig. 2). This QTL had a large contribution to the phenotypic variance, explaining 66.6% of the total. The additive effect of the Kinandang Patong allele at this QTL on RDR was 16.1%. An additional minor QTL could be identified on chromosome 3 by lowering the LOD threshold to 2.5, but, because of the low LOD score and because it accounted for <8% of the variance, it was not considered to be sufficiently important to investigate further.

Validation of the major QTL for deeper rooting using homozygous lines

To verify the genetic effects of the QTL that was detected on chromosome 9 on RDR, BC_2F_3 lines homozygous for IR64 (IR64-homo) and Kinandang Patong (KP-homo) were investigated in this region using the large baskets. Most of

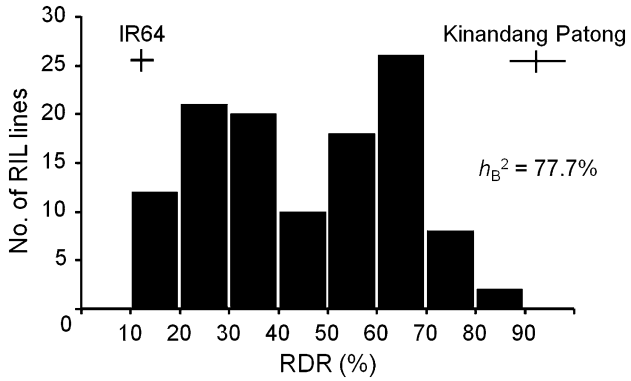

Fig. 1. Frequency distribution of the ratio of deep rooting (RDR) in the 117 RILs derived from IR64×Kinandang Patong. Vertical and horizontal lines above the bars indicate the mean and SD of each parental line. h_B^2 represents the broad-sense heritability.

the roots of IR64-homo and of IR64 elongated through the side of the basket, whereas most of the roots of KP-homo elongated through the bottom of the basket (Fig. 3A). As a result, the mean RDR of KP-homo (40.4%) was significantly larger than that of IR64-homo (2.6%) (Table 1). Longitudinal sections of the root base were also observed after removing the roots from the basket. The IR64 and IR64-homo plants tended to have shallow roots, whereas most roots of Kinandang Patong and KP-homo were elongated vertically (Fig. 3B). Consequently, it was confirmed that the Kinandang Patong allele of the RDR QTL increased deep rooting.

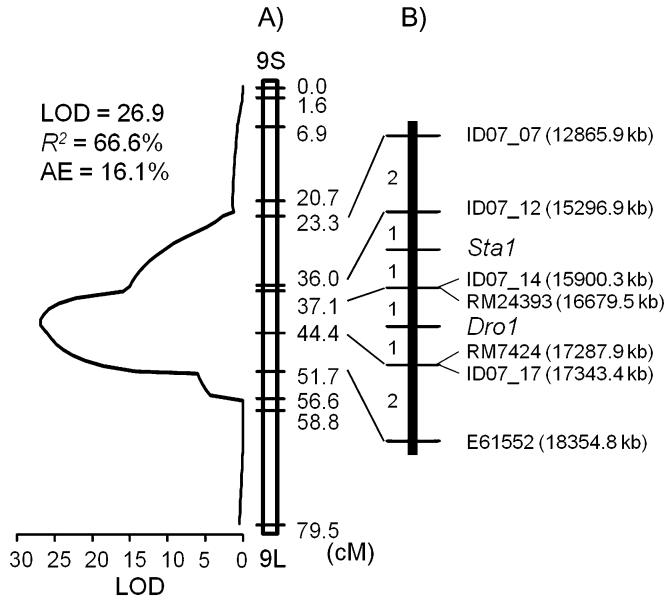

Fig. 2. The location of *Dro1* on chromosome 9. (a) Linkage map of the RILs derived from IR64×Kinandang Patong. The curve on the left shows the LOD score for the RDR QTL. R^2 indicates the percentage of the phenotypic variance that was explained. AE indicates the additive effect of the allele from Kinandang Patong relative to that from IR64. (b) Linkage map constructed from the eight BC_2F_3 recombinants. The number of BC_2F_3 lines with recombination between adjacent DNA markers is shown on the left. Names of the DNA markers are shown on the right; numbers in parentheses beside the DNA markers indicate their physical map position based on the latest version of the RAP2 database (http://rapdb.dna.affrc.go.jp/). The position of *Sta1* in the linkage map is based on data from Uga *et al.* (2010). S, short arm; L, long arm.

Fine mapping of the QTL for deeper rooting

Eight BC_2F_3 lines in which recombination occurred between the flanking markers ID07_07 and E61552 were used to map the detected QTL as a single locus. Based on progeny testing of these lines, the eight BC_2F_4 lines were classified into two groups that exhibited either shallow or deep rooting. Three lines (BC_2F_3-16-12-1, -2, and -8) showed small RDRs, ranging from 0.6% to 2.9%, whereas five lines (BC_2F_3-16-12-3 to -7) had large RDRs, ranging from 33.8% to 53.9% (Table 1). These groups corresponded to genotype classes that were homozygous for the IR64 allele and for the Kinandang Patong allele, respectively. These results clearly indicate that the RDR QTL was mapped between InDel markers ID07_14 and ID07_17 on chromosome 9 (Fig. 2). This QTL was designated *Deeper rooting 1* (*Dro1*). To define further the candidate genomic region for *Dro1*, 128 SSRs were selected in the interval between ID07_14 (15 900.3 kb) and ID07_17 (17 343.4 kb) based on the list of SSR markers described in the International Rice Genome Sequencing Project (2005). Among these markers, 17 showed polymorphism between IR64 and Kinandang Patong. Using these markers, the candidate genomic region of *Dro1* was narrowed down to the interval between RM24393 (16 679.5 kb) and RM7424 (17 287.9 kb), a distance of 608.4 kb, in the Nipponbare genome (Fig. 2).

Effect of Dro1 *on root distribution in the field*

Fine mapping showed that *Dro1* was located near *Sta1* (Fig. 2). This result indicates that the KP-homo line

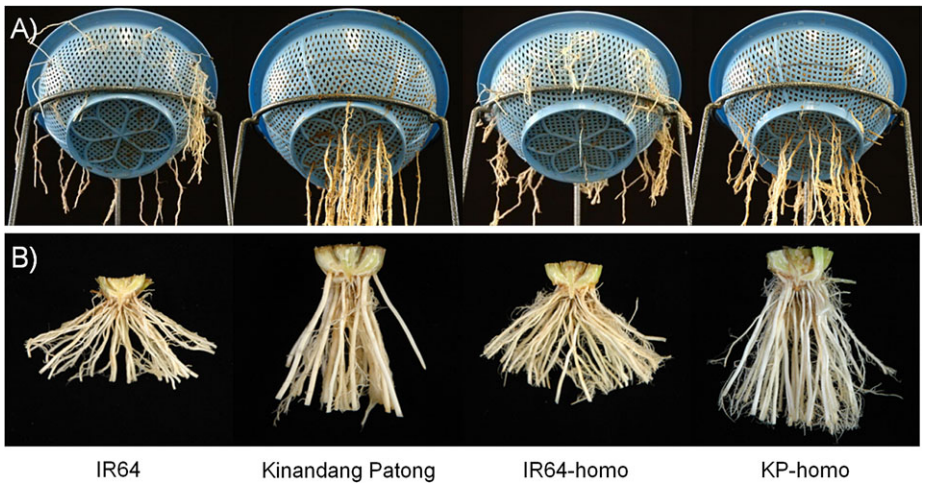

Fig. 3. Differences in the root distribution among four lines. (A) Images taken from beneath the large baskets. (B) Longitudinal sections of the root base from a typical plant in each line.

included both *Dro1* and *Sta1*. Therefore, to clarify whether *Dro1* caused deep rooting under upland field conditions, Dro1-IR64 (which had the IR64 alleles of *Dro1* and *Sta1*) and Dro1-KP (which had the Kinandang Patong allele of *Dro1* and the IR64 allele of *Sta1*) were selected using DNA markers (Fig. 4). The mean soil water potential during the growing period was –0.025 MPa at a depth of 30 cm (Supplementary Fig. S3 at *JXB* online), indicating that, on average, the rice plants were not exposed to water stress during the growing period. However, there was little rain from 25 to 70 DAS. The mean soil water potential during this period was –0.043 MPa, with a minimum of approximately –0.090 MPa. From the end of July to the end of August (45–75 DAS), mean soil water potential was –0.065 MPa, indicating that the rice plants were exposed to drought stress. Quantitative estimation of the root distribution from the core samples showed that the RDWs of Kinandang Patong in the S0, D0, and D15 samples were significantly larger than those of IR64 (Table 2). In particular, the RDW of Kinandang Patong in sample D0 was >700% of the IR64 value in this sample. It was therefore possible to quantify the difference in the root distribution between IR64 and Kinandang Patong using the core sampling method. Although Dro1-KP did not have significantly different RDWs from IR64 and Dro1-IR64 in the shallow soil, RDWs of Dro1-KP in both deep soil samples were significantly larger than those of IR64 and Dro1-IR64. These results demonstrate that the KP allele at *Dro1* conferred deeper and more vertical rooting under upland field conditions.

To investigate the effects of *Dro1* on above-ground parts of the plants, plants were harvested from each line and their morphological and yield traits were measured. Dro1-KP did not differ significantly from IR64 in culm length, panicle length, or panicle number (Table 3). Dro1-KP had significantly larger panicle weight than IR64 and Dro1-IR64, but shoot dry weight did not differ greatly among IR64, Dro1-IR64, and Dro1-KP. This suggests that the KP allele of *Dro1* did not greatly influence shoot morphological traits, but significantly improved yield under upland conditions.

Discussion

A major QTL for deeper rooting in rice

Deep rooting is a complex trait that combines root length and root growth angle. Abe and Morita (1994) reported that the root distribution in rice was shaped by a combination of the size and growth angle of the nodal roots. Although a large number of QTLs for the parameters of root size, such as root length and number, have been detected in previous studies (summarized by Courtois *et al.*, 2009), there have been no reports of QTLs for root growth angle in rice. In the present study, a QTL for root growth angle was searched for

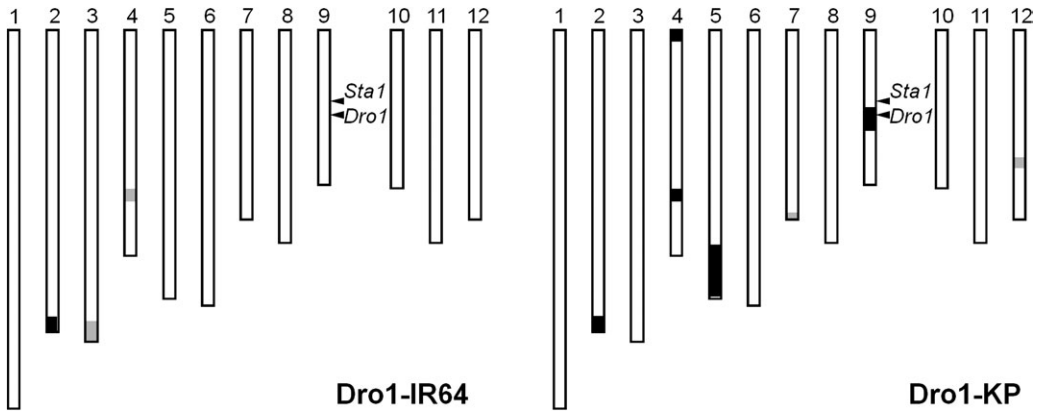

Fig. 4. Graphical genotypes of the two BC_2F_4 lines used for the evaluation of the effects of *Dro1* in the field. Chromosome numbers are indicated at the top. The arrowheads on the right of chromosome 9 show the positions of *Dro1* mapped in this study and of *Sta1* mapped by Uga *et al.* (2010). White, black, and grey boxes represent IR64 homozygous, Kinandang Patong homozygous, and heterozygous, respectively.

Table 2. Root dry weight (RDW; mg) in the upper and lower soil layers in the IR64, Kinandang Patong, Dro1-IR64, and Dro1-KP plants
Beside indicates a core sample taken from beside the hill; 15 cm distance indicates a core sample taken 15 cm from the hill.

Lines	Shallow soil (mean ±SD)		Deep soil (mean ±SD)	
	Beside (S0)	15 cm distance (S15)	Beside (D0)	15 cm distance (D15)
IR64	359.2±97.3 a	261.0±138.2 a	10.1±8.7 a	16.3±13.8 a
Kinandang Patong	629.7±218.6 b	267.1±161.1 a	71.9±30.2 b	70.2±41.0 b
Dro1-IR64	381.2±54.1 a	348.9±135.1 a	17.2±7.9 a	20.5±12.4 a
Dro1-KP	370.2±95.8 a	245.0±9.9 a	50.8±19.1 b	59.6±6.0 b

Values labelled with different letters differ significantly among the four lines (P <0.05, Tukey's multiple comparison test).

Table 3. Shoot and yield traits at harvest in the IR64, Kinandang Patong, Dro1-IR64, and Dro1-KP lines

Lines	Culm length (cm)	Panicle length (cm)	Panicle number	Shoot dry weight (g)	Panicle weight (g)
IR64	50.5±3.0 a	25.4±1.6 a,b	33.3±8.0 a	110.4±27.3 a,b	23.8±6.2 a,b
Kinandang Patong	138.8±4.6 b	20.6±1.6 c	16.3±3.3 b	123.7±24.0 b	31.7±7.8 b,c
Dro1-IR64	53.4±4.2 a	26.3±3.4 a	29.3±5.0 a	102.0±19.5 a	20.9±7.1 b
Dro1-KP	52.1±5.2 a	24.1±2.4 b	31.3±7.2 a	104.4±29.6 a,b	32.4±16.7 c

Values (given as mean ±SD) for a parameter labelled with different letters differ significantly among the four lines (P <0.05, Tukey's multiple-comparison test).

using RDR as an index. A novel major QTL on chromosome 9 that controls deep rooting was successfully identified. This QTL, *Dro1*, accounted for 66.6% of the total phenotypic variance of RDR in the RILs, suggesting that the differences in RDR between IR64 and Kinandang Patong could be mostly explained by the *Dro1* QTL.

The candidate genomic region of *Dro1* was mapped to the interval between RM24393 and RM7424 by means of linkage analysis. The relationship between the positions of *Dro1* and other root QTLs was analysed using the QTL Annotation Rice Online Database (Q-TARO, http://qtaro.abr.affrc.go.jp/; Yonemaru *et al.*, 2010). Q-TARO showed only one QTL for root dry weight in the same region as *Dro1*. This QTL was located between restriction fragment length polymorphism (RFLP) markers R79 (12 410.3 kb) and R2638 (18 491.7 kb) in doubled haploid lines derived from a cross between IRAT109 (an upland tropical *japonica* rice) and Yuefu (a lowland temperate *japonica* rice) (Li *et al.*, 2005). Courtois *et al.* (2009) summarized the QTLs for root traits reported in previous studies. They described 30 QTLs for 12 root traits located in the interval between 15 Mb and 20 Mb on chromosome 9. For deep root weight, which seems to be related to RDR, one QTL was found in the same region as *Dro1*. However, it was not known whether *Dro1* is related to either QTL reported in these previous studies because those previous QTLs covered a wide region (several Mb) that included *Dro1*. Here, *Dro1* was delimited to a region smaller than 608.4 kb in the Nipponbare genome. The Rice Annotation Project RAP2 database (http://rapdb.dna.affrc.go.jp/) predicts 54 genes in the candidate region for *Dro1*. The morphological and physiological functions of *Dro1* are not yet known. Therefore, it is difficult to identify the actual candidate gene for *Dro1* from among these many predicted genes. To do so, advanced progeny that contain recombination in the region of *Dro1* are currently being developed.

Although there have been no previous reports of a QTL associated with root angle in rice, some QTLs for root angle have been detected in F_2 populations of maize (B73)×teosinte (*Zea luxurians*) (Omori and Mano, 2007). Among them, two QTLs for root angle were located on maize chromosome 7. Comparative genome analysis has shown synteny between maize chromosome 7 and rice chromosomes 7L and 9L (Wilson *et al.*, 1999). Thus, *Dro1* might correspond to one of the QTLs for root angle located on maize chromosome 7. Cloning of *Dro1* will enable clarification of whether *Dro1* homologues exist in other crops.

Relationship between Dro1 *and* Sta1

QTL analysis in the RILs showed that *Dro1* was located near *Sta1*, which is a locus that determines the stele transversal area (Uga *et al.*, 2010). Previous studies demonstrated that root growth angle and root thickness were positively correlated (Yamazaki *et al.*, 1981; Kato *et al.*, 2006). Moreover, Morita *et al.* (1983) reported a positive correlation between root growth angle and stele diameter. In contrast, a previous study showed that the stele transversal area was not significantly correlated with RDR (Uga *et al.*, 2009). Here, delimitation of the candidate genomic region of *Dro1* clearly suggested that *Dro1* was located in a different interval from *Sta1* (Fig. 2). In general, upland rice has deeper and thicker roots than lowland rice (O'Toole and Bland, 1987). The tight linkage between *Dro1* and *Sta1* may therefore be one of the factors responsible for the positive correlation between deep rooting and thicker roots.

Effect of Dro1 *on root distribution in an upland field*

The Kinandang Patong allele of *Dro1* increased RDR in the hydroponic and pot cultures, and the homozygous line with the Kinandang Patong allele of *Dro1* showed primarily downward rooting (Fig. 3B). Because both the hydroponic and pot cultivations used in this study to identify *Dro1* used humid conditions, it was necessary to clarify whether *Dro1* would also increase RDR under rainfed upland field conditions, where plants are often exposed to water stress. Thus, the effect of *Dro1* on vertical root distribution was investigated under upland field conditions. To confirm the influence of *Dro1* on RDR under these conditions, the RDRs of IR64, Kinandang Patong, IR64-homo, and KP-homo were determined. To do so, the large baskets were planted in an upland field and the rice plants were grown under the same culture conditions as those used in a previous study (Uga *et al.*, 2009). The mean RDRs of IR64, Kinandang Patong, IR64-homo, and KP-homo were 2.0, 50.7, 1.1, and 29.8%, respectively. This showed that the Kinandang Patong allele of *Dro1* increased the RDR of IR64 under upland conditions. However, it was not clear whether the Kinandang Patong allele of *Dro1* really affects the root distribution under upland conditions.

To answer this question, the root distribution was quantified in shallow and deep soil using the core sampling method. Although RDW did not differ significantly between Dro1-KP and IR64 in the shallow soil, the RDW of Dro1-KP in the deep soil was significantly larger than that of IR64.

This means that the Kinandang Patong allele of *Dro1* increases root biomass in the deep soil under upland field conditions. Deep rooting is a beneficial strategy against drought stress in rice (Yoshida and Hasegawa, 1982; Fukai and Cooper, 1995). However, the vertical root distribution may be influenced by additional environmental factors such as the water regime, degree of soil compaction, and composition. Yoshida and Hasegawa (1982) noted that deep rooting in rice developed under upland conditions. In contrast, Ghildyal and Tomar (1982) noted that roots tended to grow deeper under flooded conditions. Kondo *et al.* (2000) reported that the vertical root distribution was not significantly affected by the intensity of water stress under upland conditions. However, these studies examined the relationship between root distribution and water regimes using rice accessions with different genetic backgrounds. Thus, it was unclear whether the vertical root distribution was affected by the water regimes or by genetic factors. The present study demonstrated that the Kinandang Patong allele of *Dro1* produced a deeper root distribution under relatively dry upland conditions. This result was reliable because IR64 and Dro1-KP, which had mostly the same genetic background (i.e. IR64), were used. However, it is also possible that the few chromosomal segments homozygous for the Kinandang Patong allele (other than the *Dro1* region) in Dro1-KP affect other root morphological traits. Further study using IR64 and an NIL of the Kinandang Patong allele of *Dro1* under different levels of water stress will clarify the relationship between *Dro1* expression and the water regime.

Although excavation is an appropriate way to measure root distribution directly in the field, this method is laborious and time-consuming with a large number of plants. Hirayama *et al.* (2007) reported that root density in the deep soil layer, measured by means of core sampling, was significantly positively correlated with the root density measured by means of excavation, in a range of different environments. They concluded that the core sampling method was a practical and reliable way to estimate root densities. Based on the findings in their report, the root distribution was estimated from differences in root mass in the soil cores in this study. It should be noted, however, that the values obtained by core sampling should be treated as estimates of the real root distribution, because the cores are taken from only part of the total root system (Kato *et al.*, 2006). To reconfirm the effect of *Dro1* on root distribution in the field, an NIL of *Dro1* will be directly investigated using the excavation method.

Effect of Dro1 *on above-ground plant traits in an upland field*

Previous studies have reported a tight relationship between root and shoot morphological changes (Yoshida *et al.*, 1982; Ekanayake *et al.*, 1985). In general, traditional upland rice cultivars are tall, with a low number of tillers, deep rooting, and thicker roots. Yoshida *et al.* (1982) reported that a deep root system was correlated with taller plants and lower tiller numbers in 1081 rice accessions. On the other hand, they also reported that plant height was not related to root depth, using two isogenic lines of Peta with different plant heights. Recently, Steele *et al.* (2006) reported that an NIL with a chromosome segment containing a QTL for root length (between RM242 and RM201) on chromosome 9 significantly increased root length and plant height under irrigated and water stress conditions. They noted that root length was related to plant height. However, because their NIL had a relatively large segment of the target QTL, it is unclear whether this phenomenon resulted from a pleiotropic effect of the QTL for root length or from a tight linkage between the QTL for plant height and the QTL for root length.

Here, shoot length and tiller number were investigated simultaneously to analyse their relationship with the QTL for RDR in the RILs. The chromosomal locations of the QTLs for shoot length and tiller number (on chromosomes 1, 2, and 3) did not coincide with the region of *Dro1* on chromosome 9 (Supplementary Fig. S2 at *JXB* online), suggesting that *Dro1* does not influence these shoot morphological traits. However, the relationship between shoot and root traits under upland field conditions and at maturity remained unclear. To clarify this relationship, shoot traits were investigated after harvesting of the above-ground parts of the rice plants grown under upland field conditions. The shoot morphological traits (culm length, panicle length, and panicle number) did not differ significantly between IR64 and Dro1-KP (Table 3). This clearly suggests that *Dro1* did not affect these three shoot morphological traits. On the other hand, the panicle weight of Dro1-KP was obviously and significantly increased compared with that of IR64 (Table 3). During the vegetative growth stage (and particularly from the end of July to the end of August, 45–75 DAS), drought stress occurred (Supplementary Fig. S3). Therefore, leaf rolling occurred in IR64 at this time (Supplementary Fig. S4). In contrast, leaf rolling was not observed in Dro1-KP plants throughout the growing period. This difference cannot be conclusively explained, but it very probably resulted from the deeper rooting of Dro1-KP plants. The IR64 plants rooted mostly in the shallow soil, and were therefore more susceptible to water stress, whereas the Dro1-KP plants also rooted in the deep soil, where water would have been more abundant, allowing them to avoid or mitigate the water stress. This may also explain why the Dro1-KP plants had a significantly larger panicle weight (Table 3). The results therefore suggest that *Dro1* is involved in drought avoidance under natural field conditions with occasional water stress. However, it is unclear whether the other regions in Dro1-KP that are homozygous for an allele from Kinandang Patong influence panicle weight. Further experiments using IR64 and the NIL of the Kinandang Patong allele of *Dro1* under controlled conditions with different levels of water stress will be needed to clarify the effectiveness of *Dro1* in drought avoidance.

Deeper rooting is a key strategy associated with avoiding drought stress. Yoshida and Hasegawa (1982) concluded

that the root length density in deeper soil was one of the factors that determined drought resistance in rice. However, they used several rice accessions with different types of shoot morphology. Although the effects of differences in shoot morphology on drought response could not be ruled out in their experiment, the present results strongly support their conclusion. *Dro1* can potentially be used to improve drought avoidance of rice by changing its rooting pattern from a shallow to a deep system, as shown in the present study. It will be necessary to clone *Dro1* so that its role in the molecular mechanisms that control root development can be better understand. The authors are currently in the process of map-based cloning of *Dro1*.

Drought stress is a serious problem not only for rice but also for crop production around the world. Once *Dro1* has been cloned, it will be interesting to seek *Dro1* homologues in other plants and to clone those homologues so their function in root development can be examined.

Supplementary data

Supplementary data are available at *JXB* online.

Figure S1. Evaluation of the root distribution in the field as determined from core samples.

Figure S2. Chromosomal locations of the QTLs for shoot length (SL) and tiller number (TN) detected in RILs derived from a cross between IR64 and Kinandang Patong.

Figure S3. Changes of soil water potential at around 13:00 h at a depth of 30 cm and precipitation during the growing period.

Figure S4. Differences in leaf condition between IR64 and Dro1-KP at around 13:00 h, 70 d after sowing.

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