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ORIGINAL PAPER

Identification of *qSOR1*, a major rice QTL involved in soil-surface rooting in paddy fields

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Abstract Specific Indonesian lowland rice (*Oryza sativa* L.) cultivars elongate thick primary roots on the soil surface of paddy fields. To clarify the genetic factors controlling soil-surface rooting, we performed quantitative trait locus (QTL) analyses using 124 recombinant inbred lines (RILs) derived from a cross between Gemdjah Beton, an Indonesian lowland rice cultivar with soil-surface roots, and Sasanishiki, a Japanese lowland rice cultivar without soil-surface roots. These cultivars and the RILs were tested for soil-surface rooting in a paddy field. We identified four regions of chromosomes 3, 4, 6, and 7 that were associated with soil-surface rooting in the field. Among them, one major QTL was located on the long arm of chromosome 7. This QTL explained 32.5–53.6% of the total phenotypic variance across three field evaluations. To perform fine mapping of this QTL, we measured the basal root growth angle of crown roots at the seedling stage in seven BC_2F_3 recombinant lines grown in small cups in a greenhouse. The QTL was mapped between markers RM21941 and RM21976, which delimit an 812-kb interval in the reference cultivar Nipponbare. We have designated this QTL *qSOR1* (*quantitative trait locus for SOIL SURFACE ROOTING 1*).

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Introduction

The efficient acquisition of water and nutrients by plant root systems is very important, because the availability of these soil resources is a limiting factor for plant growth (Lynch 1995; Gewin 2010). Many soil resources are unevenly distributed: for example, topsoil tends to retain more nutrients, especially immobile nutrients such as phosphorus (P), and less water than subsoil. Deep rooting is believed to be important for crops to improve drought avoidance in soil environments with little water (Fukai and Cooper 1995). On the other hand, shallow rooting benefits crops by enabling capture of P distributed in topsoil (Lynch and Brown 2001). In wheat (*Triticum aestivum* L.), root length density in the upper soil layer was the most important trait for improving P absorption (Manske et al. 2000). In common bean (*Phaseolus vulgaris* L.), P-efficient cultivars generated shallower basal roots and increased adventitious rooting in the topsoil (Lynch and Brown 2001). These results show that the architecture of the root system determines the ability of a plant to exploit those resources that are unevenly distributed in soil.

Cultivated rice (*Oryza sativa* L.) is grown in various environmental conditions ranging from flooded lowland fields to rainfed upland fields (Oka 1988). A wide range of genetic variation in root morphology has been observed in rice (O'Toole and Bland 1987; Lafitte et al. 2001; Uga et al. 2009). Uga et al. (2009) investigated two morphological traits (root length index and ratio of deep rooting) in 59 rice accessions. They found that *indica* cultivars from South Asia had, on average, deeper roots than the *indica* cultivars from East and Southeast Asia, possibly because of their adaptation to fields with more unstable water conditions than those of East and Southeast Asia, where fields are typically flooded. Typical upland rice produces deeper

roots than lowland rice (O'Toole and Bland 1987). The deep rooting system in upland rice may contribute greatly to drought avoidance through enhanced water uptake in deeper soil layers (Price et al. 1999). The wide range of natural variation in root morphology in rice is likely to be related to adaptation to the different soil environments in which it is grown.

Some Indonesian lowland rice cultivars belonging to ecotype Bulu showed the thickest roots and shallowest patterns of root distribution among 136 accessions that were representative of six groups defined based on isozyme markers (Lafitte et al. 2001). The authors suggested that this root phenotype was due to selection pressure within ecotype Bulu for growth in anaerobic environments. Ueno and Sato (1989) observed that ecotype Bulu accessions developed thick crown roots above the soil surface beginning at the seedling stage. They also surveyed the ability for soil-surface rooting among 56 rice cultivars including Indian ecotypes (Aus, Amon, and Boro), Indonesian ecotypes (Bulu and Tjereh), and Japanese lowland and upland rice (the five ecotypes classified by Ueno et al. 1990). Many cultivars belonging to ecotype Bulu showed soil-surface rooting, whereas other ecotypes seldom elongated their roots on the soil surface. In common lowland rice, roots forming near the soil surface (superficial roots) are generally thinner than those elongating downward. These superficial roots expand during the time from panicle initiation to the ripening stage to form a mat near the soil surface (Morita and Yamazaki 1993). Therefore, the thick soil-surface roots that develop from the seedling stage in ecotypes such as Bulu may be different from the fine superficial roots found in most rice cultivars. The ecological and physiological functions of soil-surface roots in rice are not understood in detail.

Many quantitative trait locus (QTL) analyses for root morphological traits such as maximum length, thickness, volume, and distribution have been performed in rice (reviewed by Price et al. 2002). To date, 675 QTLs related to root traits have been detected in rice (summarized by Courtois et al. 2009). Recently, Obara et al. (2010) mapped *qRL6.1*, a QTL for root length, in a 337-kb interval on rice chromosome 6 in the reference cultivar Nipponbare. Uga et al. (2010) identified *Sta1*, a QTL for stele transversal area, in 359-kb interval on rice chromosome 9 in the reference cultivar Nipponbare. Uga et al. (2011) also found *Dro1*, a major QTL for deep rooting, on rice chromosome 9 and narrowed down its candidate genomic region to within a 608-kb region on chromosome 9 in the reference cultivar Nipponbare. Yet in spite of the many genetic studies of several root morphological traits, soil-surface rooting in rice has not been genetically analyzed until now.

Here, we performed QTL analyses of soil-surface rooting using recombinant inbred lines (RILs) from a cross between a rice cultivar with soil-surface roots and a rice cultivar without soil-surface roots. Furthermore, by using advanced-backcross progeny, we validated the genetic effect of a major QTL for soil-surface roots on chromosome 7 and delimited its candidate genomic region.

Materials and methods

Plant materials

For the QTL analyses, we developed 124 F_6 RILs by the single-seed-descent method. These lines were derived from F_2 plants produced by crossing Gemdjah Beton with Sasanishiki. Gemdjah Beton is a traditional lowland cultivar (ecotype Bulu) that originated in Indonesia and grows soil-surface roots. Sasanishiki is a modern lowland cultivar from Japan that does not grow soil-surface roots.

To perform fine mapping of a QTL for soil-surface rooting that we detected on chromosome 7 (described in Results), we developed seven BC_2F_3 lines in which recombination had occurred within the region containing the target QTL. We used marker-assisted selection to select these lines from advanced-backcross progeny derived from a cross between Sasanishiki (the recurrent parent) and one RIL (GS34) in which the target QTL region was homozygous for the Gemdjah Beton (donor parent) allele. We obtained seven BC_2F_2 recombinants, and selfing of these plants produced homozygous recombinant BC_2F_3 plants. Two other lines that were homozygous for large unrecombined regions of chromosome 7 containing the target QTL (one homozygous for the Sasanishiki region, the other homozygous for the Gemdjah Beton region) were selected as isogenic controls for the linkage analysis. These nine BC_2F_3 lines were genotyped with DNA markers distributed across all chromosome regions, as described in "DNA marker analysis" below. To determine genotype classes for the target QTL in the seven BC_2F_3 recombinants, the level of soil-surface rooting of their BC_2F_4 progenies were investigated.

Scoring of soil-surface rooting in the paddy field

We scored soil-surface roots (SOR) of the RILs three times: in the F_6 population at the heading stage (August 1, 2007) and in the F_7 population at heading and ripening stages (August 4 and October 7, 2009). In April, seeds were placed in distilled water at 30°C for 2 days. Germinated seeds were then sown on seedling cell-trays (300 mm × 600 mm) and grown in a greenhouse for 4 weeks. Field experiments were conducted in the paddy fields of the Experimental Farm Station, Graduate School of Life Sciences, Tohoku University, in Kashimadai, Osaki, Miyagi,

Japan (37°28′N, 141°06′E). As water conditions affected the development of soil-surface roots (Ueno and Sato 1989), to maintain reproducibility when scoring soil-surface roots, we used a paddy field rather than an upland field in order to allow us to accurately control the water level. The field soil was classified as gray lowland soil (pH = 5.2). Inorganic fertilizer was applied to the paddy fields at 4 days before transplanting at rates of 30 kg of N, 30 kg of P, and 30 kg of K ha^{-1}. We transplanted five normal 4-week-old seedlings from each line without replication into a single row. Plants were grown at a density of one plant per hill, with 30-cm spacing between hills. At the time of scoring, we drained the water from the paddy field. Soil-surface rooting in the field was quantified by SOR score (SSOR), a visual score based on a scale of 0 to 4 (0 = no roots on soil surface [Sasanishiki type], 1 = fewer than 5 roots on soil surface, 2 = around 10 roots on soil surface, 3 = many roots on soil surface [Gemdjah Beton type]) (Fig. 1a, b). SSOR was measured for three of the five plants (excluding border plants, to avoid edge effects) from each line. SSOR values in the 2007 trial were based on a visual estimate of the average of three plants. The average score of the three plants was used as the mean value for each line within an evaluation in the 2009 trial.

Measurement of soil-surface roots at the seedling stage

We developed the 'cup method' as a simple system for evaluating soil-surface rooting. Seeds were sterilized in 70% ethanol for 30 s and then washed with flowing tap water for 3 min. The seeds were then sterilized in 2% sodium hypochlorite for 15 min. The sterilized seeds were then soaked in 0.2% PPM (Plant Preservative Mixture: Plant Cell Technology, Inc., Washington, DC, USA) at 30°C in an incubator for 2 days. We used a plastic cylindrical cup with a diameter of 37 mm and a depth of 40 mm (beaker PP, AS ONE Corporation, Osaka, Japan) to culture each plant. We made a small hole in the bottom of each cup to supply water. We filled the cups with culture soil containing chemical fertilizer (Mitsui-Toatsu no. 3, Tokyo, Japan: N 0.7 g, P 1.2 g, K 0.6 g kg^{-1}). 40 cups were put in a stainless steel tray (320 mm × 250 mm × 53 mm) drilled for drainage. Each germinated seed was sown at the center of a cup and then covered with a 10-mm layer of culture soil without fertilizer (Shibanome soil, Kikuchi Industry Co., Ltd., Tochigi, Japan). The tray was placed in a large plastic container (445 mm × 325 mm × 70 mm) and then supplied with water. The water level was maintained at 2 cm deep by supply from beneath the tray until the two-leaf-stage. From the two-leaf-stage to the time of rooting assessment, the water level was maintained at the level of the soil surface. The plants were grown in a greenhouse maintained at 20–30°C under natural daylength.

We measured soil-surface rooting of the F_6 RILs 18 days after sowing (i.e., at approximately three- to four-leaf-stage) in three trials. In each trial, three plants in each line were grown in a randomized complete block design, with one plant of each line per block. For the cup assay, soil-surface rooting was quantified by using the SOR ratio (RSOR). RSOR was defined as the number of soil-surface roots divided by the total number of primary roots of each plant, expressed as a percentage. To count the number of soil-surface roots in a cup, we carefully washed away and

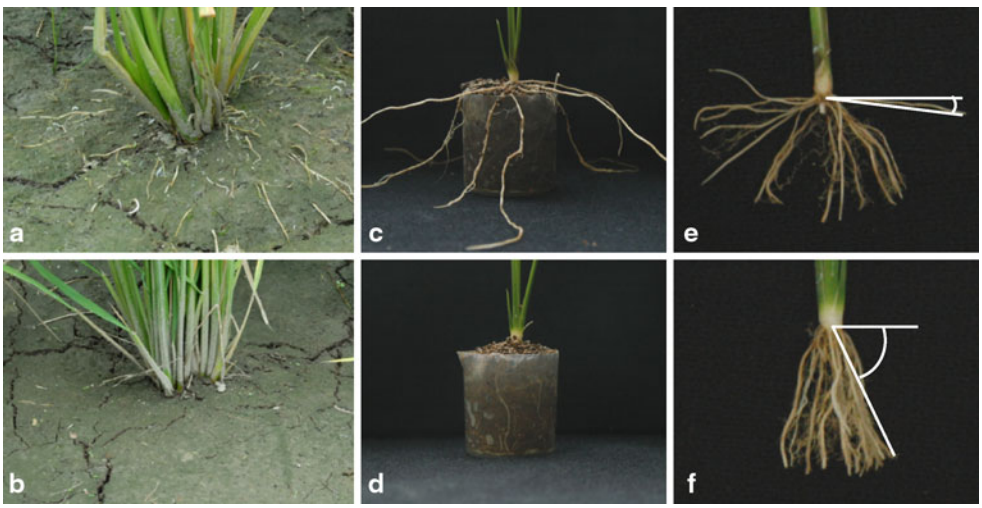


Fig. 1 Differences in soil-surface rooting traits between Gemdjah Beton (**a, c, e**) and Sasanishiki (**b, d, f**). **a, b** Image taken from the side of a hill in the paddy field. **c, d** Image taken from the side of a cup from the greenhouse assay. **e, f** Image taken from the basal part of the root after removal of roots from the cup. The *white lines* indicate the root growth angle

removed the Shibanome soil from the top of the cup, then took the cup out of the tray. We counted the number of primary roots elongated past the edge of the cup as soil-surface roots (Fig. 1c, d). Then, we washed all the roots after we assessed the soil-surface roots to count the total number of primary roots. The average of three plants was used as the mean value for each line. During the first trial, we also assessed total root number (TRN), maximum root length (MRL), plant height (PH), and tiller number (TN).

Measurement of root growth angle

For fine mapping of the SOR QTL on chromosome 7, we measured the root growth angle of BC_2F_4 plants. A total of 40 plants of each line were grown in a randomized complete block design (six or seven plants per block) by using the cup method described above. At 18 days after sowing, the roots in each cup were washed carefully, and any free water was removed from the roots with a paper towel. The root growth angle of each plant was determined by measuring the angle between the soil surface (horizontal line) and the shallowest primary root with a protractor (Fig. 1e, f).

DNA marker analysis

From the list of simple sequence repeats (SSRs) described by the International Rice Genome Sequencing Project (2005), a total of 2,124 SSR markers were tested for polymorphism between Sasanishiki and Gemdjah Beton. The RILs were genotyped with 206 SSR markers selected from the set of 515 polymorphic markers to cover all 12 chromosomes. Total DNA was extracted from leaves by the CTAB method (Murray and Thompson 1980). PCR amplifications were performed in a 5-μl reaction mixture containing 0.5 μl (20 ng) DNA, 1.0 μl 5× PCR buffer, 0.1 μl 10 mM dNTPs, 0.025 μl (5 units) KAPA2G Fast DNA Polymerase (Kapa Biosystems, Boston, MA, USA), 0.125 μl of a 20-pM mixture of forward and reverse primers (20 pM each primer type), and 3.25 μl H_2O. PCR consisted of an initial denaturation for 1 min at 95°C; followed by 35 cycles of 10 s at 95°C, 10 s at 55°C, and 1 s at 72°C; followed by a final extension for 30 s at 72°C. PCR products were separated by electrophoresis in gel consisting of 3% Agarose Type I (Sigma-Aldrich, St. Louis, MO, USA) and 1% Metaphor Agarose (Lonza Rockland, Inc., Rockland, ME, USA) at 150 V for 180 min.

For genotyping of the BC_2F_3 population, 205 SSR markers were used. These markers included all but one of the 206 markers used for the RIL genotyping and were distributed across all chromosome regions. PCR amplifications were performed as described for the RILs.

To narrow down the candidate region of the SOR QTL on chromosome 7, we selected an additional 67 SSRs in the interval between RM1365 and the end of the long arm of chromosome 7 from the list of SSR markers described by the International Rice Genome Sequencing Project (2005). Out of these 67 markers, 15 showed polymorphism between Sasanishiki and Gemdjah Beton when assayed by gel electrophoresis. Four additional SSR markers were also polymorphic, but the difference in PCR product sizes between the parental lines was too small to discriminate by gel electrophoresis. For these four markers, the PCR products were fluorescently labeled and the fragment sizes were determined on an ABI 3100 DNA sequencer by using Data Collection 2.0 and GeneMapper 3.7 software (Applied Biosystems, Foster City, CA, USA).

Statistical and QTL analyses

Linkage maps of RILs were constructed from the genotype data in MAPMAKER/EXP 3.0 software (Lander et al. 1987). Genetic distances were estimated by using the software's Kosambi map function (Kosambi 1944). Putative QTLs were detected by 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 1,000 permutations at a 5% significance level (Churchill and Doerge 1994). The additive effect and the percentage of phenotypic variance explained by each QTL (R^2) were estimated at the maximum LOD score. Based on preliminary observation of soil-surface rooting in both parental lines in the field trials, we recognized that consistent cultivation management was important for reproducibility of soil-surface rooting. Therefore, we decided to evaluate soil-surface rooting within one block (i.e., without replication), because consistent cultivation management across large numbers of plants was difficult with our evaluation method. Instead of replicating within each trial, we evaluated soil-surface rooting of the RILs without replication in three field trials. We analyzed QTLs for soil-surface rooting separately for each evaluation (trial), because each data point was obtained from an independent examination.

We calculated the broad-sense heritability (h_B^2) for each trait from the estimates of genetic (σ_G^2) and residual (σ_E^2) variances derived from the expected mean squares of the analysis of variance to understand the genetic effects of the investigated traits:

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

To compare the mean root growth angles of the seven recombinant BC_2F_4 lines, we used Dunnett's test provided by JMP version 7.0 software (SAS Institute, Cary, NC, USA). All lines were compared with homozygous line for a

large region from chromosome 7 of Sasanishiki (SA-homo) as the control. The genotypes of each line were estimated from the results of Dunnett's test at a 0.1% significance level.

Results

Phenotypic variation of soil-surface rooting among RILs

In the paddy field, Gemdjah Beton showed many soil-surface roots, whereas Sasanishiki had none (Fig. 1a, b). SOR scores (SSORs) in the RILs were distributed between the values of the two parental lines, but no RIL had a score of 3 like Gemdjah Beton (Fig. 2). In the cup test, most of the RILs had SOR ratios (RSORs) that fell between the values of the two parental lines and ranged from 0.0 to 56.3%. On the other hand, total root number and maximum root length showed transgressive segregation. Phenotypic correlations among the six traits are shown in Table 1. SSOR and RSOR showed the highest correlation, suggesting that the amount of soil-surface rooting in a paddy field (assessed by SSOR) can be estimated by RSOR in seedling plants. Total root number (TRN) did not show a significant correlation with either SSOR or RSOR. Maximum root length (MRL) correlated positively with the other five traits. These correlations indicate that the amount of soil-surface rooting (measured here by SSOR and RSOR) is likely to be associated with root length but not with total root number. Broad-sense heritabilities of SORs were intermediate to high (Table 2). Heritability estimates of the SSORs in 2009 were 0.82 and 0.66, and heritability estimates of the RSORs ranged from 0.54 to 0.72.

QTL for soil-surface root in RILs

Among 2,124 SSR markers, 515 were polymorphic between Sasanishiki and Gemdjah Beton (24.2%). The RIL linkage map, composed of 206 markers, covered almost all regions of the rice genome (Fig. 3). The total map length was 1,545.4 cM, and the average distance between markers was 7.97 cM.

Six QTL regions for SSOR were detected on chromosomes 3, 4, 6, and 7 (Table 2; Fig. 3) in the three field evaluations. The LOD thresholds and R^2 values ranged from 3.7 to 22.3 and from 6.2 to 53.6%, respectively. In particular, QTLs near RM21941 on chromosome 7 showed large R^2 values, ranging from 32.5 to 53.6%. The additive effects of the Gemdjah Beton allele at all QTLs increased SSOR by 0.23–0.69 over the value for the Sasanishiki allele.

Seven QTLs for RSOR were detected on chromosomes 1, 7, and 9 (Table 2; Fig. 3) in the three greenhouse trials. The LOD thresholds and R^2 values ranged from 3.0 to 10.1 and from 6.9 to 30.4%, respectively. The additive effects of the Gemdjah Beton allele at all QTLs increased RSOR by 3.30 to 9.14 percentage points over the value for the

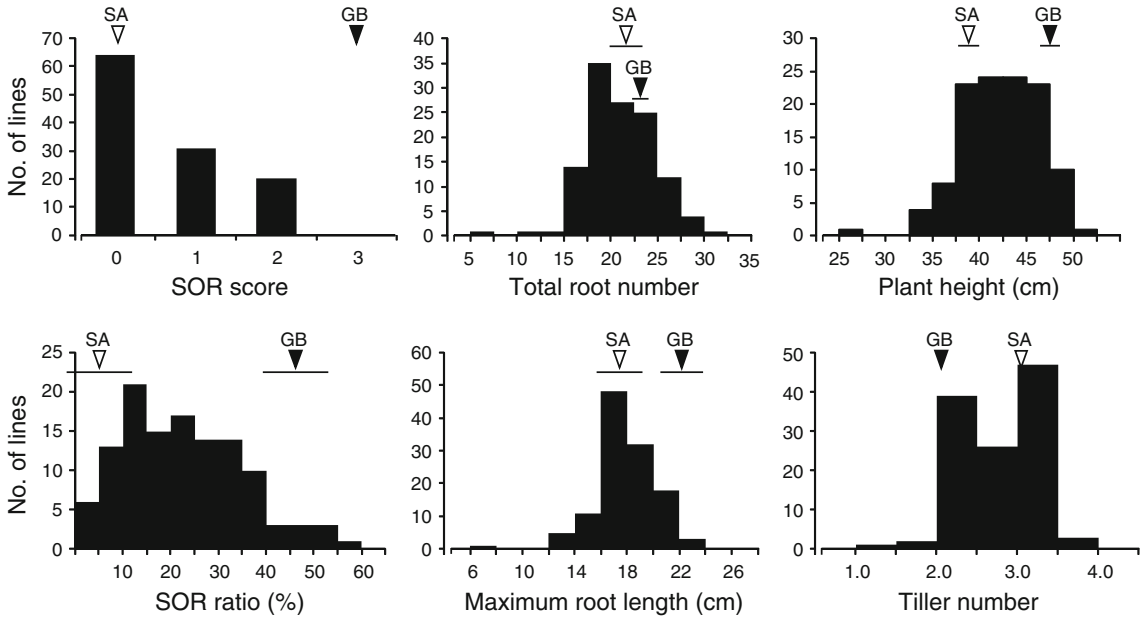


Fig. 2 Frequency distributions of four root traits and two shoot traits in RILs derived from a cross between Gemdjah Beton and Sasanishiki. *Arrowheads* and *horizontal lines* indicate the mean values and standard deviation for the parental lines. *SA* Sasanishiki, *GB* Gemdjah Beton. SOR score was assessed during the heading stage in 2007. SOR ratio, TRN, MRL, PH, and TNA were assessed during greenhouse trial 1

Sasanishiki allele. Among the seven QTLs detected, three QTLs showing moderate R^2 values (15.9 to 25.4%) were detected near RM21941 on chromosome 7, which was the same region in which major QTLs for SSOR were detected. On the other hand, three QTLs for RSOR detected on chromosome 1 were located in three different chromosome regions, although two of them overlapped slightly.

Fig. 3 Chromosomal locations of QTLs for four root traits and two shoot traits in rice. Chromosome numbers are indicated above each linkage map. Marker names are indicated to the right of each linkage map. *Arrowheads* and *boxes* to the left of each chromosome represent LOD peaks of putative QTLs and their 1-LOD support intervals (Lynch and Walsh 1998), respectively. *White*, *gray*, and *black boxes* indicate first, second and third trials, respectively, for each trait measured ▶

Table 1 Correlation coefficients among six traits in RILs derived from Gemdjah Beton and Sasanishiki

	SOR score (SSOR)[a]	SOR ratio (RSOR)	Total root number (TRN)	Maximum root length (MRL)	Plant height (PH)
SOR ratio (RSOR)[b]	0.487**				
Total root number (TRN)[b]	−0.009	0.154			
Maximum root length (MRL)[b]	0.326**	0.256**	0.252**		
Plant height (PH)[b]	0.017	0.051	0.361**	0.386**	
Tiller number (TN)[b]	−0.011	−0.012	0.413**	0.237**	0.040

** $P < 0.01$

[a] SSOR was assessed during the heading stage in 2007

[b] RSOR, TRN, MRL, PH, and TNA were assessed during greenhouse trial 1

Table 2 Putative QTLs for four root traits and two shoot traits in rice

Traits	Chromosome	Nearest marker	cM[a]	LOD	AE[b]	R^{2c}	h_B^{2d}
SOR score at field 1[e] (SSOR1)	3	RM14391	9.7	3.7	0.23	6.6	N/A
	7	RM21941	83.3	22.3	0.69	53.2	
SOR score at field 2[e] (SSOR2)	4	RM16354	2.0	4.8	0.25	7.6	0.82
	6	RM1985	0.0	3.9	0.25	6.2	
	7	RM21941	86.1	22.0	0.67	53.6	
SOR score at field 3[e] (SSOR3)	7	RM21941	85.1	12.6	0.42	32.5	0.66
SOR ratio at greenhouse trial 1 (RSOR1)	1	RM3810	171.6	3.0	3.30	6.9	0.63
	7	RM21941	81.3	7.7	5.88	21.4	
	9	RM7175	51.3	3.4	3.87	9.4	
SOR ratio at greenhouse trial 2 (RSOR2)	1	RM1349_1	120.2	4.1	5.65	9.9	0.72
	7	RM21941	84.3	10.1	9.14	25.4	
SOR ratio at greenhouse trial 3 (RSOR3)	1	RM11918	156.8	9.4	7.75	30.4	0.54
	7	RM21941	81.3	6.9	5.64	15.9	
Total root number[f] (TRN1)	1	RM3810	171.6	6.6	1.58	16.4	0.46
	4	RM3836	113.0	8.8	-1.88	22.9	
Maximum root length[f] (MRL1)	7	RM21941	85.1	5.3	0.94	15.7	0.40
Plant height[f] (PH1)	1	RM11686	145.8	3.3	1.40	11.3	0.68
	2	RM3828	67.1	4.1	1.41	11.3	
	12	RM1194	94.5	4.3	-1.64	14.1	
Tiller number[f] (TN1)	3	RM14778	52.7	3.5	-0.18	12.1	0.48

[a] Genetic distance from the end of the short arm to the QTL LOD peak

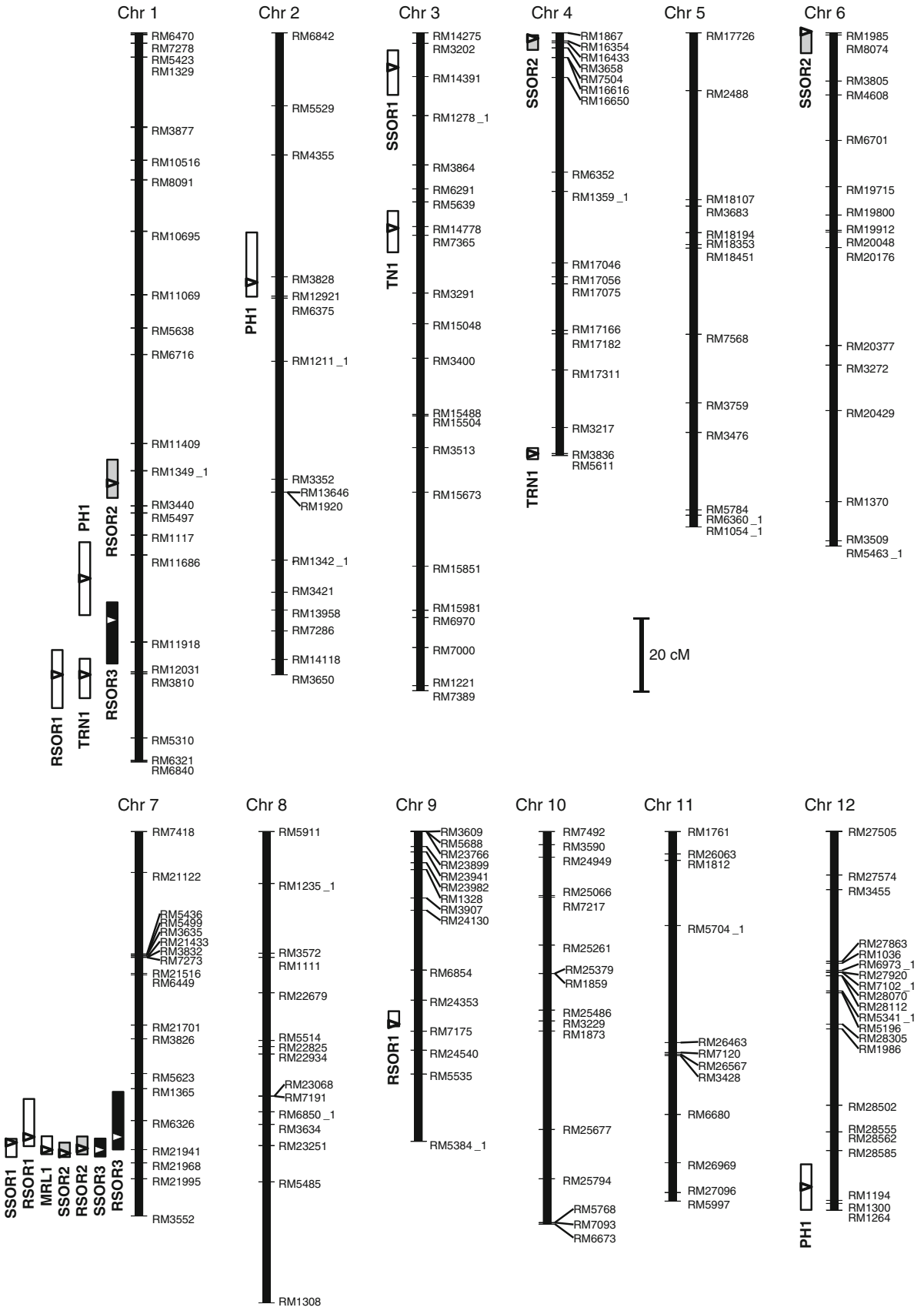
[b] Additive effect of the allele from Gemdjah Beton compared with that from Sasanishiki

[c] Percentage of phenotypic variance explained by each QTL

[d] Broad-sense heritability

[e] Fields 1, 2, and 3 correspond to evaluations at the heading stage in 2007, at the heading stage in 2009, and at the ripening stage in 2009, respectively

[f] Assessed during greenhouse trial 1 only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RM1329
RM3877
RM10516
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RM6716
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RM1349 _1
RM3440
RM5497
RM1117
RM11686
RM11918
RM12031
RM3810
RM5310
RM6321
RM6840
RSOR1
TRN1
RSOR3
RSOR2
PH1
Chr 2
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RM1264
PH1

Two QTLs for TRN were detected on chromosomes 1 and 4 (Table 2; Fig. 3). The QTL detected on chromosome 1 was located near a QTL for RSOR. Only one QTL for MRL, which had LOD threshold of 5.3 and R^2 value of 15.7%, was detected on chromosome 7, near the major QTLs for RSOR (Table 2; Fig. 3). Three QTLs for plant height (PH) and one QTL for tiller number (TN) were mapped in chromosomal regions different from the QTLs for root traits (Table 2; Fig. 3).

Validation of the major QTL for soil-surface rooting in homozygous lines

The detection of QTLs for both SSOR and RSOR on chromosome 7 near RM21941 in all field and greenhouse evaluations suggests that the QTLs for both traits were the same. To verify the genetic effect of this QTL at an early growth stage, we investigated RSOR of two BC_2F_3 lines homozygous for the candidate region of chromosome 7 from either Sasanishiki (SA-homo) or Gemdjah Beton (GB-homo) (Fig. 4) by using the cup method. Many GB-homo plants did not show soil-surface rooting as extensive as Gemdjah Beton, although some plants had roots that elongated past the edge of the cup. It was difficult to determine clear phenotypic classes with this method. We then removed the roots from each cup and directly measured root growth angle (Fig. 1e, f). SA-homo showed a mean root growth angle of 53.2°, similar to Sasanishiki (50.3°), whereas the mean root growth angle of GB-homo was 28.3° (Table 3). Thus, we confirmed that the Gemdjah Beton allele of the SOR QTL on chromosome 7 conferred shallow rooting at the seedling stage.

Fine mapping of the QTL for soil-surface rooting

We used seven BC_2F_3 lines in which recombination occurred between the flanking markers RM5623 and RM21995 to map the SOR QTL detected on chromosome 7 as a single locus. Through progeny testing, we classified the seven BC_2F_4 lines into two groups that exhibited either small or large root growth angle. Five lines (BC2F3-24-23-1 to -5) showed relatively small root growth angle, ranging from 26.9° to 33.5°, whereas two lines (BC2F3-24-23-6 and -7) had growth angles of 49.6° and 53.3°, similar to SA-homo (Table 3). These phenotypic groups were predicted to be associated with genotype classes that were homozygous for the Gemdjah Beton allele and for the Sasanishiki allele, respectively. These results clearly demonstrated that the SOR QTL was located between SSR markers RM21941 and RM21976 on chromosome 7 (Fig. 5). We designated this QTL *qSOR1* (*quantitative trait locus for SOIL SURFACE ROOTING 1*) following the nomenclature recommended by McCouch and CGSNL (Committee on Gene Symbolization, Nomenclature and Linkage, Rice Genetics Cooperative) (2008). The candidate genomic region of *qSOR1* between RM21941 and RM21976 spans 812 kb in the Nipponbare genome (Fig. 5).

Discussion

A major QTL for soil-surface rooting in rice

Soil-surface rooting is the elongation of plant roots on the soil surface that occurs from the juvenile stage to the adult stage. There have been no genetic study of soil-surface root formation in rice, although soil-surface roots have been reported in several grasses such as maize (Mano and Omori 2007), barley (Stanca et al. 2003), and rice (Ueno and Sato 1989). Ueno and Sato (1989) investigated soil-surface rooting among 56 rice accessions under three different water conditions (roots submerged in water, root submerged in water with aeration, and root not submerged).

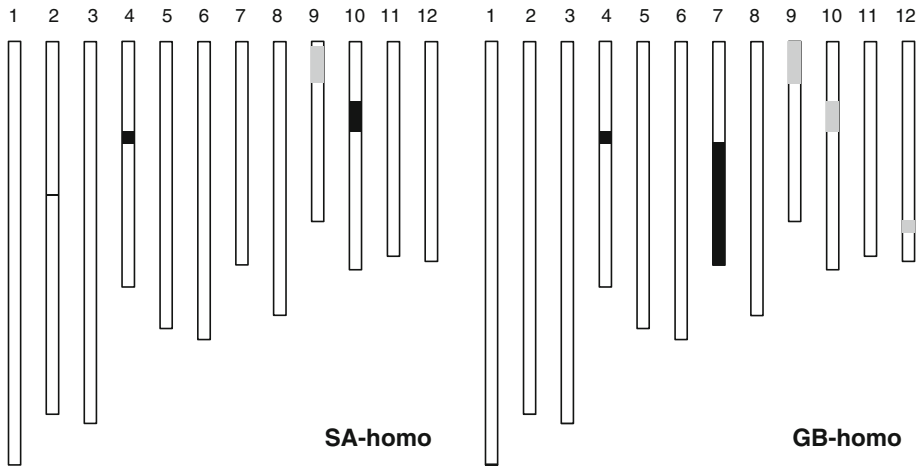

Fig. 4 Graphical genotypes of BC_2F_3 lines homozygous for each of the two *qSOR1* alleles. Chromosome numbers are indicated above each linkage map. *White*, *black*, and *gray boxes* represent regions that are homozygous for marker alleles from Sasanishiki, homozygous for marker alleles from Gemdjah Beton, and heterozygous, respectively

Table 3 Genotypes of 14 DNA markers on chromosome 7 in the BC_2F_3 lines and root growth angle in the BC_2F_4 progeny

Lines	Genotype of marker on chromosome 7 in BC_2F_3 lines[a]														Root growth angle (°) in BC_2F_4 lines				
	RM5623	RM7237	RM5847	RM1365	RM7040	RM6326	RM6362	RM21941	RM21968	RM21969	RM21976	RM21985	RM8261-02	RM21995	Mean	SD	P[b]		Predicted genotype of *qSOR1*[c]
Sasanishiki	A	A	A	A	A	A	A	A	A	A	A	A	A	A	50.3	7.1	0.83240		SA
Gemdjah Beton	B	B	B	B	B	B	B	B	B	B	B	B	B	B	12.0	9.8	<0.000001	*	GB
SA-homo	A	A	A	A	A	A	A	A	A	A	A	A	A	A	53.2	8.9	---		SA(control)
GB-homo	B	B	B	B	B	B	B	B	B	B	B	B	B	B	28.3	9.0	<0.000001	*	GB(control)
BC2F3-24-23-1	A	A	B	B	B	B	B	B	B	B	B	B	B	B	32.0	10.4	<0.000001	*	GB
BC2F3-24-23-2	A	A	A	B	B	B	B	B	B	B	B	B	B	B	26.9	10.3	<0.000001	*	GB
BC2F3-24-23-3	A	A	A	A	B	B	B	B	B	B	B	B	B	B	23.0	10.0	<0.000001	*	GB
BC2F3-24-23-4	A	A	A	A	A	B	B	B	B	B	B	B	B	B	32.2	12.5	<0.000001	*	GB
BC2F3-24-23-5	A	A	A	A	A	A	A	A	B	B	B	B	B	B	33.5	16.7	<0.000001	*	GB
BC2F3-24-23-6	A	A	A	A	A	A	A	A	A	A	B	B	B	B	49.6	8.4	0.74640		SA
BC2F3-24-23-7	A	A	A	A	A	A	A	A	A	A	A	B	B	B	53.3	8.1	1.00000		SA

[a] Genotypes of DNA markers are represented by A (white) for Sasanishiki homozygous and B (black) for Gemdjah Beton homozygous

[b] P, probability of no significant difference between control line (SA-homo) and recombinant BC_2F_3 line in Dunnett's test. * indicates significance at the 0.1% level

[c] Genotypes of *qSOR1* were predicted from the results of Dunnett's test using a 0.1% level of significance

The authors suggested that soil-surface rooting was related to the availability of oxygen, because Bulu cultivars, which produced soil-surface roots under all water conditions, tended to produce more soil-surface roots under flooding than under non-flooded conditions (Ueno and Sato 1989). Ueno and Sato (1992) also found that Bulu cultivars exhibited a gravitropic response only under light, so they suggested that soil-surface rooting was influenced by light stimulus. These reports provide evidence that soil-surface rooting is affected by several environmental stimuli, such as oxygen and light. Firm conclusions could not be drawn, however, because these studies used rice accessions having different genetic backgrounds rather than near-isogenic materials. To clarify the physiological and molecular mechanisms of soil-surface root formation, it is necessary to first understand the genetic control of soil-surface rooting. In this study, we established an evaluation method and performed QTL analysis of soil-surface rooting in rice by using RILs. We found that six chromosomal regions were associated with soil-surface rooting through investigations under paddy field and artificial conditions (Table 2; Fig. 3). Among these, the SOR QTL on chromosome 7 (*qSOR1*) was detected not only in RILs grown in the paddy field, but also in seedlings cultured in cups, and had a major effect under both sets of conditions. The broad-sense heritabilities of SORs in the RILs were intermediate (0.54) to high (0.82) (Table 2). Previous studies of QTLs for root traits reported that their heritabilities obtained in trials with multiple replicates ranged from relatively small (0.23) to high (0.84) (Yadav et al. 1997; Kamoshita et al. 2002a, b). Therefore, we concluded that our method was useful for measuring soil-surface rooting, although the heritabilities of RSORs indicated that the cup method was more heavily influenced by the environment than in the paddy field, possibly because seedlings rather than older plants were assessed in the cup method. Therefore, stable environmental control will be needed to identify minor SOR QTLs in the cup method.

Since lowland rice generally shows thin superficial roots near the soil surface that form from panicle initiation to ripening, whereas soil-surface roots are thicker and typically form from the seedling stage, *qSOR1* appears to be specifically involved in the formation of soil-surface roots. To determine the precise position of *qSOR1*, we narrowed down the candidate region by using advanced progeny. We first investigated RSOR in BC_2F_3 lines homozygous for the candidate region from either Gemdjah Beton or Sasanishiki (Fig. 4) by using the cup method. Although the GB-homo line did not always elongate roots past the edge of the cup, we observed that the primary roots elongated at a shallower angle in the cup than those of Sasanishiki (data not shown). We then measured the basal root growth angle of these plants and found that the root growth angle of GB-homo was significantly smaller than that of Sasanishiki. Based on

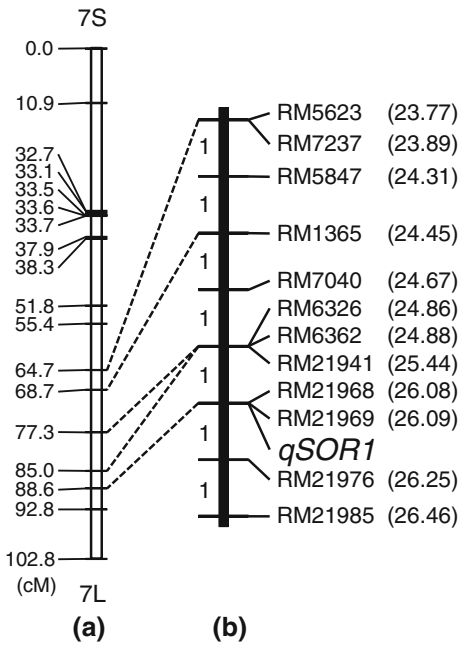

Fig. 5 Location of *qSOR1* on rice chromosome 7. **a** Linkage map of the RILs derived from Gemdjah Beton × Sasanishiki. **b** Linkage map constructed from the seven BC_2F_3 recombinants. The number of recombination units (cM) between adjacent DNA markers is shown on the *left*. Names of the DNA markers are shown on the *right*; *numbers in parentheses* beside the DNA markers indicate their physical map position (Mb) on chromosome 7 of Nipponbare

the measurements of root growth angle, the candidate region of *qSOR1* was delimited to an 812-bp region between RM21941 and R21976 (Fig. 5). The Rice Annotation Project RAP3 database (http://rapdb.dna.affrc.go.jp/) predicts 94 genes in the candidate region for *qSOR1*. The morphological and physiological functions of *qSOR1* are not yet known, so it is difficult to identify the actual candidate gene for *qSOR1* among these many predicted genes. To do so, advanced progeny that contain recombination in the region of *qSOR1* are currently being developed. We confirmed a positive correlation between root growth angle and soil-surface rooting in the parental lines (Fig. 1). But, we did not investigate relationship between them in the BC_2F_3 lines. Further study using a near-isogenic line containing the Gemdjah Beton allele of *qSOR1* (*qSOR1*-NIL) will be needed to clarify whether root growth angle at seedling stage is associated with soil-surface rooting in paddy fields.

Relationship between *qSOR1* and QTLs for other morphological traits

QTLs for total root number (TRN) were not detected near *qSOR1*, but a QTL for maximum root length (MRL) was tightly linked to *qSOR1* (Table 2; Fig. 3). We assumed that the positive correlation between SOR and MRL could be attributed either to tightly linked but distinct QTLs for the two traits or to pleiotropic effects of a single QTL. To determine the relationship between *qSOR1* and the MRL QTL, we undertook fine mapping of the MRL QTL by using the same advanced progeny we had used for linkage analysis of *qSOR1*. In those experiments, we could not map the MRL QTL to a specific interval (data not shown). This may have been because the plants grown in small cups were not suitable for evaluation of MRL or because multiple QTLs for MRL occurred in this region. To clarify the relationship between *qSOR1* and the MRL QTL, we should investigate MRL using a method such as hydroponic culture. For example, a QTL for root length of rice, *qRL6.1*, was mapped as a single locus in experiments using hydroponic culture (Obara et al. 2010). *qSOR1* was not identified in the same chromosome region as any of the QTLs for plant height (PH) or tiller number (TN), suggesting that *qSOR1* does not influence these traits. Uga et al. (2011) also reported that *Dro1*, a QTL on chromosome 9 related to root growth angle, did not influence shoot morphological traits. These results indicate that QTLs for root growth angle might be able to improve root architecture without changing aboveground traits.

Comparative analysis of QTLs for soil-surface rooting between rice and other crops

QTLs for the growth of adventitious roots on the soil surface under flooded conditions have been analyzed in maize, because this trait is one of the most important adaptations to waterlogged fields (Mano et al. 2005a, b). QTLs for adventitious roots on the soil surface were found on chromosomes 4 and 8 in F_2 populations of maize (*Z. mays* L. inbred B64) × teosinte (*Z. mays* ssp. *huehuetenangensis*) (Mano et al. 2005a), and on chromosomes 3, 7, and 8 in F_2 populations of B64 × Na4, a tropical Caribbean maize inbred (Mano et al. 2005b). Maize and teosinte cultivars having soil-surface roots can grow in lowlands where waterlogging stress is a great risk. It is unknown whether the ecological function of soil-surface rooting is the same in both rice and maize. However, we hypothesized that the soil-surface roots in different grass species are homologous organs. To test this hypothesis, we compared the chromosomal positions of SOR QTLs between rice and maize. Three SOR QTLs were detected on rice chromosome 1L in this study, and two SOR QTLs were previously detected on maize chromosomes 3L and 8L (Mano et al. 2005a, b). Comparative genome analysis has shown synteny between rice chromosome 1L and maize chromosomes 3L and 8L (Wilson et al. 1999, Schnable et al. 2009). Among the three QTLs we detected on rice chromosome 1L, two might correspond to the SOR QTLs located on maize chromosomes 3L and 8L. Maize

chromosome 7L, where one SOR QTL was located (Mano et al. 2005b), corresponds to a syntenic region of rice chromosome 7L, where *qSOR1* was mapped. Thus, *qSOR1* might correspond to the SOR QTL located on maize chromosome 7L. In common bean, four QTLs for basal root growth angle were detected in RILs derived from crosses between shallow- and deep-rooting accessions (Liao et al. 2004). We could not compare the chromosomal positions of QTLs between rice and common bean because we do not know the syntenic relationship of chromosomes between these two species. If we isolate *qSOR1* in rice, a search for homologous genes in both grass crops such as maize and dicotyledonous crops such as common bean may be possible; if so, this will help us to clarify the similarities and differences in the ecological functions of soil-surface rooting between rice and other crops.

Potential applications of QTLs for soil-surface rooting in rice

Enhancement of P acquisition efficiency is a very important breeding target in many crops, because low-fertility soils and inadequate fertilizer inputs are the main constraints to production in crop-growing areas in developing countries (Lynch 1998). In rice, P deficiency is also one of the dominant abiotic stresses limiting productivity under upland and rainfed lowland conditions (Kirk et al 1998). Almost 50% of the soils in rice cultivation areas of the world are currently P deficient (Ismail et al. 2007). In bean, basal root growth angle of young plants in growth pouches was significantly correlated with yield in field trials in low-P tropical soils (Bonser et al. 1996). Liao et al. (2004) suggested that QTLs for root growth angle could be used to facilitate selection and breeding for higher P-uptake efficiency in common bean and other crops. In rice, genetic analysis of tolerance to P deficiency has focused on root growth or root elongation under P-deficient conditions (Wissuwa et al 2002; Shimizu et al. 2008), but not on architectural features of the root system such as root growth angle. Based on studies in other crops, it seems likely that breeding for shallow rooting in rice may enhance adaptation to P-deficient soils, especially those under upland and rainfed lowland conditions. Although the Gemdjah Beton allele of *qSOR1* promotes growth of shallow roots from the juvenile to adult stages, relationship between soil-surface rooting and enhanced adaptation to P-deficient soils is not well known in rice. To verify the effects of *qSOR1* on P acquisition efficiency in a P-deficient soil, the *qSOR1*-NIL is currently being developed.

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