Breeding Science 58 : 7–14 (2008)

QTLs underlying natural variation in stele and xylem structures of rice root

Yusaku Uga*[1)], Kazutoshi Okuno[1,2)] and Masahiro Yano[1)]

[1)] *National Institute of Agrobiological Sciences*, 2-1-2 Kannondai, Tsukuba, Ibaraki 305-8602, Japan
[2)] *Present address*: *University of Tsukuba*, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8572, Japan

Rice cultivars show a wide range of variation in stele and xylem structures of the root as well as in root thickness (RTH). We identified quantitative trait loci (QTLs) for stele and xylem structures of the root by using 117 F_3 lines from a cross between the lowland rice cultivar IR64 (thin roots) and the upland rice cultivar Kinandang Patong (thick roots). QTL analysis was performed using genotype data consisting of 197 DNA markers in F_2 plants and phenotype data of the F_3 plants. Stele transversal area (STA), total area and number of late metaxylem vessels (MXA and MXN) and RTH were measured in basal cross sections of nodal roots. A total of 10 QTLs, 2 for STA, 4 for MXA, 2 for MXN and 2 for RTH, were detected on chromosomes 1, 2, 3, 9 and 10. The Kinandang Patong allele at all QTLs showed a positive additive effect on each trait, except for one QTL for MXA on chromosome 10. The phenotypic variance explained by each QTL ranged from 8.7% to 23.9%. A QTL for MXA on chromosome 9 showed the largest effect (23.9%) on total phenotypic variance. Although one QTL for STA, detected on chromosome 2, was mapped near a QTL for RTH, the other QTLs for stele and xylem structures did not map to the same chromosomal regions as the QTLs for RTH. We conclude that stele and xylem structures might be controlled by several genetic factors different from the QTLs for RTH.

Key Words: *Oryza sativa* L., root anatomy, vascular system, root thickness, quantitative trait locus.

Introduction

In rice, root traits are key to drought avoidance because they can enable the plant to avoid water stress by absorbing water deposited in the deep soil layers (Yoshida and Hasegawa 1982). Root morphological traits such as thickness, maximum length, volume and distribution were suggested to contribute to drought avoidance in the field (Yoshida and Hasegawa 1982, Fukai and Cooper 1995). A wide range of genetic variation has been observed in such traits (reviewed by O'Toole and Bland 1987). In general, the roots of upland rice cultivars are morphologically thicker and penetrate more deeply than those of lowland cultivars. These traits are controlled by multiple genes (Ekanayake *et al*. 1985). Many analyses of quantitative trait loci (QTLs) of root morphological traits have been carried out using different mapping populations (reviewed by Price *et al*. 2002a). As a result, many QTLs for rice root thickness (RTH) have been genetically identified on all chromosomes (Champoux *et al*. 1995, Price and Tomos 1997, Yadav *et al*. 1997, Ali *et al*. 2000, Zheng *et al*. 2000, Zhang *et al*. 2001, Kamoshita *et al*. 2002a, 2002b, Price *et al*. 2002b, Venuprasad *et al*. 2002, Courtois *et al*. 2003). RTH has also been targeted for improvement of root morphology by marker-assisted selection (MAS) (Steele *et al*. 2006).

Communicated by H. Kato
Received July 30, 2007. Accepted October 20, 2007.
*Corresponding author (e-mail: yuga@affrc.go.jp)

The stele (vascular cylinder), which includes the xylem and phloem of the root, is very important for the absorption and translocation of water and nutrients. In particular, xylem elements in the root are directly associated with water transport from root to shoot. Axial conductance significantly affects the rate of water uptake from the soil by a crop (Richards and Passioura 1981, Hasegawa and Yoshida 1982). The size and number of xylem vessels influence their conductivity for water transport (Kondo *et al*. 2000). Wide-diameter xylem vessels have large axial conductance with respect to water flow, enhancing water uptake compared with that by thin-diameter vessels (Fukai and Cooper 1995).

Both root anatomy and root morphology have been well studied in rice (reviewed by Morita and Nemoto 1995). Rice cultivars exhibit broad variation in stele and late metaxylem vessel (MXVII) diameters or areas (Terashima *et al*. 1987, Kondo *et al*. 2000). Kondo *et al*. (2000) compared nodal root anatomy in 12 upland and lowland rice cultivars both in hydroponic culture and under field conditions. Traditional upland *japonica* cultivars had the largest stele and MXVII diameters under both conditions. We also observed stele and MXVII differences in 61 rice accessions, including both upland and lowland rice cultivars, grown under field conditions (unpublished data), and found that transversal areas of stele and MXVII in upland rice were larger than those in lowland rice. Although a wide range of variation among cultivars has been found, QTLs for root stele and xylem structures have not been reported previously, presumably because it is more laborious and time-consuming to measure

these traits in a huge number of plants under a microscope than other root morphological traits.

The genetic relationship between QTLs for stele and xylem structures, and QTLs for RTH is not well known. Therefore, map information on QTLs for stele and xylem structures and on QTLs for RTH is required for genetic improvement of root traits associated with improved water uptake. In this study, to identify the genetic factors involving the stele and xylem structures and to clarify the relationship between them and RTH, we performed a QTL analysis of both types of traits using the F_3 population from a cross between the lowland cultivar IR64 (thin roots) and the upland cultivar Kinandang Patong (thick roots).

Materials and Methods

Plant materials and cultivation

An F_3 population consisting of 117 lines derived from a cross between IR64 and Kinandang Patong was used for the QTL analysis in this study. IR64 is a modern lowland cultivar (*indica*) developed by the International Rice Research Institute in the Philippines and widely grown South and Southeast Asia. Kinandang Patong is a traditional upland cultivar (tropical *japonica*) that originated in the Philippines. Kondo *et al*. (2000) reported that diameters of root, stele, and MXVII of Kinandang Patong were clearly larger than those of IR64. Therefore, we used this mapping population to detect QTLs underlying the differences in stele and xylem structures found between lowland and upland rice.

Rice plants were grown under upland conditions for six weeks in summer 2005 at the National Institute of Agrobiological Sciences (NIAS) in Tsukuba, Japan. A randomized complete block design was adopted with two replicates. In each block, we raised a total of 42 F_3 plants per line. Fourteen plants (seven in each block) for F_3, both parents, and F_1 were randomly selected for the measurement of root anatomical traits.

Measurement of root anatomical traits

We measured root stele and xylem structures of the main culm of the plant (Fig. 1). After fixing the main culm with roots in FAA solution (5% formalin, 5% acetic acid, 45% ethanol and 45% H_2O), we randomly selected three of the lower nodal roots of the third node (counted from the highest rooting node) and cut them out according to the procedure of Terashima *et al*. (1987) (Fig. 1A). Terashima *et al*. (1987) reported that their method could properly evaluate variations of RTH and vascular system in different varieties, so it was adopted for use in this study. A cross section of a nodal root was then cut 3 cm from the base of the root with a plant microtome (MTH-1; Nippon Medical & Chemical Instruments Co., Ltd., Osaka, Japan) and stained with toluidine blue. A digital image was taken of each cross section, and stele transversal area (STA), total transversal area and number of MXVII (MXA and MXN) and RTH were quantified under a digital microscope (VHX-200; Keyence Co., Ltd., Osaka, Japan) (Fig. 1B, 1C, 1D, 1E). To quantify the xylem structure, we focused on only MXVII, which exhibits the largest variation in xylem elements among rice cultivars (unpublished data). In general, 42 images (14 F_3 plants × 3 nodal roots) were used to calculate a mean value for each F_3 line.

Statistical analysis using phenotype data

Heritability was estimated from the variances of the parent plants and the F_1 plants, variance among the F_3 lines and variance in the F_3 line. Heritability was calculated as

$$h^2 = (3A/4 + 3D/32)/(3A/4 + 3D/32 + E),$$

where A is the sum of squares of additive effects of all genetic factors related to the traits, D is that of dominance effects of the genetic factors and E is environmental variance.

DNA marker analysis of the F_2 population

A total of 197 DNA markers distributed over the 12 rice chromosomes were used for the QTL analysis: 111 simple sequence repeat (SSR) (Akagi *et al*. 1996, Temnykh *et al*.

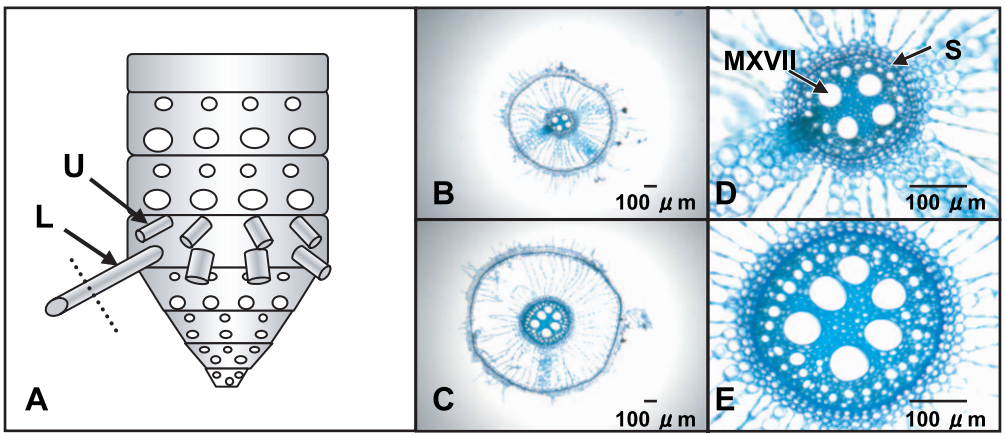

Fig. 1. Phenotypic differences in stele and xylem structures in rice root. (A) Scheme of nodal root. U, upper nodal root; L, lower nodal root. The dotted line indicates the site of the cross section. (B–E) Transverse root sections of rice plants stained with toluidine blue, showing the average value in each parent. S, stele; MXVII, late metaxylem vessel. (B) IR64; (C) Kinandang Patong; (D) IR64; (E) Kinandang Patong.

2001), 43 sequence-tagged site (STS) (Wu *et al*. 2002) and 43 insertion-deletion (InDel) markers were used for genotyping the F_2 plants. The InDel markers were constructed on the basis of information in the Rice DNA polymorphism database (Shen *et al*. 2004). The InDel markers used in this study are listed in the Appendix. Total DNA of plants was extracted from leaves by the CTAB method (Murray and Thompson 1980). PCR analysis was performed in a 5-µl reaction mixture containing 1 µl (20 ng) DNA, 0.5 µl 10 × PCR buffer, 2 mM dNTPs, 0.02 µl (5 units) *Ex Taq* DNA polymerase (Takara Bio Inc., Otsu, Japan), 0.12 µl of 20 pM solutions of both primers and 2.86 µl H_2O. PCR was carried out in 35 cycles of 15 s of denaturation at 93°C, 30 s of annealing at 55°C for SSR markers and 60°C for STS and InDel markers and 2 min of extension at 72°C. To detect polymorphism, the PCR products were electrophoresed in 3% agarose gels at 150 V for 90 min.

Construction of linkage map and QTL analysis

Linkage maps were constructed from the genotype data with MAPMAKER/EXP 3.0 (Lander *et al*. 1987). The genetic distance was estimated by using the Kosambi map function (Kosambi 1944).

Putative QTLs were detected by using the composite interval mapping (CIM) function of QTL Cartographer 2.0 (Basten *et al*. 1994). The CIM threshold was based on the results of 1000 permutation tests at the 5% level of significance (Churchill and Doerge 1994). The additive and dominance effects and the phenotypic variance explained by each QTL (R^2) were estimated at maximum LOD score. The total phenotypic variance explained by all of the detected QTLs was estimated by the multiple interval mapping (MIM) model of QTL Cartographer 2.0. To detect epistatic interactions between the detected QTLs, we performed two-way ANOVA using the genotype data of the marker nearest to each QTL.

Results

Phenotypic variation of root anatomical traits

Values of all traits in Kinandang Patong were significantly larger than those in IR64 (Fig. 1B, 1C, 1D, 1E and Fig. 2). In particular, MXA of Kinandang Patong (16661 µm^2) was almost three times that of IR64 (5942 µm^2). The values of most traits of F_1 plants were intermediate between those of the parents, but their RTH values were similar to the Kinandang Patong value, suggesting that RTH of F_1 plants could be attributed to dominance effects of the related genetic factor(s) more than to the other traits.

All traits in F_3 lines showed normal distribution within the range of the parental values (Fig. 2). Positive phenotypic

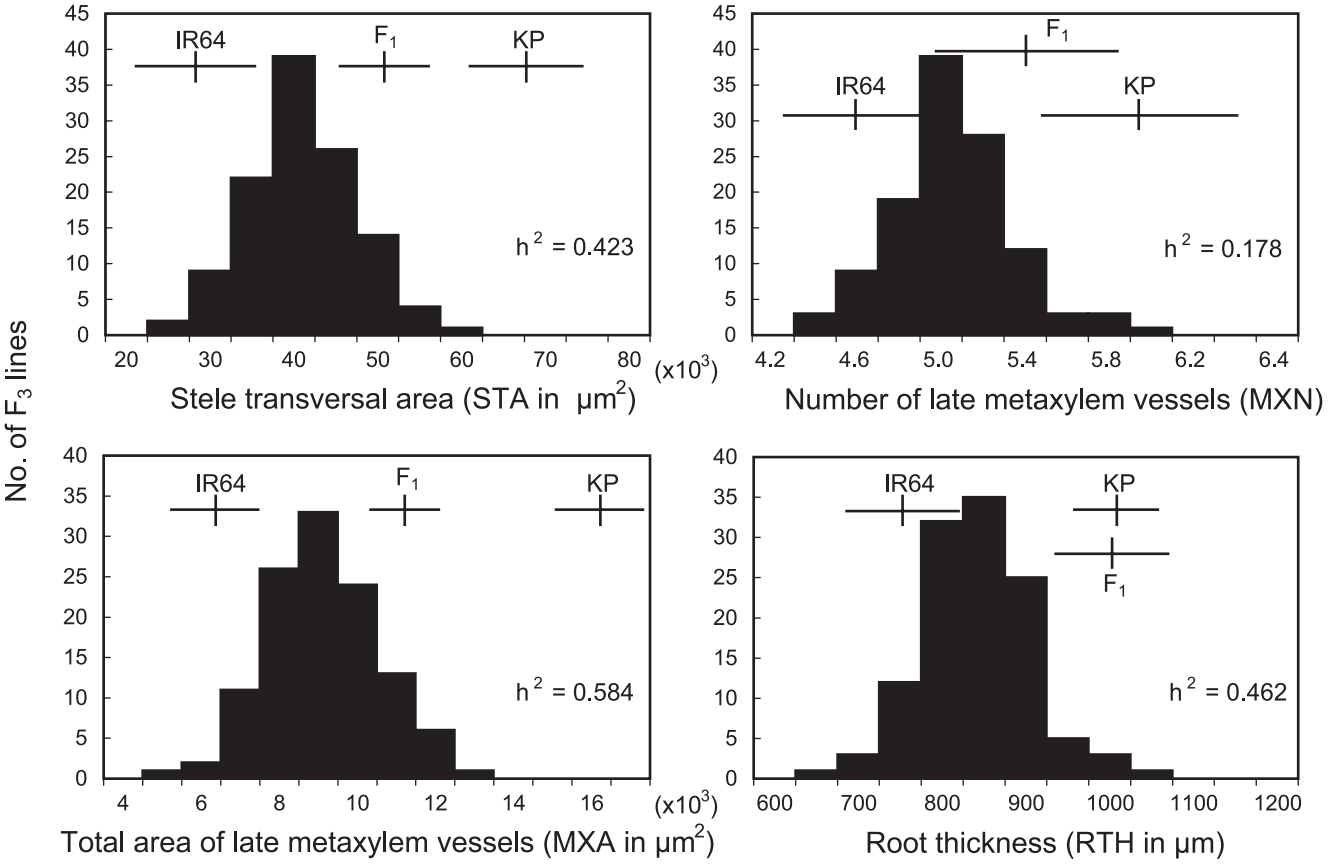

Fig. 2. Frequency distributions of four root anatomical traits in the F_3 population derived from IR64 × Kinandang Patong (KP). Vertical and horizontal bars indicate the average value and standard deviation of each line, respectively. h^2 indicates heritability.

correlations were observed between three stele and xylem structures (Table 1). In particular, a highly significant correlation was observed between MXA and STA ($r=0.923$). The RTH and three stele and xylem structures also showed a positive correlation, ranging from 0.408 to 0.879.

Heritability estimates of the four traits ranged from low (MXN, 0.178) to intermediate (MXA, 0.584) (Fig. 2).

QTL analysis of root anatomical traits

The F_2 linkage map, composed of 197 markers, covered almost the whole rice genome. The total map distance was 1539.6 cM, and the average distance between markers was 8.2 cM. We found eight QTLs for stele and xylem structures and two for RTH on this linkage map (Table 2 and Fig. 3). Two-way ANOVA revealed that no epistatic interaction occurred between the QTLs detected for each trait (data not shown).

Two QTLs for STA, *qSTA-2* ($R^2=14.7\%$) and *qSTA-9* ($R^2=16.2\%$), were detected on chromosomes 2 and 9 (Table 2 and Fig. 3). The additive effects of the Kinandang Patong alleles of the two QTLs were 3612 and 3434 μm^2, respectively, and their dominance effect values were 1123 and −88 μm^2.

Four QTLs for MXA were detected on chromosomes 2, 9 and 10 (Table 2 and Fig. 3). Their R^2 values ranged from 8.7% to 23.9%. Except for *qMXA-10*, the additive and dominance effects of the Kinandang Patong allele at all QTLs increased MXA, by from 657 to 947 μm^2 and from 48 to 300 μm^2, respectively. By contrast, the Kinandang Patong allele at *qMXA-10* decreased MXA.

Two QTLs for MXN, *qMXN-3* and *qMXN-9*, were detected on chromosomes 3 and 9 (Table 2 and Fig. 3). Their R^2 values were 13.4% and 11.9%, respectively. The additive effects of the Kinandang Patong alleles of the two QTLs were 0.14 and 0.15, respectively, and the dominance effect values were −0.04 and 0.01.

Table 1. Coefficients of correlation among four root anatomical traits in the F_3 population derived from IR64 × Kinandang Patong

	STA	MXA	MXN
MXA	0.9234**		
MXN	0.5597**	0.6112**	
RTH	0.8791**	0.7625**	0.4084**

STA, stele transversal area; MXA, total area of late metaxylem vessels; MXN, number of late metaxylem vessels; RTH, root thickness. ** $P<0.01$.

Two QTLs for RTH, *qRTH-1* and *qRTH-2*, were detected on chromosomes 1 and 2 (Table 2 and Fig. 3). The R^2 values of *qRTH-1* and *qRTH-2* were 21.0% and 19.3%, respectively. The additive effects of the Kinandang Patong alleles of the two QTLs were 41.9 and 40.1 μm, respectively, and their dominance effects were 15.9 and 21.6 μm.

Among the 10 QTLs detected in this study, some QTLs for different traits were detected in particular chromosomal regions (Fig. 3). QTLs *qSTA-9* and *qMXA-9* overlapped near InDel marker ID07_14 on chromosome 9, and *qSTA-2* and *qRTH-2* both lay near SSR marker RM262 on chromosome 2. Other QTLs were each located in a different chromosomal region.

Discussion

Many researchers have analyzed QTLs for RTH in rice because it is an important trait associated with drought avoidance (reviewed by Price *et al*. 2002a). On the other hand, no genetic analysis of stele and xylem structures in root has been performed. It may be more important to focus

Table 2. Putative QTLs for four root anatomical traits detected in the F_3 population derived from IR64 × Kinandang Patong

Traits	QTL	Chr.	Nearest marker	cM[a]	LOD[b]	A[c]	D[d]	R^2 [e]
STA	*qSTA-2*	2	RM262	0.0	7.0	3612	1123	14.7
	qSTA-9	9	ID07_14	1.2	7.6	3434	−88	16.2
								34.3[f]
MXA	*qMXA-2-1*	2	OSR9A	0.0	4.9	657	219	8.7
	qMXA-2-2	2	S5302	3.0	6.8	796	300	16.3
	qMXA-9	9	ID07_14	1.2	11.2	947	48	23.9
	qMXA-10	10	ID12_14	0.0	5.0	−673	−121	9.5
								52.3[f]
MXN	*qMXM-3*	3	RM168	1.5	5.0	0.14	−0.04	13.4
	qMXN-9	9	RM201	0.0	4.7	0.15	0.01	11.9
								26.7[f]
RTH	*qRTH-1*	1	RM3810	3.2	7.6	41.9	15.9	21.0
	qRTH-2	2	RM262	0.0	7.9	40.1	21.6	19.3
								41.3[f]

[a] Genetic distance from the QTL LOD peak to the nearest marker.

[b] LOD thresholds for CIM of STA, MXA, MXN, and RTH were 4.0, 4.0, 3.8, and 3.9, respectively.

[c] Additive effect of the allele from Kinandang Patong compared with that from IR64.

[d] Dominance effect of the allele from Kinandang Patong compared with that from IR64.

[e] Percentage phenotypic variance explained by each QTL.

[f] Percentage phenotypic variance explained by multiple QTLs.

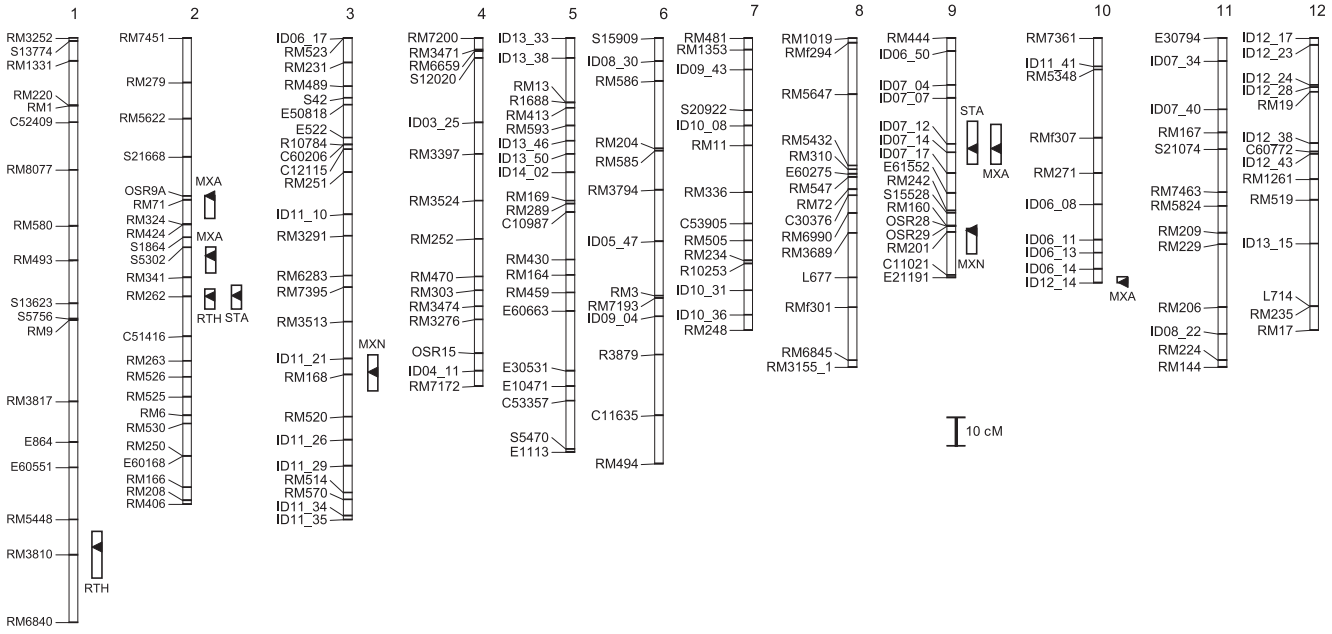

Fig. 3. Chromosomal locations of QTLs for four root anatomical traits in rice. Chromosome numbers are indicated above each linkage map. Marker names are indicated on the left side of each linkage map. Triangles and white boxes on the right of each chromosome represent LOD peaks of putative QTLs and their one-LOD support intervals (Lynch and Walsh 1998), respectively.

on stele and xylem structures than on RTH from the viewpoint of water uptake from root to shoot. If the genes affecting the development of the stele and xylem are different from those affecting RTH, we should consider improving the stele and xylem structures independently from RTH. In this study, to clarify the genetic relationship between stele and xylem structures and RTH, we analyzed QTLs of stele and xylem structures in F_2 and F_3 populations. As a result, we successfully identified eight QTLs for stele and xylem structures. Total phenotypic variance of the detected QTLs, estimated by the MIM model, was almost equivalent to the heritability of each trait in the F_3 lines. Therefore, we assumed that the major QTLs for stele and xylem structures could be found in this population. However, the accuracy of QTL detection might be limited in this population because we investigated only 14 plants in each F_3 line, which are segregated generations. To reconfirm QTL detection, we should perform further analysis using advanced backcross progeny.

The eight QTLs for stele and xylem structures were detected on chromosomes 2, 3, 9 and 10. Among them, six were found on chromosomes 2 and 9, suggesting that both chromosomal regions play a key role in developing stele and xylem structures. On chromosome 2, *qMXA-2-1*, *qMXA-2-2* and *qSTA-2* were located in the interval between OSR9A and RM262 (about 35 cM), whereas *qSTA-9* was found at ID07_14 on chromosome 9, where *qMXA-9*, which showed the largest R^2 (23.9%) among all detected QTLs, was also identified. STA was highly correlated with MXA ($r=0.923$). QTLs for several different traits showing a high level of phenotypic correlation are often detected in the same chromosomal regions (Paterson *et al*. 1991, Xiao *et al*. 1996). This QTL analysis demonstrated that the correlation between the STA and MXA can be attributed either to a tight linkage of the QTLs for the traits or to pleiotropy of one QTL. Kawata *et al*. (1979) reported that the differentiation and maturation processes of each vascular element of a nodal root in rice show close morphological correlation, which may imply that one major QTL is involved in development of both STA and MXA. Further analysis, such as fine mapping and positional cloning of the QTLs *qSTA-9* and *qMXA-9*, is needed to clarify the genetic relationship between STA and MXA during vascular system development. The two QTLs for MXN were detected in different chromosomal regions from those for STA and MXA, indicating that the number of MXVII and their total area are controlled by different genetic factors. However, MXN showed an intermediate level of correlation with MXA ($r=0.611$). When QTLs with large effects segregate simultaneously in primary populations such as the F_2 generation, it is difficult to detect QTLs with small effects (Yano and Sasaki 1997). Therefore, to reveal the genetic relationship between the MXN and MXA, fine mapping of the QTLs for both MXN and MXA using advanced backcross progeny will be necessary.

To understand the genetic relationships between stele and xylem structures and RTH, we compared the chromosomal locations of the QTLs for these traits. In the population used in this study, although we observed a high level of phenotypic correlation between stele and xylem structures and RTH (Table 1), QTLs for stele and xylem structures were not shared with the two QTLs for RTH, except for *qSTA-2* (Fig. 3). This result suggests that the genetic factors for stele and xylem structures and RTH are different. The QTL *qRTH-1* may play an important role in the development of RTH in rice because several QTLs for RTH have been

found near it in other studies (Zheng *et al*. 2000, Kamoshita *et al*. 2002a, Price *et al*. 2002b, Courtois *et al*. 2003), while only one QTL for RTH was reported near the region of the QTL *qRTH-2* (Venuprasad *et al*. 2002), according to marker positions based on physical map information published by the International Rice Genome Sequencing Project (2005). However, QTLs for stele and xylem structures were not identified in this region. On the other hand, comparison of the chromosomal locations of QTLs for RTH detected in other studies showed that *qSTA-9* and *qMXA-9* are located near regions of two RTH QTLs with small effects, detected between markers Amy3ABC and RZ228 in an IR64/Azucena population (Zheng *et al*. 2000) and at marker G385 in a Bala/Azucena population (Price *et al*. 2002b). Furthermore, in this study, although it was not statistically significant, a LOD peak (LOD score = 2.9) for RTH was observed in the interval defined by markers E61552 and RM242 on chromosome 9 near from the region detected *qSTA-9* and *qMXA-9* (data not shown). Therefore, the possibility that both stele and xylem structures and RTH are controlled by the same QTLs cannot be ruled out. To obtain a clear resolution of this problem, further analysis is required, such as fine mapping using advanced backcross progeny.

RTH is one of the most reproducible parameters among root traits, which are favorably associated with pre-heading drought avoidance (Champoux *et al*. 1995). RTH is also relatively easy to measure, even in the field (Yadav *et al*. 1997). By contrast, stele and xylem structures are laborious and time-consuming to evaluate. Therefore, many researchers interested in root morphology have focused on QTLs for RTH rather than on those for stele and xylem structures. However, our results suggest that natural variations in stele and xylem structures are generated by the combination of several alleles at QTLs different from those for RTH. This finding implies that we need to consider not only RTH but also stele and xylem structures to improve the water uptake capacity of roots. However, selection of stele and xylem structures in a large number of individuals by phenotyping is very difficult. The map information of QTLs for stele and xylem structures obtained in this study opens the way for improvement of stele and xylem structures through MAS and clarifies the genetic mechanism of their natural variation.

Acknowledgments

We thank Drs. S. Morita, J. Abe and M. Kondo for their technical advice regarding the anatomical measurement of rice roots. We also thank Dr. T. Hayashi for statistical advice regarding the genetic analysis and Dr. S. Yamanaka and the staff of the technical support section of the NIAS for field management and support during sampling. We are grateful to Dr. S. Fukuoka for constructing primers of InDel markers. This work was supported by a grant from the Ministry of Agriculture, Forestry and Fisheries of Japan (Agri-Genome Project QT-2001).

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Appendix. List of 43 InDel markers

Locus	Chromosome	PCR product in Nipponbare (bp)	Forward primer	Reverse primer	Anealing temp.
ID06_17	3	216	ttcgattcgacggtgtatca	tccattaatcgggaactcca	60
ID11_10	3	160	gtgggtccagtggcaaatag	ggtttctagcccaacattgc	60
ID11_21	3	165	gggtcccacatgtcattctc	cctctgattcgtctgcacagt	60
ID11_26	3	239	gaggcaaagctggatggtta	cccatggctgcacttatttt	60
ID11_29	3	243	tgaatcccccatagaagtgc	cccatgtgatgttgatgtgg	60
ID11_34	3	173	tgtgttgtgtggaagggaaa	tgcaagcaaccaaacacttc	60
ID11_35	3	203	cctttcttcctcctcgatcc	ggagagcagctggtgaagat	60
ID03_25	4	187	tttacacggaacttaggtcaga	tctcgcaatttacactccat	60
ID04_11	4	154	gccgatgagaatttggtcc	gttcgtaaggttcgggtgaag	60
ID13_33	5	169	gctgttggactcccgagata	tccgccctctcggtattctt	60
ID13_38	5	225	gagctgatgggatcggttttt	tagtgctcgtactgccgacctt	60
ID13_46	5	152	tgttcctctccttcctcagca	ttgctagggattgcctggag	60
ID13_50	5	155	gatgcgcgttgtcagagatg	tgtctcctctgcatgcgact	60
ID14_02	5	164	tgcaccttgtcaagcgtctc	ttttgtactgcaagggcgttag	60
ID08_30	6	189	agatgcaagcgggtatgaac	gaagcacagtcacaggacca	60
ID05_47	6	170	tcgttgagccctcatcgtct	cacccgatccagagtcttgtt	60
ID09_04	6	197	cgaaagggaaggagaaagc	agttcgtcgcacctccttt	60
ID09_43	7	248	cacccaaacacaagaaagca	attggtaattcccacgctca	60
ID10_08	7	236	ttgcctgctggctagtgaat	cgttcgcataccctatgacc	60
ID10_31	7	184	tcaccaatatgcaaccgaga	ttagcattgctttcggacct	60
ID10_36	7	192	ggtcgcctcaacttcaagag	gacaccaagcatcaactcca	60
ID06_50	9	196	cccaccctaccaatcattca	gtgttggaagaggggagatg	60
ID07_04	9	205	cgtgctaaacctggtgtaaag	ggcatagtcatgcaccctct	60
ID07_07	9	224	ggccatgaaaggtggataac	atcggagttgtgaagcaacc	60
ID07_12	9	219	ttgttgagcaatgagcgaac	ggggctctcatttcaacctt	60
ID07_14	9	174	ccatgatcaaaccacacagc	tgtcagggcaccatgactta	60
ID07_17	9	192	aaaggcagagggatctaggc	cttggcatctatcaagggttg	60
ID11_41	10	184	tggaggaattggaggatgag	tggatccggtattgaaggag	60
ID06_08	10	172	accctcgcgaatttttaagc	tgtcagtgctcggttttgtc	60
ID06_11	10	222	agggacagggatggagaagt	ctcacaagtcgatgcacaca	60
ID06_13	10	228	cgtacgctctaccacgttga	ggatggttttggagttttgg	60
ID06_14	10	247	cacgggtgaacgtaaatcct	ccttctcctttgcctttcatc	60
ID12_14	10	176	ccatggagcaagaagatggt	ggcacgtagggtgatgagat	60
ID07_34	11	216	accacacagaaatcccaagc	acaactccaggagagccaga	60
ID07_40	11	200	cagcagcagcagatcaacag	gacaacaacccctcatgaca	60
ID08_22	11	205	atgtgtatgccgtgcatgtt	gggccaaattcaatcacaac	60
ID12_17	12	172	accgtagcgttagcatggac	actacgagaatgcggtgctt	60
ID12_23	12	196	tggcttatggaagatgcaca	acgttttgctccttttcgag	60
ID12_24	12	212	ggcagaaagaataacagaccac	gaggcattttgtcaggcaat	60
ID12_28	12	250	cagctgcaacacggacttca	cgagtaccaacaaggcagca	60
ID12_38	12	187	ccctctttgccctcctgatt	tggtgttcctgcactggttg	60
ID12_43	12	243	ctcggtatagctacgttcaaatcg	cgagtgacaatcggacaattacc	60
ID13_15	12	216	tttcacgggtgcacagagatt	cgtggatctttcccatgctt	60