研究紹介

イネ地表根に関する遺伝的機構の解明

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Genetic mechanism for soil-surface roots originating from a New Plant Type rice (*Oryza sativa* L.)

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A new method of using seedling trays to evaluate root angle distribution in rice (*Oryza sativa*. L) was developed. By using this method, the root angle distributions of 97 accessions were characterized into two cluster groups ; A and B. The numbers of accessions in group A were limited, and these were categorized as shallow rooting types including soil-surface root. Group B included from shallow to deep rooting types, including both Indica and Japonica Group cultivars, lowland and upland cultivars, and landraces and improved types. An introgression line YTH16 harboring chromosome segments from a New Plant Type cultivar IR65600-87-2-2-3 with genetic background of an Indica Group rice IR 64, was included in Group A. To clarify the genetic mechanism for soil-surface rooting, quantitative trait loci (QTL) analysis was performed using hybrid populations derived from a cross between IR 64 and YTH16. A total of 8 QTLs were detected in the 3 introgressed segments on chromosomes (chr.) 2, 5 and 7. Seven chromosome segment lines (CSLs) combining these 3 QTL regions were selected from the progenies. The 2 CSLs harboring a single region (excluding the CSL with a region on chr. 5) showed high soil-surface root scores and low root vertical angles (RVA) in comparison with IR 64. Four CSLs harboring 2 or 3 regions showed high scores and low RVAs in comparison with YTH16 and the CSLs harboring a single QTL region. These results indicated that the soil-surface and shallow rooting of YTH16 was controlled by the 2 major QTLs' regions on chrs. 2 and 7, and that chr. 5 particularly played a role for supporting the effect with them.

Key words : root angle distribution, soil-surface root, New Plant Type, QTL, rice (*Oryza sativa* L.)

Introduction

Genetic improvements of root angle distribution, including soil-surface rooting and deep rooting, have been considered as important challenges in rice (*Oryza sativa* L.) yield production. For example, Hanzawa *et al.* (2013)[1], Ueno and Sato (1989)[2] mentioned that soil-surface rooting could help plants to absorb oxygen on the soil surface and avoid hypoxic conditions in waterlogged conditions. To establish breeding strategies for suitable rice cultivars adapted to waterlogged conditions, genetic variation in rice root angle distribution and genetic mechanism for soil-surface rooting will need to be clarified.

Several studies have investigated genetic variations in root angle distribution of rice cultivars. Ueno and Sato (1989)[2] evaluated the number of soil-surface roots in 56 rice cultivars in paper pots. They found that the Indonesian Japonica Group landrace ecotype Bulu formed soil-surface roots. Ueno and Sato (1992)[3] examined wide variation in the growth angles of four crown roots at 5 days after sowing on solidified agar in 130 rice cultivars by using a five-score scale. Oyanagi *et al.* (1993)[4] developed a method of measuring the growth angles of wheat cultivar roots emerging from a meshed hemispherical basket buried in the soil. Kato *et al.* (2006)[5] introduced the basket method to investigate root distribution of rice cultivars and found genetic variation in the frequencies of higher root growth angle (> 50°), defined as deeper roots, in 12 rice cultivars. On the basis of this information, Uga *et al.* (2009)[6] investigated the ratios of deeper roots in 59 rice cultivars by using the basket method. These studies focused on the frequencies of soil-surface or deeper roots in rice plants, and did not consider the whole distribution of roots in detail.

In previous studies, genetic analyses for the growth angle of roots, including soil-surface and deep rooting, have been performed. Norton and Price (2009)[7] detected 2 QTLs for growth angle of seminal roots on chrs. 6 and 11, and 4 QTLs for degree of wavy roots on chrs. 2 (2 QTLs), 3 and 11. By means of genome-wide association mapping, Bettembourg *et al.* (2017)[8] detected 15 QTLs for root growth angle in Indica Group accessions, and 40 in Japonica Group accessions. A major QTL for soil-surface rooting, *qSOR1*, was identified on chr. 7 from Gemdjah Benton[9]. A mutant gene for soil-surface rooting, *sor1*, on chr. 4 was isolated[1]. Recent studies have

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also detected 6 major QTLs for deep rooting, which is considered an opposite trait with respect to soil-surface rooting : *DRO1* on chr. 9[10, 11], *DRO2* on chr. 4[12], *DRO3* on chr. 7[13], *DRO4* on chr. 2 and *DRO5* on chr. 6[14], and *qRDR-2* on chr. 2[15]. Several of these QTLs are presumed to control the growth angle of roots of rice plants through their interactions or relationships. Norton and Price (2009)[7] found 2 epistatic interactions for growth angle of seminal roots and degree of wavy roots. The 5 major QTLs for deep rooting — *DRO1*[10, 11], *DRO2*[12], *DRO3*[13] and *DRO4* and *DRO5*[14] — were detected in the same Japonica Group cultivar Kinadang Patong. No epistatic interactions between those QTLs have been found[13, 14], but the deep rooting of Kinandang Patong was presumed to be controlled by the relationships between these several QTLs. Therefore, the root angle distribution might be also controlled by several genetic factors and their interactions or relationships.

Tomita *et al.* (2017)[16] developed a method which can be used to evaluate the growth angles of each of the crown roots at the seedling stage by allocating one of 9 scores ; they then used this method for evaluating root angle distribution in detail.

This study aimed to clarify genetic variation for root angle distribution in 97 rice accessions, using the method of Tomita *et al.* (2017)[16], and then found that an introgression line with the genetic background of Indica Group cultivar IR 64, YTH16, forms soil-surface rooting. Using hybrid populations derived from a cross between IR 64 and YTH16, QTL(s) were detected for soil-surface root scores by visual evaluation. Then, to confirm the effect(s) and the relationships of the QTLs, the scores and the root angle distribution using chromosome segment lines(CSLs) harboring different combinations of the QTL region(s) in an IR 64 genetic background were investigated.

Materials and Methods

1. Plant materials

For analysis of genetic variation in root angle distribution, a total of 97 rice accessions, including Indica and Japonica Groups, different ecosystems for rice cultivation, lowland and upland, and landrace and improved types, were used.

A total of 89 F_2 plants and F_3 family lines derived from a cross between IR 64 and YTH16 were developed at Tropical Agriculture Research Front, Japan International Research Center for Agricultural Sciences(JIRCAS), Ishigaki, Okinawa, Japan, in order to perform QTL analysis for soil-surface rooting.

Chromosome segment lines(CSLs) harboring different combination(s) of homozygous alleles for QTL region(s), were developed based on a marker-assisted selection method. Based on the genotype data, the F_4 plants harboring different combination(s) of QTL region(s) were selected from those generated from the F_3 plants, and were self-pollinated to produce F_5 and F_6 generations. Each plant was designated as a CSL.

2. Evaluations for root angle distribution and soil-surface root score

The 97 rice accessions and F_5 CSLs were used for the investigation of root angle distribution by using bottomless seedling trays following the method of Tomita *et al.* (2017)[16]. Fourteen-day-old seedlings were collected from the seedling tray, and the growth angles(°) of the crown roots in water were measured from the horizontal line of the water-surface with a protractor and classified according to a 9-score scale (10°－90°). The sum of the number of crown roots at each score on the scale on each plant was defined as the total root number (TRN). The mean of the root vertical angles (RVA) in each plant was calculated by using the following equation : RVA(°) = (Sum of scale values in all crown roots/TRN). The average value of 6 plants for each line was used as the representative data.

Degree of soil-surface rooting for each of the 89 F_2 plants and each plant of the 89 F_3 family lines, and the F_6 CSLs, were investigated by visual evaluation based on 6 scores, from 0 (no soil-surface roots) to 5 (many). The investigations of the F_2 plants were done at ripening stage ; investigations of the F_3 family lines were at maximum tiller stage, full heading stage and ripening stage ; and investigations of the F_6 CSLs were at maximum tiller stage. The mean values of 20 plants at maximum tiller and full heading stages and 10 plants at ripening stage were used as the representative data for each F_3 family line. In the F_6 CSLs, the mean values of 10 plants for each line were used as the representative data.

Soil-surface rooting at seedling stage in the F_3 family lines was also investigated using plastic trays following the method of Tomita and Fukuta(2019)[17] with a score from 0(no soil-surface roots) to 4(many). The average value of 28 plants in each family line was used as the representative data.

3. Genotyping and statistical analysis

The 97 accessions were classified on the data for the

number of roots on each score of the scale by using Ward's hierarchical clustering method[18] with the computer program JMP 11.2.0 (JMP Statistics and Graphics Guide, SAS Institute, Inc., Cary, NC, USA).

A total of 467 simple sequence repeat (SSR) markers[19] were used to survey DNA polymorphisms between IR 64 and YTH16 on 12 chromosomes. Using the polymorphism markers, the F_3 bulk DNA extracted from 10 plants in each family line was genotyped to construct a linkage map and perform QTL analysis. Linkage map was constructed using the software JoinMap 4 (Kyazma B. V., Wageningen, Netherlands). Genetic distances (cM) between the markers were estimated by using the Kosambi mapping function. Based on the linkage map, QTL analysis for soil-surface root score was performed by using composite interval mapping with Windows QTL Cartographer software (ver. 2.5.1)[20]. Putative QTL(s) were determined from the maximum logarithm of the odds (LOD) score. A LOD score >2.5 was used as threshold. The additive effect and the percentage of phenotypic variance explained by each QTL (R^2) were also estimated at the maximum LOD score.

Results

1. Variation of root angle distribution in 97 rice accessions

We found a wide variation in root angle distributions of 97 rice accessions using the seedling tray method (Fig. 1). The RVA values of these accessions varied from 52.3° to 81.2° (average 71.8°). TRN varied from 3.3 to 8.8 (average 6.1). There was no correlation between RVA and TRN ($r=-0.13$, ns).

The 97 accessions were classified into two cluster groups, A and B (Fig. 1). Six accessions ; Gemdjah Benton, NERICA4 and Trembese, IR 36, Tohoku U-3-7 and YTH16 in group A had lower RVA and higher TRN values (average 58.2° and 7.0) than those of group B. The other 91 accessions were classified into group B. The RVA and TRN of group B were 72.7° and 6.0, respectively. Group B included from shallow to deep rooting types ; including both Indica and Japonica Group cultivars, lowland and upland cultivars, and landraces and improved types. Thus, the accessions in group A were categorized as shallow rooting type including soil-surface roots.

2. QTLs for soil-surface root score

Among the accessions of group A, YTH16 was selected as soil-surface rooting line. At all growth stages, higher

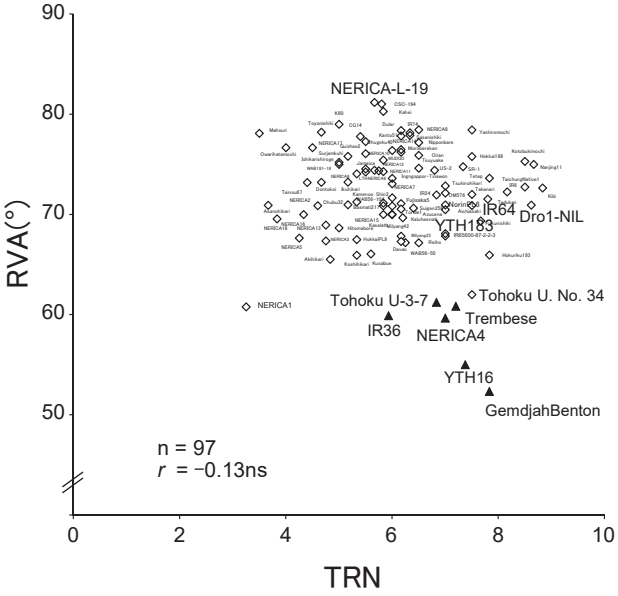

Fig. 1 Relationship between TRN and RVA in 97 rice accessions.
TRN, total root number. RVA, root vertical angle. These accessions were classified into two cluster groups ; A (closed triangles) and B (open diamonds), on the basis of data on the numbers of roots with each of nine angle scores. ns ; not significant at $P=0.05$.

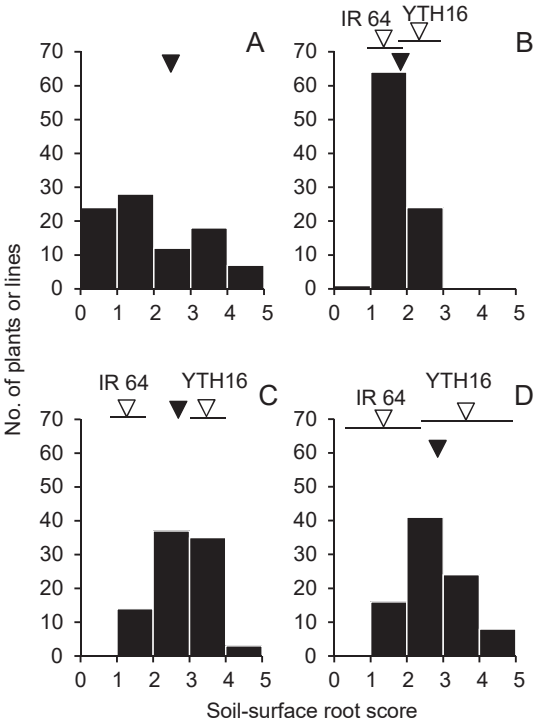

Fig. 2 Segregation of soil-surface root scores in the 89 F_2 plants and F_3 family lines.
White triangles and horizontal lines indicate the means ± SD in their parents. Black triangle indicates the mean of the hybrid population in each growth stage.
A, Ripening stage in the F_2 plants ; B, Maximum tiller stage in the F_3 family lines ; C, Full heading stage in the F_3 family lines ; D, Ripening stage in the F_3 family lines.

degrees of soil-surface root scores were found in YTH16 than IR 64 (Fig. 2). Wide continuous distributions of the scores were observed at each growth stage in the 89 F_2 plants and F_3 family lines.

Thirty-three SSR markers on chrs. 2(7 markers), 3(1)4(6), 5(3), 7(7) and 8(9) among 467 showed DNA polymorphisms between IR 64 and YTH16 (data not shown). The linkage map composed of the 32 markers, excluding the sole marker, *RM5474*, on chr. 3, covered the regions of chrs. 2, 4, 5, 7 and 8.

A total of 8 QTLs for soil-surface root scores were only detected in the regions of chrs. 2(1 QTL), 5(1) and 7(6) at the different growth stages in the 2 populations (Fig. 3, Table 1). The 6 QTLs were detected in the region on chr. 7 in the F_2 plants at the ripening stage ($qSFR7^a$), and in the F_3 family lines at seedling ($qSFR7^b$), maximum tiller ($qSFR7^c$), full heading ($qSFR7^d$) and ripening stages ($qSFR7.1^e$ and $qSFR7.2^e$). The peaks of QTLs were detected between markers of *RM6344* and *RM21950*. The LOD scores and the R^2 values were all larger than those of the other 2 QTLs on chrs. 2 and 5, except for the R^2 value of $qSFR7.2^e$. All the YTH16 alleles of the QTLs increased in score.

The other 2 QTLs, $qSFR2^e$ and $qSFR5^e$, were

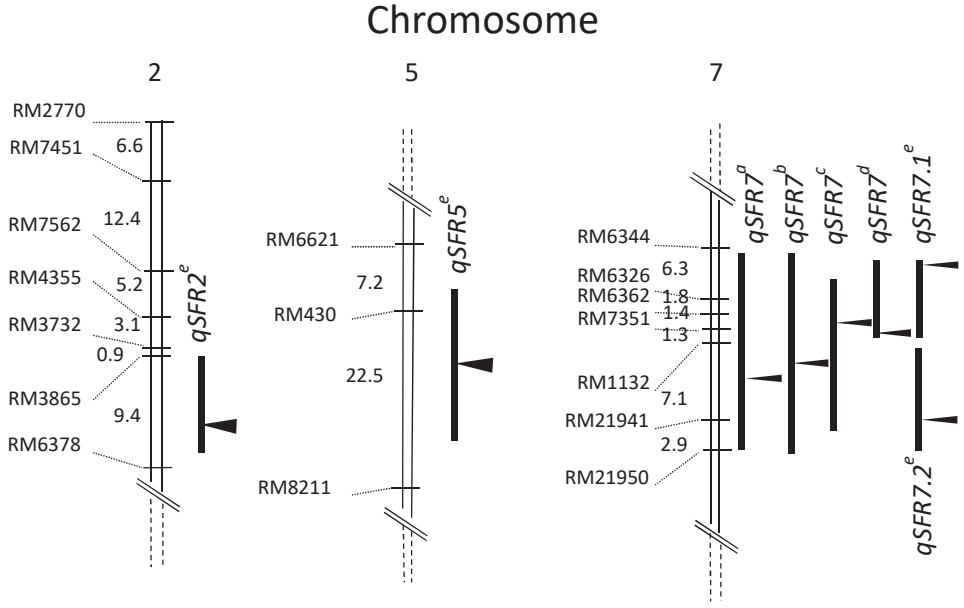

Fig. 3　QTLs for soil-surface root score detected in the 89 F_2 plants and F_3 family lines.
Genetic distances (cM) between SSR markers were calculated. Arrowheads and black bars to the right of each linkage map indicate the LOD peaks of putative QTLs and their support intervals, respectively.

Table 1　QTLs for soil-surface root score detected in the 89 F_2 plants and F_3 family lines

QTL	Chr.	Marker interval	LOD score	Additive effect	R^2 (%)	Positive allele
F_2 plants						
$qSFR7^a$	7	RM1132 – RM21941	21.2	−1.3	69.5	YTH16
F_3 family lines						
$qSFR7^b$	7	RM1132 – RM21941	24.6	−0.5	71.6	YTH16
$qSFR7^c$	7	RM6362 – RM7351	12.0	−0.4	45.8	YTH16
$qSFR7^d$	7	RM7351 – RM1132	10.3	−0.6	36.3	YTH16
$qSFR2^e$	2	RM3865 – RM6378	2.9	−0.4	10.9	YTH16
$qSFR5^e$	5	RM430 – RM8211	3.6	−0.3	20.6	YTH16
$qSFR7.1^e$	7	RM6344 – RM6326	9.2	−0.6	37.6	YTH16
$qSFR7.2^e$	7	RM21941 – RM21950	4.5	−0.5	13.5	YTH16

The marker nearest the peak LOD score is underlined. Negative and positive values of the additive effect indicate an increase or decrease, respectively, in the values of the traits with YTH16 and IR 64 alleles.
Superscript letters : a, F_2 plants at the ripening stage ; b, F_3 family lines at seedling stage ; c, F_3 family lines at maximum tiller stage ; d, F_3 family lines at full heading stage ; e, F_3 family lines at ripening stage.

detected only at ripening stage in F_3 family lines. The YTH16 alleles of these QTL increased in score. The peak of *qSFR2*^{e} was found between *RM3865* and *RM6378* on chr. 2, and the nearest marker was *RM3865*. The peak of *qSFR5*^{e} was detected between *RM430* and *RM8211* on chr. 5, and the nearest marker was *RM430*.

3. CSLs with different combinations among QTLs

To develop 7 CSLs harboring 7 different combinations of 3 QTL regions on chrs. 2, 5 and 7, the 3 regions between markers *RM7451* and *RM6873* on chr. 2, *RM430* and *RM6621* on chr. 5 and *RM7351* and *RM21950* on chr. 7 were used as the QTL regions. Based on the genotype data, a total of 7 F_4 plants were selected among the 116 F_4 individuals. Each of the F_4 plants were designated as follows : CSL(2) for a region on chr. 2, CSL(5) for a region on chr. 5, CSL(7) for a region on chr. 7, CSL(2+5) for regions on chrs. 2 and 5, CSL(2+7) for regions on chrs. 2 and 7, CSL(5+7) for regions on chrs. 5 and 7, and CSL(2+5+7) for regions on chrs. 2, 5 and 7.

Investigations of soil-surface root scores in the 7 CSLs, IR 64 and YTH16 were carried out(Table 2). The scores of YTH16 were significantly higher than those of IR 64. CSL(5) showed scores similar to those of IR 64 and these were the lowest among them. The other 6 CSLs showed scores significantly higher than those of IR 64, and they tended to show scores higher than those of YTH16. These results indicated the major single effects for soil-surface root score of the regions on chrs. 2 and 7.

The root angle distributions of the 7 CSLs in F_5, IR 64 and YTH16 were investigated (Table 2). The crown roots of IR 64 were distributed in 7 scores ranging from 30° to 90°. In YTH16, roots were distributed across all scores. The RVA of YTH16 was significantly higher than that of IR 64. The crown root scores of CSL(2) ranged from 20° to 90°, with 2 peaks at scores of 40° and 70° in the distribution. The RVA was intermediate between those of IR 64 and YTH16. The roots of CSL(5) varied in the same range of IR 64 with RVA, 64.6°, and the distribution showed 2 peaks at scores of 50° and 80°. CSL(7) showed roots distributed in all scores, with an RVA, 48.4°, and had 2 peaks, at scores of 30° and 60°. This CSL had RVA and range of root angle scores similar with those of YTH16. The RVAs of CSL(2) and CSL(7) were significantly lower than that of IR 64, and not different from that of YTH16. These results indicated that the 3 QTL regions on chrs. 2, 5 and 7 each had different effects with respect to root angle distribution, and the effects of chrs. 2 and 7 on decreasing root angles were larger than the effect of chr. 5. In CSL(2+5), CSL(2+7) and CSL(5+7), the roots were distributed in all scores, except for CSL(5+7) at 70°. The RVAs ranged from 40.2° to 47.5°. There were 2 peaks at scores of 20° and 40° in the distribution of CSL(2+5), and at 30° and 40° in that of CSL(2+7). CSL(5+7) had a mode at score of 30°. The roots of CSL(2+5+7) were found in all scores, with RVA 44.7°. The RVAs of these 4 CSLs har-boring 2 or 3 QTL regions were lower (but not significantly so) than those of YTH16 and the 3 CSLs harboring a single QTL region, and the RVAs of CSL(2+5) and CSL(5+7) were lower than that of CSL(2+7). The effects for distributions and RVAs of single QTL on chrs. 2 and 7 were changed, when the QTL on chr. 5 was combined with the other two QTLs detected. These results suggested that the shallow rooting of YTH16 was controlled by the 3 QTLs' regions, and that chr. 5 particularly played a role for supporting the effect with others.

Table 2 Soil-surface root score and root angle distributions in CSLs

Parents/CSL	Soil-surface root score				Frequency of crown roots at each score (%)									RVA (°)			
					10°	20°	30°	40°	50°	60°	70°	80°	90°				
IR 64	0.2	±	0.42	a	0.0	0.0	3.7	4.8	12.4	21.2	26.3	18.6	13.1	67.0	±	2.60	a
YTH16	2.1	±	0.32	b	1.9	13.1	9.9	12.3	28.8	18.8	2.1	1.9	11.3	49.5	±	4.00	bc
CSL (2)	2.3	±	0.48	b	0.0	8.3	14.5	22.5	10.4	7.2	19.5	9.4	8.1	53.1	±	5.32	bd
CSL (5)	0.0	±	0.00	a	0.0	0.0	6.5	4.5	21.5	15.9	18.7	21.9	11.0	64.6	±	6.70	ad
CSL (7)	2.1	±	0.32	b	4.6	12.1	20.5	13.0	8.8	14.1	10.7	7.4	8.8	48.4	±	7.43	bc
CSL (2+5)	2.3	±	0.48	b	3.3	14.6	12.7	20.9	15.9	7.9	9.3	8.9	6.5	47.5	±	11.64	bc
CSL (2+7)	2.0	±	0.00	b	3.3	10.9	16.5	14.1	22.6	18.2	9.0	3.7	1.7	46.1	±	3.14	bc
CSL (5+7)	2.2	±	0.63	b	4.2	4.2	39.5	24.7	13.1	6.3	0.0	3.9	4.2	40.2	±	4.36	c
CSL (2+5+7)	2.4	±	0.52	b	2.1	14.1	21.7	20.7	15.1	4.8	13.5	2.4	5.7	44.7	±	4.90	bc

The score and RVA are shown as the average ± SD.
Within the same column, the values denoted by the same letter are not significantly different from one another at $P=0.05$ according to the Turkey-Kramer test.

Discussion

Use of the seedling tray method[16] revealed a wide variation in root angle distribution among the 97 rice accessions ; they were classified into two cluster groups, A and B (Fig. 1). These groups showed differences in RVAs, TRNs, and root distributions. Group A consisted of six accessions : Gemdjah Benton, Trembese, YTH16, IR 36, NERICA4 and Tohoku U-3-7. Ueno and Sato (1989)[2] reported that Gemdjah Benton was an Indonesian Japonica Group cultivar and included in the ecotype Bulu ; it has soil-surface roots. Uga *et al.* (2012)[9] found a major QTL for soil-surface rooting, *qSOR1*, in Gemdjah Benton. The donor parent of YTH16 — the NPT cultivar IR 65600-87-2-2-3 — was bred from a cross between the Chinese Japonica-Group cultivar Shen Nung 89-366 and the Indonesian Japonica-Group cultivar Ketan Lumbu[21, 22]. Trembese is also an Indonesian Japonica Group cultivar[23]. IR 36 is an Indica Group cultivar bred at IRRI ; its pedigree involves 16 landraces, including an Indonesian landrace Benong[24]. These accessions in group A might harbor genetic factors originating from Indonesian Japonica Group cultivars or other upland cultivars, and these might have contributed to the shallow rooting type of this group. We found that the number of accessions in group A was limited. Thus, shallow rooting type might be limited in natural variations of rice.

A total of 8 QTLs for soil-surface root score were detected from YTH16 on the 3 regions of chrs. 2, 5 and 7 at different growth stages in the 2 populations (Fig. 3, Table 1). A QTL for soil-surface rooting, *qSOR1*, was mapped between the markers of *RM21941* (24.77 Mbp) and *RM21976* (25.59 Mbp) on chr. 7[9]. A QTL for deep rooting, *DRO3*, was also detected between the markers of *RM6885* (23.00 Mbp) and *RM5397* (23.95 Mbp) on chr. 7, originating from the Philippine Japonica Group cultivar Kinandang Patong[13]. Uga *et al.* (2015)[13] indicated that *qSOR1* and *DRO3* had different locations on chr. 7, by comparing the positions between them on a physical map. Based on the physical and linkage maps, the 6 QTLs detected on chr. 7 in this study were mapped over the region of *qSOR1*. In contrast, no QTLs were located over the regions on which *DRO3* was mapped. Thus, the QTL(s) in this study might correspond with *qSOR1*. In contrast, no QTL related to soil-surface rooting has been reported in *qSFR2*^{e} and *qSFR5*^{e}. The LOD scores and R^2 values of the 6 QTLs on chr. 7, except for the R^2 value of *qSFR7.2*^{e}, were higher than those of *qSFR2*^{e} and *qSFR5*^{e} (Table 2). Therefore, the QTL on chr. 7 played the major role for soil-surface rooting in YTH16 among them.

Using the 7 CSLs, the major single effects for soil-surface root score of the regions on chrs. 2 and 7 were demonstrated (Table 2). And 3 QTL regions might affected root angle distribution together. In particular, the effects for distributions and RVAs of single QTL on chrs. 2 and 7 were changed, when the QTL on chr. 5 was combined with the other two QTLs detected. The QTL on chr. 5 might play a role in supporting the effect of the other 2 regions on chrs. 2 and 7 in decreasing root angle score. Norton and Price (2009)[7] found 2 epistatic interactions for growth angle of seminal roots and degree of wavy/curly roots. The deep rooting of Kinandang Patong is presumed to be controlled by these several major QTLs ; *DRO1*[10, 11], *DRO2*[12], *DRO3*[13] and *DRO4* and *DRO5*[14]. This study indicated that soil-surface rooting in YTH16 is controlled mainly by the effect of the combinations of the 3 QTL regions. More detailed analysis using materials with segregation among 3 regions will be needed to confirm their relationship.

In this study, wide genetic variation in root angle distribution of rice was clarified using the method of Tomita *et al.* (2017)[17] Among the variations, an introgression line, YTH16, forms soil-surface roots. From YTH16, 3 QTL regions on chrs. 2, 5 and 7 for soil-surface rooting were found. Using 7 CSLs harboring 7 kinds of combinations of 3 QTL regions, it demonstrated that the soil-surface rooting of YTH16 is controlled mainly by the effect of the combinations of these regions. The information of the evaluation method, the genetic variation, these QTLs, the CSLs and YTH16 will be useful for genetic improvement of root architecture in rice varieties.

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