

Roles of Root Aerenchyma Development and Its Associated QTL in Dry Matter Production under Transient Moisture Stress in Rice

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Abstract: Enhanced aerenchyma development in rice under transient drought-to-waterlogged (TD-W) stress promotes root system development by promoting lateral root production. This study analyzed the quantitative trait loci (QTLs) associated with the plasticity in aerenchyma development under TD-W stress. A mapping population of 60 F₂ genotypes of chromosome segment substituted lines (CSSL) derived from CSSL47 and Nipponbare crosses were grown in rootboxes and evaluated for shoot and root growth, and aerenchyma development (expressed as root porosity). The TD-W stress was imposed starting with water saturated soil condition at sowing and then to progressive drought from 0 to 21 days after sowing (DAS) prior to exposure to sudden waterlogging for another 17 days (21 to 38 DAS). We performed simple and composite interval mapping to identify QTLs for aerenchyma development. QTL associated with aerenchyma development was mapped on the short-arm of chromosome 12 and designated as *qAER-12*. The effect of *qAER-12* on the plasticity in aerenchyma development under TD-W was significantly associated with the increase in lateral root elongation and branching. This resulted in greater root system development as expressed in total root length and consequently contributed to higher dry matter production. This *qAER-12* is probably the first reported QTL associated with aerenchyma development in rice under TD-W and is a useful trait for the improvement of the adaptive capability under fluctuating soil moisture conditions.

Key words: Aerenchyma development, Chromosome substitution lines, Quantitative trait locus, Root plasticity, Root porosity, Transient drought-to-waterlogged.

Rice (*Oryza sativa*) is grown on more than 100 million ha and about 89% of it is grown in Asia (Serraj et al., 2009). Most of Asia's rice fields are frequently exposed to different extent and types of abiotic stresses such as drought (aerobic) and waterlogging (anaerobic) conditions. These stresses are transiently recurring in the field due to the intermittent rainfall patterns and inefficient irrigation systems (Zeigler and Puckridge, 1995; Wade et al., 1998; Boling et al., 2004). The inability of the plants to acclimate to drought and oxygen (O₂) deficiency brought about by fluctuating soil moistures have been shown to reduce the dry matter production and yield in rice (Suralta and Yamauchi, 2008; Suralta et al., 2008a, 2008b; Suralta et al., 2010; Niones et al., 2012). Since rice roots are at the forefront of acclimation to fluctuating

moisture conditions, component root traits other than those contributing to drought tolerance are important for coping with the aerobic-anaerobic transitions at all growth stages of the plant.

The function of roots in deep soil is usually supported by uptake of O₂ directly from the air-filled soil pores in aerobic soil, whereas in anaerobic waterlogged soil, O₂ is supplied mainly via internal diffusion through aerenchyma (Insalud et al., 2006). Thus, the aerobic-to-anaerobic transitions brought about by the changes in soil water regimes may affect root development, physiological functions and shoot growth. On the other hand, under constant waterlogged soil, plant roots can acclimate to O₂ deficiency by developing shallow roots to maximize the efficiency of supplying atmospheric O₂ via enhanced

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Abbreviations: CSSL, chromosomes segment substitution lines; LR, lateral root; QTL, quantitative trait locus; TD-W, transient drought-to-waterlogged.

aerenchyma formation (Justin and Armstrong, 1987; Drew, 1997; Colmer, 2003; Insalud et al., 2006). The aerenchyma formation brought about by the disintegration of the root cortex provides a low resistant pathway for internal O₂ diffusion to maintain aerobic respiration and energy production, and thus to sustain nutrient absorption (Jackson and Armstrong, 1999; Evans, 2003) and other root rhizosphere activities (Vartapetian and Jackson, 1997; Wang and Yamauchi, 2006).

In rice, it is generally believed that plants can grow and acclimate to waterlogging (anaerobic) conditions by increasing the number of nodal roots with enhanced aerenchyma, which presumably is the basis for waterlogging tolerance (Colmer, 2003; Suralta and Yamauchi, 2008; Suralta et al., 2010). Colmer (2003) reported that rice plants grown in drought (aerobic) conditions have fewer but deeper nodal roots with lower root porosity, and more extensive root system development than rice grown in waterlogged (anaerobic) conditions. On the other hand, we reported that fluctuating moisture environment was stressful to rice plant (Suralta et al., 2010; Niones et al., 2012).

Our previous studies demonstrated that waterlogging after drought and vice versa significantly reduced shoot dry matter production (Suralta et al., 2010; Niones et al., 2012) and yield in rice (Niones et al., 2012). Root plasticity response in terms of promoted lateral root development and aerenchyma formation is essential for rice adaptation under soil moisture fluctuation conditions (Niones et al., 2012). Consequently, the root plasticity or the ability of plants to alter root phenotypes developmentally and functionally in response to changing environmental conditions has been suggested (O'Toole and Bland, 1987) and validated to play significant roles in plant adaptation to such kind of environment (Yamauchi et al., 1996; Bañoc et al., 2000; Siopongco et al., 2005; Wang and Yamauchi, 2006; Suralta and Yamauchi, 2008; Suralta et al., 2008a, 2008b, 2010; Niones et al., 2012).

Rice root systems are comprised mainly of lateral roots (LRs), which can be classified as L and S types. These types of LRs have been shown to differ in anatomy, morphology, developmental characteristics, carbon and nitrogen dynamics (Yamauchi et al., 1987, 1996) and the genetic control of their development (Wang et al., 2005). Morphologically, the L type LRs are long, thick and capable of branching into higher-order LR, while S-type LRs are slender, short and non-branching (Yamauchi et al., 1987). The S type LRs have a less developed vascular structure than the L type (Kono et al., 1987; Rebouillat et al., 2009).

In a series of studies, we demonstrated that the plasticity in the developmental responses of LRs to different intensities of drought stress (Yamauchi et al., 1987, 1996; Wang and Yamauchi, 2006; Kano et al., 2011; Kano-Nakata et al., 2011), rewatering after drought (Bañoc et al., 2000;

Siopongco et al., 2005), transient drought after waterlogging and vice versa (Suralta et al., 2008a, 2008b, 2010) and continuous cycles of alternate waterlogging and drought (Niones et al., 2012) contributed to the maintenance of dry matter production and yield in rice. Under fluctuating soil moisture stress (drought-to-waterlogged conditions), the plasticity in L-type LR formation was associated with higher aerenchyma development (Suralta et al., 2010; Niones et al., 2012) indicating that aerenchyma development under constant waterlogging and transient drought-to-waterlogged stress conditions is an essential trait for future varietal improvement for such kind of soil hydrologic conditions. Root traits are generally controlled by quantitative trait loci (QTL) or genes. Conventional methods currently being used for selection of adaptive root traits are not efficient due to the large effects of the environment, low heritability and high cost and difficulty in accurate measurement of these traits. Thus, we need to develop an accurate, rapid and reliable screening technique for the target root traits. The use of molecular marker-assisted selection permits rapid and accurate identification of individuals that contain gene(s) for target traits, and has been proven to accelerate rice-breeding programs in drought prone environments. Thus, the QTL associated with aerenchyma formation in rice under soil moisture fluctuation needs to be identified.

Chromosome segment substitution lines (CSSL) are genetic resources containing the major genetic background of the recurrent parent with overlapping chromosome segments of the donor parent. The CSSLs can be used to effectively identify target traits since they can reduce the confounding effects by the variation in genetic backgrounds due to other traits. Available CSSLs such as the one derived from Nipponbare and Kasalath crosses have been successfully used for the precise quantification of the contribution of root plasticity to dry matter production and yield under fluctuating soil moisture environments in rice (Suralta et al., 2008a, 2008b; Niones et al., 2012). In our previous studies, one CSSL (line 47) was selected because the growth of its shoots and roots is similar to that in Nipponbare parent under non-stressed conditions but plastic lateral root development and aerenchyma formation are promoted under short-duration transient moisture stresses (Suralta et al., 2008b, 2010) and continuous cycle of waterlogging and drought (Niones et al., 2012), which contributed to the maintenance of shoot dry matter production and yield. Genetically, we assumed that the segments from Kasalath allele introgressed into the Nipponbare genetic background in CSSL47 may regulate the formation of aerenchyma under fluctuating soil moisture at the vegetative stage. However, the graphical genetic map of CSSL47 carries ten chromosome-substituted segments (RGP, 2000). In the present study, we further separated the

substituted segments by generating backcross lines between CSSL47 and Nipponbare parent. We searched for the precise location of QTL regulating aerenchyma formation and examined its contribution to root plasticity and dry matter production under TD-W stress.

Material and Methods

1. Plant materials

A total of 60 F_2 genotypes derived from the cross between CSSL47 and Nipponbare (recurrent parent) were used for QTL mapping analysis. CSSL47 was selected from 54 CSSL populations derived from the cross of Nipponbare and Kasalath, based on the unique characteristics of the development of its root system in response to soil moisture fluctuation (Suralta et al., 2010; Niones et al., 2012). CSSL47 had 10 substituted genomic segments from Kasalath distributed across 8 chromosomes in the Nipponbare background. These substituted segments are found on chromosome 3 (between loci R1927 and R1925), chromosome 4 (between loci R2373 and C734), chromosome 6 (between loci R2147 and C235), chromosome 7 (between loci C261 and R565), chromosome 8 (between loci C1107 and R202), chromosome 10 (between loci C701 and R1629, and loci C488 and C223), chromosome 11 (between loci C447 and C3 and at R1506), and chromosome 12 (between loci G24B and R617) (NIAS, 2012). Nipponbare is a *japonica* irrigated cultivar, and was used as recurrent parent of the CSSL population (Nipponbare \times Kasalath) by the Rice Genome Research Center of the National Institute of Agrobiological Sciences, Tsukuba, Ibaraki, Japan. The F_2 mapping population was further developed at the Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, Japan.

2. Cultural condition for phenotyping

Seeds of CSSL47, Nipponbare and their F_2 genotypes were soaked in water mixed with benomyl fungicide (0.15% w/v) and washed thoroughly prior to incubation in a seed germinator at 28°C for 36 hr prior to sowing. Pre-germinated seeds of each line were sown in a soil-filled PVC rootbox (25 cm \times 2 cm \times 40 cm, $L \times W \times H$) following the method of Kono et al. (1987) and Suralta et al. (2010). The soil was pre-mixed with fertilizer containing 60 mg nitrogen (N), 80 mg phosphorus (P) and 70 mg potassium (K). The plants in the box were exposed to continuously waterlogged (control) and transient drought-to-waterlogged (TD-W) conditions. In the control, the water level was increased and maintained at 2 cm above the soil surface from 3 days after sowing (DAS) until the end of the experiment (38 DAS). The CSSL47 and Nipponbare parents were only evaluated under continuously waterlogged (control) conditions. In the TD-W, the soil inside the boxes were subjected to drought by draining the

water and maintained to target soil moisture content (SMC) of 20% for 21 days. Thereafter, the soil inside the boxes was subjected to sudden waterlogging for another 17 days (38 DAS). Target SMC was maintained at 20% (w/w) by watering every two days. The soil moisture difference between the topmost and bottom portion of soil in the root box was 0.3%, when allowed to reach 12% SMC after 14 days without watering (Kono et al., 1987). Since the plants were watered at 2-day intervals, differences in moisture between top and bottom soil in the box should have been much smaller (Suralta et al., 2010). Phenotyping of root traits were determined at 38 DAS.

3. Phenotypic evaluations

(1) Shoot traits

Plant height, number of tillers and number of leaves, were measured prior to termination of the experiment at 38 DAS. The number of tillers was manually counted. Plant height was measured using a metric ruler. Shoots were cut and placed in a paper bag and oven dried at 70°C for 48 hr and then weighed.

(2) Root porosity

The total porosity was used to estimate root aerenchyma development. The internal gas space (porosity) may not be identical with that of aerenchyma (Armstrong, 1971). This means that the size of aerenchyma may possibly be overestimated if based on porosity. However, there is no effective method available to precisely distinguish the aerenchyma from total porosity. Besides we needed to be consistent with the method used for estimating aerenchyma in our previous studies (Suralta and Yamauchi, 2008; Suralta et al., 2010; Niones et al., 2012). Two coleoptilar nodal roots were cut and used for root porosity measurement following the microbalance method (Visser and Bögemann, 2003; Suralta et al., 2010). The coleoptilar nodal roots usually develop a few days after the emergence (Yoshida, 1981) and thus assumed to be exposed to the TD-W stress. The nodal root axis was cut and divided equally into 1-cm segments acropetally from the base. The segments were cut with a sharp razor blade and gently blotted by rolling for about two sec on tissue paper using a small brush to remove adherent water. Then, to prevent water loss through evaporation, each root segment was transferred into a tube (Seal-Rite 1.5 ml graduated microcentrifuge tube, USA Scientific Inc.) with cover, which was reset to zero (tared) on a microbalance (BM-20, A&D Co. Ltd., Tokyo, Japan). The tube was closed then weighed (w_1 in mg), and then the segment was transferred to a small vial with water, kept for 30 min, and then infiltrated with water under vacuum for 30 min. After water infiltration, again the root segments were blotted dry on tissue paper and weighed in a tube (w_2 in mg). Each segment (one capsule represents one segment) was weighed and the average for all segments was calculated

for the porosity of individual segments. Using the specific weight (SW) obtained from larger samples (1.04 g ml^{-1} ; Visser and Bögemann 2003), the porosity was calculated using the equation:

$$\text{Porosity (\% v/v)} = 100 \times (w_2 - w_1) \times \text{SW} / w_2,$$

assuming water specific weight of 1.00 g ml^{-1} .

(3) Other root traits

Roots from each plant were collected at the termination of the treatment at 38 DAS. The rootboxes were sampled using the pinboard of Kono et al. (1987). The thoroughly washed sampled roots were placed in between the perforated plastic sheet and stained with 0.25% Coomassie Brilliant Blue R aqueous solution for 72 hr. The stained root samples were then rinsed with tap water and placed in a light box for digitization using a Nikon D3000 digital SLR camera (Nikon Corporation, Japan) at $2,592 \times 3872$ resolution. The stained root samples were stored in FAA (formalin: acetic acid: 70% ethanol in 1:1:18 ratio by volume) solution after digitizing for further measurements of other root traits. The total number of nodal and lateral roots (LR) was manually counted. Each nodal root was cut into 5-cm segments, keeping the lateral roots intact. The number of LRs was counted and expressed as linear frequency (number of lateral roots per unit length of root axis; Ito et al., 2006). For total root length measurement, root samples from the FAA solution were rinsed with tap water and spread on a transparent sheet without overlaps. Digital images were then taken using an Epson scanner (ES2200) at 300 dpi resolution. Total root length was analyzed using a macro program developed by Kimura et al. (1999), and Kimura and Yamasaki (2001) on the NIH image software version 1.60 (public domain released by the National Institute of Health, USA) in a computer running on Macintosh with G4 operating system. After scanning, root samples were oven dried at 70°C for 48 hr and weighed.

4. Genotyping and marker selection

(1) Marker selection

CSSL47 had 10 substituted genomic segments of Kasalath introgressed into Nipponbare. Primer pairs were selected or designed based on the substituted Kasalath segments. A total of 20 polymorphic EST and SSR markers from the Gramene (Gramene, 2005) and Rice Genome Project (RGP, 2010) were chosen for QTL mapping analysis. The following polymorphic markers were assigned in each substituted segments at: chromosome 3 (RM85), chromosome 4 (R1854, TG85, R2373), chromosome 6 (RM276), chromosome 7 (TG114, S11633), chromosome 8 (RM331, TG134), chromosome 9 (0.8cM-HhaI), chromosome 10 (TG141, RM484, RM3123, RM5471), chromosome 11 (TG148, TG152), and chromosome 12 (TG154, RM247, RM6296, TG156).

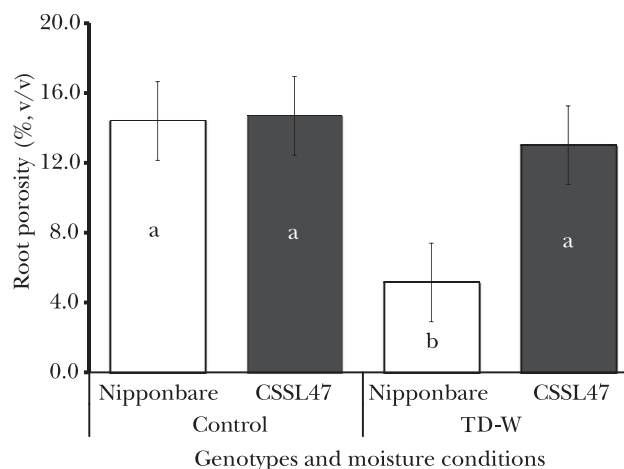


Fig. 1. The mean value of root porosity of Nipponbare and CSSL47 genotypes under continuous waterlogged (control) and transient droughted-to-waterlogged (TD-W) conditions at 38 days after sowing. Bar indicates the standard error. Data are means of three replications. The same letters in the graph represents no significant difference between the genotypes at $P < 0.05$ level in each condition.

(2) DNA extraction

Approximately 1-4 μg DNA was extracted from the fresh leaf of each genotype using the mini-preparation CTAB method (Zheng et al., 1995). The polymerase chain reaction (PCR) cocktail with a total volume of 10.0 mL/reaction was placed in costar 96-microwell PCR plates, and run in a PCR thermal cycler (MJ machines: PTC-100 thermocycler).

5. Statistical and QTL analyses

(1) Statistical analysis

The phenotypic evaluation of the Nipponbare and CSSL47 parents were laid out in a randomized complete block design with three replications. Differences between mean values of the two genotypes within treatment were compared using the least significant difference (LSD) test at $P < 0.05$ level generated using CropStat software.

(2) Construction of linkage map and QTL mapping analysis

Linkage maps were constructed from genotype data with Qgene version 4.0. The genetic distance was estimated by using the Kosambi map functions (Kosambi, 1944). The putative QTL were detected by using simple interval mapping (SIM) and composite interval mapping (CIM) functions of Qgene version 4.0 software (Joehanes and Nelson, 2008). The SIM model was employed to test the presence of associated QTL at many positions between each pair of adjacent marker loci (Lander and Bostein, 1989). The analysis point that yields the most significant association may be taken as the location of a putative QTL (Nagabhushana et al., 2006). The critical threshold value of the logarithm of odds (LOD) score was set at 2.5 to

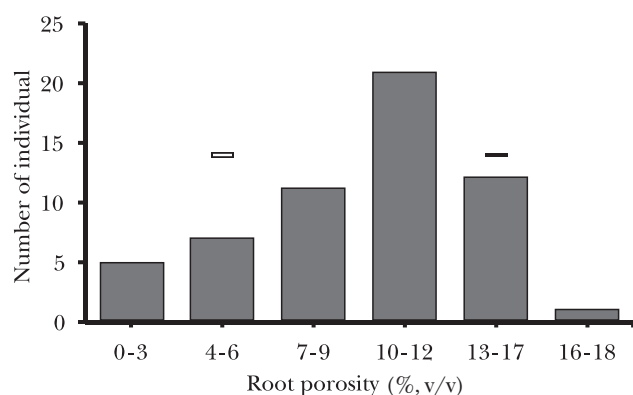


Fig. 2. Frequency distribution of F₂ CSSLs in different range of root porosity under transient drought-to-waterlogged soil conditions at 38 days after sowing. The open and solid flat boxes above the column are the means of Nipponbare (open box) and CSSL47 (solid box) genotype, respectively.

detect the QTL. The phenotypic variance explained by each QTL (R^2) was estimated at maximum LOD score.

Results

1. Phenotypic evaluation of parents

At 38 DAS, main stem leaf number was 9.8 and 9.9 in CSSL47 and Nipponbare, respectively, under both the control and TD-W conditions. The plant height of CSSL47 and Nipponbare was 58 and 56.6 cm, respectively under control conditions while it was around 64.9 cm in both genotypes under TD-W. The number of tillers per plant was 6 in both genotypes under the control condition while under the TD-W conditions, CSSL47 produced more tillers (4 per plant) than Nipponbare (2.8 per plant).

Fig. 1 shows the mean root porosity of Nipponbare and CSSL47 genotypes under the control and TD-W conditions. CSSL47 had significantly higher root porosity than Nipponbare under TD-W, though they had similar root porosity under the control condition. On the other hand, Nipponbare in TD-W had significantly lower root porosity than that in the control, but CSSL47 under TD-W had nearly the same root porosity as that in the control.

2. QTL detection

Wide phenotypic variation of the target traits between parents is important for QTL analyses. Selecting genotypes with high aerenchyma development increases the chances of detecting QTL in response to fluctuating soil moisture. The frequency distribution of aerenchyma development represented by root porosity in the F₂ population is shown in Fig. 2. A wide phenotypic variation among F₂ genotypes in aerenchyma formation under TD-W was observed, ranging from 1 to 18% (Fig. 2). This mapping population has many transgressive segregants, or lines which had greater aerenchyma development than either parent.

The linkage map of F₂ composed of 20 markers, covered

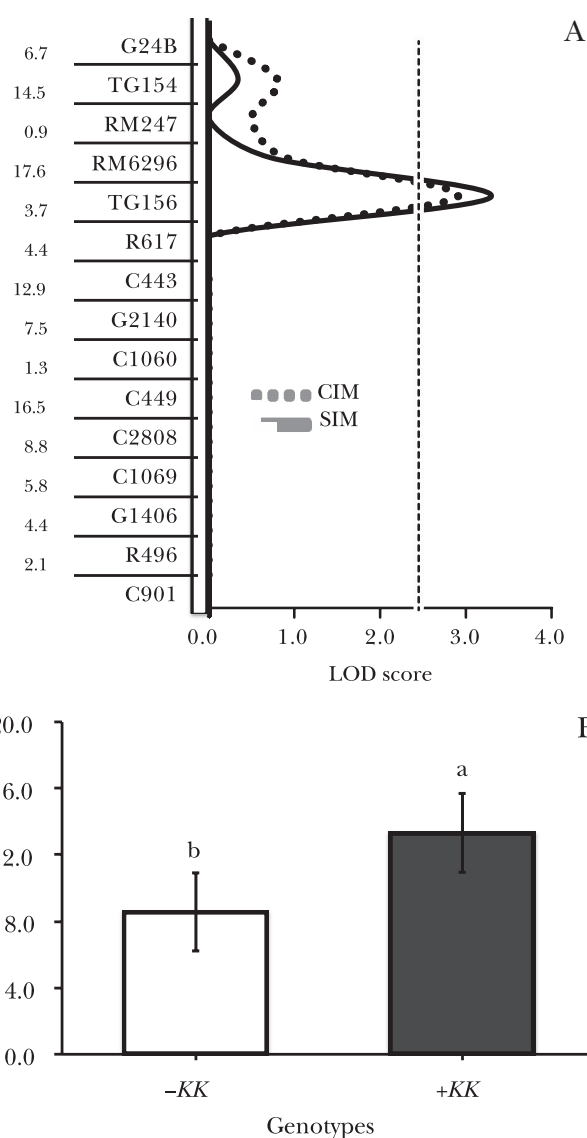


Fig. 3. (A) Molecular linkage map of CSSL F₂ population derived from CSSL47 × Nipponbare cross on chromosome 12. The LOD value was set at 2.5 to detect QTL that associated with the traits. The designations on the left side of the graph are marker name and the interval distance between markers based on Kosambi function. The LOD value of SIM was 3.29 while that of CIM was 2.90 using the Qgene version 4.0. (B) Root porosity of F₂ genotypes with (+KK) and without (-KK) substituted segment from 'Kasalath' allele on chromosome 12 grown under transient drought-to-waterlogged soil conditions. Bar on each column indicates the standard error. Different letters in the graph represent significant difference between the genotypes at $P < 0.05$.

almost all the target regions of interest as shown in the graphical map of CSSL47. A molecular linkage map was constructed using phenotypic and genotypic data derived from the F₂ mapping population. However, we specifically presented only the linkage map at the short-arm of chromosome 12, where the QTL was detected (Fig. 3A). This QTL associated with aerenchyma formation was

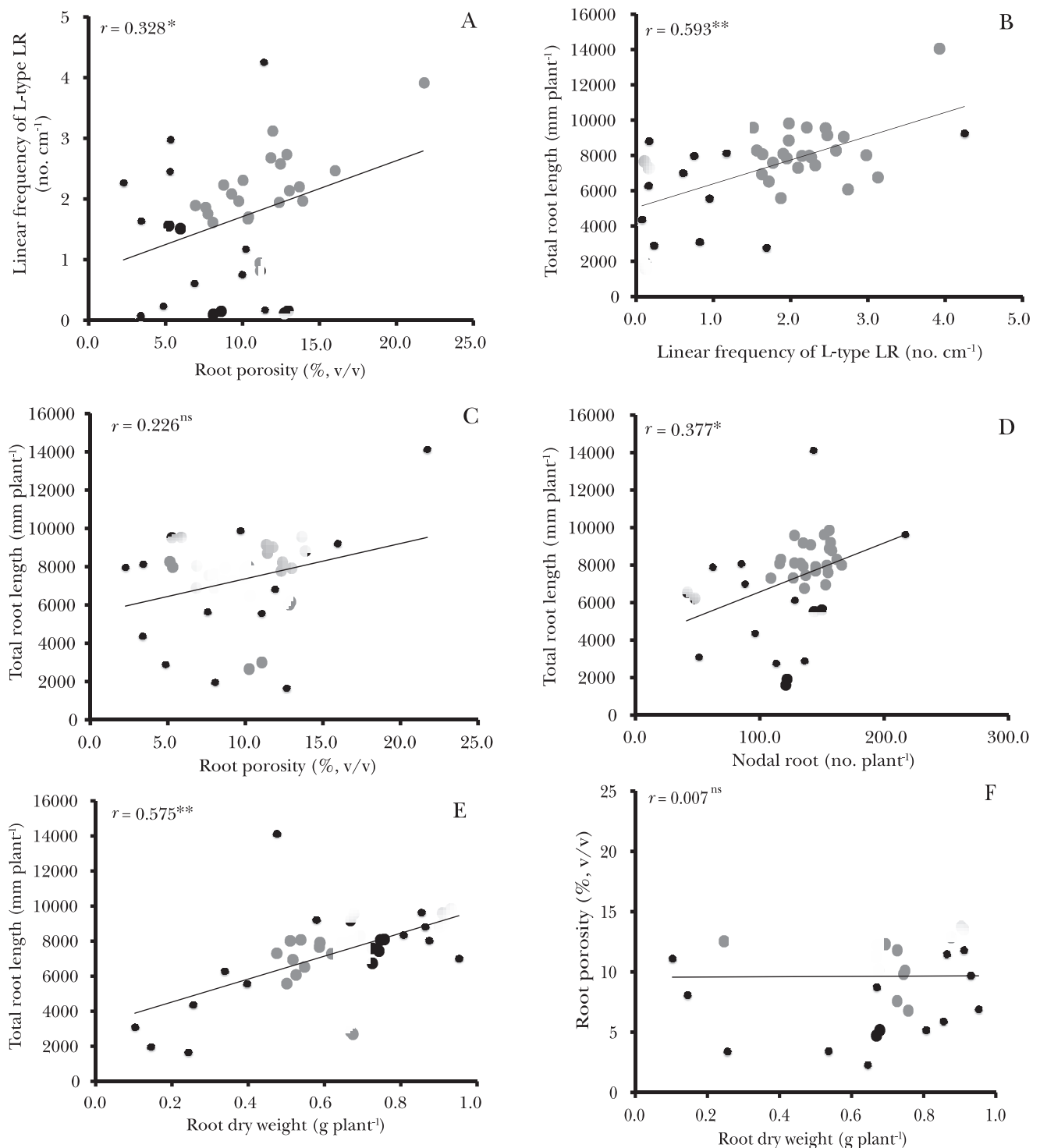


Fig. 4. Relationship between (A) root porosity and L-type lateral roots, (B) L-type lateral roots and total root length, (C) root porosity and total root length, (D) number of nodal roots per plant and total root length, (E) root dry weight per plant and total root length, and (F) root porosity and root dry weight per plant of CSSL F_2 individuals and parents grown under transient drought-to-waterlogged soil conditions at 38 days after sowing. * and ** indicate significant level at $P < 0.05$ and $P < 0.01$, respectively.

identified based on root porosity, and was designated as *qAER-12*. The *qAER-12* had a LOD value of 3.29 and 2.90 based on SIM and CIM, respectively, and was located between RM247 and TG156 loci on the short-arm of chromosome 12 (Fig. 3A). The F_2 individuals that carry

QTL on chromosome 12 were selected based on the genotype (with 'Kasalath' allele effect) ($n = 4$), and herein referred to as +KK. Additionally, those genotypes without the QTL (without 'Kasalath' allele effect) were also selected ($n = 14$), and herein referred to as -KK. The +KK

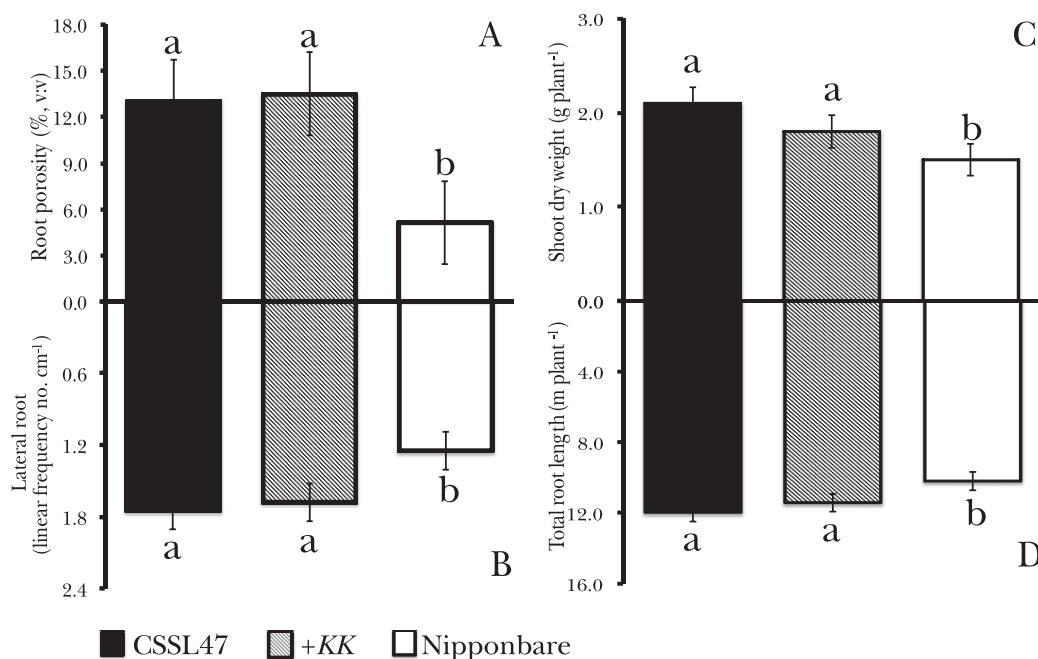


Fig. 5. Contribution of QTL from Kasalath allele on chromosome 12 to total root porosity (A), lateral root production (B), shoot dry weight (C) and total root length (D) of CSSL47, +KK and Nipponbare (recurrent parent) genotypes grown under transient drought to waterlogged stress at 38 days after sowing. The +KK are the F₂ CSSL individuals that contained a substituted segment from 'Kasalath' allele on the short-arm of chromosome 12. The bar on the graph indicates the standard error. The same letters on the graph indicate no significant difference between the genotypes at $P < 0.05$.

and -KK plants were grown and exposed to drought for 21 days followed by 17 days of sudden waterlogging (TD-W) under a soil-filled rootbox condition (Fig. 3B). The Kasalath allele significantly contributed to the increase of aerenchyma formation under TD-W. This QTL (*qAER-12*) accounted for 22.1% of the total variation in aerenchyma development. The effect of introgressed segment of Kasalath allele (+KK) on chromosome 12 enhanced the root porosity by 55.2% relative to genotypes without introgressed segment of Kasalath allele (-KK) under TD-W stress (Fig. 3B).

3. Relationship among root porosity and other root traits

Fig. 4 shows the relationship between (A) root porosity and linear frequency of L-type LR, (B) total root length and linear frequency of L-type LR, (C) root porosity and total root length, (D) nodal root number (NR) and total root length, (E) total root length and root dry weight, and (F) root porosity and root dry weight under TD-W conditions. There was a significant and positive correlation between root porosity and linear frequency of L-type LR, linear frequency of L-type LR and total root length, NR and total root length, and total root length and root dry weight under TD-W. The correlation between the root porosity and either total root length or root dry weight, however, were not significant. The results clearly showed that promoted LR production was significantly attributed

to higher root porosity (Fig. 4A) or enhanced aerenchyma development (Eissenstat, 1991; Justin and Armstrong, 1991; Raunet et al., 2006). The LR production particularly the L-type and NR showed positive and significant relationship with the total root length (Fig. 4B,D), which suggests that root aerenchyma formation have indirect influence on the root system development (Fig. 4C).

4. The effect of *qAER-12* QTL on shoot dry matter production and root system development

The shoot and root system development of +KK genotypes was compared to that of Nipponbare and Kasalath parents under TD-W stress, to further quantify the effects of *qAER-12* on LR production (Fig. 5B), shoot dry weight (Fig. 5C) and total root length (Fig. 5D). The +KK genotypes had significantly higher (162%) root porosity than Nipponbare, but the root porosity of +KK genotypes was comparable to that of CSSL47 (Fig. 5A). The root porosity of +KK genotypes was 9% (v/v) higher than that of Nipponbare indicating higher aerenchyma formation in the former than in the latter genotype. This fact strongly suggests that the QTL for plasticity in aerenchyma development under TD-W is regulated by the 'Kasalath' segment introgressed into chromosome 12. The effect of *qAER-12* was also analyzed by comparing shoot and root growth of the three genotypes (Fig. 5 B-D). The genotypes +KK and CSSL47 produced heavier shoot dry matter (1.8 g

and 2.1 g plant⁻¹, respectively) than Nipponbare (1.5 g plant⁻¹) (Fig. 5C). The total root length of +KK (11.4 m plant⁻¹) and CSSL47 (12.0 m plant⁻¹) genotypes were also greater than that of Nipponbare (10.2 m plant⁻¹) (Fig. 5D). Moreover, the LR production in +KK (1.7 LR cm⁻¹NR) and CSSL47 (1.8 LR cm⁻¹NR) genotypes were significantly higher than that of Nipponbare (1.3 LR cm⁻¹NR). The LR and total root length of +KK and CSSL47 genotypes were 34% and 12% higher, respectively, than in Nipponbare. Shoot dry weight was 20 and 40% greater in +KK and CSSL47 genotypes, respectively, than that of Nipponbare (Fig 5C) under TW-D was mainly attributed to their greater root system due to promoted L-type LR production. This result further suggests that the presence of *qAER-12* between the RM247 and TG156 loci on chromosome 12 contributed to the increase in LR production as well as total root length and subsequently contributed to the increase in SDW by 20%.

Discussion

Fluctuating soil moisture resulting from transient occurrence of waterlogging and drought is common in the rice field. This irregularity in soil moisture condition adversely affects shoot and root growth in various crops (Yamauchi et al., 1996; Azhiri-Sigari et al., 2000; Bañoc et al., 2000; Wade et al., 2000; Pardales and Yamauchi, 2003; Wang and Yamauchi, 2006; Siopongco et al., 2006, 2008, 2009; Subere et al., 2009; Suralta and Yamauchi, 2008; Suralta et al., 2008a, 2008b, 2010; Niones et al., 2012). Soil moisture fluctuation between aerobic and anaerobic conditions to various extents reduced shoot dry matter production (Bañoc et al., 2000; Siopongco et al., 2008; Suralta and Yamauchi, 2008; Suralta et al., 2008b, 2010; Kano-Nakata et al., 2011; Niones et al., 2012) and grain yield in rice (Bouman et al., 2005; Belder et al., 2005; Niones et al., 2012).

Some studies have emphasized the importance of plastic development and associated physiological responses of roots in response to fluctuating soil moisture (Suralta et al., 2010; Niones et al., 2012). Moreover, genetic variations in plastic root development as expressed in total root length and its components induced by soil moisture fluctuation have been reported (Yamauchi et al., 1996; Bañoc et al., 2000; Siopongco et al., 2008; Suralta and Yamauchi, 2008; Kano-Nakata et al., 2011; Niones et al., 2012).

Different mechanisms are required for adaptation to simple drought and constant waterlogging. For instance, the plant needs to develop a deep and extensive root system in response to drought and the capability to enhance its aerenchyma to facilitate O₂ diffusion inside the root in response to sudden waterlogging. On the other hand, shallow roots with enhanced aerenchyma, should develop rapidly and extensively during progressive drought

to acquire more water and support transpiration demand. Hence, roots have to acquire both O₂ and water for plant growth and development under fluctuating soil moisture. The inability of roots to acclimatize and respond to such transitions in soil moisture has a negative effect on plant root growth and functions, resulting in reduced dry matter production. Thus, desirable root traits needed for the plant to adapt to fluctuating soil moisture may be different from those under constant waterlogging and progressive drought stress.

Niones et al. (2012) and Suralta et al. (2010) showed evidence that root plasticity in aerenchyma development represented by root porosity attributed to the increase in LR production under fluctuating soil moisture. Furthermore, the growth stages at which genotypic differences in LR and NR production were observed, coincided with greater differences in root porosity, indicating that aerenchyma development influenced root system development under fluctuating soil moisture (Niones et al., 2012). The lateral roots generally comprise the greater proportion of the whole root system (Wang et al., 2009) and thus, the promoted LR development in response to fluctuating soil moisture directly reflects the size of the entire root system.

In rice, genotypic variation in aerenchyma development has been observed under stagnant waterlogging conditions (Colmer et al., 1998; Suralta et al., 2008a, 2008b; Mei et al., 2009), aerated conditions (Colmer et al., 1998, 2006), transient waterlogging preceded by drought conditions (Suralta et al., 2008a, 2008b, 2010) and alternate waterlogging and drought condition (Niones et al., 2012). This genotypic variation in aerenchyma development represented by porosity is the result of the variation in constitutive intercellular gas spaces in addition to aerenchyma in response to waterlogged conditions (Colmer et al., 1998; Colmer, 2003). Thus, aerenchyma may be overestimated by percent porosity. Armstrong (1971) reported that rice has root porosity of ~9% at 20 – 25 mm behind the root tips even in the absence of aerenchyma. However, there is no effective method available to precisely distinguish aerenchyma from porosity.

In this study, both CSSL47 and Nipponbare parents had similar developmental age at 38 DAS based on main stem leaf number and plant height under indicating similar genetic similarity in plant development. On the other hand, two genotypes showed significant differences in root porosity under TD-W (Fig. 1). This result confirmed our earlier studies (Suralta et al., 2010; Niones et al., 2012), which showed the significance of plasticity in root aerenchyma development in rice adaptation to soil moisture fluctuation especially during episodes drought-to-waterlogged conditions.

In this study, we identified QTL on chromosome 12

Table 1. Root traits, functional characteristics and its chromosome locations of QTLs that are commonly characterized in QTL mapping studies.

Root traits	Functional characteristics	Chromosome location	Reference
Root branching	Capacity in soil exploration	1, 6, 11	Horii et al., 2006
Total root length	Determinant of the size of root system: major determinant for water and nutrient uptake	1, 2, 4, 5, 6, 7, 9	Qu et al., 2008; Champoux et al., 1995
Root number	Potential for root system architecture and physical strength	1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 12	Qu et al., 2008; Zheng et al., 2003; Ray et al., 1996; Ali et al., 2000
Maximum root depth	Potential for absorption of soil moisture and nutrients in deeper soil layer	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11	Courtois et al., 2009
Root to shoot ratio	Assimilate allocation	1, 2, 4, 5, 6, 8, 9, 10	Price et al., 2002; Zheng et al., 2008
Root thickness	Penetration ability and hydraulic conductivity	1, 2, 3, 4, 6, 7, 8, 9, 10, 12	Qu et al., 2008; Kamoshita et al., 2002
Specific root length	Degree of branching, porosity due to aerenchyma development	1, 2, 7, 9	Zheng et al., 2003
Root penetration index	Ability to penetrate subsurface hardpan	1, 2, 3, 4, 5, 6, 11, 12	Ray et al., 1996
Root dry weight	The ability to permeate a large volume of soil	1, 6, 9, 11	Horii et al., 2006
Root aerenchyma formation	Ability for the atmospheric O ₂ transport in the root under low O ₂ deficiency (hypoxia)	12	In this study

associated with root aerenchyma (represented by root porosity) using the F₂ CSSL genotypes (Fig. 3). The analysis of SIM and CIM showed a single peak indicating that a single QTL on chromosome 12 regulated aerenchyma development under fluctuating soil moisture. The identified QTL associated with aerenchyma formation (designated as *qAER-12*) was mapped on the short-arm of chromosome 12, between the flanked markers of RM247 and TG156 loci (Fig. 3A). *qAER-12* exhibited a strong effect on aerenchyma formation in roots in response to TD-W stress. The substituted Kasalath allele (+KK genotype) on chromosome 12 contributed to the increase in aerenchyma formation as represented by a higher root porosity (153%) than that in Nipponbare under TD-W stress (Fig. 5A). This significant increase in root aerenchyma formation directly influenced lateral root development under fluctuating soil moisture (Niones et al., 2012). In addition to the capacity to form root aerenchyma, this QTL (caused by ‘Kasalath’ allele) was significantly associated with the increase in production of LR (Fig. 4A, 5B), and consequently total root length (Fig. 5D). The enhanced LR development increased root surface area and soil water extraction (Kamoshita et al., 2004; Siopongco et al., 2005, 2006; Henry et al., 2011; Kato et al., 2011), and water use (Suralta et al., 2010). Thus, root plasticity is a key trait for better adaptation to drought (Kano et al., 2011; Kano-Nakata et al., 2011) and fluctuating soil moisture conditions (Bañoc et al., 2000; Suralta et al., 2010; Niones et al., 2012).

We compared the QTL *qAER-12* identified in this study with that reported in other rice mapping populations although the latter was identified under simple drought stressed environments (Table 1). Most of the root traits that are commonly characterized in QTL mapping are related to maximum root length, root thickness below 90-cm soil depth, root penetration index, root dry weight, root branching index and root-to-shoot ratio (Champoux et al., 1995; Ali et al., 2000; Ray et al., 1996; Zhang et al., 2001; Price et al., 2002, 2006; Horii et al., 2006; Zheng et al., 2003, 2006, 2008; Qu et al., 2008; Courtois et al., 2009; Gowda et al., 2011). To determine if a common root QTL across genetic background exists, we also compared the results of the present study with those of other molecular studies. The putative QTL identified for aerenchyma development on chromosome 12 was flanked between G24B and R617 RFLP markers. The QTL associated with seminal root length was found to be on the same chromosomal location in IR1552/Azucena population (Zhang et al., 2001). Similarly, three QTLs associated with total dry weight, root dry weight and number of root are also localized in the same genomic region in Bala/Azucena population (Price et al., 2002). The majority of the above characterized QTL are expressed, however, under simple drought stress. This study suggests that the QTL *qAER-12* identified on chromosome 12 possibly regulate other root traits in addition to aerenchyma formation. The QTL associated with aerenchyma formation has not been reported under constant

waterlogging or fluctuating soil moisture stresses. To the best of our knowledge, this is the first reported QTL associated with root aerenchyma development under soil moisture fluctuation stress.

Conclusion

Our earlier studies demonstrated the significant functions of aerenchyma formation on the root system development for the adaptation to a fluctuating moisture environment (Niones et al., 2012). In those studies, we identified a line (CSSL47) having unique characteristics in maintaining root functions specifically aerenchyma development under soil moisture fluctuations (Suralta et al., 2010; Niones et al 2012). We believe that genetically one of the segments from Kasalath allele introgressed into the Nipponbare genetic background controlled the aerenchyma development (represented by root porosity) in response to soil moisture fluctuation. The presence of an introgressed segment of Kasalath allele on chromosome 12 showed a significant contribution to better adaptation to soil moisture fluctuation than the plants without the introgressed segment. The higher LR production was mainly attributed to higher aerenchyma formation (represented by root porosity) and eventually contributed to greater root system (represented by higher total root length). Greater root system development due to the plasticity in aerenchyma and LR production could contribute to higher photosynthetic activity, stomatal conductance and transpiration, and increased the dry matter production. Even though QTL of interest was identified in the existing mapping population, further development of near isogenic lines is necessary for fine mapping and more detailed genetic analysis of the said trait. This will enhance even more our understanding on the significance of aerenchyma development under a fluctuating soil moisture environment. Identification of QTL responsible for the formation of aerenchyma is essential in improving the adaptive capability of cultivars under a fluctuating soil moisture environment such as in rainfed lowland rice.

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