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REGULAR ARTICLE

QTL associated with lateral root plasticity in response to soil moisture fluctuation stress in rice

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Abstract
Background Lateral root (LR) plasticity is a key trait that plays a significant role in plant adaptation to fluctuating soil moisture stressed environments. We previously had demonstrated that promoted LR production (LR plasticity) contributed to the maintenance in shoot dry matter production and grain yield under soil moisture fluctuation (SMF) stress.
Aim To identify quantitative trait loci (QTLs) associated with LR plasticity under SMF condition and their contributions to shoot dry matter production.
Methods F_2 lines derived from Nipponbare x chromosome segment substituted line number 47 (Nipponbare/Kasalath) backcrosses were used to analyze ten substituted chromosome regions with 'Kasalath' allele that are associated with root plasticity under SMF stress.
Results We mapped two closely linked QTLs on chromosome 12 region namely *qTLRN-12* at seedling stage and *qLLRN-12* at vegetative stage. Under SMF conditions, *qTLRN-12* found at the flanking markers between TG154 and RM247 is responsible for the plasticity in total LR number while *qLLRN-12* detected at the flanking markers between RM6296 and TG156 is associated with plasticity in L-type LR production. Kasalath genome contributed the corresponding alleles for increasing the mentioned root traits that resulted in a significant increase in shoot dry matter production under SMF stress.
Conclusion We identified two QTLs associated with LR plasticity on chromosome 12 which significantly contributed to the greater root system development and maintenance of total dry matter production under SMF stress.

Keywords Chromosome segment substitution lines (CSSL) · Drought · Lateral roots · Quantitative trait loci (QTL) · Root plasticity · Waterlogging

Abbreviations

CSSL	Chromosome segment substitution lines
CWL	Continuous waterlogging
LR	Lateral root
LLRN	L-type lateral root number
QTL	Quantitative trait loci
RDW	Root dry weight
RSR	Root to shoot ratio
SMF	Soil moisture fluctuation stress
TRL	Total root length

Introduction

Water deficit and varying degrees of waterlogging are important abiotic stresses that adversely impact rice

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production especially in the rainfed lowlands (Boling et al. 2004; Yadav et al. 2011) where rainfall patterns are erratic. The inability of rice plants to acclimate to SMF stress often resulted to reduced dry matter production and grain yield (Suralta et al. 2010; Niones et al. 2012).

Root plasticity, which is defined as the plant's ability to alter its root phenotype in response to changing environmental conditions (O'Toole and Bland 1987), plays a significant role in plant adaptation. We have accumulated experimental evidences through series of root studies demonstrating the functional role of root plasticity in the plant adaptation to drought and SMF (Yamauchi et al. 1996; Wang and Yamauchi 2006). In rice, root plasticity such as promoted lateral root (LR) development in response to different intensities of drought stress (Yamauchi et al. 1987; 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 waterlogged and drought stress (Niones et al. 2012) has been quantified as an important trait for maintaining dry matter production and yield.

The lateral roots make up most of the root system of rice in terms of number and length (Yamauchi et al. 1987). The LRs are classified into L- and S-types, and generally differ in anatomy, morphology, developmental characteristics, carbon and nitrogen dynamics (Yamauchi et al. 1987; Yamauchi et al. 1996) and genetic control of development (Wang et al. 2005). Such phenomenon is termed heterorhizy. The S-type LRs have less developed vascular structure than the L-type LRs (Kono et al. 1987; Rebouillat et al. 2009). For example, in one-month-old rice plant, on the average, the L-type LRs were 30 mm long and 159 μm thick, and were capable of branching into higher-order LRs. The S-type LRs on the other hand, had an average length and thickness of 7.6 mm and 80 μm, respectively, and were non-branching (Yamauchi et al. 1987). As such, by paying special attention to the heterorhizy, we have shown that the L-type LR plasticity is one of the key traits for plant adaptation to SMF (Bañoc et al. 2000; Suralta et al. 2010; Niones et al. 2012).

Root traits are generally controlled by many quantitative trait loci (QTL) or sets of genes. Currently available marker-assisted selection is less efficient for root traits due to the interaction with the environment and low heritability, than it is for other traits such as disease resistance that have relatively higher heritability. Recent progress of molecular genetic research on rice root provide methods of dissecting genetic and molecular mechanism of QTLs or genes that control root development (Courtois et al. 2009; Courdert et al. 2010; Price 2006; Rebouillat et al. 2009). This research progress may increase the efficiency of marker assisted selection for root traits, which is critical for breeding of new rice ideotypes for fluctuating soil moisture condition. When studies attempting to identify the QTLs that regulate plasticity of lateral roots and understanding its underlying genetic mechanism for responses to fluctuating soil moisture stresses, it would be quite useful to pay special attention to the heterorhizy.

The use of chromosome segment substitution lines (CSSLs) derived from Nipponbare×Kasalath cross has been useful in demonstrating the roles of root plasticity in the maintenance of dry matter production and grain yield under SMF (Suralta et al. 2010; Niones et al. 2012, 2013). CSSL47 is a substitution line that was selected from a population of 54 CSSLs derived from Nipponbare x Kasalath cross for its unique root system development especially in response to SMF (Suralta et al. 2010; Niones et al. 2012). Under constant drought and waterlogged conditions, the shoot and root system development of CSSL47 were comparable with those of Nipponbare. Under SMF, however, CSSL47 showed better growth performance than Nipponbare. This was due to the greater ability of CSSL47 than Nipponbare in promoting root elongation and production of L-type LRs under SMF, which maintained higher stomatal conductance and photosynthesis, resulting in higher shoot dry matter and yield (Suralta et al. 2010; Niones et al. 2012).

The genotypic map of CSSL47 shows that 8 out of its 12 chromosomes have substituted segments from the Kasalath genome (NIAS 2012). We assumed that genetically, one of these substituted segments regulates L-type LR plasticity in the substitution line and consequently contributes to the maintenance in dry matter production under SMF. In this study, therefore, we used F_2 mapping population (Nipponbare/CSSL47) to identify the QTL(s) for the plasticity of L-type LRs, and quantify the contribution of QTL(s) to shoot dry matter production under SMF stress.

Material and methods

Plant materials

A F_2 mapping population derived from Nipponbare x CSSL47 (Nipponbare/ Kasalath) backcross was used in

this study. CSSL47 was selected due to its ability to produce more L-type LRs relative to Nipponbare parent under SMF stress (Suralta et al. 2010; Niones et al. 2012). The CSSL47 genetic map profile carries 10 substituted chromosome segments from Kasalath (Fig. 1) (NIAS 2012). The F_2 mapping population was developed at the Graduate School of Bioagricultural Sciences, Nagoya University, Japan. To break and further segregate the substituted segments in CSSL47 into different lines, the CSSL47 was backcrossed with Nipponbare (as female parent) to generate F_1 lines. Then, 10 F_1 plants were grown and selfed to produce enough F_2 seeds for this study. Because quantitative analysis (phenotyping) of root traits in this study requires destructive sampling, the 305 F_2 lines were randomly divided and assigned for each growth stage as follows: 155 lines were grown up to seedling stage grown under hydroponics culture condition, 54 lines grown up to vegetative stage under soil-filled root box condition and 96 lines were grown up to heading stage under soil bed field conditions.

The parents and F_2 seeds were soaked in water containing benomyl fungicide (0.15% *w/v*), washed thoroughly and incubated in a seed germinator at 28°C for 36 h prior to sowing. Pre-germinated seeds of each set of F_2 lines and their parents (Nipponbare and CSSL47) were grown in hydroponics, soil filled rootbox and watertight experimental bed conditions. The Nipponbare and CSSL47 parents were grown under control (waterlogged) and SMF stress conditions, while the F_2 lines were grown under SMF conditions only.

Experimental treatments and growing conditions

SMF treatment at seedling stage A set of 155 F_2 lines were grown in hydroponics and initially exposed to O_2-deficient conditions from the day of sowing to 7 days after sowing (DAS) and then transferred to simulated 'drought' condition for another 7 days (8-14 DAS). Oxygen deficiency was induced by mixing water with agar (0.1% *w/v*) and flushed with N_2 gas to reduce O_2 concentration in the water to 0.14–0.16 ppm (Suralta et al. 2008a, 2008b). This solution was not aerated and kept 'stagnant' during the growing period. The addition of 0.1% agar in the water solution prevents turbulence, thus simulating the slow gas movement found in waterlogged soils (Wiengweera et al. 1997). On the other hand, 'drought' treatment was induced by adding polyethylene glycol (PEG 6000; 4% *w/v*) to achieve a water potential of -0.13 MPa (Suralta and Yamauchi 2008; Suralta et al. 2008a). Polyethylene glycol has no apparent toxic effects under well-aerated condition (Ober and Sharp 2003; Ogawa et al. 2005) but can mimic the drying effects of the soil environment. In this experimental set up, only the Nipponbare and CSSL47 parents were subjected to both continuous well-aerated (control) and transient O_2 deficient and drought conditions while the selected F_2 lines were subjected to the latter treatment only. The experiment was terminated at 14 DAS and the phenotyping for root traits was carried out subsequently.

SMF treatment at vegetative stage Three pre-germinated seeds of each 54 F_2 lines and parents were sown in a polyvinyl chloride (PVC) root boxes (25 cm× 2 cm×40 cm, L×W×H) filled with soil following the method of Kono et al. 1987 and Suralta et al. 2010. The soil was pre-mixed with fertilizer containing 60 mg nitrogen, 80 mg phosphorus and 70 mg potassium. The seedlings in each box were thinned to one at 3 DAS. The parents Nipponbare and CSSL47 were grown under continuous waterlogging (control) and SMF conditions (waterlogged-to-drought) conditions while the selected F_2 lines were grown under SMF condition only. For continuous waterlogging (control), the water level in the root box was maintained at 2 cm above the soil surface. For SMF treatment, the soil inside the boxes was subjected to waterlogging from 0 to 17 DAS. Thereafter, the soil was not re-watered and was subjected to progressive drying until its moisture content reached 20% (SMC, w/w) at 21 days after imposition of drought treatment (38 DAS). Boxes were weighed daily using a digital balance to record the wetness of the soil. The SMC (% by weight) in each box was calculated as the ratio between water weight (difference between the wet weights of the soil excluding the box on a given day) and the dry weight (2.9 kg) soil. This SMC was maintained by adding water every two days at the soil surface. 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 we observed a 2-day watering interval, differences in moisture between the top and the bottom soil in the box should have been smaller (Suralta et al. 2010). Plant sampling was carried out at 38 DAS.

SMF treatment at heading stage A set of 96 F_2 lines and parents were initially sown in black plastic trays.

Healthy, 18-day-old seedlings were transplanted at one seedling per hill at a spacing of 20 cm between hills in a watertight experimental bed. The waterproof experimental bed for controlling soil moisture conditions was established under a rain-out shelter at Nagoya University experimental farm (lat. 35°6'42"N, long. 137°4'57"E). The bed size was 3.6 m wide, 6.6 m long and 0.3 m deep. The soil used in this experiment was sandy loam, which was carefully and uniformly distributed into the fabricated concrete bed covered with plastic sheets. Two grams of complete fertilizer were applied per hill. The transplanted seedlings were allowed to acclimatize for 10 days under well-watered conditions before subjecting them to SMF (continuous cycles of alternate waterlogging and drought) and continuous waterlogging (CWL, control). Under SMF, the soil bed was first waterlogged for 14 days and thereafter, rewatering was withheld until the soil water potential (pF) dropped and reached to -30 kPa. Thereafter, rewatering was carried out by bringing the water level back to 5 cm above the soil surface. Under CWL treatment, the water level was maintained at 5 cm above the surface throughout the duration of the experiment. The SMF treatment was initiated during the 7th leaf stage and repeated 6 times before reaching the heading stage. The parent Nipponbare and CSSL47 were grown under CWL and SMF conditions while the selected F_2 lines were grown under SMF condition only. Plant samplings were carried out during the heading stage (87 DAS).

Soil water potential was regularly recorded from nine random points using a soil tensiometer (Daiki soil and moisture, Daiki Rika Kogyo Co., Japan) installed at 20 cm soil depth.

Phenotyping

Shoot parameters In the watertight soil bed experiment, shoots were cut at heading stage, placed in paper bags and oven dried at 70°C for 48 hours and weighed.

Root parameters Sampling and measurement of the roots from the hydroponics set-up were carried out following the method of Suralta et al. 2008a. The number of lateral roots along the seminal root axes was manually counted and expressed as linear frequency of lateral root number according to types (L and S).

In the root box experiment, root system sampling was carried out following the methods of Kono et al. 1987 and Suralta et al. 2010. The root samples were placed between two perforated plastic sheets, washed with running water and stained with 0.25% Coomassie Brilliant Blue R aqueous solution for 72 hours for clearer contrast. The stained root samples were then rinsed with tap water and placed in a light box and digitized using a Nikon D3000 digital SLR camera (Nikon Corporation, Japan) at 300 dpi resolution.

In the watertight soil bed experiment, root sampling was carried out similar to that of Niones et al. 2012 and Kano et al. 2011 using a monolith stainless cylinder (15 cm diameter×20 cm height) (Kang et al. 1994). The roots contained inside the monolith after extraction was collected and washed with running water before storing in FAA (formalin: acetic acid: 70% ethanol in 1:1:18 ratio by volume) solution for further measurements.

The total number of nodal roots (NRs) and LRs were manually counted. Two coleoptile nodal root axes that emerged in the vicinity of seminal root were cut into 5-cm segments, keeping the LRs intact. The number of LRs was counted and expressed as linear frequency (number of LRs per unit length of root axis, Ito et al. 2006). For the total root length (TRL) measurements, the FAA-stored root samples 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. TRL 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). After scanning, root samples were oven dried at 70°C for 48 hours before the root dry weight (RDW) was recorded. The root to shoot ratio (RSR) was calculated as the ratio between the shoot dry weight and RDW. Specific root length (SRL) was computed as the ratio between the TRL and RDW.

The root plasticity in F_2 lines was computed directly as the difference in measured traits between F_2 lines and Nipponbare plants grown under SMF treatments. Since the F_2 lines used in this study were segregating and thus no two lines were genetically identical, it was not possible to grow and compare the same genotype under CWL and SMF conditions. However, we expected that since these lines contain the major genetic background of Nipponbare but with more specific substituted

segments from the Kasalath alleles, and therefore, these lines would perform similarly with Nipponbare under CWL but would show greater growth performance than Nipponbare under SMF. And thus, the growth performance of F_2 lines relative to Nipponbare under SMF can give direct estimates of root plasticity.

Genotyping and marker selection

Marker selection CSSL47 has major genetic background of Nipponbare with 10 substituted genomic segments from Kasalath distributed across 8 chromosomes (Fig. 1). 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). Primer pairs or markers were selected and designed based on the substituted Kasalath segments on CSSL47 genotype. A total of 20 polymorphic markers from Gramene 2005 and rice genome project (RGP 2000) were assigned and distributed across 8 chromosome regions for QTL mapping analysis (Fig. 1).

DNA extraction At seedling stage or prior to the imposition of SMF treatments in each growing conditions, approximately 1–4 μg DNA was extracted from fresh leaves of each F_2 plant and parents using the CTAB method (Zheng et al. 1995). The PCR was conducted following standard PCR protocols (Horii et al. 2006).

Statistical analysis

The parents (Nipponbare and CSSL47) were replicated three times under both CWL and SMF conditions, while the F_2 lines were grown without replication under SMF treatment only. Analysis of variance (ANOVA) was performed for detecting differences between parents and between water treatments. The treatment means were compared using least significant difference (LSD) test at $P<0.05$ levels.

Furthermore, the contributory effects of the identified QTLs in relation to the shoot and root development were analyzed at heading stage (soil bed system) only. The F_2 lines carrying Nipponbare homozygous or without substituted segment of Kasalath (**-K** alleles effect) lines and with substituted segment Kasalath (**+K** allele effect) lines on chromosome 12 region were selected and grouped for the analysis of QTL effect. The **+K** group of genotypes was selected based on flanking markers between RM247 and TG156 loci (with 'Kasalath' allele) on chromosome 12 region with Nipponbare background on the rest of chromosome regions. Out of 96 F_2 lines, 14 F_2 lines with **-K** substituted segment and 12 F_2 lines with **+K** substituted segment on the chromosome 12 region were grouped accordingly based on the genotypic data. The mean values of each group were

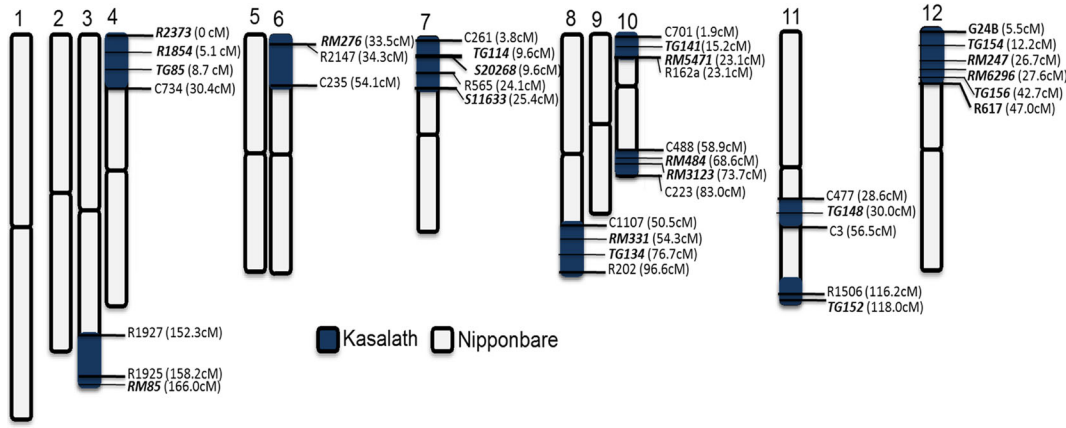

Fig 1 The graphical map of CSSL47 showing the Nipponbare background with substituted chromosome segments from the Kasalath genome (NIAS 2012). The *solid white square* represents Nipponbare segment while the *solid blue square* represents Kasalath segment. The designation on the right of the substituted segments is the flanking genetic marker with the chromosome position in the parenthesis (RGP 2000). All marker names in **bold** were used in the QTL mapping analysis

compared with the mean of those in Nipponbare using the pairwise comparison at 5% level of significance.

Construction of linkage map and QTL analysis

Linkage maps were constructed from genotypic data with Qgene software version 4.0. The genetic distance was estimated using the Kosambi map functions (Kosambi 1944). The putative QTLs were detected using the composite interval mapping (CIM) functions of the Qgene software version 4.0 (Joehanes and Nelson 2008). The 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 critical threshold value of the logarithm of odds (LOD) score was set at 2.5 to detect the QTL. The phenotypic variance explained by each QTL (R^2) was estimated at maximum LOD score.

Results

Phenotypic evaluation of parents

The root system development between the CSSL47 and Nipponbare genotypes was generally comparable under control conditions regardless of growth stages (Table 1 and Fig. 2). However, under SMF, the root system of CSSL47 showed significantly higher values for most traits at the seedling (hydroponics system), vegetative (soil filled root box system) and heading (soil bed system) stages than Nipponbare (Table 1). Specifically, TRL was significantly greater in CSSL47 than in Nipponbare at the vegetative and heading stages. The total LR production was also significantly greater by 26% in CSSL47 than in Nipponbare at the seedling stage. The number of L- and S-type LRs of CSSL47 at vegetative and heading stages were

Table 1 Root growth of Nipponbare and CSSL47 genotypes under control conditions and soil moisture fluctuation stress at seedling, vegetative and heading stage

Traits	Control		**Soil Moisture fluctuation**	
	CSSL47	Nipponbare	CSSL47	Nipponbare
A. Seedling stage (14 DAS)				
TLRN (no. cm^{-1})	10.2 a	11.7 a	**10.4 a**	**8.5 b**
B. Vegetative stage (38 DAS)				
TRL (m $plant^{-1}$)	121.8 a	125.7 a	**53.0 a**	**48.1 b**
NRN (no. $plant^{-1}$)	72.0 a	73.7 a	**54.5 a**	**43.2 b**
L-LRN (no. cm^{-1})	1.1 a	1.3 a	**1.3 a**	**1.1 b**
S-LRN (no. cm^{-1})	7.5 a	7.6 a	**10.3 a**	**7.6 b**
TLRN (no. cm^{-1})	9.2 a	9.2 a	**12.3 a**	**9.5 b**
RDW (g $plant^{-1}$)	0.4 a	0.4 a	0.2 a	0.2 a
SRL (m RL g^{-1}RDW)	33.8 a	32.3 a	30.0 a	30.3 a
RSR	0.3 a	0.2 a	0.1 a	0.2 a
C. Heading stage (87 DAS)				
TRL (m $plant^{-1}$)	176.2 a	165.2 a	**237.9 a**	**221.1 b**
NRN (no. $plant^{-1}$)	440.7 a	422.0 a	341.3 a	349.8 a
L-LRN (no. cm^{-1})	1.9 a	1.8 a	**1.9 a**	**1.8 b**
S-LRN (no. cm^{-1})	4.6 a	3.3 a	**3.1 a**	**2.9 b**
TLRN (no. cm^{-1})	7.0 a	5.9 a	**5.3 a**	**4.9 b**
RDW (g $plant^{-1}$)	**3.1 b**	**5.3 a**	**4.2 b**	**5.9 a**
SRL (m RL g^{-1}RDW)	36.3 a	33.0 a	**52.0 a**	**41.1 b**
RSR	**0.2 b**	**0.3 a**	**0.1 b**	**0.2 a**

(n DAS), number of days after sowing were plants are sampled. *TRL,* total root length; *NRN*, nodal root number; *RDW,* root dry weight; *SRL*, specific root length; *L-LRN*, L-type linear lateral root number; *S-LRN*, S-type linear lateral root number*; TLRN*, total lateral root number; *RSR*, root to shoot ratio. In a row within each trait and soil moisture treatments, means followed by the same letters are not significantly different between genotypes at $LSD_{0.05}$ level. The ***bold italic values*** indicated to have significant different between genotypes

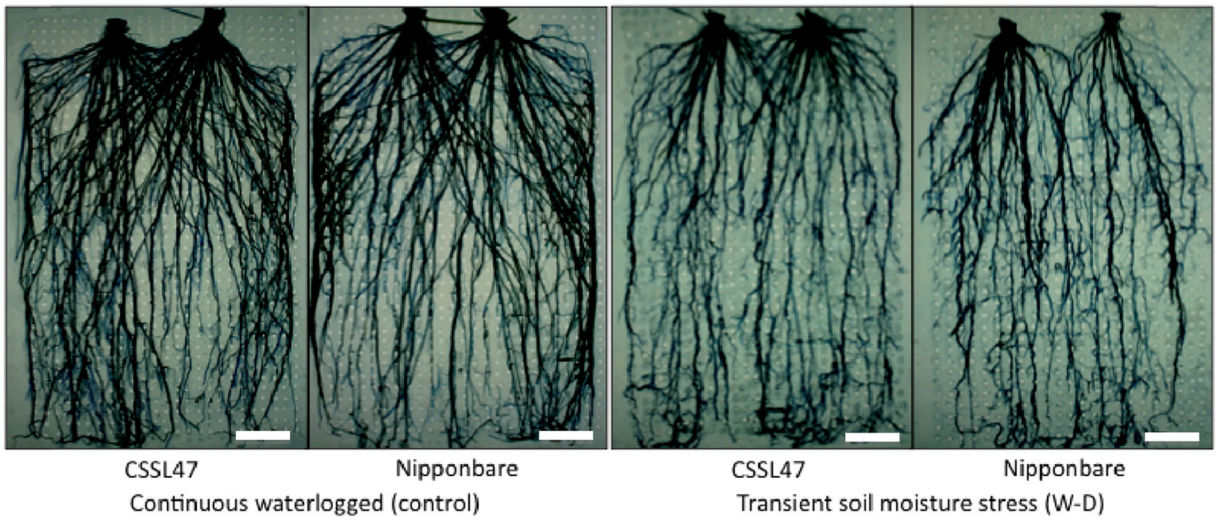

Fig. 2 The root system development of CSSL47 and Nipponbare genotypes sampled during vegetative growth stage. The plants were exposed to control (continuous waterlogged) and soil moisture fluctuation stress (waterlogged-to-drought) conditions in soil filled rootboxes for 38 days. The scale of white bars of each plate (below on the right corner) is 5 cm

significantly higher by 12% (0.34 LR no. cm^{-1}) and 27.8% (2.90 LR no. cm^{-1}), respectively, than Nipponbare (Table 1). Furthermore, CSSL47 showed a significantly greater SRL than Nipponbare at heading stage only. On the other hand, RDW and RSR were significantly higher in Nipponbare than in CSSL47 at heading stage both under control and SMF conditions.

Frequency distribution of root traits

A wide phenotypic variation of F_2 lines in LR development was observed under SMF regardless of growth stage. The LRN at the seedling stage (hydroponics system) ranged from 6.0 to 16.0 cm^{-1} NR axis (Fig. 3a) while at the vegetative stage (soil filled root box system), the L-type LRs production ranged from 0.16 to 3.07 cm^{-1} NR axis (Fig. 3b). Furthermore, at heading stage (soil bed system), the LR production regardless of types ranged from 1.0 to 7.0 cm^{-1} NR axis (Fig. 3c). This F_2 mapping population also showed many transgressive segregants that had either higher or lower LR development than either of the parents.

Detection of QTL associated with root plasticity

The linkage map of F_2 lines composed of 20 markers, covering almost all of the target regions of interest based on the graphical map of CSSL47 (Fig. 1). A molecular linkage map was constructed using the phenotypic and genotypic data of this mapping population especially on chromosomes 12 (2 QTLs), where co-location of QTLs associated with lateral root plasticity were detected (Table 2 and Fig. 4).

At seedling stage (hydroponics system), a QTL for TLRN (*qTLRN-12*) was detected at the flanking markers between TG154 and RM247 on chromosome 12. The *qTLRN-12* position was at 25.5 cM on the short-arm of chromosome 12 region (Table 2 and Fig. 4). It had a LOD value of 13.8 with the increase effects on LR production from 'Kasalath allele'. This QTL accounted for 57.5% of the total variation of LRs at seedling stage (Table 2).

At vegetative stage (soil filled root box system), a QTL controlling L-LRN (*qLLRN-12*) was detected on chromosomes 12 region (Fig. 4) with LOD score of 3.5 (Table 2). The *qLLRN-12* was located at the flanking markers between RM6296 and TG156 with the increase effect from 'Kasalath allele' that controls the L-type LR production. The *qLLRN-12* position was at 42.2 cM on the short-arm of chromosome 12 region, which accounted for 14.9% of the total variation of LRs at the vegetative stage (Table 2). The introgressed Kasalath segment on chromosome 12 enhanced the production of L-type LRs by 50% relative to Nipponbare under SMF.

At heading stage (soil bed system), apparent QTLs for LLRN (*qLLRN*) as well as that of TLRN were detected on chromosome 8 and 12 regions,

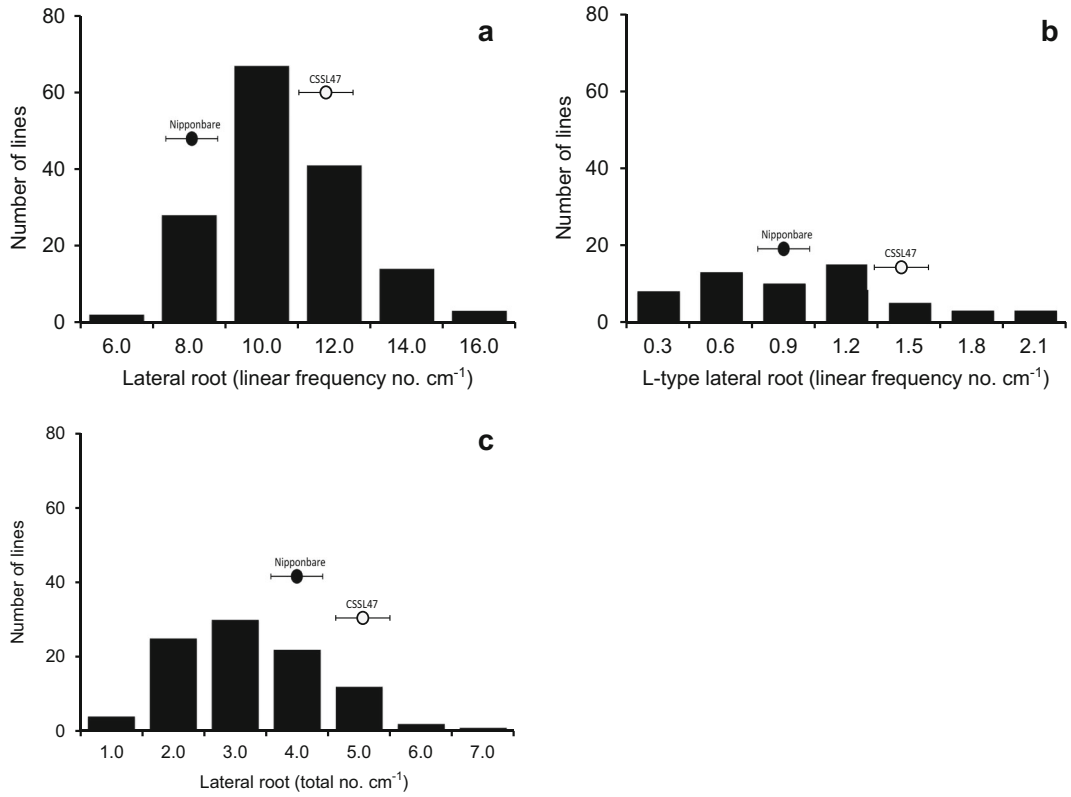

Fig. 3 Frequency distribution of the linear frequency of lateral root (LR) number at seedling stage (hydroponics system) (**a**), linear frequency of L-type LR at vegetative stage (soil-filled rootbox system) (**b**), and linear frequency total LR number at heading stage (soil bed system) (**c**) of F_2 CSSLs grown under moisture fluctuation stress. The values above the bars are the means of Nipponbare (solid circle) and CSSL47 (open circle) genotype±standard error

respectively, although their LOD score (1.5 and 1.2) fell short on the threshold value of 2.5 (Table 2). The *qLLRN-8* was located at the flanking markers between RM331 and TG134 loci while *qTLRN-12* was between RM6296 and TG156 loci. Both QTLs had increased effect from 'Kasalath allele' that controls the L-type and total LR production which accounted for 7.2% and 9.6%, respectively, of the total variations of LRs at the heading stage (Table 2).

Table 2 QTLs associated with LR root plasticity under soil moisture fluctuation stress at seedling, vegetative and heading stage

Trait	Stage	Growth Condition	QTL	Chr	Flanking markers	QTL Position (cM)	Prob.	[a]LOD	PVE (%)	F	Increase effect
Lateral root number	Sdlng	HC	*qTLRN*-12	12	TG154- RM247	25.5	0.000***	13.8	57.5	0.11	Kasalath
	Veg	RC	*qLLRN*-12	12	RM6296-TG156	42.2	0.011*	3.5	14.9	8.76	Kasalath
	Hdg[b]	FC	*qLLRN*-8	8	RM331-TG134	54.3	0.009**	1.5	7.3	6.78	Kasalath
	Hdg[b]	FC	*qTLRN*-12	12	RM6296-TG156	43.5	0.021*	1.2	9.6	5.48	Kasalath

Chr, chromosome; *qTLRN*, QTL of total lateral root number; *qLLRN*, QTL of L-type lateral root number; *PVE*, phenotypic variance explained by each QTL. *, ** and ***, significant at $P_{0.05}$, $P_{0.01}$ and $P_{0.001}$ level. [a] LOD value of each marker in the interval analysis. QTL was detected at LOD value of 2.5. *HC*, hydroponic condition; *RC*, rootbox condition; *FC*, field condition. Sdlng, seedling stage; Veg, vegetative stage; Hdg, heading stage. [b] Although the QTL is significant but the LOD value falls short of the threshold level

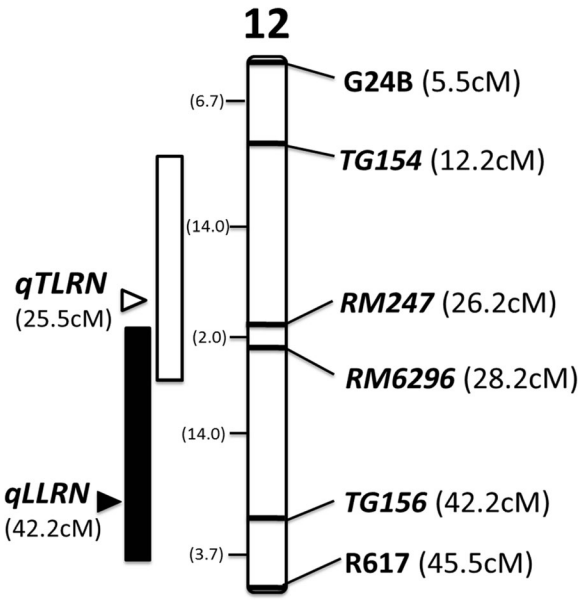

Fig. 4 Molecular linkage map of CSSL F_2 population derived from Nipponbare/ CSSL47 cross and the possible location of the QTLs on Chromosome 12 region. The LOD value was set at 2.5 to detect QTLs that are associated with the traits. Bars on the left side are the location of the QTLs on total lateral root number, ***qTLRN*** (white bar) and linear frequency of L-type lateral root number, ***qLLRN*** (solid bar). Arrowheads indicate the approximate location of the QTLs that were identified at vegetative stage under soil-filled rootbox, and at seedling stage under hydroponics conditions. The value in the parenthesis (n) on the left side of the chromosome are the interval distance (cM) between markers based on Kosambi function, while on the right side of the chromosome are the marker's name and its position

Effects of the QTLs on root system development and shoot dry matter production

We found two co-located QTLs associated with root plasticity on chromosome 12 regions (Fig. 4). However, we specifically show the effect of QTLs for LR (*qLLRN-12* and *qTLRN-12*) with the increase effect contributed by the 'Kasalath' allele on chromosome 12 regions at heading stage (soil bed system) only. The F_2 lines that carry QTL on chromosome 12 (with 'Kasalath' allele effect) were selected and grouped based on genotypic data (n=12) (herein referred to as '+***K*** genotype'). On the other hand, those F_2 lines without the QTL (without 'Kasalath' allele effect) on chromosome 12 were also selected and grouped (n=14) (herein referred as '–***K*** genotype'). The shoot growth and root system development of+***K*** and –***K*** genotypes were compared to those of Nipponbare parent under SMF stress, to quantify the effects of QTLs on the number of L type lateral roots, total root length and shoot dry weight (Table 3). The+***K*** genotype produced significantly greater LLRN (5.8%), TRL (75.4%) and shoot dry weight (39.5%) than Nipponbare. On the other hand, the –***K*** genotype produced comparable LLRN, TRL and shoot dry matter with Nipponbare.

Discussion

Soil moisture fluctuation resulting from transient waterlogged-to-drought and vice versa is common in rainfed lowland rice field. Our previous studies have emphasized the significance of plastic LR development and associated physiological responses of roots for plant adaptation to SMF stress (Yamauchi et al. 1996; Wang and Yamauchi 2006; Suralta et al. 2010; Niones et al. 2012). Niones et al. 2012 and Suralta et al. 2010 showed evidence that plasticity in LR development contributed to the increase in root system development based on the total root length under SMF. CSSL47 and Nipponbare parents had similar developmental age based on main stem leaf number when grown under CWL. However, under SMF, these two genotypes significantly differed in root development specifically the LR production at all growth stages (Table 1 and Fig. 2). This result validated our earlier studies demonstrating the significance of LR

Table 3 The contribution of QTLs from Kasalath (***K***) allele on chromosome 12 to shoot dry weight (SDW), L-type lateral root number (LLRN) and total root length (TRL) in response to soil moisture fluctuation stress relative to Nipponbare parent at heading stage

Genotype	SDW (g plant^{-1})	TRL (m plant^{-1})	LLRN (no. cm^{-1})	Number of F_2 lines
Nipponbare	28.9	221.3	1.70	-
CSSL47	37.4 *	237.9 *	1.89*	-
+ **K** genotype	40.3 **	388.2 **	1.81*	12
- **K** genotype	28.7 ^{ns}	183.3 ^{ns}	1.71^{ns}	14

* and **, significantly different from Nipponbare parent at 5 and 1% level, respectively; ^{ns}, not significant

plasticity for rice plant adaptation to SMF (Suralta et al. 2010; Niones et al. 2012). Notably, CSSL47 exhibited faster LR development particularly the L-type as compared to Nipponbare at all growth stages.

Using the above rice mapping population, two QTLs located on the chromosome 12 region (*qTLRN-12*, *qLLRN-12*) were significantly associated with LR development at seedling and vegetative stages (Table 2 and Fig. 4). These QTLs were co-located on the short-arm of chromosome 12 at the flanking markers between RM6296 and TG156, and between TG154 and RM247 loci. The *qTLRN-12* attributed a large QTL effect (57.5%) to the total number of LR expressed during seedling stage (14DAS, hydroponics system) while *qLLRN-12* had a 14.9% QTL effect for L-type LR expressed during vegetative stage (38DAS, soil-filled rootbox system) (Table 2). Unexpectedly, the association of these QTLs controlling LR development in response to SMF was not observed during the heading stage (soil bed system). Although, the QTL associated with the total number of LRs showed a peak on chromosome 12 region at heading stage (soil bed system), its LOD value fell short of the threshold value (<2.5), and thus was excluded as a major QTL (Table 2). There are several possible explanations for the observed differential expression of QTLs at different growth stages. Firstly, the expression of root plasticity at different stages under SMF may be influenced by QTL x environment interaction as has also been reported in other studies (Qu et al. 2008; Paterson et al. 2003; Price et al. 2002; Kamoshita et al. 2002a). Secondly, the QTL for lateral root plasticity is possibly growth stage dependent. This observation was reported in other root studies that found a growth stage-specific expression of different QTLs controlling TRL, RSR, root number and root thickness traits under constant drought stress or a progressive drought stress with a certain maintained soil moisture level (Qu et al. 2008; Price et al. 2002; Champoux et al. 1995). Lastly, we were not able to detect the QTL for LLRN and TLRN because their LOD values (1.5 and 1.2, respectively) fell short to the set threshold value of 2.5, although we found significant variations in LLRN and TLRN at the heading stage among the phenotyped set of F_2 lines (data not shown).

The *qLLRN-12* and *qTLRN-12* exhibited a strong effect on lateral root development in response to SMF stress. The substituted 'Kasalath' allele (+***K*** genotype) on chromosome 12 significantly contributed to the increase in LR development, specifically L-type LR production (53%) compared with those of Nipponbare and -***K*** genotypes under SMF condition (Table 3). This significant increase in LR production directly reflects and influences the size of the entire root system under fluctuating soil moisture conditions (Yamauchi et al. 1987; Wang et al. 2009; Suralta et al. 2010; Niones et al. 2012). The enhanced LR development particularly the L-type LR (contributed by 'Kasalath' allele) contributed to the maintenance of greater root system for better adaptation under drought (Kano et al. 2011; Kano-Nakata et al. 2011) and fluctuating soil moisture (Bañoc et al. 2000; Suralta et al. 2010; Niones et al. 2012) conditions because of its effect on increasing the root surface area for soil water extraction and water use (Kamoshita et al. 2000, 2004; Siopongco et al. 2005, 2006; Kato et al. 2011; Henry et al. 2011; Kobata et al. 1996; Suralta et al. 2010). Consequently, this contributed to the increase in shoot dry matter production (Kano et al. 2011; Kano-Nakata et al. 2011; Bañoc et al. 2000; Suralta et al. 2010; Siopongco et al. 2006) and yield (Niones et al. 2012) under SMF stress.

Most of the root traits in response to water stress that are commonly characterized in QTL mappings were associated to maximum root length, root thickness below 90-cm soil depth, root penetration index, RDW, root branching index, root number and root thickness and RSR (Champoux et al. 1995; Yadav et al. 1997; Ali et al. 2000; Zhang et al. 2001; Price et al. 2002; Babu et al. 2003; MacMillan et al. 2006; Steele et al. 2006; Zheng et al. 2006; Horii et al. 2006; Kamoshita et al. 2002a, 2002b). Conversely, the QTLs *qLLRN-12* and *qTLRN-12* associated with root plasticity identified in this study were different from those reported in other root trait studies, although the latter was identified specifically under constant drought stress conditions. Moreover, in contrast with the earlier findings on QTL of root traits in response to water stress (Zheng et al. 2003; Huang et al. 2004; Horii et al. 2006; Courtois et al. 2009; Gowda et al. 2011), the QTLs associated with LR development identified in this study were located on different chromosome regions. Our findings suggest that the QTL associated with the plasticity on LR development under SMF may be completely different from those that are expressed under constant drought stress. As the expression of these QTLs or genes is dependent on the type of water stress (SMF, progressive drought), timing (growth stage) and intensity, it is unlikely that we can find similar QTLs reported in the earlier findings as none of the previous researches had attempted to identify QTL associated with root responses as plant

adaptation under SMF, which is a common water stress under rainfed lowland conditions. The plasticity in lateral root development under SMF and mild drought is widely observed in various genotypes/cultivars (Bañoc et al. 2000; Kano et al. 2011; Kano-Nakata et al. 2011; Suralta et al. 2010). It is therefore likely that the QTLs or genes for lateral root plasticity may not be specifically unique in Kasalath. Our findings also suggest that the QTL *qLLRN-12* and *qTLRN-12* mapped on chromosome 12 possibly regulate other root traits. The presence of the QTL (+***K*** genotype) contributed by 'Kasalath' segment on chromosome 12 promotes L-type LR development, which was responsible for the increase in TRL and subsequent increase in SDW (Table 3).

In conclusion, our earlier studies have demonstrated the significant functions of lateral root production on the root system development for the adaptation to fluctuating moisture environment (Niones et al. 2012). This study has shown two closely linked QTLs on chromosome 12 region (*qTLRN*-12 and *qLLRN*-12) at seedling and vegetative stage, respectively. These QTLs were found at the flanking markers between TG154 and RM247, and between RM6296 and TG156, respectively. These QTLs are most likely positioned at 25.5 cM (*qTLRN-12*) and 42.2 cM (*qLLRN-12*), respectively on the short-arm of chromosome 12 region. Individual lines carrying introgressed 'Kasalath' segment (+**K** genotypes) on chromosome 12 have contributed to the root system development and shoot dry matter production greater than either Nipponbare parent or lines without introgressed 'Kasalath' segment under SMF stress. Greater root system development mainly due to the plasticity in LR production could contribute to higher photosynthetic activity, stomatal conductance and transpiration, and an increase in dry matter production (Niones et al. 2012; Kano et al. 2011; Suralta et al. 2010). Although the QTLs of interest were identified in the mapping population used in this study, further development of near isogenic lines is still needed for fine mapping to enhance the understanding on the genetic mechanisms controlling LR development under SMF environment. In addition, this study showed that L-type LR development is the main attribute in the plasticity of the entire root system development and thus the key trait for broader adaptation to SMF, which is totally different from those traits (i.e. root length, deep root ratio) reported in previous studies. In a series of our studies with special attention to lateral root heterorhizy, we have demonstrated the significant functional roles of these QTLs under SMF. These QTLs associated with LR plasticity are essential to improve the adaptive capability of cultivars under fluctuating soil moisture environment such as those in rainfed lowlands.

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